PHARMACOKINETICS OF FLORFENICOL IN SERUM AND SYNOVIAL FLUID SAMPLES AFTER REGIONAL INTRAVNEOUS PERFUSION IN THE DISTAL LIMB OF CATTLE

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PHARMACOKINETICS OF FLORFENICOL IN SERUM AND SYNOVIAL FLUID SAMPLES AFTER REGIONAL INTRAVNEOUS PERFUSION IN THE DISTAL LIMB OF CATTLE

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CHAPTER I

INTRODUCTION

Background

Lameness is an important problem in cattle operations due to its effects on animal health and production as well as animal welfare.[1] The etiology of lameness is often multifactorial. Regardless of the cause, chronic lameness frequently leads to decreased reproductive performance,[2-5] decreased milk production[6,7] and premature culling.[8-10] The incidence and prevalence of lameness in dairy cattle have received considerable investigation.[11-16] A recent study reported a lameness prevalence of 24.6% in Minnesota dairy cows.[17] In one study in dairy cows in England, lameness accounted for 27% of the health costs.[18] Similar studies in beef cattle are limited; however, the 1999 National Market Cow and Bull Beef Quality Audit found an increased incidence of lameness in culled beef cows and bulls compared to the 1994 National Non-Fed Beef Quality Audit.[19] The causes of lameness in cattle have been recently reviewed.[20,21] A systematic compilation of the literature on lameness has also been published.[22]

Infectious processes such as osteomyelitis, septic arthritis, and tenosynovitis, collectively known as deep digital sepsis (DDS), are among the most debilitating causes of lameness, often resulting in significant loss of function or destruction of the animal.[23] These conditions often occur secondary to more common causes of lameness such as sole ulcers and interdigital necrobacillosis[20,24,25] and may or may not be associated with

significant cellulitis of local soft tissues. Antimicrobial therapy alone is generally ineffective for DDS and must be accompanied by surgical therapy including debridement, drainage and lavage, and stabilization of affected tissues.[23, 26-31] In addition, systemic antibiotics may need to be administered for a long period of time to eliminate infection. A treatment period of four to six weeks is generally recommended.[23,32-35] Penetration of antibiotics into bone does not appear to be a limiting factor as numerous antibiotics reach therapeutic levels in normal bones and joints after systemic administration.[36] Reasons for the limited success of antibiotic therapy alone include the presence of necrotic material, poor vascular perfusion, the presence of inflammatory mediators, entrapping of bacteria in fibrin, and the presence of biofilm or glycocalyx surrounding foreign material or surgical implants.[28,37-41] Treatment options for orthopedic infections in cattle[23,31,42] and horses[28,30] have been reviewed.

Regional intravenous antibiotic perfusion (RIVP) of the distal limb may offer several advantages over systemic antibiotic administration when treating cases of DDS. The advantages include higher concentrations of antibiotic at the site of infection and reduced potential for systemic toxicity due to lower systemic drug concentrations.[40,43,44] Other potential advantages include decreased duration of antibiotic therapy and reduced drug costs.

Florfenicol is a phenolic antibiotic that inhibits the 50S subunit of the bacterial ribosome.[45] Florfenicol is approved for use in the treatment of bovine respiratory disease complex and foot rot in cattle[a]. Although labeled for intramuscular or subcutaneous

[a] www.nuflor.com

administration, florfenicol can be safely administered intravenously.[46-50] These factors make florfenicol a logical choice for evaluation in the application of regional antibiotic perfusion.

Objective

The objective of this study was to define the pharmacokinetics of florfenicol in digital blood, metatarsophalangeal synovial fluid, and jugular blood samples following regional intravenous administration of 2.2 mg/kg florfenicol to the distal limb of cattle. If adequate concentrations can be achieved and maintained, administration of florfenicol in this manner may be a useful tool in the treatment of infectious conditions involving the deep structures of the foot of cattle.

Florfenicol Pharmacology

Florfenicol is a prescription antibiotic labeled for use in the United States for the treatment of cattle, pigs, catfish, and salmon. It is a derivative of thiamphenicol, in which the hydroxyl group has been replaced by fluorine (Figure 1). Like thiamphenicol, florfenicol lacks the p-nitro group which is thought to cause the dose-independent, irreversible bone marrow suppression associated with chloramphenicol. Substitution of the 3-hydroxyl group with fluorine results in a molecule that is not susceptible to chloramphenicol transacetylases.[51] In cattle, florfenicol is approved for the treatment of bovine respiratory disease caused by *Mannhaemia haemolytica*, *Pasteurella multocida*, and *Histophilus somnus* and for the treatment of infectious pododermatitis caused by *Bacteroides melaninogenicus* and *Fusobacterium necrophorum*. Florfenicol is labeled for intramuscular administration at 20 mg/kg repeated in 48 hours or subcutaneous

administration once at 40 mg/kg (NADA 141-063[b]). In Canada, florfenicol is also approved for the treatment of infectious keratoconjunctivitis caused by *Moraxella bovis*.

Florfenicol has a broader spectrum of activity than chloramphenicol and thiamphenicol and is typically considered bacteriostatic. Similar to chloramphenicol, florfenicol has high bioavailability and wide tissue distribution. In cattle, florfenicol is excreted primarily by the kidney as unchanged drug.[50]

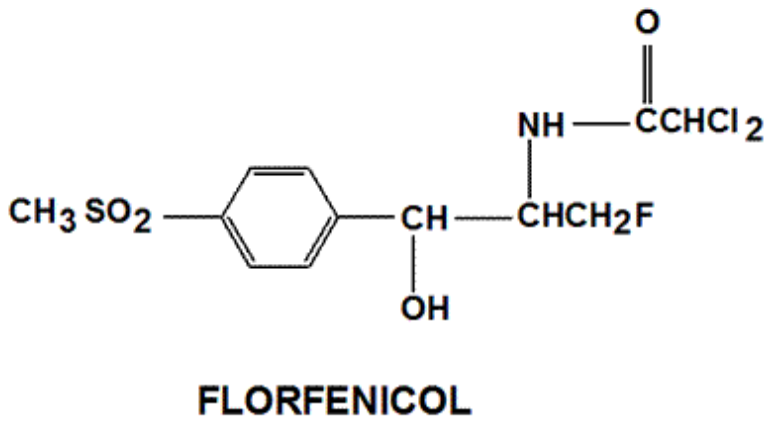

Figure 1. Chemical structure of florfenicol. Image obtained from www.umesc.usgs.gov/aquatic/drug_research/florfenicol/florfenicol_structure_400.gif

[b] http://www.fda.gov/cvm/FOI/141-063s011499.pdf

Extralabel Drug Use in Food Animals

Using antibiotics in regional perfusion systems constitutes extra-label drug use. The Animal Medicinal Drug Use Clarification Act of 1996 (AMDUCA) allows for the use of Food and Drug Administration (FDA) approved drugs in an extra-label manner as long as certain conditions are met. The reader is referred to FDA's AMDUCA website[c] for more information. In the veterinary literature, aminoglycosides are the most common antibiotics used in regional perfusion for the treatment of orthopedic infection in species other than cattle. Although not prohibited by AMDUCA, the use of aminoglycosides in food animals is discouraged due to the need for extended withdrawal periods.[52] Also, several antibiotics that have reportedly been used in local delivery systems are prohibited for use in food animals. The reader is referred to the Food Animal Residue Avoidance Databank (FARAD)[d] for a current list of drugs prohibited for extra-label use in food animals. Due to the limited availability of antibiotics suitable for regional perfusion in food animals, the potential use of florfenicol in regional perfusion warrants investigation. Extra-label use of florfenicol is not prohibited as long as the provisions of AMDUCA are followed. The FDA has established a tolerance level of 3.7 parts per million for the florfenicol metabolite, florfenicol amine, in cattle liver. No tolerance level has been established in milk so any detectable residue is considered a violation.[e]

[c] www.fda.gov/cvm/amducatoc.htm

[d] www.farad.org/

[e] http://www.cfsan.fda.gov/~acrobat/mi-06-5.pdf

CHAPTER II

REVIEW OF LITERATURE

Pharmacokinetics of Florfenicol

The pharmacokinetics of florfenicol have received considerable investigation in a variety of animal species. The broad application of this antimicrobial is an indication of its utility in veterinary medicine. The pharmacokinetics of florfenicol have been described in swine,[53-56] camels,[57] goats,[57-59] sheep,[57,60,61] elk,[62] chickens,[63-65] muscovy ducks,[66] rabbits,[67,68] loggerhead sea turtles,[69] rhesus macaques,[70] horses,[71] atlantic salmon,[72,73] catfish,[74] cod,[75] koi carp,[76] red pacu,[77] white spotted bamboo sharks,[78] and cattle.[47-50,79-84]

Selected pharmacokinetic parameters from several studies investigating the florfenicol pharmacokinetics in pigs are presented in Table 1. Jiang et al.[53] studied the pharmacokinetics of florfenicol in pigs following intravenous (IV), intramuscular (IM), and oral (PO) dosing of 20 mg/kg florfenicol where as Voorspoels et al.[54] reported the florfenicol pharmacokinetics in pigs following IM and PO doses of 15 mg/kg. Additionally, Jiang et al.[53] examined the effect of feed intake on the pharmacokinetics of orally administered florfenicol. Fasted pigs exhibited a higher peak concentrations (Cmax) where as fed pigs exhibited a longer mean residence time. The Area Under the Curve (AUC) and elimination half life were not different between the two groups. Liu[55] compared the pharmacokinetics of florfenicol in healthy pigs and pigs experimentally

infected with *Actinobacillus pleuropneumoniae*. These authors found no statistical differences in pharmacokinetic parameters between the healthy and diseased pigs. In two separate studies,[53,55] the Cmax achieved after PO dosing was higher than that achieved after IM dosing. In another study, Liu et al.,[56] reported the tissue pharmacokinetics of florfenicol in pigs. This study found the highest florfenicol concentrations in the kidney and lung while liver and muscle exhibited the longest elimination half lives.

Several investigators have evaluated the pharmacokinetics of florfenicol in small ruminant species. The results of these studies are summarized in Tables 2-4. Atef et al.[58] and Ali et al.[57] describe the pharmacokinetics of florfenicol after IV and IM administration at 20 mg/kg to goats. Both studies report good tissue distribution and bioavailability as well as a prolonged elimination half life after IM administration when compared to IV administration. In another study, Atef et al.[59] found significant differences in pharmacokinetic parameters depending on the assay method used. These authors administered florfenicol at 20 mg/kg through both IV and IM routes. The samples were then divided and analyzed using High Pressure Liquid Chromatography (HPLC) and a microbiologic assay. For the IV study, the HPLC assay revealed significantly higher initial concentrations and AUC values, lower Volume of Distribution at steady state (Vd_{ss}) and Clearance (Cl) values, and longer elimination half lives compared to the microbiologic assay. The HPLC assay also revealed significantly higher values for AUC and bioavailability for the IM study. These findings indicate that care should be taken when comparing results obtained by different analytical methods. Jianzhong et al.[60] described larger AUC values and longer elimination half lives for florfenicol administered to sheep at 20 mg/kg and 30 mg/kg by IV and IM routes when

compared to values reported for goats[59]. Lane et al.[61] reported values of 206 ± 31 μg·h/ml, 34.7 ± 9.6 hr, and 65.0 ± 12.2 % for AUC, elimination half life, and bioavailability respectively, following subcutaneous (SQ) dosing of 40 mg/kg florfenicol once daily for three days in sheep. The data presented from this study in Table 3 were obtained after the first SQ dose so that more accurate comparisons could made with other values in the table. The pharmacokinetics of florfenicol in goats, sheep, and camels were compared by Ali et al.[57] These authors state that while the data from the three species are similar, the rate and extent of absorption appear lower in camels compared to sheep and goats. Evaluation of the pharmacokinetics of florfenicol following administration of 40 mg/kg SQ to North American Elk revealed a shorter elimination half life compared to cattle, leading the authors to recommend once daily dosing of florfenicol in this species.[62]

The pharmacokinetics of florfenicol in broiler chickens and Muscovy ducks have been defined. A summary of the pharmacokinetic parameters reported in these studies is reported in Tables 5 and 6, respectively. Afifi et al.[63] defined the pharmacokinetics of florfenicol in chickens following IV, IM, and PO doses of 30 mg/kg. These authors also reported tissue concentrations of florfenicol following multiple IM and PO doses of 30 mg/kg. They found that the kidney and bile had the highest concentrations and that florfenicol could not be detected in any tissue except bile after 72 hours. Florfenicol could not be detected in bile after 96 hours. Shen et al.[65] described the pharmacokinetics of florfenicol in chickens following IV, IM and PO doses of 15 mg/kg and 30 mg/kg. These authors found that florfenicol exhibited very high bioavailability following both IM and PO dosing but that it was rapidly eliminated, leading the authors to recommend twice daily dosing to maintain adequate concentrations. Infection with *Escherichia coli*

had little effect on the pharmacokinetics of florfenicol in broiler chickens.[64] *Pasteurella multocida* infection in Muscovy ducks resulted in lower serum florfenicol concentrations and shorter elimination half lives.[66] In this study, both healthy and diseased birds exhibited greatly increased elimination half lives compared to values reported for chickens.

Two reports describe the pharmacokinetics of florfenicol in rabbits.[67, 68] Selected pharmacokinetic parameters from these studies are presented in Table 7. El-Aty et al.[68] reported the pharmacokinetics of florfenicol in rabbits following IV, IM, and PO doses of 30 mg/kg. Following IV administration, florfenicol was rapidly eliminated and could only be detected up to 10 hours after administration. Interestingly, this study described absorption rate-dependent elimination, also known as "flip-flop" kinetics, following IM and PO administration. Park et al.[67] described the pharmacokinetics of florfenicol and its major metabolite, florfenicol amine, in rabbits following IV and PO administration of 20 mg/kg.

McKellar et al.[71] described the pharmacokinetics of florfenicol in horses and ponies after IV, IM, and PO doses 22 of mg/kg. The pharmacokinetic data from this study are presented in Table 8. These authors reported that florfenicol was well absorbed following both IM and PO dosing with the PO dose resulting in higher, but shorter lived, concentrations compared to IM dosing. All of the horses in this study developed loose stool following florfenicol administration causing the authors to discourage use of florfenicol in horses until further safety studies could be conducted.

The pharmacokinetics of florfenicol have been described in a number of aquatic species under a variety of circumstances. Species studied include atlantic salmon,[72,73]

korean catfish,[74] cod,[75] koi carp and threespot gourami,[76] red pacu,[77] and white-spotted bamboo sharks.[78] Selected pharmacokinetic parameters from these studies are presented in Table 9. Florfenicol depletion studies have been conducted in atlantic salmon[73] and channel catfish[85] for the purposes of establishing appropriate withdrawal periods. Stamper et al.[69] described the pharmacokinetics of florfenicol in loggerhead sea turtles. This study had a very small number of experimental units and data from this study are not presented.

Cook et al.[70] described the pharmacokinetics of florfenicol in rhesus macaques following an IM dose of 50 mg/kg. The data from this study was presented as individual animal data, and means, standard deviations, and standard errors were not provided. Median values were presented in the text. Data from this study are not presented. These authors concluded that administration of florfenicol to rhesus macaques at a dose of 50mg/kg IM every 48 hours should be an effective treatment for common infectious conditions.

Several investigators have described the pharmacokinetics of florfenicol in cattle. The pharmacokinetic parameters reported in these studies are presented in Table 10. In 1986, Varma et al.[50] described the pharmacokinetics of florfenicol in veal calves following a dose of 22 mg/kg administered IV, PO to fed calves, and PO to fasted calves. Calves that had been fasted for 12 hours prior to drug administration had higher serum concentrations, shorter time to Cmax, and higher bioavailability than calves that had been fed immediately prior to drug administration. Adams et al.[49] reported the pharmacokinetics of florfenicol after IV administration of 11 mg/kg in veal calves. This study also reported the pharmacokinetics and tissue concentrations of florfenicol

following oral doses of 11 mg/kg every twelve hours for seven doses.[49] The IV study demonstrated rapid distribution and elimination of florfenicol while the multiple dose PO study found a prolonged absorption phase in two calves that resulted in these calves exhibiting "flip-flop" kinetics. Florfenicol was found in the highest concentrations in the urine, kidney, and bile. Bretzlaff et al.[47] described the pharmacokinetics of florfenicol in non-lactating dairy cattle following an IV dose of 50 mg/kg. These authors also evaluated the effect of florfenicol on phagocytosis by blood neutrophils and found that, at all concentrations tested, florfenicol inhibited phagocytosis by neutrophils. They stated that the clinical significance of this finding was unknown. Soback et al.[48] described the pharmacokinetics of florfenicol following IV, IM and intra-mammary (IMM) doses of 20 mg/kg in lactating cows. Interestingly, florfenicol was more rapidly absorbed and reached higher serum concentrations following IMM dosing compared to IM dosing. These authors also describe absorption rate-dependent, or "flip-flop" kinetics following IM administration indicating that the drug is absorbed slowly from the IM administration site. Following IV and IM administration of 20 mg/kg florfenicol to feeder calves, Lobell et al.[84] reported a median bioavailability of 78.5%. The plasma and cerebrospinal fluid (CSF) pharmacokinetics of florfenicol following an IV dose of 20 mg/kg have been described by de Craene et al.[46] These authors reported a maximum CSF concentration of 4.67 μg/ml achieved 2 hours after dosing, indicating that florfenicol effectively crossed the blood-brain barrier. Varma et al.[86] described the pharmacokinetics of florfenicol following a SQ dose of 40 mg/kg and two IM doses of 20 mg/kg administered 48 hours apart. Florfenicol was absorbed rapidly after SQ administration and the single SQ dose resulted in an AUC value similar to that obtained after the two IM doses.

Table 1. Reported pharmacokinetic parameters for florfenicol in swine.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Pigs	20	IV	Non	HPLC	90.1 ± 6.5			0.2 ± 0.0	1.5 ± 0.2	6.7 ± 1.6		Jiang, 2006[53]
Pigs	20	IM	Non	HPLC	84.3 ± 18.9	3.5 ± 0.5	1.0 ± 0.4			17.2 ± 3.0	96.9 ± 20.8	Jiang, 2006[53]
Pigs	20	PO	Non	HPLC	132.1 ± 17.6	9.9 ± 2.4	1.5 ± 0.6			10.0 ± 2.1	148.5 ± 26.2	Jiang, 2006[53]
Pigs	15	IM	###	HPLC	110.6 ± 31.5	7.3 ± 6.0	2.3 ± 1.2			14.6 ± 7.2		Voorspoels, 1999[54]
Pigs	15	PO	###	HPLC	103.4 ± 17.8	14.8 ± 2.6	3.0 ± 0.9			5.5 ± 4.2		Voorspoels, 1999[54]
Pigs	20	IV	###	HPLC	66.17 ± 7.13			0.3 ± 0.0	1.0 ± 0.1	2.6 ± 0.5		Liu, 2003[55]
Pigs	20	IV*	###	HPLC	64.9 ± 14.4			0.3 ± 0.1	1.2 ± 0.4	2.9 ± 0.8		Liu, 2003[55]
Pigs	20	IM	1 comp	HPLC	68.6 ± 10.4	3.2 ± 0.6	0.9 ± 0.3			14.3 ± 2.7	103.7 ± 26.8	Liu, 2003[55]
Pigs	20	IM*	1 comp	HPLC	79.6 ± 26.8	4.0 ± 0.8	0.8 ± 0.3			13.9 ± 4.1	122.8 ± 44.4	Liu, 2003[55]
Pigs	20	PO	2 comp	HPLC	65.9 ± 12.8	10.8 ± 2.7	1.4 ± 0.7			12.4 ± 7.3	99.6 ± 19.3	Liu, 2003[55]
Pigs	20	PO*	2 comp	HPLC	73.3 ± 14.7	8.1 ± 2.7	1.9 ± 0.5			16.5 ± 4.2	112.9 ± 8.8	Liu, 2003[55]
Pigs	20	IM	Non	HPLC	87.2					13.6		Liu, 2002[56]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral, * indicates pigs were infected with *Actinobacllius pleuropneumoniae*. For model: Non = Noncompartmental analysis, 1 comp = One-compartment modeling, 2 comp = Two-compartment modeling, ### indicates that model type was not specified. For assay: HPLC = high performance liquid chromatography.

Table 2. Reported pharmacokinetic parameters for florfenicol in goats.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Goat	20	IV	2 comp	MBA	6.3 ± 0.5			3.3 ± 0.3	3.4 ± 0.3	0.9 ± 0.1		Atef, 2000[58]
Goat	20	IM	2 comp	MBA	4.1 ± 0.2	0.9 ± 0.0	1.2 ± 0.1			2.2 ± 0.1	65.7 ± 3.4	Atef, 2000[58]
Goat	20	IV	2 comp	MBA	74.1 ± 7.9			0.3 ± 0.0	0.6 ± 0.1	1.2 ± 0.2		Ali, 2003[57]
Goat	20	IM	2 comp	MBA	58.7 ± 7.5	1.2 ± 0.1	1.13 ± 0.13			2.123 ± 0.183	60.9 ± 5.9	Ali, 2003[57]
Goat	20	IV	2 comp	MBA	6.3 ± 0.5			3.3 ± 0.3	3.4 ± 0.3	0.9 ± 0.5		Atef, 2001[59]
Goat	20	IV	2 comp	HPLC	39.1 ± 5.3			0.6 ± 0.1	1.7 ± 0.1	2.6 ± 0.2		Atef, 2001[59]
Goat	20	IM	2 comp	MBA	4.1 ± 0.2	0.9 ± 0.0	1.2 ± 0.1			2.2 ± 0.1	65.7 ± 3.4	Atef, 2001[59]
Goat	20	IM	2 comp	HPLC	33.7 ± 2.0	2.4 ± 0.6	1.6 ± 0.2			2.7 ± 0.2	86.2 ± 1.1	Atef, 2001[59]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular. For model: 2 comp = Two-compartment modeling. For assay: HPLC = high performance liquid chromatography, MBA = microbiologic assay.

Table 3. Reported pharmacokinetic parameters for florfenicol in sheep.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Sheep	20	IV	2 comp	MBA	62.5 ± 6.6			0.3 ± 0.0	0.7 ± 0.1	1.3 ± 0.1		Ali, 2003[57]
Sheep	20	IM	2 comp	MBA	49.6 ± 5.5	1.0 ± 0.1	1.4 ± 0.2			2.3 ± 0.2	65.8 ± 6.7	Ali, 2003[57]
Sheep	20	IV	3 comp	HPLC	76.3 ± 9.1			0.3 ± 0.0	1.7 ± 0.1	18.8 ± 6.8		Jianzhong, 2004[60]
Sheep	30	IV	3 comp	HPLC	119.2 ± 2.1			0.3 ± 0.0	1.7 ± .0	18.7 ± 1.9		Jianzhong, 2004[60]
Sheep	20	IM	1 comp	HPLC	68.0 ± 0.6	4.1 ± 0.3	1.5 ± 0.2			10.3 ± 1.1	89.0	Jianzhong, 2004[60]
Sheep	30	IM	1 comp	HPLC	102.0 ± 1.9	7.0 ± 1.6	1.34 ± 0.34			9.6 ± 2.8	85.5	Jianzhong, 2004[60]
Sheep	40	IV	Non	HPLC	107.0 ± 18.0	86.7 ± 45.1	0.1 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	1.1 ± 0.0		Lane, 2004[61]
Sheep	40	SQ*	Non	HPLC	42.3 ± 13.4	2.6 ± 1.1	2.0 ± 1.0			34.7 ± 9.6	40.2 ± 16.2	Lane, 2004[61]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, SQ = subcutaneous. SQ* = SQ dose administered daily for three days. Data presented are those obtained after the first dose, prior to the second dose. For model: Non = Noncompartmental analysis, 1 comp = One-compartment modeling, 2 comp = Two-compartment modeling, 3 comp = Three-compartment modeling. For assay: HPLC = high performance liquid chromatography, MBA = microbiologic assay.

Table 4. Reported pharmacokinetic parameters for florfenicol in dromedary camel and North American elk.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Camel	20	IV	2 comp	MBA	60.61 ± 7.0			0.3 ± 0.0	0.9 ± 0.1	1.5 ± 0.2		Ali, 2003[57]
Camel	20	IM	2 comp	MBA	41.9 ± 5.8	0.8 ± 0.1	1.5 ± 0.1			2.5 ± 0.3	69.2 ± 7.8	Ali, 2003[57]
Elk	40	SQ	Non	HPLC	134.0 ± 89.0	3.7 ± 1.9	4.2 ± 1.7			44.0 ± 15.0		Alcorn, 2004[62]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, SQ = subcutaneous. For model: Non = Noncompartmental analysis, 2 comp = Two-compartment modeling. For assay: HPLC = high performance liquid chromatography, MBA = microbiologic assay.

Table 5. Reported pharmacokinetic parameters for florfenicol in broiler chickens.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Chicken	30	IV	2 comp	MBA	1140.0 ± 60.0			1.6 ± 0.2	5.1 ± 0.7	2.9 ± 0.1		Afifi, 1997[63]
Chicken	30	IM	###	MBA	1100.0 ± 20.0	3.8 ± 0.2	1.7 ± 0.1			3.4 ± 0.2	96.6 ± 6.5	Afifi, 1997[63]
Chicken	30	PO	###	MBA	630.0 ± 20.0	3.2 ± 0.2	1.1 ± 0.7			1.8 ± 0.2	55.3 ± 3.7	Afifi, 1997[63]
Chicken	15	IV	2 comp	HPLC	14.8 ± 2.3			1.0 ± 0.2	5.0 ± 1.1	2.8 ± 0.7		Shen, 2003[65]
Chicken	30	IV	2 comp	HPLC	29.5 ± 4.5			1.0 ± 0.2	3.5 ± 1.0	3.0 ± 1.2		Shen, 2003[65]
Chicken	15	IM	1 comp	HPLC	14.0 ± 2.5	3.5 ± 1.1	0.6 ± 0.4			2.5 ± 1.2	95.0	Shen, 2003[65]
Chicken	30	IM	1 comp	HPLC	28.8 ± 5.6	6.8 ± 1.4	0.7 ± 0.3			2.4± 0.3	98.0	Shen, 2003[65]
Chicken	15	PO	1 comp	HPLC	14.2 ± 2.5	4.4 ± 1.7	0.9 ± 0.4			1.7 ± 0.4	96.0	Shen, 2003[65]
Chicken	30	PO	1 comp	HPLC	27.6 ± 8.8	5.8 ± 2.4	1.4 ± 0.4			2.3 ± 0.5	94.0	Shen, 2003[65]
Chicken	30	IV	2 comp	HPLC	41.7 ± 6.9			0.7 ± 0.1	1.2 ± 0.4	2.7 ± 0.5		Shen, 2002[64]
Chicken	30	IV*	1 comp	HPLC	40.9 ± 6.0			0.8 ± 0.1	1.0 ± 0.2	2.0 ± 0.2		Shen, 2002[64]
Chicken	30	IM*	1 comp	HPLC	35.6 ± 7.2	8.9 ± 1.2	0.8 ± 0.5			2.2 ± 0.2	87.0	Shen, 2002[64]
Chicken	30	PO*	1 comp	HPLC	29.0 ± 6.6	7.9 ± 3.0	1.1 ± 0.4			1.7 ± 0.3	71.0	Shen, 2002[64]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral. For model: 1 comp = One-compartment modeling, 2 comp = Two-compartment modeling, ### indicates that model type was not specified. For assay: HPLC = high performance liquid chromatography, MBA = microbiologic assay. * indicates that birds were infected with *Escherichia coli*.

Table 6. Reported pharmacokinetic parameters for florfenicol in Muscovy ducks.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Ducks	30	IV	2 comp	MBA				0.6 ± 0.0	5.1 ± 0.1	7.2 ± 0.1		El-Banna, 1998[66]
Ducks	30	IV*	2 comp	MBA				2.0 ± 0.1	13.2 ± 0.8	5.9 ± 0.2		El-Banna, 1998[66]
Ducks	30	IM	###	MBA		3.0 ± 0.0	1.2 ± 0.0			7.4 ± 0.1	73.0 ± 0.6	El-Banna, 1998[66]
Ducks	30	IM*	###	MBA		1.3 ± 0.1	1.1 ± 0.0			5.2 ± 0.2	77.2 ± 0.8	El-Banna, 1998[66]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular. For model: 2 comp = Two-compartment modeling, ### indicates that model type was not specified. For assay: MBA = microbiologic assay. * indicates that birds were infected with *Pasteurella multocida*.

Table 7. Reported pharmacokinetic parameters for florfenicol in rabbits.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Rabbit	20	IV	Non	LC/MS	32.0 ± 3.0			0.6 ± 0.1	0.9 ± 0.2	0.9 ± 0.2		Park, 2007[67]
Rabbit	20	PO	Non	LC/MS	23.8 ± 5.0	8.0 ± 2.8	0.9 ± 0.4			1.4 ± 0.6	76.2 ± 12.0	Park, 2007[67]
Rabbit	30	IV	Non	MBA	98.1 ± 14.3			0.3 ± 0.1	0.6 ± 0.9	1.5 ± 0.5		El-Aty, 2004[68]
Rabbit	30	IM	Non	MBA	86.6 ± 12.0	21.7 ± 2.6	0.5 ± 0.0			3.0 ± 1.5	88.3 ± 17.4	El-Aty, 2004[68]
Rabbit	30	PO	Non	MBA	49.0 ± 13.1	15.1 ± 4.5	0.5 ± 0.0			2.6 ± 1.7	50.8 ± 14.3	El-Aty, 2004[68]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral. For model: Non = Noncompartmental analysis. For assay: LC/MS = liquid chromatography/mass spectrometry, MBA = microbiologic assay.

Table 8. Reported pharmacokinetic parameters for florfenicol in horses and ponies.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Horse	22	IV	2 comp	HPLC	64.2 ± 9.6			0.4 ± 0.1	0.7 ± 0.2	1.8 ± 0.9		McKellar, 1996[71]
Horse	22	IM	###	HPLC	52.0 ± 0.3	4.1 ± 1.2	1.3 ± 0.5				81.0	McKellar, 1996[71]
Horse	22	PO	###	HPLC	53.5 ± 0.2	13.8 ± 4.8	1.1 ± 0.5				83.3	McKellar, 1996[71]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral. For model: 2 comp = Two-compartment modeling, ### indicates that model type was not specified. For assay: HPLC = high performance liquid chromatography.

Table 9. Reported pharmacokinetic parameters for florfenicol in various fish species.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Altlantic salmon	10	IV	2 comp	HPLC	116.3			0.1	1.1	12.2		Martinsen, 1993[72]
Altlantic salmon	10	PO	1 comp	HPLC	112.0	4.0	10.3				96.5	Martinsen, 1993[72]
Altlantic salmon	10	IV	2 comp	HPLC	133.0			0.1	1.3	14.7		Horsberg, 1996[73]
Altlantic salmon	10	PO	1 comp	HPLC	139.9	9.1	6.0				99.0	Horsberg, 1996[73]
Korean catfish	20	IV	Non	LC/MS	269.6 ± 51.7			0.1 ± 0.0	1.1 ± 0.1	11.1 ± 1.1		Park, 2006[74]
Korean catfish	20	PO	Non	LC/MS	246.6 ± 14.7	9.6 ± 0.4	8.0			15.7 ± 2.6	92.6 ± 10.1	Park, 2006[74]
Cod	10	IV	2 comp	HPLC	573.0			0.0	1.1	43.0		Samuelson, 2003[75]
Cod	10	PO	1 comp	HPLC	524.0	10.8	7.0			39.0	91.0	Samuelson, 2003[75]
Koi carp	25	IM	Non	HPLC	444.4	18.0	24.0			13.9		Yanong, 2005[76]
Koi carp	50	PO	Non	HPLC	262.6	12.3	3.0			7.6		Yanong, 2005[76]
Threespot gourami	50	IM	Non	HPLC	188.6	28.0	3.0			2.5		Yanong, 2005[76]
Threespot gourami	50	PO	Non	HPLC	27.5	2.6	5.0			6.6		Yanong, 2005[76]
Red pacu	10	IM	###	HPLC		1.1 ± 0.1	3.0			4.3 ± 1.0		Lewbart, 2005[77]
White-spotted bamboo shark	40	IM	Non	HPLC	5248.9 ± 2537.6	11.9 ± 1.5	54.0 ± 19.0			269.8 ± 135.9		Zimmerman, 2006[78]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral. For model: Non = Noncompartmental analysis, 1 comp = One-compartment modeling, 2 comp = Two-compartment modeling, ### indicates that model type was not specified. For assay: HPLC = high performance liquid chromatography, LC/MS = liquid chromatography/mass spectrometry.

Table 10. Reported pharmacokinetic parameters for florfenicol in cattle.

Species	Dose (mg/kg)	Route	Model	Assay	AUC (µg·h/ml)	Cmax (µg/ml)	Tmax (hr)	Cl (L/kg/hr)	Vdss (L/kg)	Elimination t1/2 (hr)	F (%)	Reference
Cattle	20	IV	2 comp	HPLC	97.5 ± 18.0			0.2 ± 0.1	1.0 ± 0.2	3.2 ± 1.0		de Craene, 1997[46]
Cattle	22	IV	2 comp	HPLC	126.2			0.2	0.8	2.9		Varma. 1986[50]
Cattle	22	PO fed	1 comp	HPLC	72.4	9.4 ± 1.1	3.4 ± 0.7				65.0	Varma. 1986[50]
Cattle	22	PO fast	1 comp	HPLC	105.6	11.3 ± 4.0	2.5 ± 0.7				88.0	Varma. 1986[50]
Cattle	50	IV	3 comp	HPLC				0.2 ± 0.0	0.6 ± 0.0	3.2		Bretzlaff, 1987[47]
Cattle	11	IV	2 comp	HPLC	63.9			0.2	0.9	3.7		Adams, 1987[49]
Cattle	11	PO*	1 comp	HPLC	62.5	5.7 ± 0.3	3.6 ± 0.7			3.7	88.9	Adams, 1987[49]
Cattle	11	PO**	1 comp	HPLC	112.4	8.2 ± 1.3	3.4 ± 0.5			5.6		Adams, 1987[49]
Cattle	20	IV	3 comp	HPLC	89.5			0.2	0.8	2.7		Lobell, 1994[84]
Cattle	20	IM	###	HPLC	70.7	3.1	3.3			18.3	78.5	Lobell, 1994[84]
Cattle	20	IV	Non	HPLC	128.4 ± 29.6			0.2 ± 0.0	0.4 ± 0.1	3.0 ± 0.4		Soback, 1995[48]
Cattle	20	IM	Non	HPLC	56.2 ± 18.3	2.3	3.0			12.5 ± 3.6	38.0 ± 14.0	Soback, 1995[48]
Cattle	20	IMM	Non	HPLC	67.4 ± 19.0	6.9	6.0			3.9 ± 1.8	54.0 ± 18.0	Soback, 1995[48]
Cattle	20	IM	###	###	226.6	8.9	3.6					Varma, 1998[85]
Cattle	40	SQ	###	###	224.5	5.4	5.3					Varma, 1998[85]

AUC = Area under the Concentration vs. time curve, Cmax = maximum concentration, Tmax = time to maximum concentration, Cl = Clearance, Vdss = Volume of distribution at steady state, F = bioavailability. For route: IV = intravenous, IM = intramuscular, PO= oral, SQ = subcutaneous, IMM = intramammary. For model: Non = Noncompartmental analysis, 1 comp = One-compartment modeling, 2 comp = Two-compartment modeling, 3 comp = Three-compartment modeling. ### indicates that model and/or assay type was not specified. For assay: HPLC = high performance liquid chromatography. * indicates data obtained after first dose, ** indicates data obtained after seventh dose in multiple dose study.

Tourniquet Use

Tourniquet use is common in human surgery to reduce blood loss and provide a bloodless surgical field.[87] The application of regional anesthesia distal to a tourniquet is also practiced.[88] In veterinary medicine, tourniquets are used for similar purposes. Tourniquets are routinely applied in the application of intravenous regional anesthesia[89,90] and regional antibiotic delivery[91,92] in a variety of species including horses,[93] cattle,[91,94] sheep,[95] dogs,[96] and cats.[97] Tourniquet use in veterinary surgery has been reviewed.[98]

Application of a tourniquet to a limb interrupts blood flow and induces ischemia distal to the tourniquet.[99] The physiologic effects of tourniquet application to the limb have been investigated in cattle[100,101] and horses.[102,103] Singh et al.[100] studied the effects of a tourniquet applied proximal to the elbow and left in place for 90 minutes in buffalo calves. They found that the tourniquet induced a significant decrease in pH and an increase in carbon dioxide concentration in the limb distal to the tourniquet. They state that the pH returned to baseline values 5 minutes after tourniquet release but then increased above baseline values and remained elevated throughout the study period (150 minutes). These authors also state that oxygen exchange in the limb tissue was impaired throughout the study period. These findings led them to conclude that tourniquet application for periods of 90 minutes was not safe in buffalo calves. In a similar study involving cattle, Chawla et al.[101] investigated the effects of a tourniquet placed proximal to the elbow and left in place for 60 minutes. The findings of this study were similar to those of Singh et al.[100] leading the authors to conclude that tourniquet application in buffaloes for 60 minutes is unsafe. Scott et al.[102] investigated the effects of a tourniquet applied to the distal limb of horses for 120 minutes. Tourniquet application resulted in a

decrease in pH, an increase in potassium concentrations, and a decrease in hematocrit in the tourniqueted limb. Interestingly, these values all returned to normal within 15 minutes after tourniquet removal. Sandler et al.[103] evaluated the vascular responses of the equine thoracic limb following tourniquet application. These authors demonstrated reactive hyperemia following tourniquet release and found that both blood flow and blood pressure returned to normal within 10 minutes of tourniquet removal. The findings of Chawla et al.[101] and Singh et al.[100] are interesting when compared to the findings of Scott et al.[102] and Sandler et al.[103] and in the light of the common clinical use of tourniquets in bovine practice. Several authors have described regional intravenous anesthesia[89,90,94,104-111] and regional antibiotic perfusion[91,112] in cattle. All of these studies involve the use of some sort of tourniquet and none of them report adverse reactions to the tourniquet.

Although tourniquet use is common, it is not without risks. Reported complications in humans include pain associated with tourniquet application,[113,114] deep venous thrombosis,[115,116] vasospasm,[117] nerve injury,[118,119] and pulmonary embolism.[120-122] Tourniquet application has also been shown to result in increased systemic blood pressure.[123-125]

There is controversy in the literature concerning the affect of tourniquet application on the development of deep venous thrombosis (DVT). While some studies have concluded that tourniquet use increases the development of DVT,[115,116] others have found no correlation between tourniquet use and the risk of DVT.[126,127] Deep venous thrombosis has been reported in cattle following regional antibiotic delivery.[128,129] Steiner et al.[128] reported venous thrombosis in two out of fifteen cows following

intravenous regional administration of benzyl penicillin. The authors of this report attributed the complications to the high doses of penicillin rather than to the use of the tourniquet. Kofler et al.[129] reported venous thrombosis in two cows but only one of these cows had undergone regional perfusion.

Persistent lameness has been reported following regional intravenous anesthesia of the forelimb of buffalo calves.[111] In this study, the tourniquet was placed proximal to the elbow and left in place for over one hour. All of the animals eventually recovered.

The tourniquet pressure and the length of time the tourniquet is applied are thought to be the most important contributing factors in the development of complications to tourniquet use. A variety of pressure and application times have been reported in both the human and veterinary literature. Reported tourniquet pressures in humans and veterinary patients range from 250-800 mmHg. Several authors recommend a maximum pressure of 100 mmHg greater than systolic blood pressure[88,130]. Controlled tourniquet pressure is difficult to achieve in large animal clinical practice because pneumatic tourniquets are rarely used in these settings. Several authors[89,90,94,104-106] have performed regional intravenous anesthesia to the distal limb of cattle using rubber tourniquets without any control of tourniquet pressure. None of these authors report adverse reactions to the tourniquet use.

Three hours has been reported to be the maximum safe duration of tourniquet ischemia in human surgery.[131,132] The application of a tourniquet above the elbow of cattle for 90 minutes[100] and 60 minutes[101] have been described as unsafe. For the purpose of regional antibiotic or anesthetic delivery, tourniquet times in current veterinary practice rarely exceed one hour. Also, in current large animal practice, the tourniquet is

typically applied to the proximal or mid-metatarsal region where there is much less risk of muscle damage.

Regional Limb Perfusion in Large Animal Veterinary Medicine

Regional limb perfusion may be performed via intravenous (RIVP) or intraosseous (RIOP) routes. Regional limb perfusion with antimicrobial agents was reported in humans in the late 1950's and early 1960's for the treatment of chronic osteomyelitis.[133] Regional intravenous perfusion is easily performed on the distal limb by placing a tourniquet on the limb and infusing the antibiotic distal to the tourniquet. Higher on the limb, the affected area may be isolated by placing a tourniquet proximal and distal to the area to be infused. It is thought that increased intravascular pressure resulting from the tourniquet distends venules and allows the antibiotic to gain access to the capillaries through back diffusion into bone and soft tissues. The antibiotic may be infused into any accessible vein in the target area with the dorsal common digital vein being the most easily accessible and commonly used vein in the distal limb of cattle.

This technique has been well described in the equine literature[92,134-137] and a recent review[93] of regional limb perfusion in horses has been published. The pharmacokinetics of cefazolin[112] and ceftiofur[91] after RIVP in cattle have been defined. Both of these studies demonstrated antibiotic concentrations above therapeutic levels for commonly encountered pathogens. Additionally, no adverse effects of RIVP were observed. Even though these studies demonstrate that cefazolin and ceftiofur should be effective in treating orthopedic infections, both studies were pharmacokinetic studies utilizing normal animals. Clinical reports of this technique in cattle are rare; however,

the equine literature contains several successful reports of the use of regional limb perfusion (RLP) for the treatment of orthopedic infections. Recently, the synovial fluid pharmacokinetics of ceftiofur after RIVP in the horse have been described.[137]

Aminoglycosides are a common choice for RLP in horses.[138] In one study,[41] experimentally infected antebrachiocarpal joints were treated with RIVP of gentamicin sulfate or systemic intravenous gentamicin injection. Regional intravenous perfusion resulted in significantly higher gentamicin concentrations in synovial fluid compared to systemic administration. Also, culture of synovial fluid and synovial membrane was negative for two out of three horses undergoing RIVP while bacteria were isolated from all three horses undergoing systemic administration of gentamicin. Another study[134] reported the successful treatment of osteomyelitis associated with surgical implants using amikacin administered via RIVP.

Complications associated with RIVP are uncommon. In one report,[128] two out fifteen cows undergoing RIVP of the distal limb suffered severe thrombosis of all vessels distal to the tourniquet. The thrombosis was attributed to extremely high concentrations of benzylpenicillin. Another report[129] describes two cows with thrombosis of distal limb veins but only one of these cows had received RIVP.

Regional limb perfusion may also be performed via an intraosseous route. This technique is useful when intravenous access is difficult to achieve or maintain.[139] The technique is relatively easy to perform and involves either the insertion of a catheter into a hole drilled into the medullary cavity[139-142] or the insertion of a cannulated bone screw with a catheter adapter.[134,135,143]

Regional intravenous perfusion and RIOP have been compared.[141,143] In both studies, both RIVP and RIOP resulted in concentrations of amikacin well above recommended therapeutic levels. However, RIVP resulted in higher concentrations in synovial fluid when compared to RIOP. The pharmacokinetics of vancomycin after both RIVP and RIOP in the horse have been recently described;[144,145] however, this antibiotic cannot be used in food animals and will not be discussed further. Regional intraosseus perfusion has been shown to be effective in treating clinical cases,[134,140] and can be performed without general anesthesia.[142] There are no known published reports of the use of RIOP in the treatment of clinical cases in food animals; however, the technique has been used experimentally in pigs.[146-148]

CHAPTER III

METHODOLOGY

Animal Selection

This study utilized six adult mixed breed beef cows ranging from 6-10 years of age and 445 kg to 688 kg body weight. Two cows exhibited minor *Bos indicus* breed characteristics. The cows were identified with individual ear tags labeled with single letters. No clinical signs of lameness were evident at the beginning of the study. Throughout the study, the cows were housed in stalls in the Veterinary Teaching Hospital and provided free choice grass hay and water.

Catheter Placement

The cows were sedated with 25 mg xylazine[f] IV and restrained in lateral recumbency in a hydraulic tilt chute (Figure 2). The distal aspect of the left rear limb, beginning at the mid-metatarsus, was clipped and cleaned with chlorhexidine scrub. A rubber tourniquet was placed tightly around the mid-metatarsus. Anesthesia of the distal limb was accomplished using a ring block around the mid-metatarsus with 2% lidocaine[g] just distal to the tourniquet. The claws were covered with a sterile glove and the skin over the distal limb was prepared in sterile fashion.

[f] Xylazine 20 Injection, 20 mg/ml, The Butler Company, Dublin, OH

[g] Lidocaine 2% Injectable Solution, The Butler Company, Dublin, OH

Figure 2. Photograph of a cow restrained on a hydraulic tilt table in preparation for catheter placement.

Venipuncture of the dorsal common digital vein was performed using an 18 gauge 2.5 cm needle. A sterile guide wire was placed through the needle into the vein and the needle was removed. A stab incision was then made over the vein with a #15 scalpel blade using the wire as a guide. An 18 gauge, 4.8 cm catheter[h] was then fed over the wire

and into the vein. The wire was then removed and a T-port[i] with injection cap[j] was then placed on the catheter. The catheter and T-port were sutured into place using 2-0 nylon suture. The same procedure was repeated for the plantar vein of the lateral digit (PVLD).

The metatarsophalangeal joint was then catheterized using a 20 gauge epidural infusion catheter[k]. Arthrocentesis was performed over the craniolateral aspect of the joint using an 18 gauge 3.8 cm needle. An injection cap was placed on the needle and 30-50 mls of sterile saline was infused into the joint through a 19 gauge butterfly catheter[l] placed in the injection cap. As the joint was distended, the caudolateral aspect of the joint was palpated to identify the joint pouch. A 0.5 cm stab incision was made over the joint pouch with a # 15 blade and a 19 gauge 9 cm Touhy needle was placed in the caudolateral joint pouch. This needle was placed in the stab incision and held at an angle of approximately 30 degrees lateral to the median plane of the limb and 15 degrees caudal to the dorsal plane of the limb. The needle was advanced until it entered the

[h] 1.3 x 48 mm Intravenous Catheter, BD Insyte, Franklin Lakes, NJ

[i] Non-DEHP T Connector, Medex, Dublin, OH

[j] Prepierced Reseal Male Adapter Plug-Short, Hospira©, Forest Lake, IL

[k] Periflex® Continuous Epidural Anesthesia Set, B. Braun Medical, Bethlehem, PA

[l] Surflo® Winged Infusion Set, 19 gauge 0.75 inch thin walled needle with 12 inch tubing, Terumo© Medical, Somerset, NJ

distended joint. A curved 16 gauge 10 cm needle was placed through the skin approximately 4 cm proximal to the stab incision, advanced under the skin, and exited through the stab incision. This needle was used to create a subcutaneous tunnel through which the catheter tubing could be placed. The catheter tubing was passed approximately 1.5 cm into the joint through the Touhy needle. The Touhy needle was removed and the catheter tubing was shortened to the desired length. The free end of the tubing was passed through the 16 gauge needle and the needle was removed. The tubing was pulled through the subcutaneous tunnel until it was no longer exposed at the stab incision. An injection cap was placed on the catheter tubing and the catheter was secured to the skin using 2-0 Nylon in a Chinese finger cuff pattern. Figure 3 shows a foot fully instrumented with catheters in the dorsal common digital vein, the plantar vein of the lateral digit and the metatarsophalangeal joint. A light bandage was placed over the catheters for protection. Sedation was reversed when needed using tolazoline[m] at 1 mg/kg IV. All catheters were placed at least 24 hours prior to beginning the study.

Dosage Calculation

Selecting the appropriate dose is one of the uncertainties associated with the use of RIVP. The dose of 2.2 mg/kg (1mg/lb) used in this study was determined by weighing the distal limb (distal to mid-metatarsus) of one cow and calculating a dose for this weight using a dose of 40mg/kg. The dose of 2.2 mg/kg of whole body weight was chosen because it provided between 2 and 3 times the high end of the label dose (40 mg/kg) for the weight of the distal limb. At this dose, the volume of florfenicol delivered was small (ranging from 3.3 to 5.0 ml) and could be readily administered.

[m] Tolazoline Injectable Solution, 100 mg/ml, Lloyd Laboratories, Shenandoah, IA

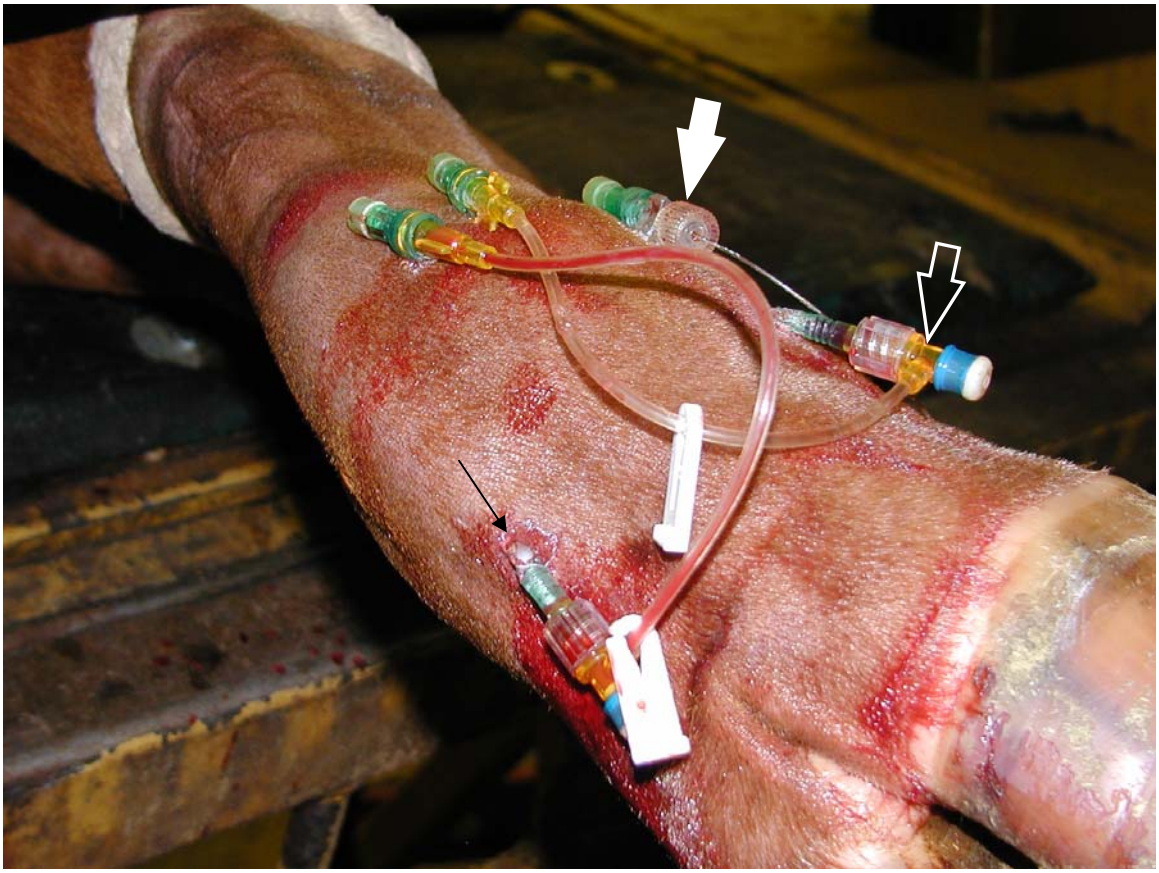

Figure 3. Photograph of bovine foot showing placement of catheters in the dorsal common digital vein (small arrow), the plantar vein of the lateral digit (large, open arrow), and the metatarsophalangeal joint (large, closed arrow).

Drug Administration and Sample Collection

Each cow was sedated with 25 mg xylazine IV and restrained in lateral recumbency in the hydraulic tilt chute. A pneumatic tourniquet[n] was placed around the limb at the mid-metatarsus. Prior to each sample collection, 3 ml of blood and 0.2 ml of synovial fluid were collected and discarded. The synovial catheter had a volume of approximately 0.1 ml. A 3 ml sample of blood was collected from the plantar vein of the lateral digit and both IV catheters were flushed with 3 ml of heparinized saline. A 0.5 ml sample of synovial fluid was collected from the metatarsophalangeal joint catheter. These first samples served as the time 0 samples. The tourniquet was inflated to 300 mmHg. Florfenicol[o] was administered at 2.2 mg/kg body weight into the dorsal common digital vein (time 0). The catheter was not flushed after florfenicol administration. The volume of the catheter and T-port was determined to be 0.6 ml so an additional 0.6 ml of florfenicol was added to the dose to account for the volume of the catheter since the catheter was not flushed. A 3 ml blood sample and a synovial fluid sample ranging from 0.3- 0.5 ml were collected as described above at 15, 30 and 45 minutes post florfenicol administration. Systemic blood samples were collected at the same time points from the left jugular vein via venipuncture. The tourniquet was removed after the 45 minute sample and the cow was allowed to stand. Sample collection continued at 1, 1.5, 2, 4, 8, 12, 18, and 24 hours after florfenicol administration. After the 45 minute sample, all remaining samples were collected with the cows standing in a chute. Blood samples

[n] Portable Tourniquet System, Delfi Medical Innovations Inc., Vancouver, BC, Canada

[o] Florfenicol Injectable Solution, 300 mg/ml, Schering Plough Animal Health, Omaha, NE

were collected in plain glass tubes and synovial fluid samples were placed in 0.5 ml plain plastic cryotubes[p]. Synovial fluid samples were labeled and placed on ice immediately after collection. Blood samples allowed to clot at room temperature and serum was harvested by centrifugation. Serum was placed in 1.5 ml plain plastic cryotubes[q]. Samples that could not be centrifuged within 2 hours of collection were refrigerated and all serum was harvested within 18 hours of collection. Florfenicol recovery rates greater than 99% have been demonstrated in plasma and cerebrospinal fluid samples stored at room temperature for 24 hours.[46] Synovial fluid samples that were contaminated with blood were centrifuged, and the supernatant was collected.[91,112] Synovial fluid samples with significant blood contamination were from cow A only. Serum and synovial fluid samples were frozen at -20˚ C until analysis could be performed.[46] Florfenicol is stable in plasma samples subjected to three freeze-thaw cycles at -20˚ C over a 2 month period.[149]

Sample Analysis

The serum and synovial joint fluid samples were assayed for florfenicol using reverse-phase high pressure liquid chromatography (HPLC) with ultraviolet (UV) detection. The laboratory used other published references as a guide[48,49,83,84], but added some modifications to the procedure to produce an assay adapted for the fluids collected from cattle in this study. The HPLC system consisted of a quaternary pump and degasser[r], automated sampler[s], and UV detector[t]. Plasma extraction was accomplished

[p] Microcentrifuge tube, 0.5 ml, SCI Dynamics, Adelphia, NJ

[q] Microcentrifuge tubes, 1.5 ml, SCI Dynamics, Adelphia, NJ

[r] Agilent 1000 series solvent delivery system.. Agilent Technologies Wilmington, DE

[s] Agilent 1000 series autosampler. Agilent Technologies Wilmington, DE

with solid phase hydrophilic-lipophilic balanced (HLB) extraction cartridges[u], conditioned with 1 mL methanol, followed by 1 mL distilled water. After the addition of 200 µL of serum sample to the cartridge, it was washed with 1.0 mL distilled water:methanol (95:5). The eluent was discarded. The final elution was achieved with the addition of 1.0 mL methanol into a clean glass tube. The eluate was evaporated in a hot water bath (45°C) under nitrogen for 20-25 minutes and reconstituted with 200 µL of mobile phase.

A reverse phase stable bond 4.6 mm x 15 cm C-8 column[v], heated to 40°C, achieved separation. The mobile phase consisted of 70% water and 30% acetonitrile. The UV detector was set to a wavelength of 223 nm. The volume for each injection was 20 µL. Retention time for florfenicol was 4.5-5.0 minutes. Chromatograms were integrated with computer software.[w]

A stock solution of florfenicol was prepared by dissolving a pure analytical reference standard of florfenicol[x] in acetonitrile at a concentration of 1 mg/mL and stored in the refrigerator. The analytical reference standard solution was used to make calibration standards and to fortify quality control (QC) samples. The 1 mg/mL stock solution was further diluted serially with distilled water to concentrations ranging from 1000 µg/mL to 3.91 µg/mL. Standard curves for plasma analysis were prepared by fortifying 200 µL of pooled bovine serum with 20 µL of the diluted stock solutions to

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[t] Agilent 1000 series variable wavelength detector (VWD). Agilent Technologies Wilmington, DE

[u] Oasis HLB solid phase extraction cartridges. Waters Corporation, USA.

[v] Zorbax Eclipse XDB-C8 4.6 mm x 15 cm column. Agilent Technologies Wilmington, DE.

[w] Agilent 1100 series Chemstation software, Agilent Technologies, Wilmington, DE.

[x] Florfenicol reference standard donated by Schering Plough Corporation.

make eleven calibration standards (including zero) of florfenicol in plasma ranging from 100 μg/mL to 0.195 μg/mL. Unfortified cattle serum was used as a blank and to verify that the assay contained no interfering compounds. The fortified calibration samples were processed and prepared exactly as described for the incurred samples. For each day's run, a fresh set of calibration and blank samples were prepared. Calibration curves of peak height versus concentration were calculated by use of linear-regression analysis. All calibration curves were linear with a R^2 value of 0.99 or higher. Limit of quantification for florfenicol in cattle serum was 0.195 μg/mL, which was determined from the lowest point on a linear calibration curve that produced an appropriate signal-to-noise ratio. The laboratory used guidelines published by the United States Pharmacopeia (2006).

The synovial fluid samples were prepared in the same manner as that of the serum samples, except for slight modification. Because synovial fluid is viscous, processing is difficult in extraction cartridges. Therefore, hyaluronidase (10 μL) was added to each sample prior to processing followed by vortexing. The synovial fluid samples were then processed in the same manner as the serum. For the calibration samples, blank (untreated) synovial fluid was collected from cattle with no evidence of lameness or gross musculoskeletal pathology that were presented to the college's necropsy service. This synovial fluid was fortified (spiked) with florfenicol in the same manner as the serum samples to prepare calibration samples. The calibration range was the same as for the serum samples.

Pharmacokinetic Analysis

Serum and joint fluid concentrations of florfenicol after the injection into the digital vein were analyzed using a computer program[y]. A non-compartmental analysis (NCA) that does not assume any compartmental structure was used for the analysis because this was not a standard intravenous bolus injection and calculation of compartmental parameters would have been subject to error. Calculation methods were from published methods.[150,151]

For the NCA, the AUC from time 0 to the last measured concentration, defined by the limit of quantization (LOQ), was calculated using the log-linear trapezoidal method. The AUC from time 0 to infinity was calculated by adding the terminal portion of the curve, estimated from the relationship C_t/λ_Z, to the $AUC_{0\text{-}Ct}$, where λ_Z is the terminal rate constant of the curve, and C_t is the last measured concentration point. The percent of the $AUC_{infinity}$ that was extrapolated using the trapezoidal rule was calculated by use of the following equation:

$$\% \text{ AUC Extrapolated} = (AUC_{infinity} - AUC_{0 \text{ to } Ct})/AUC_{infinity} \times 100.$$

Values for the maximum concentration after dosing (C_{MAX}) and time to maximum plasma concentration (T_{MAX}) were taken directly from the data. Half-lives were calculated from the terminal slope: T½ = ln 2.0/(terminal rate constant), where ln 2.0 is the natural logarithm of 2.0. Traditional pharmacokinetic parameters such as apparent volume of distribution and systemic clearance (CL) were not calculated for digital serum or synovial fluid samples because this was a regional administration of the drug, and

[y] WinNonLin. Version 5.0.1 Pharsight Corporation, Mountain View California.

these are whole-body parameters. For the jugular serum samples, clearance (Cl/F) and volume of distribution at steady state (VD_{SS}/F) were adjusted for bioavailability.

Statistical Analysis

Data were analyzed using PC SAS Version 9.2[z]. The experimental design was a randomized complete block design with repeated measures. The cow was considered the blocking variable, and location the main unit factor. Time was the repeated measures factor. Because of normality and heterogeneity of variance problems associated with the response variable, a natural log(x+1) transformation was used to stabilize and normalize the data. PROC MIXED in SAS was used to conduct the analysis of variance, and an autoregressive period 1 covariance structure used to model the intra-location covariances across time. An LSMEANS statement with a SLICE option was also used to evaluate the simple effects of time given location and location given time. If the test of simple effects was significant, pairwise t tests using the DIFF option was used to separate the means.

[z] SAS Institute, Cary, NC

CHAPTER IV

FINDINGS

Florfenicol Concentrations and Pharmacokinetics

No lameness or other adverse effects were observed throughout the study period. Samples could not be collected from the digital vein for cow D after the 4 hour sample or cow K at the 18 hour sample due to loss of the catheter. The 24 hour sample for cow K was collected via venipuncture of the PVLD. Also, synovial fluid samples were not collected from cow A at the 0.75 and 1 hour sampling times due to difficulties with the catheter.

The concentration versus time profiles of florfenicol for the digital blood (DIG), synovial fluid (SYN), and systemic blood (JUG) are shown in Figures 4-6 respectively. The mean peak concentrations for the DIG, SYN, and JUG samples were 714.8 ± 301.9, 39.2 ± 29.4, and 5.9 ± 1.4 μg/ml, respectively. At 0.25 hours post infusion (HPI), florfenicol concentration in DIG samples was significantly higher than either SYN or JUG samples. At 0.5 and 0.75 HPI, concentrations at all sampling locations were significantly different. After 8 HPI, no significant differences existed between sampling locations. The mean florfenicol concentrations for each sampling location analyzed by sampling time are presented in Table 11. Table 12 shows the mean florfenicol concentrations for each sampling location analyzed by sampling location. For the DIG samples, the florfenicol concentration was significantly different at each time point up to the 8 hour sample. For the SYN samples, a significant increase in florfenicol concentration was observed at the 0.5 hour time point and by 4 hours the concentration decreased to a level equal to the initial sample at 0.25 hours. Significant decreases were

seen at 4, 8 and 12 hours, after which, significant differences were not observed. For the JUG samples, a significant decrease in florfenicol concentration was seen at 8 hours. Further significant differences were not observed.

The mean pharmacokinetic parameters of florfenicol for DIG, SYN, and JUG samples are presented in Table 13. Individual animal pharmacokinetic parameters for DIG, SYN, and JUG samples are presented in Tables 14-16, respectively. The individual animal data are included because of obvious differences among the individuals. Two of the animals, cow A and cow F, had lower drug concentrations in the digital vein. The same animals had lower concentrations in the joint fluid.

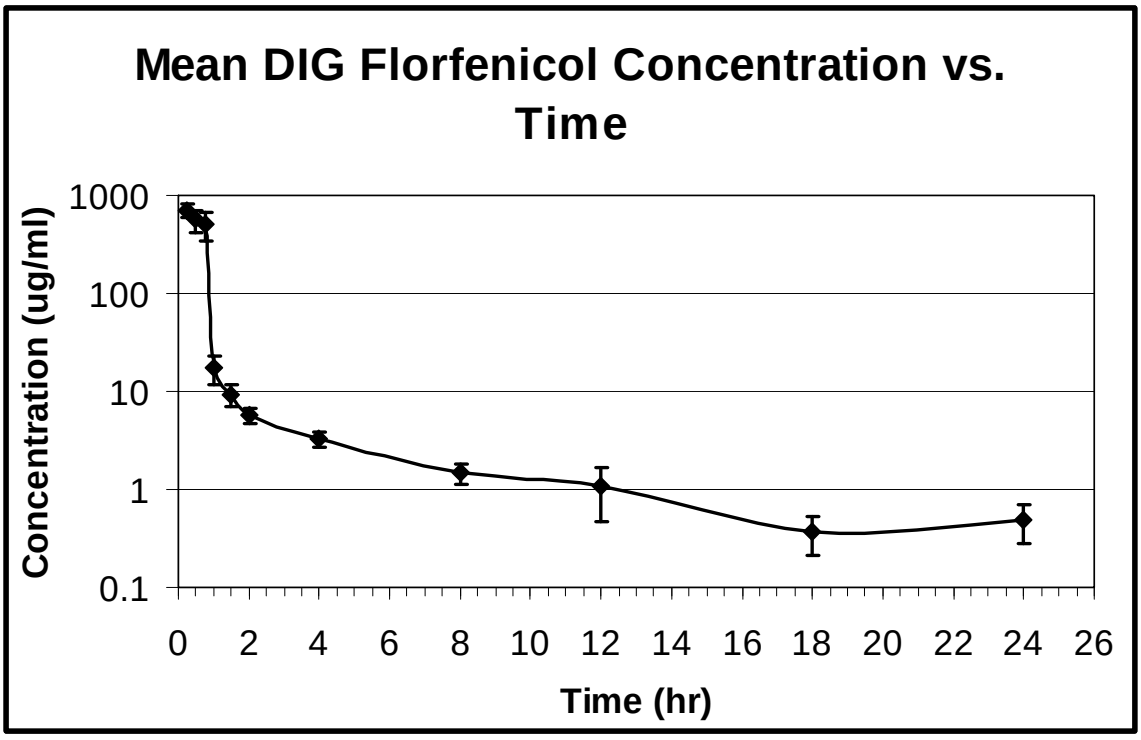

Figure 4. Mean ± SE florfenicol concentration vs. time for digital serum samples following intravenous regional perfusion of 2.2 mg/kg florfenicol.

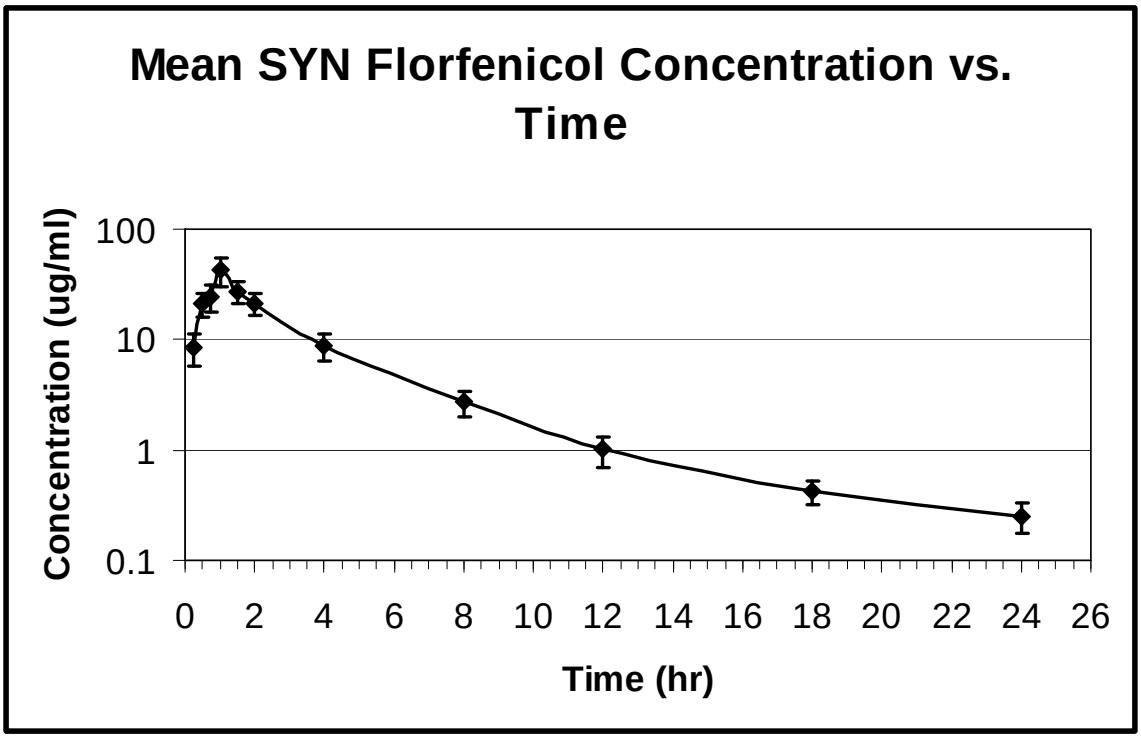

Figure 5. Mean ± SE florfenicol concentration vs. time for synovial fluid samples following intravenous regional perfusion of 2.2 mg/kg florfenicol.

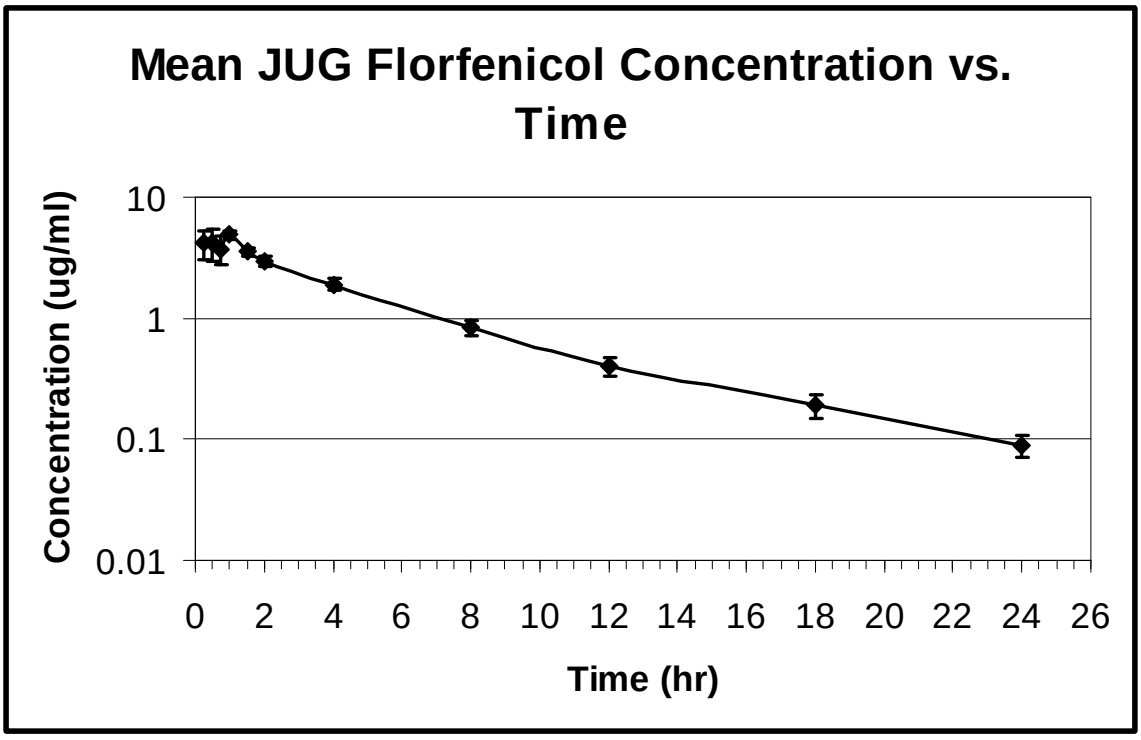

Figure 6. Mean ± SE florfenicol concentration vs. time for jugular serum samples following intravenous regional perfusion of 2.2 mg/kg florfenicol.

Table 11. Mean ± standard error florfenicol concentration (μg/ml) for DIG, SYN, and JUG samples at each time point.

	Time (hours)										
Sample	**0.25**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**4.00**	**8.00**	**12.00**	**18.00**	**24.00**
DIG	$706.15^{a} \pm 120.84$	$568.43^{a} \pm 145.17$	$508.65^{a} \pm 167.59$	$17.41^{a} \pm 5.51$	$9.30^{b} \pm 2.39$	$5.69^{b} \pm 0.96$	$3.26^{ab} \pm 0.58$	$1.49^{a} \pm 0.38$	$1.07^{a} \pm 0.67$	$0.38^{a} \pm 0.20$	$0.49^{a} \pm 0.25$
SYN	$8.58^{b} \pm 2.87$	$21.03^{b} \pm 4.81$	$24.33^{b} \pm 7.28$	$42.58^{a} \pm 13.83$	$27.54^{a} \pm 6.38$	$21.59^{a} \pm 4.98$	$8.83^{a} \pm 2.37$	$2.71^{a} \pm 0.70$	$1.01^{a} \pm 0.32$	$0.42^{a} \pm 0.10$	$0.25^{a} \pm 0.07$
JUG	$4.18^{b} \pm 1.20$	$4.22^{c} \pm 1.29$	$3.75^{c} \pm 1.0$	$4.87^{b} \pm 0.46$	$3.57^{b} \pm 0.27$	$2.95^{b} \pm 0.28$	$1.90^{b} \pm 0.21$	$0.83^{a} \pm 0.12$	$0.40^{a} \pm 0.07$	$0.19^{a} \pm 0.04$	$0.09^{a} \pm 0.02$

Values with different superscripts in each column are significantly different ($P<0.05$).

Table 12. Mean ± standard error florfenicol concentration (μg/ml) for DIG, SYN, and JUG samples at each time point.

	Time (hours)										
Sample	**0.25**	**0.50**	**0.75**	**1.00**	**1.50**	**2.00**	**4.00**	**8.00**	**12.00**	**18.00**	**24.00**
DIG	$706.15^{a} \pm 120.84$	$568.43^{c} \pm 145.17$	$508.65^{c} \pm 167.59$	$17.41^{d} \pm 5.51$	$9.30^{e} \pm 2.39$	$5.69^{e} \pm 0.96$	$3.26^{f} \pm 0.58$	$1.49^{g} \pm 0.38$	$1.07^{gh} \pm 0.67$	$0.38^{h} \pm 0.20$	$0.49^{gh} \pm 0.25$
SYN	$8.58^{b} \pm 2.87$	$21.03^{a} \pm 4.81$	$24.33^{a} \pm 7.28$	$42.58^{a} \pm 13.83$	$27.54^{a} \pm 6.38$	$21.59^{a} \pm 4.98$	$8.83^{b} \pm 2.37$	$2.71^{c} \pm 0.70$	$1.01^{d} \pm 0.32$	$0.42^{d} \pm 0.10$	$0.25^{d} \pm 0.07$
JUG	$4.18^{ab} \pm 1.20$	$4.22^{ab} \pm 1.29$	$3.75^{ab} \pm 1.0$	$4.87^{a} \pm 0.46$	$3.57^{ab} \pm 0.27$	$2.95^{ab} \pm 0.28$	$1.90^{b} \pm 0.21$	$0.83^{c} \pm 0.12$	$0.40^{c} \pm 0.07$	$0.19^{c} \pm 0.04$	$0.09^{c} \pm 0.02$

Values with different superscripts in each row are significantly different ($P<0.05$).

Table 13. Mean ± std dev pharmacokinetic parameters for DIG, SYN, and JUG samples following regional intravenous perfusion of 2.2 mg/kg florfenicol.

Sample	Elimination rate (1/hr)	Half-life (hr)	Tmax (hr)	Cmax (µg/ml)	AUC (0 to C_t) (hr·µg/ml)	AUC (0 to ∞) (hr·µg/ml)	AUC % extrap (%)	Cl/F (L/kg/hr)	Vd_{ss}/F (L/kg)
DIG	0.22 ± 0.15	4.09 ± 1.93	0.29 ± 0.10	714.79 ± 301.93	485.47 ± 270.17	488.14 ± 272.53	0.46 ± 0.41		
SYN	0.19 ± 0.04	3.81 ± 0.81	0.88 ± 0.38	39.19 ± 29.42	112.97 ± 54.76	113.82 ± 54.71	1.01 ± 0.88		
JUG	0.15 ± 0.02	4.77 ± 0.67	0.63 ± 0.31	5.90 ± 1.37	22.57 ± 6.72	23.1 ± 6.91	2.55 ± 0.62	0.10 ± 0.04	0.56 ± 0.21

Table 14. Pharmacokinetic parameters from DIG samples for each individual cow following regional intravenous perfusion of 2.2 mg/kg florfenicol.

Serum Collected from Digit							
Parameter	**Units**	**Cow A**	**Cow D**	**Cow E**	**Cow F**	**Cow G**	**Cow K**
Elimination rate	1/hr	0.19	0.51	0.15	0.21	0.10	0.16
Half-life	hr	3.61	1.36	4.53	3.34	7.28	4.40
Tmax	hr	0.25	0.25	0.25	0.25	0.50	0.25
Cmax	ug/mL	171.96	860.87	918.03	540.04	907.68	890.18
AUC (0 to C_t)	hr*ug/mL	104.18	659.25	654.45	188.18	742.97	563.77
AUC (0 to ∞)	hr*ug/mL	104.45	663.91	655.13	188.33	751.87	565.17
AUC % extrapolated	%	0.26	0.70	0.10	0.25	1.18	0.25

Table 15. Pharmacokinetic parameters from SYN samples for each individual cow following regional intravenous perfusion of 2.2 mg/kg florfenicol.

Synovial Fluid							
Parameter	**Units**	**Cow A**	**Cow D**	**Cow E**	**Cow F**	**Cow G**	**Cow K**
Elimination rate	1/hr	0.15	0.20	0.22	0.14	0.18	0.24
Half-life	hr	4.50	3.39	3.15	4.98	3.95	2.90
Tmax	hr	0.50	0.75	1.00	0.50	1.50	1.00
Cmax	ug/mL	9.13	48.03	51.27	18.63	19.89	88.23
AUC (0 to C_t)	hr*ug/mL	38.07	158.05	189.32	74.80	104.88	112.69
AUC (0 to∞)	hr*ug/mL	39.03	158.32	190.42	75.50	106.63	113.01
AUC % extrapolated	%	2.44	0.17	0.58	0.93	1.65	0.28

Table 16. Pharmacokinetic parameters from JUG samples for each individual cow following regional intravenous perfusion of 2.2 mg/kg florfenicol.

Serum Collected from Jugular Vein							
Parameter	**Units**	**Cow A**	**Cow D**	**Cow E**	**Cow F**	**Cow G**	**Cow K**
Elimination rate	1/hr	0.13	0.20	0.15	0.13	0.15	0.13
Half-life	hr	5.21	3.55	4.54	5.26	4.78	5.27
Tmax	hr	0.25	0.50	0.50	0.50	1.00	1.00
Cmax	ug/mL	7.03	5.32	7.15	5.72	3.54	6.66
AUC (0 to C_t)	hr*ug/mL	27.93	21.34	30.58	23.95	20.18	11.45
AUC (0 to ∞)	hr*ug/mL	28.65	21.58	31.35	24.47	20.92	11.66
AUC % extrapolated	%	2.51	2.84	2.47	2.11	3.57	1.77
Vd_{area}	L/kg	0.56	0.51	0.45	0.67	0.72	1.43
Cl/F	L/kg/hr	0.07	0.10	0.07	0.09	0.10	0.19
Vd_{ss}/F	L/kg	0.39	0.48	0.40	0.43	0.77	0.87

Regional intravenous administration of florfenicol into the dorsal common digital vein produced very high drug concentrations in the digital serum. These concentrations remained high until the tourniquet was removed, at which time the concentration decreased rapidly. Even though the concentration of florfenicol decreased after tourniquet removal, the concentration remained above 1 μg/ml for 12 hours. The pharmacokinetics of florfenicol after systemic intravenous administration have been reported. An initial concentration of 65.68 μg/ml was reported following administration of 22 mg/kg body weight to veal calves.[50] Another study reported an initial concentration of 39.7 μg/ml following administration of 20 mg/kg to feeder calves.[84] The initial mean DIG concentration of 706.15 μg/ml following a much smaller total dose of florfenicol in this study demonstrates the effectiveness of RIVP in achieving high local drug concentrations. The mean DIG florfenicol concentration at 24 hours was numerically greater than the concentration at 18 hours however; the difference was not statistically significant.

Administration of florfenicol via RIVP in this study produced high concentrations of florfenicol in the metatarsophalangeal joint. The mean peak SYN concentration was 39.19 μg/ml, and time to maximum concentration was 0.88 hours. This peak concentration occurred after removal of the tourniquet indicating that absorption of florfenicol into the joint continued to occur after tourniquet removal in several cows. Florfenicol concentration remained above 1 μg/ml for 12 hours and was 0.25 μg/ml at 24 hours. Synovial fluid concentrations of florfenicol have not been previously reported.

The concentrations of florfenicol detected in the JUG samples remained low throughout the study. Florfenicol concentrations in JUG samples slowly decreased until

tourniquet removal, after which time there was a slight increase followed by a gradual decrease in florfenicol concentration.

Elimination half-life and withdrawal information

The elimination half life for florfenicol observed in this study was slightly longer than other reports in the literature. Reported elimination half-lives for florfenicol following intravenous administration to cattle include 2.87,[50] 3.0,[48] and 2.65[84] hours. In this study, the mean half lives of florfenicol in DIG, SYN, and JUG samples were 4.09, 3.81, and 4.77 hours, respectively. Following intramuscular administration, the elimination half life of florfenicol ranges from 12.5[48] to 18.3[84] hours. Following intramuscular administration at a dose of 20 mg/kg, florfenicol has a meat withdrawal time of 28 days. Based on these data, administration of florfenicol via RIVP should not result in a prolonged withdrawal period compared to the current label recommendations.

CHAPTER V

CONCLUSION

Clinical Application

The data produced by this study indicated that RIVP administration of florfenicol should be a useful tool in the treatment of deep digital sepsis in cattle. Most digital infections in cattle involve a mixed bacterial population including *Arcanobacterium pyogenes* and *Fusobacterium necrophorum.*[38,152] A study[153] involving 445 cattle with appendicular skeletal infections found that *A. pyogenes* was the most common bacterial isolate. Florfenicol is effective against both of these organisms. The New Animal Drug Application for florfenicol (NADA-141-063) reports an MIC_{90} for *F. necrophorum* of 0.25 μg/ml. A study of 49 *A. pyogenes* isolates from cattle and pigs reported an MIC_{90} of 1.56 μg/ml.[154] A study of 16 *A. pyogenes* isolates from white-tailed deer reported an MIC_{90} of 0.5 μg/ml.[155] The florfenicol concentrations from the DIG samples remained above the MIC_{90} for *A. pyogenes* for a minimum of 8 hours and above that for *F. necrophorum* for a minimum of 18 hours. For the SYN samples, the concentration remained above the MIC_{90} for *A. pyogenes* for a minimum of 8 hours and above that for *F. necrophorum* for the full 24 hour sampling period.

Mycoplasma bovis is a common cause of infectious arthritis in calves, especially following an episode of respiratory disease.[156] Minimum Inhibitory Concentrations and Minimum Mycoplasmacidal Concentrations (MMC) of florfenicol against *M. bovis* have been reported.[157] That study reported an MIC_{90} of 16 μg/ml, an MMC_{50} of 16 μg/ml, and an MMC_{90} of 32 μg/ml. Only danofloxacin was more effective than florfenicol. Regional intravenous perfusion of florfenicol produced synovial concentrations that

exceeded both the MMC_{50} and the MMC_{90} for *M. bovis*. Mycoplasmal arthritis typically occurs in the larger joints such as the carpus, elbow and stifle. Regional perfusion can easily be performed for the carpal or tarsal joints but is difficult to perform on the larger, more proximal joints.

In order to be ideally suited for use in RIVP, an antibiotic should have bactericidal, concentration dependent activity. Florfenicol is typically described as a time-dependent, bacteriostatic antibiotic. However, recent research has demonstrated concentration dependent, bactericidal activity for florfenicol.[45] Unfortunately, the organisms included in this study were respiratory tract pathogens and did not include *F. necrophorum* or *A. pyogenes.* At this time, it is unknown if florfenicol possesses concentration dependent activity against these organisms. Even if the activity of florfenicol is time-dependent against these target organisms, this study demonstrates that RIVP administration of florfenicol could be useful in the treatment of digital infections in cattle. Florfenicol concentrations remained above the MIC_{90} of *A. pyogenes* for 8 hours in both the DIG and SYN samples and above the MIC_{90} of *F. necrophorum* for at least 18 hours in the DIG samples and for 24 hours in the SYN samples.

Regional perfusion of florfenicol is best performed via venipuncture rather than through an indwelling intravenous catheter. Due to the poor solubility of florfenicol in water (less than 2 mg/ml[aa]), flushing a catheter with heparinized saline or other aqueous solution following florfenicol administration will result in precipitation of the florfenicol and obstruction of the catheter. Dilution of florfenicol with sterile water combined with

[aa] http://www.fda.gov/cvm/FOI/141-063EA.pdf

a solubilizing agent, dimethyl formamide, has been described.[67,68] This compound is a carcinogen[158] and causes birth defects,[159] therefore it cannot be used in food animals.

Summary

In summary, RIVP of florfenicol at a dose of 2.2 mg/kg resulted in high drug concentrations in both the serum and synovial fluid of the distal limb while maintaining low systemic drug concentration. Florfenicol demonstrates concentration dependent killing activity against some pathogens.[45] The most likely pathogens encountered in cases of deep digital sepsis include *Fusobacterium necrophorum* and *Arcanobacterium pyogenes.*[38] The concentrations of florfenicol achieved in the distal limb in this study greatly exceeded the published MIC of these pathogens. In addition, the concentration of florfenicol in synovial fluid remained above the MIC of *F. necrophorum* for up to 24 hours. These findings indicate that RIVP of florfenicol should be useful in the treatment of deep digital sepsis whether the activity of florfenicol against these pathogens is concentration dependent or time dependent.

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VITA

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Candidate for the Degree of

Master of Science

Thesis: PHARMACOKINETICS OF FLORFENICOL IN SERUM AND SYNOVIAL FLUID SAMPLES AFTER REGIONAL INTRAVNEOUS PERFUSION IN THE DISTAL LIMB OF CATTLE

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Scope and Method of Study:

Six healthy adult cows were used in this study. Intravenous catheters were placed in the dorsal common digital vein (DCDV) and the plantar vein of the lateral digit (PVLD), and an indwelling intrasynovial catheter was placed in the metatarsophalangeal (MT) joint of the left rear limb. Samples from the left jugular vein (JUG) were collected via venipuncture. A pneumatic tourniquet was placed on the mid-metatarsus. Florfenicol was administered at 2.2 mg/kg into the DCDV. Samples from the PVLD (DIG samples), JUG (JUG samples) and MT joint (SYN samples) were collected at 15, 30, and 45 minutes. After tourniquet removal, samples were collected at 1, 1.5, 2, 4, 8, 12, 18, and 24 hours post infusion. Florfenicol analysis was performed by high performance liquid chromatography.

Findings and Conclusions:

The pharmacokinetic parameters of florfenicol following regional intravenous perfusion (RIVP) are shown (table).

	Sample		
Parameter	DIG	SYN	JUG
Cmax (μg/ml)	714.79 ± 301.93	39.19 ± 29.42	5.90 ± 1.37
AUC (hr*μg/ml)	488.14 ± 272.53	113.82 ± 54.71	23.10 ± 6.91
T1/2 (hrs)	4.09 ± 1.93	3.81 ± 0.81	4.77 ± 0.67

RIVP of florfenicol produced high concentrations in DIG and SYN samples while the concentration in JUG samples remained relatively low. RIVP of florfenicol may be useful in the treatment of infectious processes involving the distal limb of cattle.

ADVISER'S APPROVAL: Robert N. Streeter