AN INVESTIGATION OF INTRAPERITONEAL PROCAINE PENICILLIN G

ADMINISTRATION IN LACTATING DAIRY COWS

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Alan L. Chicoine

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List of Abbreviations

AABP	American Association of Bovine Practitioners
ADI	Acceptable Daily Intake
AMDUCA	Animal Medicinal Drug Use Clarification Act (US)
AMR	Antimicrobial resistance
AOAC	Association of Official Analytical Chemists
AUC	Area under the curve
AVMA	American Veterinary Medical Association
CFIA	Canadian Food Inspection Agency
CgFARAD	Canadian global Food Animal Residue Avoidance Databank
Cl_S	Systemic clearance
C_{max}	Maximum drug concentration
CVDR	Centre for Veterinary Drug Residues
CVMA	Canadian Veterinary Medical Association
ELDU	Extralabel Drug Use
F	Bioavailability
FDA	Food and Drug Administration (US)
FSIS	Food Safety Inspection Services (US)
HPLC	High performance liquid chromatography
IM	Intramuscular
IMM	Intramammary
IP	Intraperitoneal
IV	Intravenous
LOD	Limit of detection
LOQ	Limit of quantification
MIC	Minimum inhibitory concentration
MRL	Maximum residue limit
NCIMS	National Conference of Interstate Milk Shipments (US)
NOEL	No observed effect level
PD	Pharmacodynamics
PK	Pharmacokinetics
PMO	Pasteurized Milk Ordinance (US)
PPG	Procaine Penicillin G
SC	Subcutaneous
SCC	Somatic cell count
STOP	Swab test on premises
T_{max}	Time to maximum drug concentration
TMR	Total mixed ration
$T_{1/2\ elim}$	Elimination half-life
VCPR	Veterinarian-client-patient relationship
V_D	Volume of distribution
WCABP	Western Canadian Association of Bovine Practitioners
WCVM	Western College of Veterinary Medicine
WDI	Withdrawal interval
WDT	Withdrawal time

Chapter 1: Introduction and Literature Review

1.1 Thesis Introduction

Performing surgery on cattle is a significant part of a large animal veterinarian's duties. A large number of these surgical procedures involve entering the animal's abdominal cavity, whether for a Caesarian section, fixing a displaced abomasum, performing an exploratory laparotomy, or repairing an umbilical hernia.   Many of these surgical procedures are performed on farm rather than in a veterinary clinic. Unfortunately, this entails operating in conditions that are less than optimal, as a hygienic surgical environment may not be possible. Unhygienic conditions may result in post-surgical infections that threaten the production and well-being of the animal. In an effort to prevent the deleterious effects of a post-surgical infection, many veterinarians administer perioperative antimicrobials, sometimes directly into the abdominal cavity. This intraperitoneal (IP) use of antimicrobials has not been well studied in animals in general and cattle in particular. Pharmacokinetic differences between IP and other routes of administration are not known for most antimicrobials in cattle. As well, meat and milk drug residues kinetics are unknown after IP administration.   Whether IP antimicrobials inflame or irritate the abdominal cavity and peritoneum is unknown. Finally, no evidence exists to verify that IP antimicrobial use prevents postoperative infections in cattle. Although the author utilized IP antimicrobials routinely while performing bovine surgery, no information was available to support or refute this practice. These unanswered questions raise a larger issue: is IP antimicrobial use during bovine surgery justifiable? This thesis examines part of this broad subject by describing the plasma kinetics, milk and meat residues, and safety profile after IP administration of procaine penicillin G in lactating dairy cows.

1.2 Extra-label Drug Use (ELDU)

Using any medication in a manner different from the specific label directions constitutes extra-label drug use (ELDU). This includes altering the dosage, route, or duration of therapy; or using the product on a species or for a condition not specified on the drug label. Antimicrobials currently approved for use in cattle have label instructions for various routes of administration, including intramuscular (IM), subcutaneous (SC), intravenous (IV), oral, topical, or some combination of these routes. However, no product is labeled for IP use. Therefore administration of antimicrobials by the IP route in cattle constitutes ELDU, and evaluating IP use in cattle requires an understanding of the issues surrounding ELDU.

1.2.1 ELDU Legislation and Guidelines

Because of the limited number of veterinary pharmaceuticals and label indications available, ELDU is essential to the modern practice of veterinary medicine. Under the federal Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994, the United States recognizes a veterinarian's ability to perform ELDU, providing certain criteria are met (CVM, 2002). Canada's Veterinary Drug Directorate (VDD) concurs, stating that ELDU is not in violation of the Food and Drugs Act and Regulations (VDD, 2005). However, the necessary conditions required prior to ELDU are not explicitly stated, except that ELDU must not result in violative drug residues. The Canadian Veterinary Medical Association (CVMA) and other provincial and species-specific veterinary associations support ELDU guidelines similar to those presented in AMDUCA, though not codified as explicitly (CVMA, 2002).

1.2.2 Animal Medicinal Drug Use Clarification Act

The passage of AMDUCA formally recognizes the practice of ELDU, but only if certain conditions are met (CVM, 2002). The first provision states that acceptable ELDU requires using an approved animal or human drug. It must only be performed on the order of a licensed veterinarian within the context of a veterinary-client-patient relationship (VCPR). Another important, yet often overlooked, condition is that ELDU is limited to cases when the health of the animal is threatened or suffering or death may result from failure to treat. Growth promotion and reproductive therapy are not acceptable reasons for ELDU. Regarding ELDU in food-producing animals, further conditions must also be met, including:

- There is no approved animal drug that is labeled for this particular use that is likely to be clinically effective.

- The veterinarian has made a careful diagnosis and evaluation of the conditions for which this ELDU will occur.

- A substantially extended withdrawal period has been established prior to marketing food products from animals treated by ELDU, and no illegal drug residues will occur.

1.2.3 CVMA Extra-label Drug Use Guidelines

Although ELDU in Canada is not codified in federal law as in the United States, the CVMA position statement on extra-label drug use in animals contains similar themes to AMDUCA (CVMA, 2002). It encourages Canadian veterinarians to use and prescribe approved veterinary drugs as per the label instructions. However, it recognizes that ELDU is justified "if the prescribing veterinarian has scientific evidence that it is in the best interests of the animal", and does not contravene any provincial regulation. Although few, if any, provincial veterinary acts specifically regulate ELDU in animals, determining the appropriateness of ELDU in specific

situations will inevitably fall on the leadership of each provincial veterinary medical association. The CVMA guidelines also reiterate that only veterinarians, in a valid VCPR, are qualified to order ELDU in animals. As well, veterinarians must "educate themselves and their clients on appropriate withdrawal times and the liabilities associated with non-approved use".

1.2.4 WCABP Extra-label Drug Use Guidelines

The Western Canadian Association of Bovine Practitioners (WCABP) has published their own position statement on ELDU for bovine practitioners (WCABP, 2005). Like the CVMA position statement, it does not spell out specific provisions for acceptable ELDU. Rather, it reiterates the necessity of ELDU in bovine practice, the importance of veterinary supervision and a VCPR, and the veterinarian's liability if violative residues occur after ELDU.

1.2.5 IP Drug Use in Relation to ELDU

As stated earlier, because no antimicrobial is labeled for IP use this practice is by definition ELDU. But does IP administration meet the aforementioned criteria for acceptable ELDU? Because the Canadian guidelines are non-specific (only stating "Scientific evidence that ELDU is in the best interest of the animal"), this practice is easier to evaluate using the definitions outlined in AMDUCA. Since IP antimicrobial use is only likely to occur during bovine abdominal surgery, which should only be performed by a licensed veterinarian, the criteria for a veterinary order and valid VCPR are met. As well, since there are no animal drug products specifically labeled for either IP use or surgical prophylaxis, the directive to use an "approved product on-label" first has also been met. However, if one strictly applies the criteria for proper ELDU, IP antimicrobial use may not be acceptable. It is debatable if IP antimicrobial "prophylaxis" constitutes justifiable ELDU, as the health of the animal may not be threatened if IP treatment is withheld. If the veterinarian suspects surgical contamination and possibly

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infection, other antimicrobials are labeled for the treatment of this condition. For example, IM or IV injections of short-acting oxytetracycline are labeled for the treatment of peritonitis, and IM injections of procaine penicillin G are labeled for the treatment of wound infections. One could argue that either drug may be clinically ineffective for these conditions if given by IM injection postoperatively, or that intraoperative IP antimicrobial use is theoretically more effective (though no data currently exists to prove this). The other reason IP antimicrobial use contravenes ELDU guidelines is that no scientific evidence is available on which to base meat or milk withdrawal intervals. Therefore, even if withdrawal intervals longer than the label IM withdrawal times are recommended by the veterinarian, there is no guarantee that violative residues will not occur.

1.3 Drug Residues in Food Animals and Drug Residue Testing

Before discussing the kinetics of antimicrobial residues in meat and milk after IP administration in cattle, a general understanding of issues surrounding drug residues in food is required. Animal agriculture promotes its food products as safe and nutritious for the consumer. An important component of this "wholesome" message is the underlying assumption that nothing "unwholesome" is present. Food products contaminated with drug residues (including antimicrobials) are considered a public health hazard by both regulatory agencies and the public at large. Adverse reactions (hypersensitivity), continued emergence of antimicrobial resistance, and carcinogenicity or teratogenicity have been linked to antimicrobial residues in food. As a result, producers have proactively adopted prudent use guidelines in an effort to minimize violative drug residues. As well, regulatory agencies actively screen for drug residues in food of animal origin. In conjunction with these groups, veterinarians play an important role ensuring residues of medications used on farm do not reach the consumer.

1.3.1 *Negative consequences of antimicrobial residues in food of animal origin*

Risk to human health: Adverse drug reactions, such as hypersensitivity or allergic reactions, are possible if susceptible individuals consume contaminated meat or milk products. Approximately 5-10 percent of people are hypersensitive to penicillin (Dayan, 1993). Allergic reactions including skin rashes, hives, asthma, and possibly anaphylactic shock are possible if susceptible individuals ingest small quantities of penicillin (Wicher et al., 1969; Lindemayr et al., 1981; Kanny et al., 1994) Although there is potential for adverse reactions after penicillin residue ingestion, the number of confirmed cases is very small (Sundlof, 1989; Dewdney et al., 1991; Dayan, 1993) Emergence of antimicrobial resistance in human enteric bacteria caused by exposure to antimicrobial residues in food of animal origin is another concern (Tollefson, 2006). Antimicrobials present in food are thought to put selective pressure on antimicrobial-susceptible enteric bacteria, thus favouring resistant strains. Finally, some antimicrobials are banned for use in food producing animals due to the potentially catastrophic effects of drug residues. For example, chloramphenicol causes irreversible aplastic anemia in a small proportion of individuals. Other antimicrobials banned for use in food animals, such as metronidazole and nitrofurazone, are carcinogenic or teratogenic in laboratory species (Dowling, 2006).

Public perception: While the true risk to humans of antimicrobial residues in food is debatable, the public considers any drug residue in food as unhealthy or unsafe. Publicity of drug residues in meat or milk products results in decreased consumer confidence and threatens international trade. Animal agriculture and government agencies therefore have a vested interest to ensure food products containing drug residues do not reach either domestic or international markets.

Manufacturing/food processing: Antimicrobials in milk cause difficulty for the milk processor who purchases milk from the farmer. Antimicrobials hinder the production of dairy products and may result in reduced quality butter, yogurt, and cheese (Jones, 1999; Molina et al., 2003; Payne et al., 2006)

1.3.2 Common Drug Residue Violations in Cattle

Regulation of milk and dairy drug residue testing in Canada is done on a provincial basis, and results of residue testing are not readily available. Actual testing protocols, such as frequency and types of antimicrobial testing, vary with jurisdiction and individual processors. A limited residue surveillance program is conducted by the Canadian Food Inspection Agency (CFIA). However, this program only analyzes products already marketed for sale after provincial and/or processor testing and does not account for any residue violations found prior to marketing. Therefore, the results of federal testing do not reflect the true incidence of antimicrobial residues in milk and dairy products. In comparison, mandatory test reporting is required by American state regulatory agencies under the National Conference on Interstate Milk Shipments (NCIMS). Residue violations resulted in the discard of 76 million pounds of milk in 2002/2003. The most common drug residue violations in milk in both Canada and the United States are for ß-lactam residues, including penicillin, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur (NCIMS, 2003). See Chapter 2 for more information on drug residue testing in milk.

Residue detection in beef falls under federal jurisdiction in both Canada (CFIA) and the United States (Food Safety Inspection Service, FSIS, a division of the United States Department of Agriculture). Suspect carcasses are tested using rapid test kits (such as the Swab Test on Premises, STOP) in the slaughter plant. General surveillance of beef distributed for sale also

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occurs. For example, in 2003/2004 in Canada, 811 beef samples were tested for drug residues with 98.1% cleared for sale (CFIA, 2006). If specific antimicrobial residues are suspected in certain groups of animals, targeted surveillance is used. Veal calves are notorious for aminoglycoside residues (especially neomycin), and are tested more frequently for these drugs (Gibbons et al., 1996; JAVMA, 2004).

1.3.3 Reasons for Drug Residue Violations in Meat and Milk

Drug residues in milk are most often caused by improper use of antimicrobials for the control of mastitis. The odds that a violative antimicrobial residue will be found in bulk tank milk increases with increasing somatic cell count (SCC) status of the herd (Sargeant et al., 1998; Ruegg & Tabone, 2000; Saville et al., 2000) As the SCC is an indicator of the prevalence of mastitis within a herd, high SCC herds generally have more cows with mastitis requiring antimicrobial therapy, thus increasing the chance of milk residue violations. Mastitic cows are routinely treated with antimicrobials in order to lower the SCC to acceptable levels. Another predictor of increasing milk residue violations is large farm size, possibly due to miscommunication between multiple farm workers (van Schaik et al., 2002). Specific reasons for antimicrobial residue violations in milk (such as insufficient communication between barn staff, incomplete treatment records, and poor cow identification) can all result in failure to observe the label withdrawal time (Kaneene & Ahl, 1987). Drug residues in meat are caused by similar factors, again resulting in failure to observe the withdrawal time after drug administration (Paige et al., 1999).

1.3.4 Meat and milk withdrawal time determination

Before approving a new food animal drug, regulatory authorities establish an acceptable daily intake (ADI). The ADI represents a quantity of drug a person can ingest daily over a lifetime without appreciable risk to their health. ADIs are determined from the No Observed Effect Level (NOEL), which is the highest quantity of drug tested on laboratory animals that showed no adverse effects (including morphology, carcinogenicity, reproductive safety, lifespan, etc.). The ADI is calculated by dividing the NOEL by a safety factor, usually between 100 and 1000 (Baynes et al., 1999). The total ADI is allotted to meat and milk using different food consumption factors, to give each type of food a maximum allowable drug concentration. Foods consumed in small amounts or infrequently are allowed greater drug concentrations than those foods consumed often or in large quantities (such as milk). This maximum quantity of drug is known in Canada and the European Union as the maximum residue limit" (MRL). The MRL is calculated such that daily intake of food containing drug residues at the MRL will result in a total daily consumption of residues in quantities at or below the ADI. ADIs are based on the total residue of a chemical present in food (parent compound and all metabolites), whereas MRLs are based on a single, measurable marker residue (which may be the parent compound or any of its metabolites).

Once the MRL for a particular tissue or milk has been established, a withdrawal time (WDT) can be determined. The label WDT for a drug is the amount of time required after treatment for tissue or milk residues to fall below the MRL in 99% of animals, with 95% confidence. WDTs can only be determined by performing tissue or milk residue depletion studies. This requires taking tissue or milk samples at numerous time points after drug administration and constructing a residue depletion curve. Note the difference between the label

WDT as determined by regulatory agencies and a withdrawal interval (WDI) recommended by a veterinarian after ELDU, which should be longer than the WDT. Because WDTs are based on the 99th percentile of animals with 95% probability, residue depletion studies with small sample sizes or large variability are likely to result in longer withdrawal times. An extended meat WDT is not a major financial penalty for beef producers, as keeping cattle alive for a few extra days before slaughter is not terribly inconvenient. However, prolonged milk withdrawal times are a major economic hardship for dairy farmers. Assuming average milk production of 30 L/cow/day and a milk payment of $0.50 per litre, each day of milk discard costs the producer $15 in lost revenue. Therefore, dairy producers may not comply with excessively conservative WDI recommendations.

1.3.5 The role of the CgFARAD

Because veterinarians must routinely engage in ELDU, they are obligated to determine appropriate meat and milk WDI for the producer. Many veterinarians do not have appropriate pharmacokinetic training or cannot access the necessary scientific literature to make valid withdrawal estimations. It is for these reasons that the US Food Animal Residue Avoidance Databank (FARAD), and its Canadian global offshoot (CgFARAD) were created. Maintaining a comprehensive database containing pharmacokinetic data from scientific literature, proprietary drug trials, and international regulatory agencies allows staff pharmacologists to make scientifically-based withdrawal estimates for individual cases. A central database can also recognize when gaps exist in the literature. For example, the CgFARAD has received WDI requests after IP use of penicillin in cattle. After examining the database and performing a literature search, little scientific information was available to support any WDI recommendation.

The lack of kinetic information after IP antimicrobial administration in cattle was the major reason for conducting the experiments in this thesis.

1.3.6 Drug residue testing in milk and meat

Milk testing: As stated earlier, testing for antimicrobial residues in milk is performed mainly by the processing plant to which the milk is delivered. In Saskatchewan, before milk producers may ship milk to the processing plant they agree that no detectable drug residues will be present in their milk. Numerous tests are available to screen for antimicrobial residues however, and each test has different sensitivities for individual compounds. Therefore, results of a test used on-farm may not agree with results from a different test at the processing plant. Most rapid, on-farm screening tests are microbial growth inhibition assays, although ELISA-based tests are also available. Rapid screening test results are generally assessed visually and therefore give a qualitative "yes or no" answer. Processing plants may employ more expensive and bulky semi-quantitative equipment systems that rely on immuno- or microbial receptor assays. Screening tests are validated only for use with bulk tank or tanker-truck milk samples. No kits are currently validated for testing milk from individual cows. Although milk screening tests can be extremely sensitive, they are not quantitative and cannot confirm a specific drug residue. They are therefore designated Codex Level III methods, suitable for screening large numbers of samples quickly. If federal regulatory agencies conducted milk testing, positive samples on a preliminary screening test would be subjected to further testing methods, which quantify (Codex Level I or II methods) and uniquely identify (Codex Level I only) the drug present. Due to the short shelf-life of dairy products, this level of testing is not feasible as products would expire before conclusive results are available. Because rapid milk screening tests are not quantitative, they cannot be used to establish a withdrawal time after drug administration.

Meat: Pre-slaughter testing for meat antimicrobial residues is more difficult than milk testing, as historically only post-slaughter tests were available. Without a pre-slaughter test, a producer cannot ascertain when a treated animal will no longer contain violative meat residues. The recently introduced Meatsafe[TM] ß-lactam test kit, a rapid immunoassay test, uses urine drug concentration to predict kidney residue concentrations and can be used for antemortem testing.

1.3.7 IP antimicrobial use and drug residues

The IP administration of antimicrobials in cattle cannot be justified if the ensuing drug residue kinetics are not known. An understanding of how a residue depletion experiment is properly conducted is required before attempting to determine these kinetics. For example, appropriate study design of an IP antimicrobial residue depletion experiment requires knowledge of factors involved in WDT determination, such as sample size, timing, and variability. The MRL of each antimicrobial in specific matrices, as well as the sensitivity of commonly used rapid test kits, is required for a reasonable WDT estimation. An appropriate understanding of rapid screening test uses and limitations is necessary before they can be used effectively to screen for drug residues after IP administration. Further information regarding milk residue testing is contained in Chapter 2 of this thesis.

1.4 Antimicrobial Efficacy and Perioperative Use

The broader issue of IP antimicrobial use appropriateness necessitates an understanding of proper antimicrobial use in general. The following is a brief summary of current antimicrobial uses in bovine medicine, predictors of antimicrobial efficacy, negative consequences of antimicrobial use in cattle, and information specific to perioperative use.

1.4.1 Antimicrobials licensed for use in cattle

Many older antimicrobials (such as penicillin and tetracyclines) are available to beef and dairy producers as over the counter drugs, which do not require a veterinary prescription. These products often contain multiple broad label indications based on regulatory approval granted long ago. Newer antimicrobials (such as ceftiofur, florfenicol, tilmicosin, and tulathromycin) are subject to more rigorous regulatory analysis before approval; their label claims can be limited to specific pathogens that cause a particular disease (such as bovine respiratory disease caused by *Mannheimia hemolytica*). Table 1 specifies the common injectable antimicrobials currently licensed in Canada for use in beef or dairy cattle, their indications, and routes of administration. Note extra-label administration of other products not licensed for use in cattle occurs.

1.4.2 Antimicrobial pharmacokinetics and pharmacodynamics

Predicting success or failure when treating an infectious process requires an understanding of numerous host/bacteria/drug factors, collectively known as pharmacokinetics and pharmacodynamics (PK/PD).

Pharmacokinetics (PK): The processes of drug absorption, distribution, metabolism, and elimination (ADME) determine a drug's PK profile. These depend on the physical and chemical characteristics of the drug as well as host physiology and pathology. Primary PK parameters depend only on the physiological process of ADME and include systemic clearance (Cl_S), volume of distribution (V_D), and bioavailability (F). Secondary PK parameters are derived from primary parameters and include maximal plasma drug concentration (C_{max}), time to maximal concentration (T_{max}), elimination half-life ($T_{1/2\ elim}$), and area under the plasma concentration versus time curve (AUC).

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Pharmacodynamics (PD): This is the pharmacological effect of the drug in the body; antimicrobial PD measures a drug's efficacy in killing or inhibiting a particular bacterial species. Antimicrobial PD parameters include the minimum inhibitory concentration (MIC), which is the lowest concentration of drug required to inhibit *in vitro* antimicrobial growth on an agar culture or microtitre plate. Note that only certain methods of antimicrobial susceptibility testing (such as multiple broth or agar dilutions or the Etest®, but not the Kirby-Bauer disc diffusion method) can be used to determine MIC values. In some cases generalizations can be made about the efficacy of certain antimicrobials against a particular bacterial species (such as the resistance of *E. coli* to penicillin). However, the heterogeneity of bacterial resistance genes and their expression within a bacterial population means that a range of MICs will occur for an antimicrobial when tested on large number of a bacterial isolates from a particular species.

PK/PD integration: Once the host-drug PK and drug-pathogen PD are known, the PK/PD parameters can be integrated and modeled to make predictions of antimicrobial efficacy. For example, if the plasma drug concentrations over a defined dosage interval are established (PK), but drug concentrations never reach the lowest quantity required to inhibit microbial growth during *in vitro* susceptibility testing (MIC, PD), it is unlikely that this antimicrobial dose will be efficacious in treating an infection with this bacterial isolate. Current PK-PD models do not perfectly predict antimicrobial efficacy, however. Because most infections are located in the extracellular or interstitial fluid, plasma drug concentrations form the basis of antimicrobial PK and are good surrogate markers of infection site drug concentrations and clinical efficacy (Gunderson et al., 2001). Plasma drug concentrations are not representative of drug concentrations at the site of all infectious diseases, however. For example, macrolides such as tilmicosin and tulathromycin have low plasma concentrations but very high lung and intracellular

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concentrations, and are thus excellent choices for treating bovine pneumonia despite poor plasma PK/PD analyses (USP, 2003). On the other hand, drugs with a low V_D may not penetrate fibrinous pneumonic lung tissue and may be ineffective for treating lower respiratory tract infections despite achieving high plasma concentrations.

Antimicrobial PK/PD: Antimicrobials are generally classified into two PK-PD categories: time-dependent and concentration-dependent. Time-dependent antimicrobials are most efficacious when the length of time the drug concentration at the site of infection is maintained above the pathogen's MIC is maximized (the PK/PD parameter T > MIC). For classic time-dependent antimicrobials such as penicillins and cephalosporins, maximum clinical efficacy occurs when T > MIC is 50-80% of the dosing interval, although other recommendations range from 40-100% (Gunderson, Ross et al., 2001; McKellar et al., 2004; Toutain & Lees, 2004). Maximizing the drug concentration for a short period is not as efficacious as maintaining lower concentrations (but still above the MIC) for longer periods. Therefore, efficacy of time-dependent antimicrobials is generally improved by increasing the dosing frequency, rather than increasing the dose. Concentration-dependent antimicrobials are more efficacious when the drug concentration at the site of infection is maximized, even if for shorter periods of time. The ratios C_{max}:MIC and AUC:MIC are used as PK/PD parameters for concentration-dependent drugs. Ratios of C_{max}:MIC of $\geq$ 8-10 and AUC:MIC of $\geq$ 100-125 have been recommended for fluororoquinolones and aminoglycosides (McKellar, Sanchez Bruni et al., 2004). For concentration-dependent antimicrobials, increasing the dose is typically more effective than increasing the dosing frequency.

1.4.3 Adverse effects of antimicrobial use in cattle

Direct adverse effects: Antimicrobial use can cause a wide range of adverse reactions or toxic effects in animals. Although relatively rare in cattle, cutaneous hypersensitivity reactions have been reported after using ceftiofur and streptomycin (Gauchia et al., 1996; Tyler et al., 1998); potentiated sulfonamides and tetracyclines can cause anaphylaxis, especially after IV administration (CVP, 2005). Renal damage can occur with repeated aminoglycoside or tetracycline doses, or if sulfonamides crystallize in the urinary tract (USP, 2003). Tilmicosin administered by IV infusion is cardiotoxic, and IV tetracyclines can cause cardiovascular dysfunction and collapse due to hypotension (Gyrd-Hansen et al., 1981). A very common deleterious effect of some antimicrobials such as florfenicol and ampicillin is an alteration in microbial gut flora (especially in calves), resulting in diarrhea (USP, 2003; CVP, 2005).

Antimicrobial resistance (AMR): The continued use of antimicrobials inevitably results in selection pressure favouring resistant organisms. Even if the surviving AMR species are non-pathogenic, plasmid-mediated transmission of AMR genes to pathogenic bacteria is a primary concern. Resistant bacterial species are a risk not only for the animal who harbours them, but also for humans who may be exposed to these resistant species when eating animal-derived food products. Regulatory agencies in the US currently require a risk assessment of AMR-emergence when reviewing new animal antimicrobial submissions. Drug sponsors must demonstrate the rate and extent of development of AMR enteric bacteria in the animal's gastrointestinal tract following exposure to a new animal drug (Tollefson, 2004). Similar data is likely to be required in the future for new Canadian drug submissions as well.

Production and financial effects: Using antimicrobials in cattle has direct consequences for the producer. Milk and meat withdrawal times must be observed, which may result in lost revenue (discarded milk) or increased expenditures (extra feeding costs before slaughter). The risk of incurring violative drug residues must be considered, which is especially punitive for dairy producers. Irritating antimicrobial formulations such as long-acting oxytetracycline can cause injection site lesions, reducing the value of the carcass for the slaughter plant. Finally, direct and indirect expenditures (the price of medication and labour costs) are incurred every time antimicrobials are used.

These adverse possibilities when using antimicrobials in cattle require the veterinarian and producer to continually perform cost/benefit analyses. Are the consequences of infection severe for the animal (such as clostridial infections) or the producer's finances (such as mastitis)? What is the likelihood of treatment success? What are the risks of adverse drug reactions or toxicity? Will this use contribute to AMR emergence? What is the withdrawal period and cost of the drug?

1.4.4 Perioperative use of antimicrobials

Because IP antimicrobial administration in cattle is only likely to occur during abdominal surgery, a brief discussion of the current principles governing perioperative antimicrobial use is warranted. Any time surgery is performed there is a possibility of post-surgical infection. Healthy young patients with a competent immune system are unlikely to suffer infectious surgical complications. Proper surgical site preparation, good surgical technique, sterile equipment and operating conditions, and minimal surgery time reduce the risk of postoperative infection (Brumbaugh, 1990; Nicholson et al., 2002; Giguere, 2006). Some surgical procedures have greater risk of postoperative infection despite the best efforts of surgical staff. Human

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surgeries are divided into four classes based on the risk of surgical site infection, with a similar scheme applied to veterinary surgery (Table 2). Prophylactic use of antimicrobials is also used routinely in both human (Bratzler & Houck, 2005) and veterinary medicine (Brown et al., 1997; Whittem et al., 1999; Weese & Halling, 2006) to reduce the likelihood of postoperative infections. Current guidelines include the following recommendations (Gyssens, 1999; Zelenitsky et al., 2002; Bratzler & Houck, 2005; Howe & Boothe, 2006):

- Use perioperative antimicrobials only when needed

- Select the proper drug based on known or predicted efficacy against the likely pathogens to be encountered

- Drug administration should occur *before* microbial contamination occurs, ideally at induction of anesthesia; and plasma drug concentrations should remain above the probable pathogen MIC throughout the operation but no longer.

The evidence that prophylactic antimicrobials reduce surgical infections in people is generally well accepted (Bratzler & Houck, 2005), although data from animal trials is mixed. Some studies show efficacy (Haven et al., 1992; Whittem, Johnson et al., 1999; Eugster et al., 2004) but others demonstrate little or no benefit (Vasseur et al., 1985; Klein & Firth, 1988; Brown, Conzemius et al., 1997). It may be inappropriate to compare results from multiple trials when the type and duration of surgery, skill of the surgeons, and local conditions differ between trials. The optimal prophylactic antimicrobial dose regimen is not conclusively known. However, high plasma antimicrobial concentrations during incision and throughout the surgery are thought to prevent infections by reducing intraoperative bacterial counts below a critical threshold. The patient's immune system can then adequately respond to the remaining pathogens. Prolonged duration of post-surgical antimicrobial therapy does not enhance success (Haven, Wichtel et al., 1992; Gyssens, 1999; Eugster, Schawalder et al., 2004; Giguere, 2006).

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Perioperative antimicrobial data is useful for bovine veterinarians as well, as surgery is a routine part of large animal veterinary practice. Some procedures such as castrations and dehorning are relatively simple with little risk of infection. More invasive abdominal surgery, including caesarian section, left or right displaced abomasum correction, exploratory laparotomy, and omphalophlebitis surgery, can be complicated by infectious processes; post-surgical infection rates for these procedures are reported to be 5 – 15% (de Kruif et al., 1987; Seger et al., 1994; Desrochers et al., 1996; Bedard et al., 2001; Desrochers, 2005). Notable differences between bovine surgery and routine human or small animal surgery include the following:

Location: Whereas nearly all human and small animal surgery is performed in sterile operating rooms with adequate staff, bovine surgery is generally performed in a chute in the veterinary clinic or on-farm, or outdoors with the cow restrained to a fence, tree, or tractor. Sterility is exceptionally difficult to achieve in these situations as dust, flies, or manure may contaminate the surgery.

Anesthesia: Bovine abdominal surgery is performed under local or regional anesthesia, not general anesthesia. Therefore the animal is more likely to move and cause breaks in sterility during surgery.

Anatomy and physiology: Abdominal surgery on cattle can be physically demanding on the surgeon because of the large size of the abdominal cavity and weight of the organs, increasing the risk of contamination. Conversely, the ability of cattle to produce fibrin and wall off abdominal infections minimizes the negative systemic effects of peritoneal contamination.

Further details on perioperative antimicrobial use by bovine veterinarians are presented in Chapter 3.

1.5 The Intraperitoneal (IP) Route of Drug Administration

Before presenting data from IP antimicrobial experiments in cattle, a general review of the IP route of administration is warranted. Medications administered by IP infusion and resulting pharmacokinetics and efficacy are discussed.

1.5.1 Anatomic considerations

The IP (also known as intra-abdominal) route of administration involves delivery of a substance directly into the abdominal cavity, but not into an abdominal organ. The skin, subcutaneous tissue, abdominal musculature and peritoneum must be penetrated before reaching the abdominal cavity. A ventral midline approach is often used in lab and small animals while in dorsal recumbency. Medications can be administered IP in standing cows through the left or right paralumbar fossa. Organs that may come in contact with the medication using this approach include the abomasum, rumen, small or large intestines, cecum, uterus, and bladder. The liver, omasum, and reticulum are generally located too far cranially to come in contact with IP infusions when using a paralumbar approach.

1.5.2 Uses for IP administration

Human: Administration of IP antimicrobials is well documented in the literature for abdominal surgery and peritoneal dialysis. ß-lactams (including piperacillin, cephalothin, cephazolin, ceftazidime, and cefoperazone) and aminoglycosides (kanamycin, gentamicin) are frequently mentioned (Ericsson et al., 1978; Stephen & Loewenthal, 1979; Okuda et al., 1986; Yelon et al.,

1996; Sinswat et al., 2000; Wallet et al., 2000; Sisterhen et al., 2006). Reports of IP clindamycin, lincomycin, vancomycin, and metronidazole use also exist (Stephen & Loewenthal, 1979; Saha, 1985; Schwartz et al., 1986; Sisterhen, Stowe et al., 2006). Other classes of drugs besides antimicrobials have been administered by IP infusion, including the antifungals amophotericin B and flucytosine and the anti-inflammatory/anti-endotoxic compound taurolidine (Arthur et al., 2004; Schneider et al., 2005).

Cattle: Experimental trials in cows have used IP infusions of oxytetracycline in saline, ampicillin/cloxicillin and kanamycin/penicillin preparations designed for intramammary use, and sodium ampicillin / ampicillin anhydrate (Fensterbank, 1976; Klein et al., 1989; Gitzel & Grunder, 1994; Klein et al., 1994). Other compounds that have been deposited intraperitoneally in cattle include calcium EDTA (for the treatment of vanadium poisoning) and semen (for insemination). (Lopez-Gatius, 2000; Gummow et al., 2006)

Other Animals: Various IP antimicrobials have been studied in other animal species, including dogs, rats, rabbits, and fish (Wieriks & Schornagel, 1971; Fry et al., 1986; Bruno, 1989; Ablan et al., 1991; Fairgrieve et al., 2006).

1.5.3 IP Pharmacokinetics

The abdominal cavity has a large surface area and is highly perfused, in theory this should allow for rapid drug absorption. Depending on the site of drug deposition within the abdomen, medication absorbed into the portal bloodstream passes through the liver before reaching the systemic circulation. This potentially results in hepatic metabolism of drug before reaching the systemic circulation, known as "first-pass effect" (Benet, 1990). Conversely, drugs absorbed into the parietal peritoneal blood vessels will not be subject to a first-pass effect.

Kinetic data after IP administration compared to other routes of administration is not available for most drugs. Results from one bovine PK trial with IP administration include a T_{max} of 20 min for sodium ampicillin and 1.0 h for ampicillin anhydrate (Klein, Firth et al., 1989). Rapid absorption was found in one IP kanamycin study in humans, evidenced by a T_{max} of 15 min (Ericsson, Jr. et al., 1978). Other human trials with various antimicrobials had a T_{max} range of 0.5-5 h (Okuda, Katoh et al., 1986; Schwartz, Kowalsky et al., 1986; Sisterhen, Stowe et al., 2006).

1.5.4 Efficacy of IP use

The principle behind IP antimicrobial use during surgery is to achieve high local drug concentrations in the abdomen, where the infection/contamination occurs. However, the lack of randomized trials with positive and negative controls (preoperative IV antimicrobial therapy and IP saline, respectively) makes it difficult to evaluate IP antimicrobial efficacy. One retrospective study in cows showed a significantly lower rate of post-surgical infection after IP administration compared to cows given no antimicrobials (Klein, van der Velden et al., 1994). No comparisons of intraoperative IP versus preoperative intravenous (IV) antimicrobials are available in cattle. The efficacy of IP antimicrobials in reducing infections after human abdominal surgery is inconclusive. Some studies demonstrate efficacy (Yelon, Green et al., 1996), while others show no benefit over other treatment methods (Salvati et al., 1988; Schneider, Sack et al., 2005). One trial in rabbits found an abdominal lavage containing a cephalosporin was more efficacious than saline alone for treating peritonitis, but only if the bacterial contamination was severe and the antimicrobial was administered promptly after contamination (Ablan, Olen et al., 1991). An IP chlorhexidine lavage in mice reduced mortality after cecal puncture compared to cephalosporin and lactated ringer's lavage groups (Bondar et al., 2000).

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1.5.5 Contraindications of IP use

The IP route is generally considered non-irritating and safe in human medicine, although safety may be dependent on the antimicrobial formulation. Human IP medications are all IV formulations thoroughly diluted in an appropriate lavage/dialysis solution. Veterinary literature does contain warnings about IP drug use, including chemical irritation of the peritoneum and adhesion formation after use of tetracycline, neomycin, and streptomycin (Withrow & Black, 1979). There was evidence of peritonitis in cows after IP infusions of ampicillin anhydrate but not sodium ampicillin (Klein, Firth et al., 1989). Other antimicrobials and formulations have not been evaluated for irritability or safety in cattle.

1.6 Project Goals

There is a lack of information in the scientific literature regarding the incidence, kinetics, residues and efficacy of IP antimicrobial use in cattle. A series of research projects were designed to provide the data necessary to answer these questions:

1. Review and update on-farm drug residue test kit data for dairy producers and veterinarians.

2. Before embarking on IP pharmacokinetic (PK) or clinical trials, information was needed on the incidence of perioperative IP use by bovine veterinarians. Although anecdotal reports of IP use are common, both through CgFARAD contacts and the American Association of Bovine Practitioners (AABP) listserve, information was needed to confirm that this practice does frequently occur. This data could also be used to focus IP kinetics / efficacy experiments on the proper antimicrobials and doses. A survey of Western Canadian Bovine Practitioners was

created to better understand perioperative antimicrobial use in general, with specific emphasis on IP administration.

3. If it was determined that IP antimicrobial administration occurs frequently, a PK trial would be performed on the appropriate product(s). Plasma drug concentrations, milk and meat residue depletion, and adverse drug reactions would be determined.

A project determining the clinical efficacy of IP antimicrobial administration would also be required to fully evaluate this practice. However, financial and time restraints meant an efficacy trial was beyond the scope of this MSc thesis. Nonetheless, we anticipated the novel data produced in the planned experiments would provide invaluable insight into the practice of IP antimicrobial use in cattle.

Table 1.1 Commonly used parenteral antimicrobials licensed in Canada for use in beef and/or dairy cattle

Drug Class	Generic Drug	Trade names	Rx OTC	Beef / dairy	Label Indications	Route
ß-lactams	Penicillin	Numerous	OTC	Both	Respiratory disease Wound infections Foot rot Metritis Mastitis	IM IMM
	Ampicillin	Polyflex	Rx	Both	Respiratory disease Enteritis	IM, SC
	Ceftiofur	Excenel	Rx	Both	Respiratory disease Foot rot	IM, SC
	Cephapirin	Metricure	Rx	Both	Metritis	IU
		Cefa-Lak	OTC	Dairy	Mastitis	IMM
	Cloxacillin	Dry-clox	Rx	Dairy	Mastitis	IMM
Tetracyclines	Oxytetracycline	Numerous	OTC	Both	Respiratory disease Foot rot Mastitis Metritis Enteritis Peritonitis Clostridial disease Joint infections	IM, IV
Macrolides	Erythromycin	Erythro-200 Erythro-36 Gallimycin	OTC	Both	Respiratory disease Foot rot Mastitis	IM, IMM
	Tylosin	Tylan-200	OTC	Beef	Respiratory disease Metritis	IM
	Tilmicosin	Micotil	Rx	Beef	Respiratory disease	SC
Lincosamides	Pirlimycin	Pirsue	OTC	Dairy	Mastitis	IMM
Phenicols	Florfenicol	Nuflor	Rx	Beef	Respiratory disease Foot rot Keratoconjunctivitis	IM, SC
Fluoroquinolones	Enrofloxacin	Baytril	Rx	Beef	Respiratory disease	SC
	Danofloxacin	A180	Rx	Beef	Respiratory disease	SC
Sulfonamides, potentiated	Sulfadoxine + trimethoprim	Trivetrin Borgal	Rx	Both	Respiratory disease Enteritis Foot rot	IM, IV
Aminoglycosides	Gentamicin	Gentocin	Rx	Both	Metritis	IU
	Streptomycin	Ethamycin	OTC	Both	Respiratory disease Leptospirosis	IM
	Spectinomycin	Adspec	OTC	Beef	Respiratory disease	SC
Iodides	Sodium iodide	Sodide	OTC	Both	Actinomycosis Actinobacillosis	IV

Rx = prescription only, OTC = over the counter

IM = intramuscular, SC = subcutaneous, IV = intravenous, IMM = intramammary,

IU = intrauterine

Product monographs obtained from Compendium of Veterinary Products, 9th Ed. (CVP, 2005)

Table 1.2 Classification of surgical wounds based on probability of contamination and risk of surgical site infection (Giguere, 2006).

Classification	Criteria	Approx. Risk
Clean	Non-traumatic; sterile technique; respiratory, GI, genitourinary tracts not entered.	<5%
Clean-contaminated	Elective surgery on respiratory, GI, or genitourinary tracts with minimal contamination; minor break in sterility.	5-10%
Contaminated	Contamination from GI tract; surgery into region already infected; major break in sterility; chronic open wound surgery.	10-20%
Dirty	Encounter purulent material during surgery; perforation of respiratory, GI, or genitourinary tracts before surgery; penetrating trauma >4 h old.	>20%

Chapter 2: Milk Residue Testing

The dairy industry actively promotes milk and dairy products as safe and nutritious, and veterinarians play an important role ensuring residues of medications used on farm do not end up reaching the consumer. Milk contaminated with antimicrobials is considered a public health hazard because of possible adverse reactions and antimicrobial resistance. Approximately 5-10 percent of people are hypersensitive to penicillin (Dayan, 1993) and can potentially suffer allergic reactions if they ingest small quantities, including dermal reactions, asthma, or anaphylactic shock (Wicher, Reisman et al., 1969; Lindemayr, Knobler et al., 1981; Kanny, Puygrenier et al., 1994)   Despite these potential risks, confirmed cases of allergic reaction to penicillin in milk are extremely rare, and the residue issue is more a perceived than real problem (Sundlof, 1989; Dewdney, Maes et al., 1991; Dayan, 1993). Another concern with antimicrobial residues in milk is a possible shift in antimicrobial resistance patterns in human enteric bacteria (Tollefson, 2006). Although one can argue the relative risks (or lack thereof) of antimicrobial milk residues, what cannot be argued is the public demand for "wholesome" and "safe" food. Any publicity of drug residues in dairy products weakens consumer confidence, and the dairy industry and government agencies proactively ensure contaminated milk does not reach the market. From the milk processor's perspective, antimicrobials also interfere with the manufacture of dairy products; concentrations of 1 ppb delay starter activity for cheese, butter, and yogurt. These "inhibitors" also decrease the acid and flavor production associated with butter manufacture, reduce the curdling of milk, and cause improper ripening of cheeses (Jones, 1999; Molina, Molina et al., 2003; Payne, Craigmill et al., 2006).

2.1 Drug Violations in Milk

In Canada, regulation of milk and dairy products is done on a provincial basis. Results of residue testing are not readily available, and testing protocols may vary with jurisdiction and individual processors. In the province of Saskatchewan alone, the value of milk discarded due to antimicrobial drug residues is estimated to be $200,000 per year (Christensen, D.; personal communication). A limited residue surveillance program is conducted by the Canadian Food Inspection Agency (CFIA). In 2003/2004, 1,507 milk and cheese products were tested for antimicrobial residues with no positives detected (CFIA, 2004). In comparison, mandatory test reporting is required by American state regulatory agencies under that National Conference on Interstate Milk Shipments. The Pasteurized Milk Ordinance (PMO) requires all bulk milk tankers to be sampled and analyzed for animal drug residues before the milk is processed. In addition, a minimum of four samples from pasteurized fluid milk and milk products must be tested from each plant every six months and each producer must be tested at least four times every six months (FDA, 2004). In 2002/2003, over 4 million tests were conducted for antimicrobials, with 2945 (less than <0.1%) positive (NCIMS, 2004). The violations resulted in the discard of 76 million pounds of milk. The vast majority of residue violations were due to ß-lactam antibiotics, with a few sulfonamide and tetracycline violations.

Surveys indicate that improper use of drugs in the control of mastitis is the major source of residues in milk. The odds that a violative antimicrobial residue will be found in bulk tank milk increases with increasing somatic cell count (SCC) status of the herd (Ruegg & Tabone, 2000; Saville, Wittum et al., 2000). The SCC is an indicator of the prevalence of mastitis within a herd and such infections are routinely treated with antimicrobials in order to lower the SCC to acceptable levels. High SCC is also associated with poor management practices; improper

antimicrobial use practices may contribute to the increased odds of a residue violation. Drugs administered for dry cow therapy are unlikely to cause drug residues if milk is not shipped for the first four days after calving, if dry periods are longer than six weeks, and if dry cows are not accidentally milked.

2.2 Maximum Residue Limits and Withdrawal times

When a drug is approved for use in a food animal species, regulatory authorities establish an acceptable daily intake (ADI). The ADI represents a level of daily intake of a drug which, during an entire lifetime, is without appreciable risk to the health of the consumer. The ADI is used to determine the maximum concentration of a marker residue in edible tissues, honey, milk, or eggs that is legally permitted or recognized as acceptable. In Canada and the European Union these acceptable concentrations are termed "maximum residue limits" (MRLs), and "tolerances" in the US. The MRL is calculated such that daily intake of food containing drug residues at the MRL will result in a total daily consumption of residues in quantities at or below the ADI. The ADI is based on the total residue of a chemical present in food (parent compound and all metabolites), whereas MRLs are based on a single, measurable marker residue (which may be the parent compound or any of its metabolites). In establishing MRLs, consumption factors for the various foods are taken into account. Therefore, foods consumed infrequently or in small amounts are allowed greater MRL values than those foods likely to be consumed daily or which represent a major component of the diet. Because of differences in consumption factors, MRLs may differ between countries; even if ADIs are equivalent (see Table 1) (VDD, 2003). An example is ceftiofur, which has a Canadian MRL of 100 ppb in milk versus 50 ppb in the US. Canadian MRLs can be found at http://www.hc-sc.gc.ca/dhp-mps/vet/mrl-lmr/mrl-

lmr_versus_new-nouveau_e.html. Note that not all drugs licensed for use in dairy animals in Canada have an established MRL in milk, such as oxytetracycline (OTC) and cloxacillin. In these cases, any drug residue detected constitutes a residue violation.

The milk withdrawal time (WDT) for a drug with a lactating dairy cow claim is based on the time required after treatment for milk residues to fall below the MRL in 99% of animals, 95% of the time. Differences may occur between label milk WDTs for the same product in Canada and the US. In Canada there is no assumption regarding dilution of drug residues in the bulk tank, so when establishing a WDT milk from any individual cow must be below the legal MRL. The Food and Drug Administration in the US assumes that no more than one-third of the milk in the bulk tank will come from treated cows. Therefore the label WDT is determined so that the milk from any treated cow will be less than 3 times the legal MRL (FDA, 1994). Veterinarians and producers must remember that US WDTs do not apply in Canada, as evidenced by intramammary pirlimycin (Pirsue®, Pfizer Animal Health) which has a 48 h milk withdrawal time in Canada but only 36 h in the US (CVP, 2005; CVP, 2005). The milk WDT is not the point at which residues can no longer be detected. Currently, milk WDTs are established using a quantitative chemical test as the milk screening tests do not have the required analytical characteristics to establish official WDTs.

2.3 Elimination Kinetics

Drug elimination from a cow typically follows a characteristic pattern, in that the amount of drug eliminated per unit of time is proportional to the amount of drug present (1st order or linear kinetics). Because of this logarithmic nature of drug depletion, doubling a drug dose is not a major cause of residue violations, as doubling a dose only adds one elimination half-life to the

withdrawal time (Figure 2). However, giving label doses to a sick or geriatric cow significantly increases the risk for having a residue violation even if the label withdrawal time is followed. Pathology such as renal or hepatic dysfunction, dehydration, or hypoproteinemia can alter drug distribution and clearance. These changes may prolong the elimination half life ($T_{1/2\ ELIM}$ = 0.693 x V_D / Cl_S). Variation in the half life can have profound effects on the WDT (Figure 2).

Improper injection techniques can cause violative residues even with label dosages. If slowly-absorbing formulations like procaine penicillin G are injected between muscle groups or given subcutaneously, the absorption is delayed (Papich et al., 1993). Most drugs will be absorbed more quickly and completely if given intramuscularly in the cow's neck rather than the hindquarter muscles. Injecting excessive drug volumes per site can also result in a decreased absorption rate and elongated withdrawal time.

2.4 Drug Residue Testing

Drug residues in milk can be detected by several methods. Most rapid, on-farm screening tests are microbial growth inhibition assays such as the Charm Farm Cowside (Charm Sciences Inc., Lawrence, Massachusetts, USA) and Delvotest SP (DSM Food Specialties, Delft, The Netherlands), or ELISA-based tests such as the IDEXX SNAP series (IDEXX Laboratories, Inc.,Westbrook, Maine, USA). More expensive and bulky equipment used at processing plants includes the Charm II system (an immuno- or microbial receptor assay) and the Charm ROSA immunoassay (Charm Sciences Inc.). Some screening test results can be assessed visually (a qualitative "yes or no"), while other systems incorporate semi-quantitative detectors.

Rapid antimicrobial screening tests are validated only for use with bulk tank or tanker-truck milk samples. Despite brand names that may include the term "cowside", none of the tests

are currently validated for testing milk from individual cows. Likewise, no screening test is validated for sheep or goat milk, although certain tests appear adequate for this purpose including IDEXX SNAP ß-lactam, Delvotest, Charm Farm Cowside, and Charm II test kits (Zeng et al., 1998; Althaus et al., 2003). To become certified by the Association of Official Analytical Chemists (AOAC), milk residue tests must pass rigourous evaluations for sensitivity, repeatability, and robustness. Each test has a ninety percent sensitivity level (90/95) for specific drugs. This is the estimated lowest concentration of that drug in milk that will give a positive result on 90% of truly positive samples, with 95% confidence (19 times out of 20). For a test kit to be AOAC certified, this 90/95 number must be below the MRL (Europe) or tolerance (US) of the jurisdiction where the test is sold. As well, drug residues at the MRL concentration must be detected with 100% sensitivity (all truly positive samples will test positive). Because Canadian and American MRLs are not identical, some screening tests designed for the US market may not be ideal for use in Canada. For example, the Charm Farm Cowside test has a sensitivity of 300 ppb for oxytetracycline (OTC) in milk, the same as the US tolerance. However, no MRL exists for OTC in milk in Canada and other tests can detect OTC at far lower concentrations, such as the IDEXX SNAP tetracycline test which can detect concentrations at or below 30 ppb. Therefore a Canadian dairy producer using the Charm Cowside test could inadvertently ship OTC-contaminated milk that may be detected if the plant uses a different test.

Because 90/95 levels must be below the MRL, screening tests can produce a positive result when the drug concentration is below the legal MRL. These "subviolative" positive test results are positive test results on a milk sample in which the actual drug concentration is at or above the detectable concentration of the test, but below the established MRL. With all of the tests, there is a characteristic response curve, which means that as the drug concentration

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increases in the milk, there is a corresponding increase in the percentage of positive tests until a plateau is reached and all samples test positive. Even if two different tests have the same 90/95 results at the MRL, the responses at less than MRL concentrations can differ. In the example in Figure 2, two test kits have almost exactly the same sensitivity level (7.5 ppb for Test A and 7.7 ppb for Test B). But at the low penicillin concentrations, Test B gives a significantly greater percentage of positive test results. In Canada, depending on the provincial regulations and the contract between the producer and the processing plant, the label WDTs and legal MRLs for drugs used in dairy cattle are essentially meaningless. If provincial law or the contract states that there shall be no drugs in the milk, then the provincial authority or the processor is free to use any validated residue detection test, even if its 90/95 sensitivity level is far below what was determined to be safe for human consumption (the legal MRL). This is problematic for some drugs like ceftiofur and cephaprin (intrauterine formulations) that have zero milk WDTs on the label, but for which screening test sensitivity is far below the MRL that was used to establish the zero WDT. When evaluating the values for a screening test, the sensitivity is the concentration of the drug in the milk that the test will detect at the 90/95 level. If the sensitivity of the SNAP Beta-lactam test is 5.4 ppb for ceftiofur, then it will correctly detect 90% of samples that actually contain 5.4 ppb ceftiofur, 95% of the time. But it is possible for the SNAP Beta-lactam test to detect as little as 1 ppb of ceftiofur in the milk on occasion – a subviolative positive at 1/100th of the legal MRL of 100 ppb. Also, rapid testing methods incorporating semi-quantitative visual detectors will give a range of actual readings at any single drug concentration. For example with Test B and a sample truly containing 6 ppb, repeating the test could give a range of readings from 4 to 12 ppb. To determine the actual drug concentration of a sample, a truly quantitative method must be used such as high performance liquid chromatography (HPLC).

The issue with subviolative positives becomes more complicated when using multi-residue tests such as the beta-lactam or sulfonamide screening tests. Each multi-residue test detects one or more drugs at concentrations below their respective MRL, but is not ideal for detecting all drugs (especially cloxacillin). When testing for a known or suspected drug in milk, it is best to use a test that is designed specifically for that drug. When testing milk from cows where the treatment history is unknown, it is better to use a multi-drug screening test. However, a positive result on a multi-drug test will not identify which specific drug is present.

The rejection of subviolative but "safe" milk is an economic issue for dairy producers, who may not understand how they can use an approved drug according to label directions, follow the label WDT, and still have a residue violation. The regulatory authorities and processors know that these testing methods will result in a very small percentage of milk being dumped for testing positive, even though the drug residues are safe for human consumption (below the MRL) (CVM, 1996). Identifying the specific drug and quantity present in a milk sample requires more specific chemical analysis, such as HPLC or mass spectrometry. This is not feasible for every milk sample testing positive with a rapid screening test; due to the time and expense of witholding a positive milk tanker from the food supply until conclusive results are obtained. The authorities accept the imprecision of the screening tests for the sake of the public good and the efficient delivery of milk products to consumers.

2.4 Considerations When Choosing and Using Milk Residue Tests

When recommending antimicrobial screening tests to clients, the sensitivity, cost, availability, and ease of use of the test must be considered. In most cases, the choice should be a kit that tests as sensitive as possible, at least to the same concentration as the test that the

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regulator or processor is using. Different processors may use different tests, so it is important to know which method the processor is using. In Table 1, commercially available screening tests that are readily used "cowside" are compared according to their sensitivities for specific antimicrobials against the MRL values in the United States, Canada and the European Union.

High levels of natural inhibitors are present in mastitic milk and in colostrum; they can cause false positive results in the microbial growth inhibition assays, such as Charm Farm Cowside or Delvtotest SP. Heat treatment of milk to 82°C for 5 min inactivates natural inhibitors and can be used to prove false-positive results in the microbial growth inhibition assays (Kang et al., 2005). High concentrations of milk protein and milk fat can adversely affect antimicrobial residue test performance, but the degree of the effect depends upon the analytical method of the screening test (Andrew, 2000). Higher concentrations of immunoglobulins and milk protein can also cause false positives with screening tests used on samples from recently freshened heifers or cows (Andrew, 2001).

Because rapid milk residue tests will only detect certain antibiotics or classes of antimicrobials, veterinarians should review farm drug use with their clients so an appropriate test can be recommended. If more than one drug is given, a single test may not be adequate to ensure that the milk is free of detectable residues. Despite the fact that test kits are not validated for individual animals, milk from all sick or dehydrated cows that have been treated with antimicrobials should be tested, even if label instructions were followed. If there is no suitable on-farm test available, the producer should ask the processor to check a milk sample before adding milk from a treated cow to the bulk tank. Rapid screening tests do not detect drugs other than antimicrobials; however, the CFIA does carry out quantitative testing for other drugs and chemicals, including flunixin, ivermectin, phenylbutazone, and pesticides.

Most of the screening tests use a color comparison against a control to indicate a positive or a negative test result. The test operator must be able to distinguish between intensities of color for accurate results. While they tend to be expensive, we recommend purchasing the electronic reader if it is available with a screening test. Otherwise, any milk with a questionable result should be discarded and the cow re-sampled at the next milking. Clients must be warned that screening tests are not meant to shorten the official milk WDT. As well, producers should not dilute "positive" milk by adding it to the bulk tank milk as the actual amount of drug present cannot be quantified with the screening test. One cannot be certain that diluting the positive milk will be sufficient, and the entire bulk tank may end up contaminated. During our recent penicillin studies, one dose of 21,000 IU/kg procaine pen G administered via intraperitoneal infusion resulted in penicillin concentrations in milk of nearly 400 ppb 6 h later (see Chapter 4 for further details). Even if diluted with milk from 100 untreated cows, the bulk tank penicillin concentration (4 ppb) would still be detected by most screening tests.

Figure 2.1 Milk Residue Depletion Curves
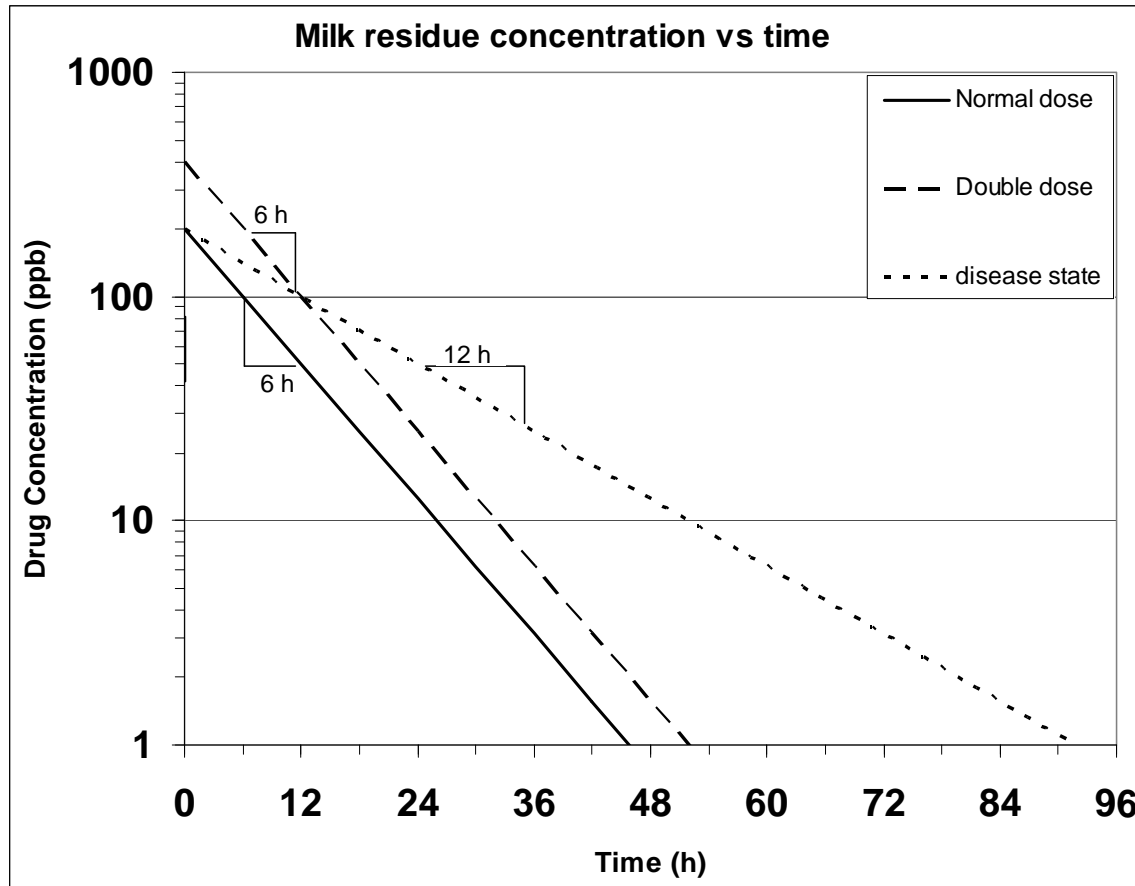
For the normal dose in a healthy cow (solid line), drug concentration in milk decreases by one-half every 6 h. Assuming the MRL is 1 ppb, the drug will have a WDT of approx. 46 h. Doubling the dose (--- --- ---) results in the same half-life (6 h). The WDT will be one half-life longer (approx. 52 h). If disease processes affect drug distribution or clearance, a longer half-life may result (- - - -). Although the dose was the same as the normal treatment, doubling the elimination half-life effectively doubles the withdrawal time, in this case to 92 h.

Figure 2. **Test Sensitivity Curves**
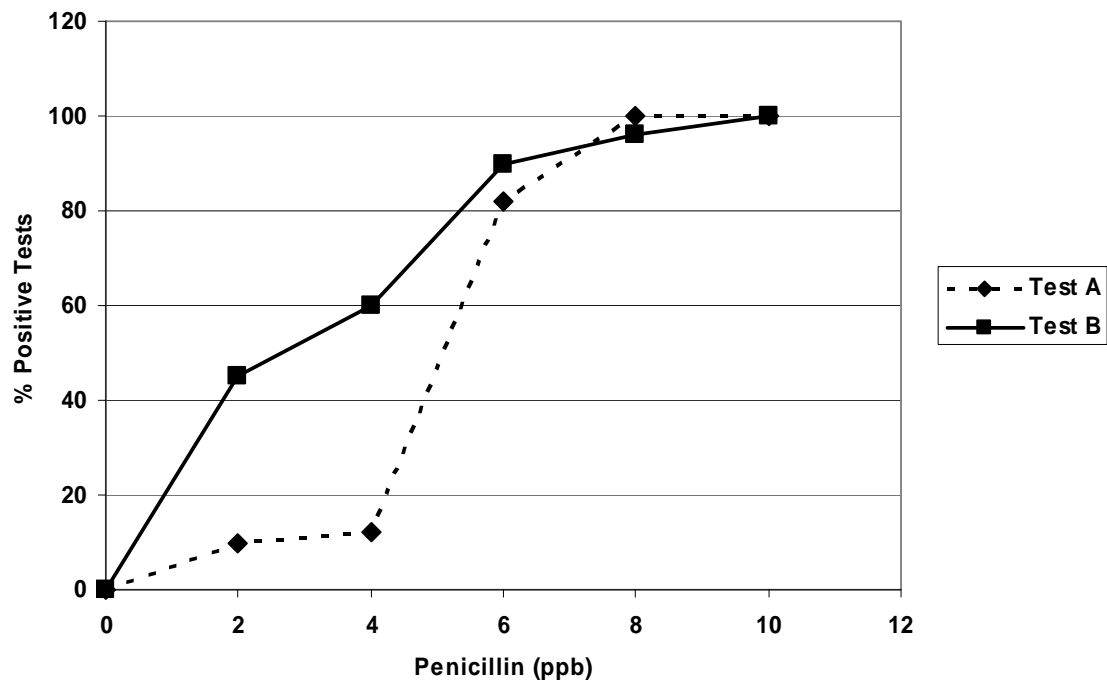
Test A and Test B have similar 90/95 sensitivities at penicillin concentrations of 10 ppb, but Test B is more likely to have subviolative positive results at concentrations less than 10 ppb.

Table 2.1 Comparison of commercially available on-farm screening tests for antimicrobials (as of December 2006)

Drug	Test	Sensitivity (ppb)	US MRL (ppb)	CDN MRL (ppb)	EU MRL (ppb)
Amoxicillin	Charm Farm Cowside	6	10	NE	4
	Delvotest P	6			
	IDEXX SNAP B-lactam	7.3			
Ampicillin	Charm Farm Cowside	5	10	10	4
	Delvotest P	5.8			
	IDEXX SNAP B-lactam	5.8			
Ceftiofur	Charm Farm Cowside	300	50	100	100
	Delvotest P	50-70			
	IDEXX SNAP B-lactam	5.4			
Cephapirin	Charm Farm Cowside	10	20	20	10
	Delvotest P	7			
	IDEXX SNAP B-lactam	11.7			
Chlortetracycline	Charm Farm Cowside	300	300	NE	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	30			
Cloxacillin	Charm Farm Cowside	30	10	NE	30
	Delvotest SP	20			
	IDEXX SNAP B-lactam	50			
Dicloxacillin	Charm Farm Cowside	25	NE	NE	30
	Delvotest P/SP	100			
	IDEXX SNAP B-lactam	50			
Erythromycin	Charm Farm Cowside	150	50	50	40
	Delvotest SP	250			
Gentamicin	Charm Farm Cowside	300	30	NE	100
	IDEXX SNAP Gentamicin	30			
	Delvotest SP	400			
Lincomycin	Charm Farm Cowside	200	150	NE	150
	Delvotest SP	400			
Neomycin	Delvotest P	150	150	NE	500
Novobiocin	Delvotest P/SP	600	100	NE	100
Oxytetracycline	Charm Farm Cowside	300	300	NE	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	30			
Penicillin	Charm Farm Cowside	4	5	10*	4
	Delvotest SP	2.7			
	IDEXX SNAP B-lactam	3.0			
Pirlimycin	Charm Farm Cowside	200	400	400	100
	Delvotest SP	50			
Polymixin B	Delvotest P	30	0	4	NE
Sulfonamides	Charm Farm Cowside	20-200	10	10	100
	Delvotest SP	100			
Tetracycline	Charm Farm Cowside	100	300	NE	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	20			
Tilmicosin	Charm Farm Cowside	100	0	NE	40
Tylosin	Charm Farm Cowside	100	50	NE	50
	Delvotest P/SP	100			

NE: no legal maximum residue limit established, *MRL value is in IU/mL

Chapter 3: A survey of antimicrobial use during bovine abdominal surgery by Western Canadian veterinarians

3.1 Abstract

Members of the Western Canadian Association of Bovine Practitioners were surveyed regarding their use of antimicrobials in bovine abdominal surgery.   Perioperative antimicrobials were used in 100% of abdominal surgeries by 96 of 98 respondents. Although postoperative administration was the most common perioperative period for antimicrobial use, intraoperative intraperitoneal (IP) use was reported by more than half of the veterinarians surveyed. Procaine penicillin G and oxytetracycline were the most commonly administered perioperative antimicrobials.

3.2 Introduction

The Canadian global food animal residue avoidance databank (CgFARAD) has received many requests for meat and milk withdrawal recommendations after perioperative extra-label use of antimicrobials, including the intraperitoneal (IP) or intra-abdominal infusion of antimicrobials. Due to the unhygienic operating conditions often encountered during ambulatory surgeries, many veterinarians choose to administer perioperative antimicrobials. Gram-positive staphylococci, Gram-negative enteric bacteria, and anaerobes are all potential contaminants during ambulatory bovine surgery. In human medicine, current guidelines call for prophylactic antimicrobials to be selected based on predicted efficacy against probable pathogens and administered before microbial contamination occurs (Gyssens, 1999; Zelenitsky, Ariano et al., 2002). The success of preoperative antimicrobial prophylaxis has also been demonstrated in bovine surgery (Haven, Wichtel et al., 1992). However, due to withdrawal times required for most antimicrobials used

in cattle, presurgical antimicrobials may be withheld if the prognosis is uncertain and the animal is to be salvaged for slaughter. Following successful corrective surgery, practitioners may opt to administer antimicrobials directly into the abdomen.

The IP route is employed in human abdominal surgery and peritoneal dialysis, with cephalosporins and aminoglycosides commonly used (Ericsson, Jr. et al., 1978; Okuda, Katoh et al., 1986; Yelon, Green et al., 1996). All reports cite antimicrobial formulations suitable for intravenous use that are thoroughly diluted in appropriate lavage/dialysis solutions. The efficacy of IP antimicrobials in reducing infections after human abdominal surgery has not been proven decisively. The kinetics of IP antimicrobials in humans are variable, with maximum plasma concentrations occurring from 15 min - 5 h after administration for various antimicrobials (Ericsson, Jr. et al., 1978; Okuda, Katoh et al., 1986; Schwartz, Kowalsky et al., 1986). Intraperitoneal antimicrobials are also used in animal species, including dogs, rabbits, and fish (Fry, Trachtenberg et al., 1986; Bruno, 1989; Ablan, Olen et al., 1991; Fairgrieve, Masada et al., 2006). One trial in rabbits found that an abdominal lavage containing a cephalosporin was more efficacious than saline alone for treating peritonitis, but only if the bacterial contamination was severe and the antimicrobial was administered promptly after contamination (Ablan, Olen et al., 1991). Experimental trials in cows have used IP infusions of oxytetracycline in saline and ampicillin/cloxicillin and kanamycin/penicillin preparations designed for intramammary use (Fensterbank, 1976; Gitzel & Grunder, 1994; Klein, van der Velden et al., 1994). One retrospective study showed a lower rate of post-surgical infection after IP administration compared to cows not given antimicrobials (Klein, van der Velden et al., 1994). However, comparisons against pre-operative intravenous (IV) prophylaxis are lacking.

Although the IP route is generally considered non-irritating and safe in human medicine, the formulation of the antimicrobial may play an important role. There is evidence of peritonitis in cows after IP infusions of an ampicillin anhydrate formulation, but not of sodium ampicillin (Klein, Firth et al., 1989). Other antimicrobials and formulations have not been evaluated for peritoneal inflammation or safety. Another issue that has not been addressed in cattle is withdrawal times after IP administration. Because this practice is extra-label, practitioners have contacted CgFARAD for advice regarding meat and milk withdrawal times. Unfortunately, insufficient data is available in the literature to develop an informed withdrawal interval estimate. The CgFARAD has received anecdotal reports of cows with penicillin-positive milk samples for weeks after perioperative IP use, although cows given ampicillin by IP infusion had positive milk tests for only 24-96 hours, depending on the formulation (Klein, Firth et al., 1989). Tissue residue depletion kinetics in cows are not available, although a 1955 study found residues in muscle, kidney, and liver in bulls given IP oxytetracycline one hour before slaughter (Kersey et al., 1955). As well, trials using IP injections of oxytetracycline and macrolides in salmon found detectable drug residues in various tissues up to 8 weeks later (Bruno, 1989; Fairgrieve, Masada et al., 2006).

The frequency of IP antimicrobial administration during bovine surgery has not been reported. Before pursuing a kinetic trial to evaluate this practice, we surveyed members of the Western Canadian Association of Bovine Practitioners (WCABP) on their antimicrobial use during bovine abdominal surgery. The objective was to determine the frequency and specifics of perioperative antimicrobial use by bovine veterinarians, with special emphasis on IP use.

3.3 Materials and Methods

Survey Design

In June of 2005, surveys were mailed to all 240 members of the WCABP, a voluntary membership organization representing mixed and large animal veterinarians practicing beef and/or dairy medicine in the 4 Western Canadian provinces. Surveys were mailed along with pre-paid return envelopes as part of the WCABP quarterly newsletter. The survey was designed by two of the authors (AC, PD) and reviewed by one clinician at the Western College of Veterinary Medicine large animal clinic. Respondents were asked to record the percentage of cattle undergoing abdominal surgery they treated with antimicrobials at each of three surgical periods (preoperative, postoperative, or intraoperative IP). Although data regarding IP use was the primary motivation for the survey, preoperative and postoperative antimicrobial use questions were included to give perspective on IP use compared to other time periods of perioperative antimicrobial use. Veterinarians were also asked to list which drug(s) they commonly used at each surgical time frame and what dose and route of administration were used. Trade or generic names of antimicrobials were accepted. Because IP use of antimicrobials is extra-label, meat and milk withdrawal intervals (WDI) recommended by the veterinarian after IP use were also asked. See Figure 1 for a sample survey response.

Statistical Analysis

As the data were non-normally distributed, a Kruskal-Wallis one-way analysis of variance was used to test for significant differences between frequency of antimicrobial use for each surgical period (α=0.05). Post-hoc comparisons were performed with Wilcoxan Rank Sum tests and Bonferroni's correction. Data was analyzed using a commercial software package (Statistix Version 8, Analytical Sotfware, Tallahassee, FL).

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3.4 Results

Response rate and data tabulation: Survey responses were received for 6 weeks after mailout. The survey response rate was 40.8% (98/240). As responses were anonymous, no specific follow-up with non-respondents or respondents was possible. A general follow-up to all survey recipients was not performed. Drugs were categorized according to their generic formulation, regardless of brand. Frequencies of antimicrobial administration were combined into the following categories: Never (0%), Rarely (1-24%), Sometimes (25-74%), and Frequently (75-100%). Since there were few respondents that identified use as either 25 – 50 % or 50 – 74% these responses were combined in the 'Sometimes' category.

Perioperative antimicrobial use: Of the 98 respondents, 96 treated all cattle with some type of perioperative antimicrobial. Seventy-nine of 98 respondents administered postoperative antimicrobials to at least 75% of their surgical patients. Intraoperative IP antimicrobial use was reported by 54 of 98 respondents at least some of the time, with 30 of 98 respondents using IP antimicrobials frequently ($\geq$ 75% of their surgeries). Forty-four of 98 respondents never utilized IP antimicrobials. Those using IP antimicrobials reported a frequency of administration ranging from 1 – 100% of surgeries. Preoperative antimicrobials were administered frequently by 18/98 respondents, while a slight majority (52/98) of respondents never administered preoperative antimicrobials before bovine abdominal surgery. There was a significant difference ($p < 0.001$) between the frequency of postoperative administration versus preoperative and IP administration. No significant difference between the frequency of preoperative and IP administration was observed.

Types and doses of antimicrobials used: The antimicrobials predominantly used perioperatively by the survey respondents are shown in Figure 2. Overall, penicillin and oxytetracycline were the most commonly used antimicrobial at each perioperative period. Other antimicrobials used include chlorhexidine (IP, n=1), nitrofurazone (IP, n=1), and sulfamethazine (IP, n=1; postoperative, n=1).   Doses of preoperative and postoperative antimicrobials closely followed manufacturer's label recommendations. Doses of the IP antimicrobials used predominantly were similar to the label IM/SC dose (n=28), with 8 respondents using more and 3 using less than the label dose.

IP meat and milk withdrawal intervals: Withdrawal intervals (WDIs) recommended by respondents after IP administration of various antimicrobials are shown in Figures 3a and 3b. WDIs ranged from 3-14 days (n=14) for penicillin in milk, 3-60 days (n=5) for oxytetracycline in milk, and 5-120 days (n=51) for various antimicrobials in meat. Thirteen of 14 respondents recommended a milk WDI of 96 hours or more after using penicillin IP, and 5/5 recommended 72 hours or more after IP oxytetracycline LP use. Meat WDIs were $\geq$ 10 days for 23/25 respondents who used IP penicillin, and 9/11 respondents recommended $\geq$ 18 days after IP oxytetracycline use.

3.5 Discussion

The purpose of this survey was to develop a better understanding of the current perioperative antimicrobial practices of bovine veterinarians in Western Canada, with specific emphasis on intraperitoneal (IP) use. Members of the Western Canadian Association of Bovine Practitioners (WCABP) were chosen to receive this survey as they were deemed representative of bovine practitioners in Western Canada. Although approximately 27% of veterinarians in

Western Canada performing bovine work are members of the WCABP, we felt this was a representative sample of bovine veterinarians. Few veterinary clinics have more than one WCABP member, so clustering of responses by clinic would be minimized in this study. Potential biases may be present in the survey results. Antimicrobial usage by WCABP members may not mirror that of non-WCABP bovine practitioners, or that of the WCABP members who did not respond to the survey. A lack of central information on other veterinarians practicing bovine medicine in Western Canada precluded sending surveys to non-WCABP members.

Interpretation of survey results was also limited by other factors. Veterinarians who routinely use perioperative antimicrobials may have been more likely to respond than non-users. The percentage of surgeries receiving antimicrobial treatment was likely a "best guess" and may have been under- or over-estimated. As respondents were instructed to write their specific antimicrobial choices and dose regimens, answers were sometimes ambiguous. Respondents were not asked information on personal and practice details that might affect antimicrobial usage, such as veterinary school attended, year of graduation, or how their perioperative regimen was established. However, despite these difficulties a number of observations can be made from the data.

Perioperative antimicrobial use is routine during bovine abdominal surgery, as nearly all respondents treated all of their bovine abdominal surgeries with an antimicrobial. The most common surgical period for antimicrobial administration in this group of respondents was postoperatively, despite limited evidence of efficacy compared to preoperative administration (Haven, Wichtel et al., 1992). It is unknown why practitioners continue to favour this practice when other alternatives appear more effective. Perhaps the evidence of limited efficacy has not reached practitioners or empirical surgical success leads them to believe postoperative therapy is

beneficial. One possible reason for the relatively low frequency of preoperative antimicrobial use is the unwillingness to incur a withdrawal time if the surgical prognosis is poor and the animal will instead be sent to slaughter. Some drugs used preoperatively in this survey are long-acting formulations (such as oxytetracycline LA or benzylpenicillin) that do not achieve therapeutic plasma concentrations by the time surgery is underway (Papich et al., 1994; Craigmill et al., 2004).

Intraperitoneal (IP) antimicrobial use also occurred during bovine abdominal surgery. Though the frequency of IP use in individual animal surgeries cannot be reliably estimated from this survey, enough respondents indicated frequent IP use (30/98 responses use it during $\geq$ 75% of their surgeries) to conclude that this practice is not an isolated occurrence. Possible rationales for IP use include ease of administration, perceived quicker absorption than intramuscular or subcutaneous injections, and a perceived local antimicrobial effect. However, IP kinetic data is limited and a local (peritoneal) antimicrobial effect has not been proven.

The majority of WDIs recommended after IP antimicrobial administration were longer than the manufacturer's label withdrawal time (WDT) after intramuscular (i.m.) use of the same drug. Respondents were not specifically asked whether on-farm drug residue milk tests were used, or if CgFARAD was contacted for a withdrawal recommendation. A large number of "not applicable" responses were given for milk withdrawal recommendations, presumably because the surgeries were performed on beef cattle.

Procaine penicillin G and oxytetracycline were the most commonly administered perioperative antimicrobials. However, some clearly inappropriate drugs were administered perioperatively as well. These include the IP use of neomycin (inappropriate spectrum of

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activity, chemical irritation, and prolonged residues due to renal accumulation), nitrofurazone (banned in food producing animals), and chlorhexidine (chemical irritation).

Perioperative antimicrobial use was practiced by the majority of WCABP members who responded to this survey. Postoperative administration was the most common perioperative period for antimicrobial use, but IP use was reported to occur in at least some surgeries by more than half of the respondents. Information on IP antimicrobial use provided by this survey has guided the specific direction of the authors' ongoing IP antimicrobial pharmacokinetic trials.

Figure 3.1 Perioperative antimicrobial survey sample response.

The Canadian gFARAD receives inquiries for appropriate withdrawal times for meat and milk after administration of antibiotics for bovine abdominal surgery. Little data exists to determine proper withdrawal times in these circumstances. We are surveying WCABP members about their peri-operative antibiotic use. This information will help us design studies to validate "cowside" tests and propose appropriate withdrawal times. We would greatly appreciate it if you would take the time to fill out this form and send it back in the postage-paid envelope. For more information, contact **Dr Patricia Dowling at 306-966-7359 or trisha.dowling@usask.ca**

1. How frequently do you perform abdominal surgery (for example Caesarean-section, displaced abdomen, and exploratory laparotomy) on bovine patients?

    _____ <10 surgeries per year _____✓_____ 50-100 surgeries per year _____ 10-50 surgeries per year _____ >100 surgeries per year

2. What percentage of cows do you treat with **PRE-operative** antibiotics? _____O_____ %

Which antibiotic(s) do you commonly use?

Drug (Brand name/Generic name)	Dose	Route

3. What percentage of cows do you treat with **POST-operative** antibiotics? _____100_____ %

Which antibiotic(s) do you commonly use?

Drug (Brand name/Generic name)	Dose	Route	Duration
Procaine Pen G.	20 000 iu/kg I.M	4days	

4. What percentage of cows do you treat with **INTRA-abdominal** (intra-peritoneal) infusion of antibiotics? _____~75_____ %

Which antibiotic(s) do you commonly use, and what withdrawal time(s) do you recommend?

Drug (Brand name/Generic name)	Dose	Milk withdrawal	Meat withdrawal
Procaine Pen G.	20 000 iu/kg	5 days	usually not an issue

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Figure 3.2 Frequency of perioperative antimicrobial administration by type of antimicrobial and time of administration
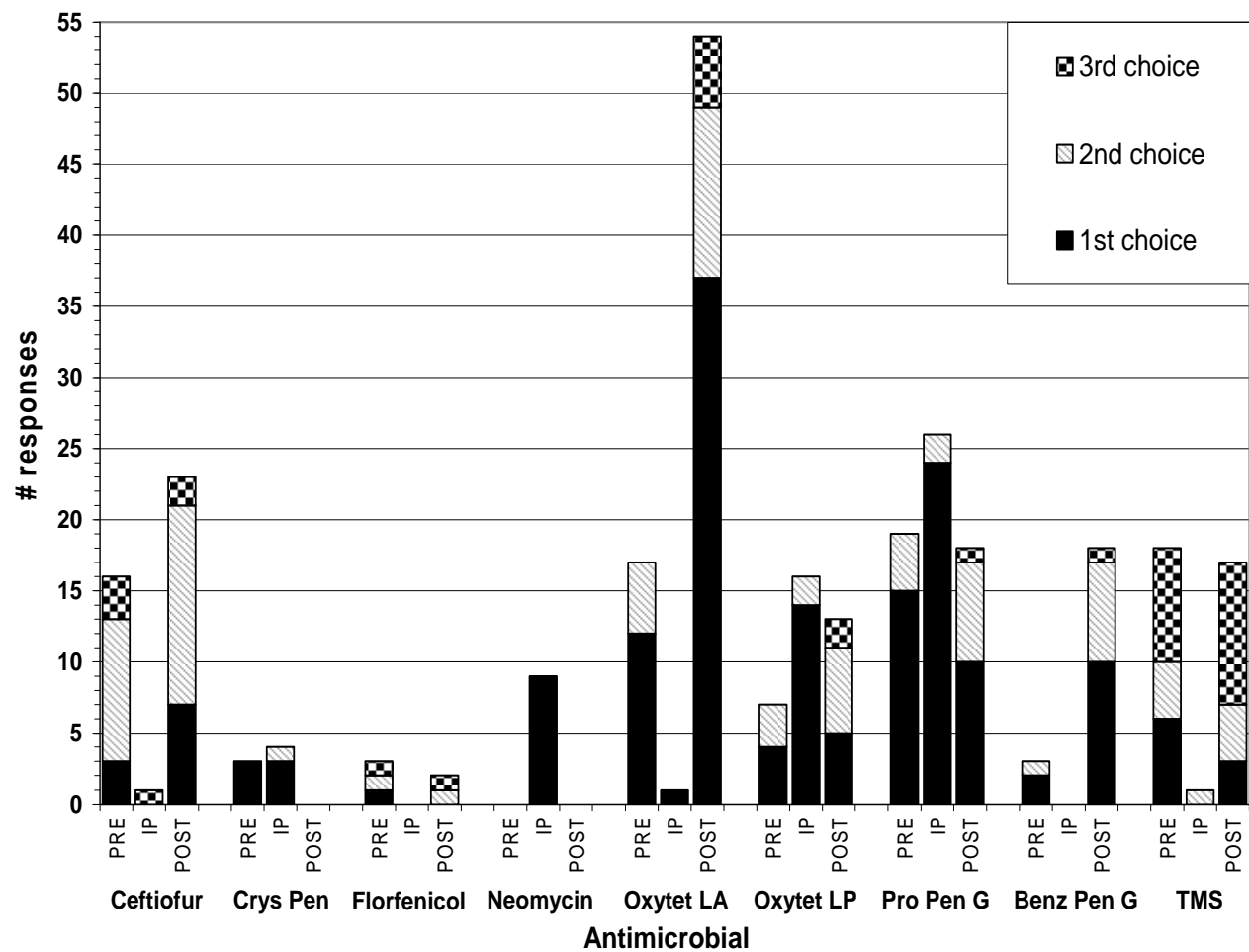
PRE = preoperative, IP = intraperitoneal, POST = postoperative
Crys Pen = Crystalline penicillin G
Oxytet LA = Oxytetracycline (long-acting formulation)
Oxytet LP = Oxytetracycline (short-acting formulation)
Pro Pen G = Procaine Penicillin G (short-acting)
Benz Pen G = Benzathine + Procaine Pencillin G (long-acting)
TMS = Trimethoprim/Sulfonamide combination

Figure 3.3 Milk and meat withdrawal intervals (WDIs) recommended by veterinarian after IP use of primary antimicrobials
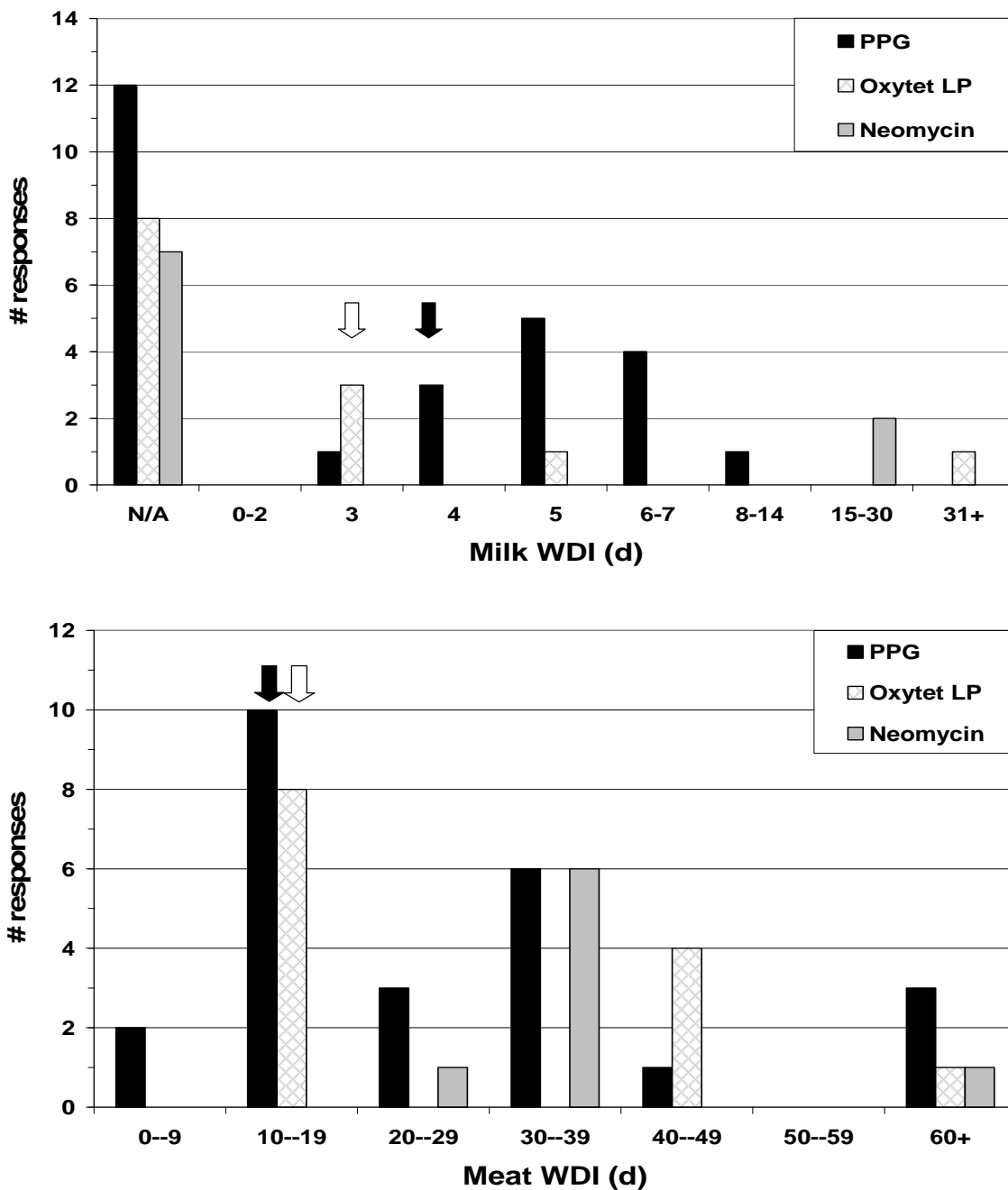
PPG = procaine penicillin G

Oxytet LP = oxytetracycline LP (short acting)

Vertical arrows indicate manufacturer's label withdrawal time (WDT) after intramuscular (IM) administration. Empty arrow = oxytetracycline LP: WDT, 3 d (milk), 18 d (meat). Solid arrow = procaine penicillin G: WDT, 4 d (milk), 10 d (meat). Neomycin is not labeled for parenteral use in cattle and does not have an established milk or meat withdrawal time. N/A = not applicable (no milk WDI given for beef cattle).

Chapter 4: Pharmacokinetics and residues after IP administration of procaine penicillin G in lactating dairy cows

4.1 Abstract

This study describes the pharmacokinetic profile of procaine penicillin G after intraperitoneal (IP) administration in 8 lactating dairy cows. Procaine pencillin G (PPG, 21,000 IU/kg) was deposited into the abdominal cavity of each cow following an incision in the right paralumbar fossa. Blood and milk samples were taken over the following 10 days, at which point the cows were euthanized. Plasma, milk, muscle, liver, and kidney penicillin concentrations were determined by HPLC, with a limit of detection (LOD) of 5 ppb for plasma and milk samples. Noncompartmental methods were used to analyze plasma kinetics. The mean pharmacokinetic parameters (± s.d.) were: C_{max}, 5.5 ± 2.6 µg/mL; T_{max}, 0.75 ± 0.27 h; $AUC_{0-\infty}$, 10.8 ± 4.9 µg*h/mL; MRT, 2.2 ± 0.9 h. All milk from treated cows contained penicillin residues for a minimum of 3 milkings (31 h) and maximum of 5 milkings (52 h) after administration. Concentrations of penicillin G in all muscle, liver, and kidney samples taken 10 days post-administration were below the limit of detection. Necropsy examinations revealed foci of hemorrhage on the rumenal omentum of most cows but peritonitis was not observed. Systemic inflammation as determined by altered leukograms and fibrinogen was noted in one cow. The results of this study demonstrate that IP procaine penicillin G is absorbed and eliminated rapidly in lactating dairy cows.

4.2 Introduction

Caesarian sections, left or right displaced abomasums, exploratory laparotomies, and umbilical hernia repairs are common indications for abdominal surgery in bovine practice. The risk of surgical infection is elevated after on-farm surgery due to the nature of abdominal surgery

(clean-contaminated, contaminated, or dirty); and the non-sterile operating conditions encountered. In addition to observing proper surgical site preparation, minimal surgery time, and good surgical technique, veterinarians can administer perioperative antimicrobials in an effort to reduce post-surgical infections (Brumbaugh, 1990; Desrochers, 2005). In veterinary teaching hospitals, post-surgical infection rates of 5-15% for bovine abdominal surgeries are reported (de Kruif, van den Brand et al., 1987; Seger, Grunert et al., 1994; Desrochers, St-Jean et al., 1996; Bedard, Desrochers et al., 2001; Desrochers, 2005). "On-farm" rates are likely higher. Gram-positive staphylococci (from skin or environment), Gram-negative enteric bacteria (from fecal contamination), and anaerobes (from necrotic tissue) are potential contaminants during bovine surgery. Unfortunately, cultures and susceptibilities of the bacteria causing post-surgical infections are not routinely performed.

Current guidelines in human and veterinary medicine recommend prophylactic antimicrobials based on predicted efficacy against probable pathogens with administration *before* microbial contamination occurs (Gyssens, 1999; Zelenitsky, Ariano et al., 2002; Giguere, 2006; Howe & Boothe, 2006). The benefits of preoperative versus postoperative administration have also been demonstrated in bovine surgery (Haven, Wichtel et al., 1992), where one preoperative dose of intravenous (IV) penicillin was as efficacious as the preoperative dose plus a 7-day postoperative course of intramuscular (IM) penicillin in reducing post-rumenotomy complications. Both groups had lower rectal temperatures and fewer abscesses than non-treated animals. If an infection becomes established however, a longer course of antimicrobial therapy is warranted. Calves undergoing contaminated umbilical hernia surgery were less likely to have post-operative infections if treated with IM penicillin and dihydrostreptomycin for 4 days postoperatively rather than just one (Klein & Firth, 1988).

Using preoperative antimicrobials in surgical cases where the prognosis is uncertain precludes the possibility of salvaging the animal for slaughter because a withdrawal time must be observed. In such cases, practitioners may administer antimicrobials directly into the abdomen (intraperitoneal, IP) upon successful completion of the abdominal surgery. The IP route is commonly employed in human abdominal surgery and peritoneal dialysis, using ß-lactams (cephalothin, cephazolin, ceftazidime) and aminoglycosides (kanamycin, gentamicin) (Ericsson, Jr. et al., 1978; Stephen & Loewenthal, 1979; Okuda, Katoh et al., 1986; Yelon, Green et al., 1996; Sinswat, Wu et al., 2000; Sisterhen, Stowe et al., 2006). Other reports mention IP use of macrolides, vancomycin, metronidazole, and the antifungals amophotericin B and flucytosine (Saha, 1985; Schwartz, Kowalsky et al., 1986; Arthur, Drew et al., 2004).

Because the frequency of IP antimicrobial use in cattle has not previously been reported, the authors surveyed bovine veterinarians in Western Canada. Perioperative antimicrobial use was widespread (96/98 respondents), with 54/98 practitioners surveyed using IP antimicrobials occasionally. Of those, 30/98 used IP antimicrobials in $\geq 75\%$ of their abdominal surgeries (Chicoine, 2007). Procaine penicillin G and oxytetracycline LP were the most commonly used IP antimicrobials. Veterinarians assumed IP antimicrobial administration is safe and that the drug is absorbed faster than IM or SC injections, though little PK data in cattle is available to support this assumption. The kinetics of IP antimicrobials in humans are variable, with maximum plasma concentrations occurring from 15 minutes to 5 hours after administration for various antimicrobials (Ericsson, Jr. et al., 1978; Okuda, Katoh et al., 1986; Schwartz, Kowalsky et al., 1986).

Bovine experimental trials using IP infusions of oxytetracycline in saline and intramammary (IMM) ampicillin/cloxacillin and kanamycin/penicillin preparations have been

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performed (Fensterbank, 1976; Gitzel & Grunder, 1994; Klein, van der Velden et al., 1994).

One retrospective study showed a lower rate of post-surgical infection after IP antimicrobial administration compared to untreated cows (Klein, van der Velden et al., 1994). Unfortunately, comparisons against pre-operative intravenous (IV) prophylaxis are not available. IP antimicrobials are also used in other animal species, including dogs, rats, rabbits, and fish (Wieriks & Schornagel, 1971; Fry, Trachtenberg et al., 1986; Bruno, 1989; Ablan, Olen et al., 1991; Fairgrieve, Masada et al., 2006). The efficacy of IP antimicrobials in reducing infections has not been proven decisively; some studies demonstrate efficacy while others show no benefit over irrigation with saline alone. One trial in rabbits found that an abdominal lavage containing a cephalosporin was more efficacious than saline alone for treating peritonitis, but only if the bacterial contamination was severe and the antimicrobial was administered promptly after contamination (Ablan, Olen et al., 1991).

Because IP antimicrobial administration constitutes extralabel drug use, practitioners have contacted the Canadian global Food Animal Residue Avoidance Databank (CgFARAD) for advice regarding meat and milk withdrawal times. Unfortunately, insufficient data is available in the literature to develop a withdrawal interval estimate. The CgFARAD has anecdotal reports of cows with penicillin-positive milk samples for weeks after IP penicillin administration, although cows given ampicillin by IP infusion had positive milk tests for only 24-96 hours, depending on the formulation (Klein, Firth et al., 1989). Tissue residue depletion kinetics in cattle after IP antimicrobial administration are not available, but residues were detected in muscle, kidney, and liver in bulls given IP oxytetracycline one hour before slaughter (Kersey, McMahan et al., 1955). Trials using IP injections of oxytetracycline and macrolides in salmon found detectable drug residues in various tissues up to 8 weeks later (Bruno, 1989; Fairgrieve, Masada et al., 2006).

The purpose of this study was to determine the plasma pharmacokinetics of penicillin in lactating dairy cows after the administration of 21,000 IU/kg procaine penicillin G via IP infusion. Milk and meat penicillin residues would also be determined and any adverse reactions evaluated.

4.3 Materials and methods

Animals: A total of 9 cull, lactating Holstein cows were purchased from a local dairy farm in 3 groups, with 3 cows per group. Cows were culled for reproductive failure (n=6), decreased milk production (n=2), or lameness (n=1). Cows were housed in stanchions at the Western College of Veterinary Medicine (WCVM) large animal clinic for the duration of the trial and were fed a diet of alfalfa/grass hay and total mixed ration (TMR). Water was freely available. The mean age and days in milk (n=8) were 5.2 ± 1.6 y (range 2.3 – 7.9 y) and 292 ± 184 d (range 38 -642 d). Mean milk production during the current lactation had been 20.5 ± 5.3 kg/d. The cows' estimated weights (using a weight tape measure) ranged from 500 – 700 kg (mean 650 kg). Cows were milked twice daily with a portable vacuum milking machine for the duration of the trial. All cows were free of mastitis for at least 3 months before the trial began. No cows used in the study had undergone prior abdominal surgery. The study protocol was approved by the University of Saskatchewan Animal Care Committee.

Drug administration: IP penicillin administration was designed to mimic a typical dose during bovine abdominal surgery. A paravertebral infusion of lidocaine, 40 mL per site at the cranial border of L1 – L3 was used for local anesthesia of the right flank. The right paravertebral fossa was clipped and aseptically prepared for surgery. For cows A, B, and C, a 3 cm incision was made in the skin followed by insertion of a sterile teat canula through the abdominal muscle

layers to minimize surgical trauma. The cannula was inserted through the peritoneum, negative

air pressure was ascertained audibly, and 21,000 IU/kg (13.1 mg/kg) procaine penicillin G (Pen

Vet 300, Rafter 8 Products, Calgary, AB, Canada) was infused through the cannula. After

determining that penicillin concentrations were negligible in milk and plasma of cow A (but

easily detectable in cows B and C), it was speculated that the penicillin may have been infused

into the rumen instead of the abdominal cavity. The blind teat cannula approach was replaced in

cows D – I by a full surgical incision through the muscle layers and peritoneum, with

visualization of the abdomen before penicillin was infused (Figure 4.1). Peritoneum, muscle,

and skin layers were closed with routine surgical methods.

Blood collection: A jugular catheter was placed in each cow prior to surgery to facilitate blood

collection.   Blood was drawn into a syringe and transferred into 50 mL polypropylene centrifuge

tubes containing 500 IU sodium heparin. Samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24,

36, 48, 60, and 72 h following the IP administration of penicillin. The blood samples were

refrigerated for < 8 h until centrifuged at 1500 X g for 15 m. Plasma was harvested and stored at

-20°C until analysis. Samples were analyzed within 21 days of collection. Blood samples were

also submitted for complete blood count and/or chemistry profile before treatment and at various

times from 1 – 4 days post treatment to document any inflammatory response.

Milk collection: Samples were taken from the total milk collected from each cow after routine

morning and evening milkings. Blank milk samples were collected from each cow prior to

penicillin administration. Milk was stored at -20°C until analysis.

Tissue samples: Ten days after treatment, cows were euthanized via captive bolt gun followed

by rapid IV potassium chloride administration. Necropsies were performed by a pathologist at

the Prairie Diagnostic Services at the WCVM. During necropsy, multiple samples of muscle, liver, and kidney were obtained from each cow and stored at -20°C until analysis.

Drug Analysis: Penicillin concentrations were determined in all samples by HPLC with ultraviolet detection at 325 nm using an adapted version of previously published protocols (Boison et al., 1991; Boison et al., 1992; Boison et al., 1994).   Penicillin V was used as the internal standard for all assays. Calibration curves were prepared by fortifying blank plasma, milk, muscle, liver, and kidney samples with known concentrations of penicillin G and penicillin V (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). Two hexane flushes were performed on milk samples to extract fat present in the milk. Protein denaturation on all samples was performed with 5% sodium tungstate and 0.17 M sulfuric acid, followed by washing with 20% sodium chloride and vacuum filtering through a GF/B filter. Samples were cleaned by solid phase extraction using Bond Elut C18 extraction cartridges (Varian Inc, Lake Forest CA, USA) preconditioned with methanol, water, and a 2% sodium chloride solution. After penicillin extraction the cartridges were washed with the same sodium chloride solution and water. Penicillin was eluted with 1.0 mL elution solution (5% 0.2 M phosphate buffer / 35% water / 60% ACN) into clean glass tubes. 1.0 mL derivitizing reagent (containing 1,2,4 Triazole and 0.01 M $HgCl_2$) was added to the eluent and placed in a 65°C water bath for 30 minutes.

An HP1100 apparatus was used, consisting of a pump system equipped with an automatic injector (50 – 100 μL / sample) and a UV variable-wavelength monitor at 325 nm. Separation was achieved by reverse-phase column (Inertsil C8, 5 μm, 150 × 4.6 mm, GL Sciences, Torrance, CA, USA). The mobile phase consisted of a mixture of 28% acetonitrile and 72% 0.05 M phosphate buffer and a flow rate of 1.2 mL/min was used.   Calibration curves were linear between 5 – 100, 100 – 1000, and 1000 – 10000 ng/μL for plasma, 5 – 100 and 100 – 500 ng/μL

for milk, and 40 – 400 ng/µL for tissues, with a coefficient of determination (r^2) greater than 0.99

for each curve. Appendix 1 describes the complete protocol for penicillin determination in milk.

The limits of detection and quantification (LOD and LOQ) were approximately 5 and 15 ppb

respectively, based on measured signal:noise ratios. The original tissue protocol was previously

validated at the Centre for Veterinary Drug Residues (Saskatoon, SK).

A milk sample from each cow was qualitatively assayed for penicillin residues

immediately following each milking using IDEXX SNAP beta-lactam test kits with visual

inspection (90/95 level, 3.1 ppb; IDEXX Laboratories Inc., Westbrook, ME, USA).

Pharmacokinetic Analysis: Penicillin concentrations were analyzed using a commercial PK

software program (WinNonlin, Version 2.1; Pharsight Corporation, Mountain View, CA, USA).

A noncompartmental model was used to analyze the data.   Peak concentration in plasma (C_{max})

and time to peak concentration (T_{max}) were determined using observed values. The apparent

terminal rate constant, λ_z, was determined by linear regression of the last 7-8 points on the

terminal phase of the logarithmic plasma concentration versus time curve. The area under the C-

T curve until the final plasma sample ($AUC_{0\text{-}24h}$) was determined using the linear trapezoidal

rule. The total area under the curve extrapolated to infinity ($AUC_{0\text{-}\infty}$) was calculated by adding

the $C_{24h\,obs}/\lambda_z + AUC_{0\text{-}24h}$. The terminal half life ($T_{1/2\lambda}$) was calculated as $\ln2/\lambda_z$. Clearance

(Cl_B/f) was determined by the dose divided by $AUC_{0\text{-}\infty}$ The apparent volume of distribution

(V_z/f) was calculated by clearance divided by λ_z. The mean residence time (MRT) was

calculated as the area under the moment curve extrapolated to infinity ($AUMC_{0\text{-}\infty}$) / $AUC_{0\text{-}\infty}$.

4.4 Results

Plasma penicillin kinetics: No adverse effects were observed immediately after IP penicillin administration in any cow. The log plasma penicillin concentration versus time graph is shown in Fig. 4.2. The pharmacokinetic parameters for each cow are presented in Table 4.1.   The mean ($\pm$ s.d.) C_{max} and T_{max} were 5.5 ± 2.6 µg/mL and 0.75 ± 0.27 h, respectively. The mean $AUC_{0-\infty}$ was 10.8 ± 4.9 µg*h/mL. The average $MRT_{0-\infty}$ and $T_{1/2\lambda}$ were 2.2 ± 0.9 h and 1.6 ± 1.0 h respectively.

Milk penicillin residues: Average milk production per cow over the first 5 days post-treatment was 8.5 ± 3.1 kg/day. Each cow's milk tested positive for drug residues using the SNAP ß-lactam test kits for a minimum of three 12-h milking intervals after IP penicillin administration (median, 4; range, 3-5, see Table 4.2). Quantitative milk penicillin concentrations determined by HPLC are shown in Fig. 4.3. The mean $\pm$ s.d. maximum milk penicillin concentration was 222 ± 100 ppb. Milk residues were detectable by HPLC for at least two 12-h milking intervals (median, 3; range, 2-4).

Tissue penicillin residues: No penicillin residues were detected in any muscle, liver, or kidney samples taken at necropsy (10 d post-treatment).

Safety and irritation: Ante-mortem evidence of inflammation was determined by altered leukograms (neutrophilia) and decreased plasma protein/fibrinogen ratios ($\leq$ 10:1 was used as evidence of inflammation). Only one animal (Cow C) met these criteria at any time post-IP infusion. However, a pre-treatment neutrophilia with degenerative left shit and protein/fibriniogen ratio of 9:1 indicated a prior inflammatory process occurring in this cow. Serum chemistry abnormalities included mildly elevated creatine phosphokinase and aspartate

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aminotransferase enzymes in some cows as the trial progressed. At necropsy, all cows had mild

to moderate, focal hemorrhage on the greater omentum overlying the rumen (Figure 4.4).

Peritonitis or adhesions were not observed in any animal, and no penicillin was grossly visible in

the abdomen.

4.5 Discussion

Data from only 8 cows were used for kinetic analysis because penicillin was not detected

in any tissue, plasma or milk from one animal (cow A).   The absorption and elimination of

procaine penicillin (PPG) was rapid after IP administration in 8 lactating dairy cows. Although

direct comparisons with historic data can be misleading, the mean time to maximum plasma

concentration (T_{max}) of 0.75 ± 0.27 h was quicker than values previously reported in the literature

for cattle given PPG by other routes. A T_{max} of 5.3 to 6.0 h was reported for steers dosed with

24,000 – 66,000 IU/kg PPG via IM or SC injections (Papich, Korsrud et al., 1993), compared to

0.5 – 2.0 h after 20,000 IU/kg IM or SC injections in cows (Conlon et al., 1993). Other studies

reported a mean T_{max} of 1.5 – 2.0 h using 30,000 IU/kg IM in calves (Bengtsson et al., 1989;

Bengtsson et al., 1991) and 2.0 – 7.1 h after various IM doses in lactating cows (Dubreuil et al.,

2001). A population pharmacokinetic approach estimated a T_{max} of only 1.14 h after IM

administration of procaine penicillin (Craigmill, Miller et al., 2004).

Intraperitoneal administration also resulted in higher maximum plasma concentrations

(C_{max}) than values obtained in other studies after IM administration with similar doses. Mean

C_{max} after IP infusion was 5.5 μg/mL versus mean IM values of 0.99 and 1.74 μg/mL (Papich,

Korsrud et al., 1993; Dubreuil, Daigneault et al., 2001). The mean elimination half life ($T_{1/2\ elim}$)

of PPG after IP infusion was shorter than that previously reported for IM injections (1.6 vs 7.95

h). The prolonged IM half-life is due to procaine-mediated vasoconstriction which slows the absorption rate and thus influences elimination kinetics (Craigmill, Miller et al., 2004). Whether procaine affects penicillin absorption after IP administration in cows is not clear, though an IP sodium ampicillin $T_{1/2\ elim}$ reported earlier (Klein, Firth et al., 1989) was similar to the PPG $T_{1/2\ elim}$ in this study. This suggests procaine does not significantly delay IP absorption. The shorter T_{max} after IP infusion may also be due to the large surface area for drug absorption within the abdomen compared to an IM injection.

Of interest in this study was the large inter-animal variability in C_{max} (range, $1.2 - 8.8$ µg/mL). One hypothesis is that the exact location of PPG deposition after IP infusion is not uniform, as drug may settle on the greater omentum, small intestines, rumen, uterus, or peritoneum. Each anatomic site has its own local circulation possibly influencing the rate and extent of PPG absorption.

The question of whether intraoperative IP penicillin use in cattle is a rational therapy is debatable. Overall, evidence for prophylactic antimicrobials reducing surgical infections in animals is mixed. Some studies have demonstrated efficacy (Haven, Wichtel et al., 1992; Whittem, Johnson et al., 1999; Eugster, Schawalder et al., 2004) while others have shown no benefit (Vasseur, Paul et al., 1985; Brown, Conzemius et al., 1997). The type and duration of surgery, surgical technique, and local conditions are important prognostic factors for the development of infection. Prophylactic antimicrobial efficacy requires high plasma antimicrobial concentrations (including ß-lactams) at the onset of surgery, which prevents infection by keeping intraoperative bacterial counts below a critical threshold. The duration of post-surgical therapy does not influence outcome (Haven, Wichtel et al., 1992; Gyssens, 1999; Eugster, Schawalder et al., 2004; Giguere, 2006). Therefore surgical prophylaxis guidelines

recommend plasma drug concentrations greater than the probable pathogen MIC before and throughout the surgery, but not once surgery is completed. This is contrary to the idea of penicillin as a time-dependent antimicrobial when treating established infections, whereby maximum effect occurs when plasma concentrations remain above the pathogen MIC for a prolonged period (T > MIC) (McKellar, Sanchez Bruni et al., 2004). Suitable plasma penicillin concentrations can be achieved at the time of surgery with preoperative IV administration, but this route is not commonly used by bovine veterinarians (Chicoine, 2007). If preoperative IV antimicrobials cannot be administered, the goal of antimicrobial prophylaxis should be to reach suitable systemic drug concentrations as soon as possible after surgery has begun. Therefore, the antimicrobial therapy with the quickest rate of absorption should be most effective in preventing post-surgical infection. Once an infection is established however, a longer course of therapy is required where traditional PK/PD predictors of efficacy (such as T > MIC) will apply.

The rapid absorption and elimination after IP infusion of procaine penicillin create a plasma concentration versus time profile intermediate between those of IV and IM administration. The swift absorption after IP administration predicts this route will be more efficacious than a single post-operative dose of IM penicillin in preventing postoperative infections. However, IP administration still does not comply with the antimicrobial prophylaxis recommendation of plasma concentrations > MIC at the time of incision. Therefore pre-operative IV administration should be more effective than intraoperative IP infusion. An argument made by some practitioners is that IP antimicrobials have a local effect in the abdomen, working directly at the site of infection. The rapid absorption from the abdominal cavity refutes this theory. If one assumes that the likely pathogens in bovine abdominal surgery are coliforms (especially in unsanitary surgical conditions), then penicillin should not be an

effective therapy due to the inherent antimicrobial resistance of coliforms. However, Gram-positive and anaerobic infections could be prevented by penicillin. The pharmacokinetics of PPG after IP administration determined in this trial cannot be extrapolated to other antimicrobials or doses. As the formulation of each drug differs, rapid absorption and elimination cannot be assumed and therefore IP administration of other antimicrobials cannot be recommended at this time.

Although perioperative use of IM penicillin could be argued as label therapy (for treatment of wound infections in cattle), IP use is definitely extralabel. AMDUCA specifically states that any extralabel drug in food animals requires extended withdrawal intervals and must not result in violative residues. After administration of 21,000 IU/kg PPG by IP infusion, milk residues were not detectable by HPLC or ELISA for any animal by 72h. This is similar to the Canadian 96h milk withdrawal time after on-label IM use of the same dose. One difference between the study and general populations was the low milk production of the cull cows used in this trial (8.5 ± 3.1 kg/cow/day). However, low milk production correlates with reduced drug clearance and greater milk residues (Whittem, 1999), therefore the rapid excretion of PPG after IP infusion should be applicable to high producing dairy cows. Although milk from cow C tested positive for ß-lactam residues using the IDEXX SNAP test, residues could not be detected by HPLC. We suspect this cow's milk had some intrinsic component that bound penicillin, as penicillin V internal standard added to this milk was also undetectable.

No penicillin residues were detected in any tissue at 10 d post-treatment. As our small sample size precluded a full tissue residue depletion study, we euthanized at the label withdrawal time after IM administration of this same PPG dose. The rapid decline in plasma concentrations

and lack of detectable tissue residues support a minimum withdrawal interval recommendation of 10 d.

Although no evidence of severe gross or clinical pathological changes was noted after IP administration of PPG in these cows, these findings do not prove that IP infusion is safe. The cause or clinical significance of the mild-moderate hemorrhage seen on the omentum is not known. Although possibly an incidental finding at necropsy, the consistency of the lesion raises suspicion that penicillin infusion, or possibly general abdominal surgery, may be the cause. Cows with a previous history of abdominal surgery were excluded from the study. Unfortunately, in an effort to maximize the number of penicillin-treated cows, no negative or IP-saline treated control animals were used. Negative controls are required to specifically determine if abdominal surgery or IP penicillin is responsible for the hemorrhagic omentum. There was evidence of peritonitis in cows after IP infusions of an ampicillin anhydrate formulation, but not sodium ampicillin (Klein, Firth et al., 1989). Other antimicrobials and formulations have not been evaluated for irritability or safety in cattle. Although the IP route is generally considered non-irritating and safe in human medicine, peritoneal reactions may depend on the formulation of the antimicrobial. When IP infusions are performed in people an IV antimicrobial formulation thoroughly diluted in a lavage/dialysis solution is used. Other antimicrobials such as tetracycline, neomycin, and streptomycin can cause chemical peritonitis or adhesions in animals when administered IP (Withrow & Black, 1979).

This study found rapid absorption and elimination of PPG after IP administration of 21,000 IU/kg in lactating dairy cows, with no evidence of prolonged meat or milk drug residues or serious adverse reactions. Prospective clinical trials are required to determine if this practice minimizes post-surgical abdominal infections in cattle and is a rationale therapeutic choice.

Figure 4.1 Intraperitoneal (IP) administration of penicillin through an incision in the right paralumbar fossa.
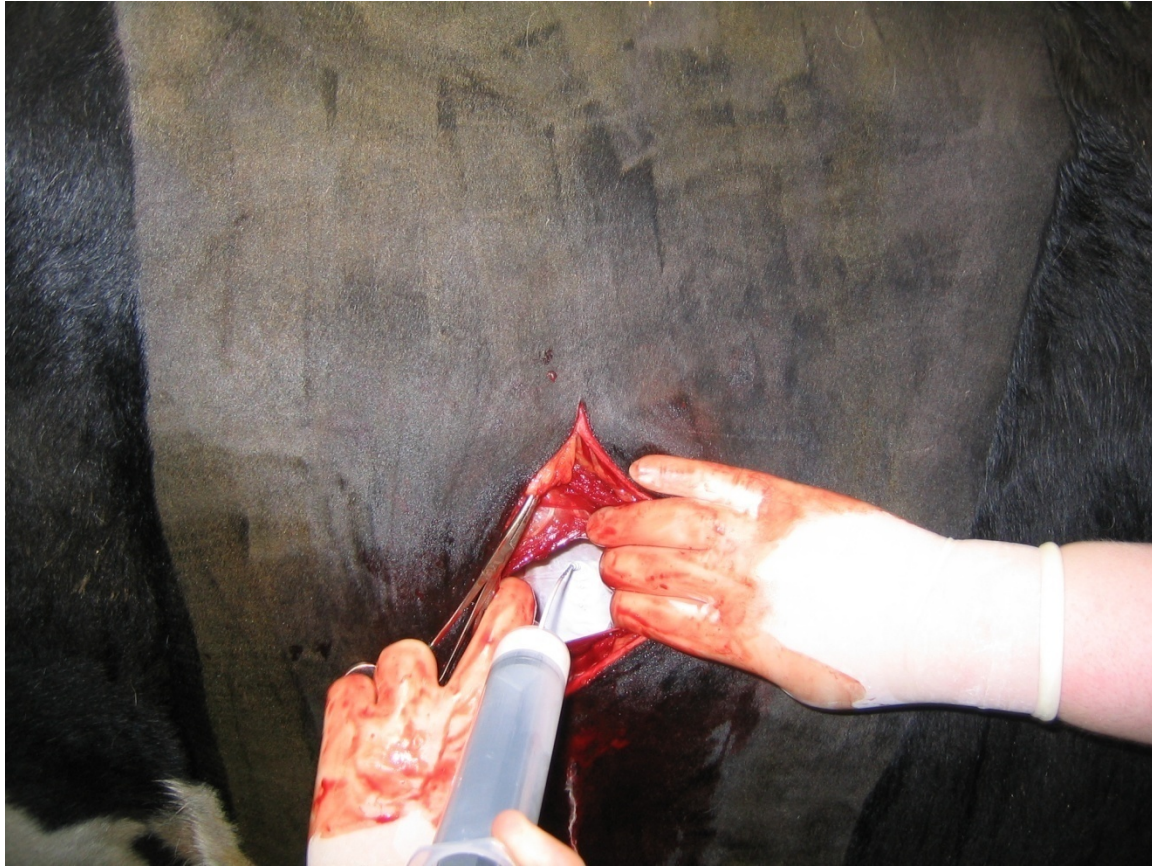
Table 4.1 Plasma pharmacokinetic parameters determined by noncompartmental analysis after IP administration of 21,000 IU/kg procaine penicillin G in 8 lactating dairy cows.

Parameter	Cow B	Cow C	Cow D	Cow E	Cow F	Cow G	Cow H	Cow I	Mean ± SD
C_{max} (μg/mL)	3.1	1.2	3.8	5.6	8.8	7.9	7.1	6.1	5.5 ± 2.6
T_{max} (h)	0.50	1.0	0.50	0.50	1.0	1.0	0.50	1.0	0.75 ± 0.27
λ (1/h)	0.53	0.45	0.49	0.52	1.11	0.18	0.51	0.52	0.54 ± 0.26
$AUC_{0-\infty}$ (μg*h/mL)	5.3	2.89	9.2	9.6	13.5	16.4	15.4	14.0	10.8 ± 4.9
$AUMC_{0-\infty}$ (μg*h^2/mL)	9.3	6.1	18.1	17.2	17.9	68.2	30.6	31.3	24.8 ± 19.6
MRT (h)	1.8	2.1	2.0	1.8	1.3	4.2	2.0	2.2	2.2 ± 0.9
$T_{1/2\ elim}$ (h)	1.3	1.5	1.4	1.3	0.62	3.9	1.4	1.3	1.6 ± 1.0
Cl_B/f (mL/h/kg)	2.5	4.6	1.4	1.4	0.97	0.80	0.85	0.93	1.7 ± 1.3
V_z/f (L/kg)	4.7	10.2	2.9	2.6	0.87	4.5	1.7	1.8	3.6 ± 2.9

Figure 4.2 Plasma penicillin concentration (µg/mL) vs time after IP administration of 21,000 IU/kg procaine penicillin G in 8 lactating Holstein cows. Bottom graph: mean (± s.d.) plasma penicillin concentrations vs time.
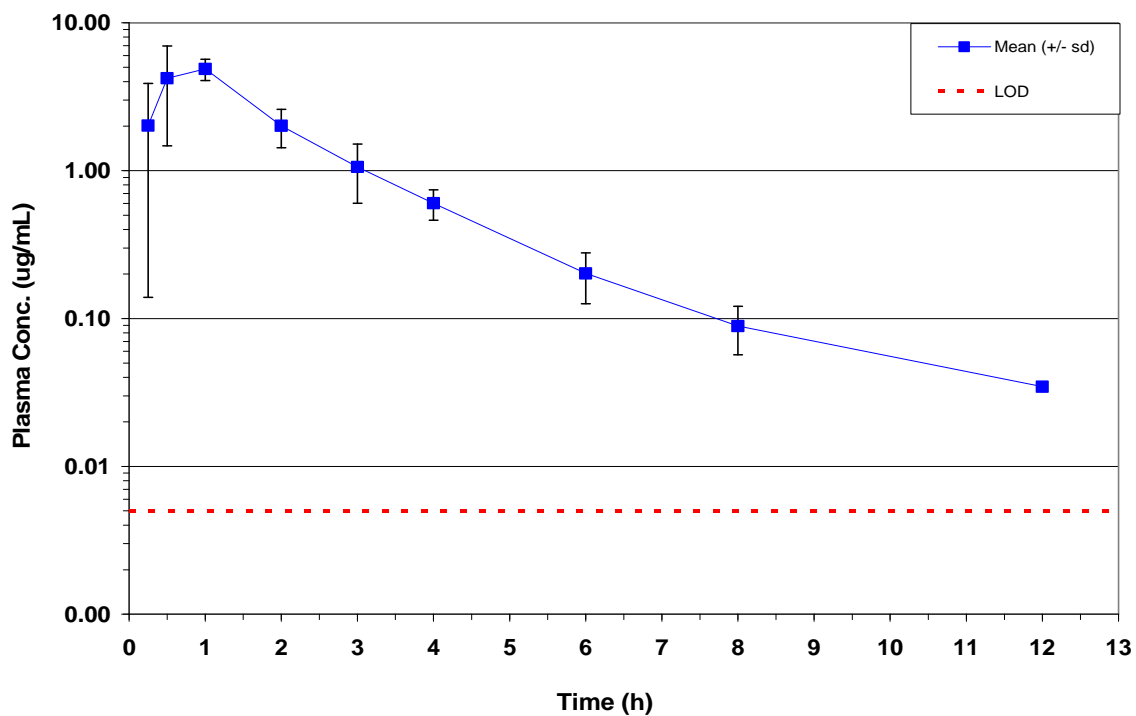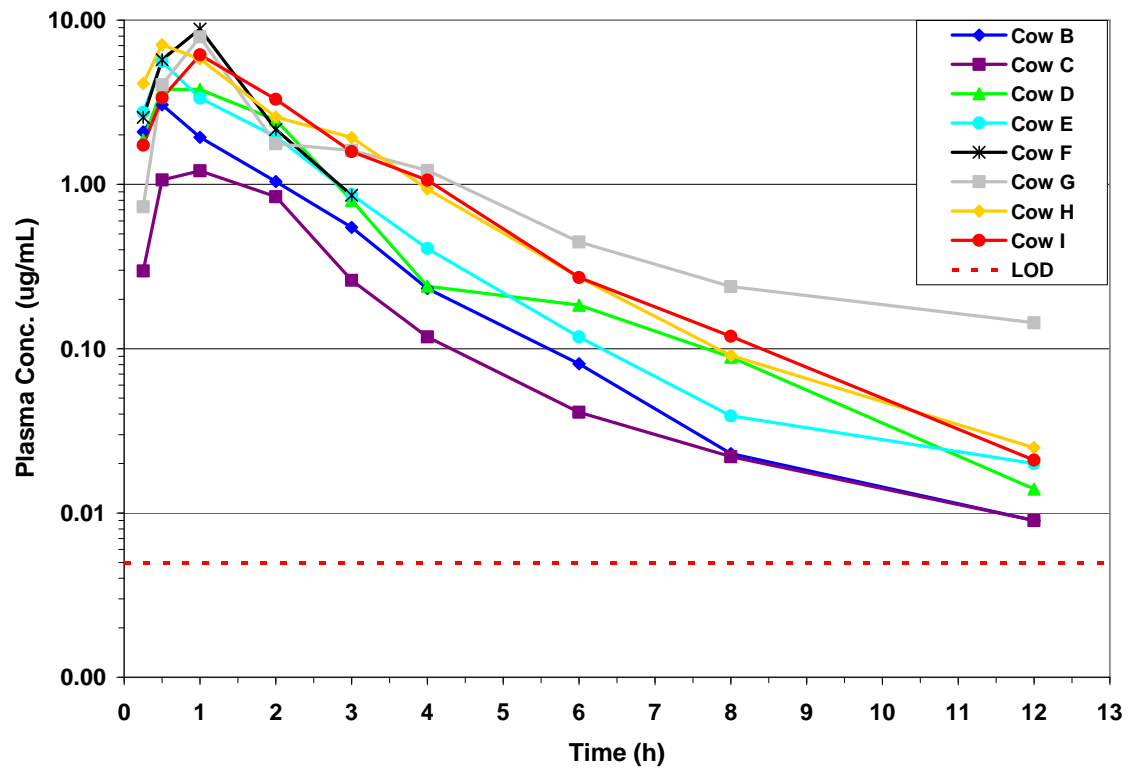
Table 4.2 IDEXX SNAP ß-lactam test results at each 12 h milking interval after IP administration of 21,000 IU/kg procaine penicillin G in 8 lactating Holstein cows.

Milking Number	Cow B	Cow C	Cow D	Cow E	Cow F	Cow G	Cow H	Cow I
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	-	-	+	+	+	+	+	+
5	-	-	-	+	+	+	-	-
6	-	-	-	-	-	-	-	-

Figure 4.3 Milk penicillin residues (ppb) vs time after IP administration of 21,000 IU/kg procaine penicillin G in 8 lactating Holstein cows.
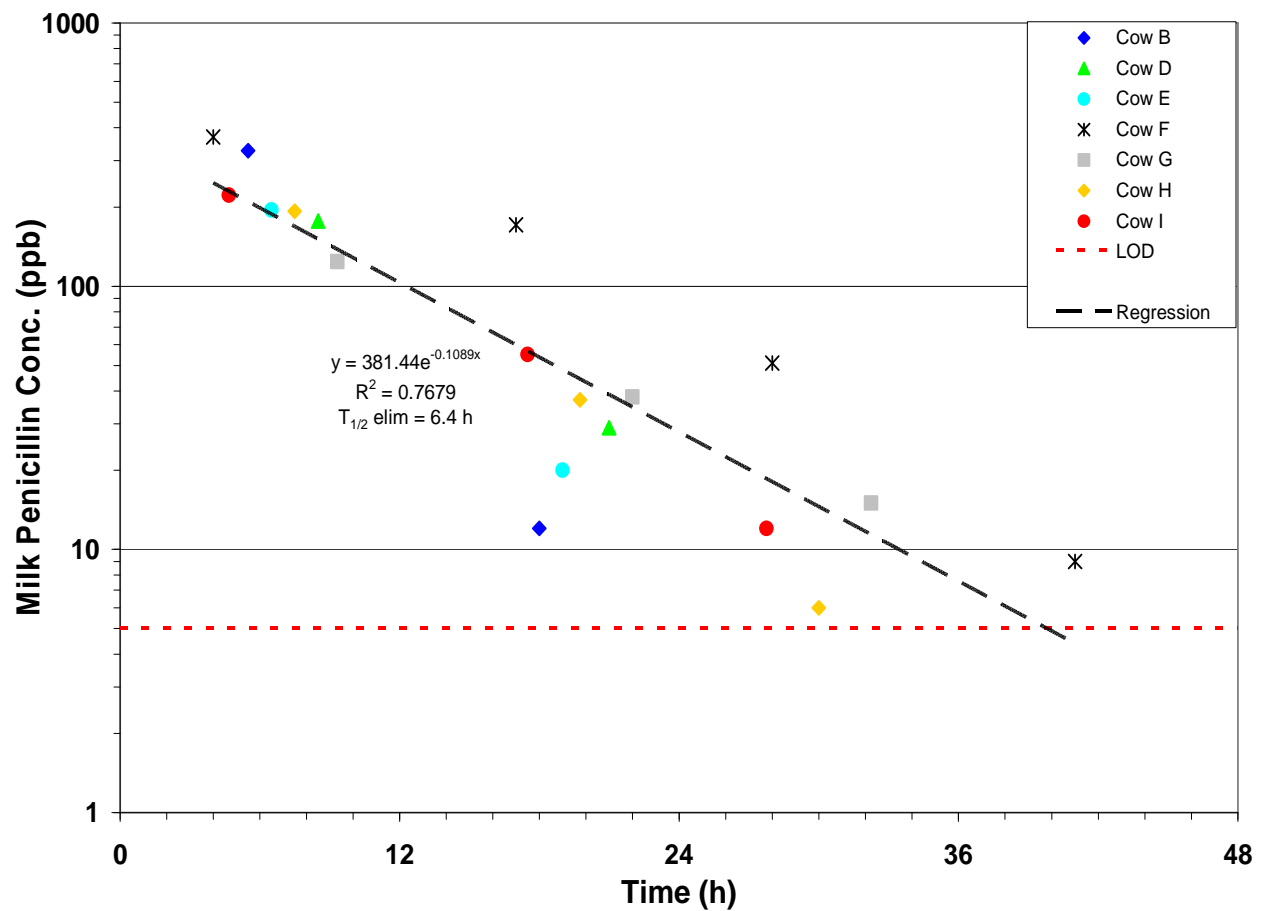

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Figure 4.4 Photograph of typical focal hemorrhage on the greater omentum overlying the rumen 10 days after IP infusion of 21,000 IU/kg PPG.

Appendix 4.1 Summary of protocol used for penicillin residue determination in bovine milk (developed Jan/Feb 2006, revised Mar 2007)

Penicillin Extraction:

1. Standard curve 100-500 ppb
Dispense 10 mL of known "blank" milk (can use 3.25% milk fat/homogenized milk or whole milk) into 6 * 50 mL disposable PVDF centrifuge tubes. Spike each sample with 200 μL of a 20 μg/mL Pen-V working standard solution (this will give a final concentration of 400 ng/mL Pen V internal standard). Add the following volumes of Pen-G using a 20 μg/mL working standard solution:

    0 μL = 0 ppb
    50 μL = 100 ppb
    100 μL = 200 ppb
    150 μL = 300 ppb
    200 μL = 400 ppb
    250 μL = 500 ppb

    Vortex tubes for 10 seconds.

Unknown milk samples: All milk samples will be undiluted and contain 10 mL milk. Spike each sample with 200 μL of a 20μg/mL Pen-V working standard solution to give 400 ng/mL Pen-V internal standard. Vortex tubes for 10 s.

2. Add 2 mL hexane to each tube. Vortex for 10 seconds.

3. Centrifuge tubes for 5 minutes at 1500 X G. During centrifugation time, proceed to condition C18 SPE cartridges (step 14).

4. Aspirate hexane and "solid" layer from tubes using a disposable glass pipette with vacuum suction.

5. Add another 2 mL hexane to each tube, vortex, and centrifuge.

6. Again aspirate hexane layer from tubes. Ensure at least 5 mL "milk" remains after aspiration.

7. Pipette 5 mL of post-hexane extraction milk into a clean 50 mL PVDF centrifuge tube. Discard remaining milk.

8. Add 20 mL distilled water, 5 mL 5% sodium tungstate, and 5 mL 0.17 M sulfuric acid to the milk. Shake by hand for 10 seconds.

9. Centrifuge at 1500 X G for 10 minutes.

10. Aspirate any hexane bubbles from the top of the supernatant.

11. Vacuum filter the supernatant through a 5.5 cm GF/B filter paper into a 125mL sidearm flask. For successful filtration, pre-wet the filter paper with distilled water and ensure solution flows through centre of the filter.

12. Pour filtrate into a clean 50mL PVDF centrifuge tube. Add 10mL of a 20% NaCl solution to the filtrate and vortex for 5 seconds.

13. Centrifuge at 1500 X G for 10 minutes.

14. Prepare a set of Bond-Elut C18 SPE columns with 75 mL reservoirs on the vacuum block. Condition C18 SPE columns by flushing with 20mL methanol, 20mL water, and 10mL 2% NaCl.

15. Pour supernatant into reservoir. (If C18 SPE columns are becoming clogged, an additional filtration step can occur here).

16. Ensure a flow rate of approx. 1 drop/sec. Vacuum suction will likely be required, but do not exceed -20 mmHg. After sample is loaded on the C18 SPE column, wash the column with 10mL 2% NaCl and follow with 10mL distilled water. Continue drawing air through the column for a further 3 minutes.

17. Remove the reservoir and adaptor from the SPE column. Elute the penicillins from the column with 500μL Elution Solution into a 10 mL glass test tube. Vacuum suction will be required.

18. Add 500 μL Derivatizing Reagent to the eluate and vortex. Place the tube in a 65°C heating block for 30 minutes.

19. Allow the tubes to stand for 10 minutes at room temperature. Filter a sample aliquot through an Acro filter into an HPLC sample vial.

20. LC parameters:
    - Sample volume = 50 μL
    - Flow rate = 1.2mL/min
    - Detector wavelength = 325 nm
    - Run time = 10 minutes
    - Column temp = 27°C

Chapter 5: Conclusions and Further Discussion

5.1 Overall summary

The experimental results discussed in this thesis—the incidence, kinetics, milk and meat residues, and safety of IP penicillin use in cattle—are part of a larger central question: Is IP use of antimicrobials justified during bovine abdominal surgery? Frankly, I began this investigation skeptical of the prophylactic or therapeutic claims attributed to IP antimicrobial use by veterinarians. The possible adverse effects of IP use, such as prolonged milk and meat residues and peritonitis, were also cause for apprehension. We realized that justifying this practice would require demonstrating IP antimicrobial use was actually effective in preventing postoperative infections, but was beyond the scope of an MSc project. However, other important questions concerning IP use remained. Is IP administration common during bovine surgery, and if so, which drugs are used? What are the plasma pharmacokinetics after IP administration, and do they predict efficacy? What are reasonable meat and milk withdrawal times after IP antimicrobial use? And is this practice safe for the animal?

The survey results presented in Chapter 3 confirm that perioperative antimicrobial use is nearly universal among Western Canadian bovine practitioners. The use of IP antimicrobials is common during bovine abdominal surgery (more than half of the veterinarians reported using IP antimicrobials at least occasionally), though not as routine as postoperative IM or SC therapy. Procaine penicillin G was the most commonly used IP drug so it was selected for the ensuing pharmacokinetic and drug residue experiment. Milk and meat withdrawal recommendations after IP administration were generally longer than the corresponding label intramuscular (IM) withdrawal time.

The pharmacokinetic results described in Chapter 4 demonstrate that IP procaine penicillin is rapidly absorbed in lactating dairy cows, with a T_{max} occurring within 0.5 – 1.0 h. Elimination from plasma was also rapid, as most plasma samples were below the limit of detection by 24 h. The first milking after IP administration had the highest penicillin concentrations, with rapid declines in subsequent milkings. Milk samples tested positive using a standard on-farm antimicrobial test kit for 3-5 milkings. No penicillin residues were detected in any tissue 10 days after administration. Necropsies did not reveal serious peritonitis or adhesions, though diffuse focal hemorrhage on the omental surface was present in most cows. The major finding from this study was the rapid rise and decline in plasma penicillin concentrations. This result is similar to the only other study examining IP antimicrobial use in cattle (Klein, Firth et al., 1989), in which sodium ampicillin (but not ampicillin anhydrate) concentrations rapidly declined after an early peak.

These conclusions can be summarized as follows. We know that extra-label IP antimicrobial use does occur in Western Canadian veterinary practices. Procaine penicillin is rapidly absorbed and excreted after IP administration. Milk and meat residue violations should not occur if the IM dose is given by IP infusion, as long as reasonable withdrawal intervals are followed. The label withdrawal times after IM procaine penicillin administration (96 h for milk, 10 d for meat) are appropriate. Intraperitoneal procaine penicillin may cause mild abdominal irritation, but this is not likely clinically relevant. Because current surgical prophylaxis guidelines predict antimicrobial efficacy based on plasma concentrations > MIC immediately before and during surgery, I predict the rapid absorption after IP procaine penicillin administration will result in equal to or greater efficacy than a postoperative IM dose in preventing post-surgical infections in cattle.

Finally, these results are pertinent to the larger issue of IP antimicrobial appropriateness. Many of the reasons for my initial skepticism—the unknown pharmacokinetics, residue depletion, and safety of this practice—were refuted by the results of these trials. This data does not demonstrate that IP antimicrobial use in cattle is unjustified, and it is certainly more rational than some other common perioperative antimicrobial practices.

5.2 Relevance of this Research

What is the overall impact of this research on veterinarians and the broader scientific community? These results are applicable to relatively few disciplines; but the conclusions are useful to veterinarians, pharmacokineticists, and residue chemists.

5.2.1 Extra-label drug use guidelines

As stated in the introduction to this thesis, administering drugs by IP infusion constitutes extra-label drug use (ELDU). Although an accepted part of veterinary practice in North America, numerous agencies have created guidelines to ensure ELDU is prudent and justified. IP drug use meets some of these criteria, such as performance only under the direction of a valid veterinarian-client relationship. Conformity with other ELDU guidelines could not be evaluated because information about IP use was lacking. Data from our experiments addresses some of these deficiencies. The major thrust of ELDU regulations for food animals under both AMDUCA and CVMA guidelines is the avoidance of violative drug residues and risk to human food safety. Previously no residue depletion data was available to create appropriate withdrawal recommendations, but the rapid elimination of procaine penicillin after IP infusion in our study allows confidence in using label IM withdrawal times. This would satisfy Canadian ELDU guidelines, but AMDUCA requires another condition before ELDU is employed, that the health

or welfare of the animal is jeopardized and no other drug product used on label will be effective for this condition. Because clinical efficacy trials were not performed, we cannot evaluate IP penicillin effectiveness in preventing post-surgical infections against other licensed antimicrobials used on-label. However, the Center for Veterinary Medicine exerts regulatory discretion during enforcement; though this specific question is unresolved it does not incriminate IP penicillin use as imprudent. So long as adequate withdrawal periods (which are now known) are followed, IP penicillin use appears to comply with guidelines regulating ELDU in North America.

5.2.2 *Preventing meat and milk penicillin residue violations*

Procaine penicillin is an over-the-counter (OTC) drug that can be purchased from any veterinary clinic, feed store, or even pet stores without a veterinary prescription. It is accessible, inexpensive, and effective and thus routinely used. Administration of IP penicillin should only performed during abdominal surgery by a veterinarian, therefore constituting an exceedingly small proportion of the total penicillin use in cattle. The vast majority of drug residue violations are a result of simply failing to follow the withdrawal time before shipping milk or meat, not because the withdrawal time was unknown. Therefore, the relatively rare occurrence of IP penicillin use (compared to total penicillin use) is unlikely to be a significant source of penicillin meat or milk residue violations. So what was the point in determining residue depletion kinetics? Because IP administration is ELDU, the veterinarian is responsible for any violative drug residues that may be incurred when using this route, and must determine an appropriate withdrawal interval for the producer. No data previously existed to predict a withdrawal interval with any confidence. This meant veterinarians either had to recommend excessively conservative intervals (a financial burden especially for dairy farmers), or risk a residue violation

with shorter times. Knowledge of IP penicillin milk and meat residue depletion may not make the food supply much safer, but it will reduce unnecessary milk discard and keep a few veterinarians from regulatory action.

5.2.3 Relevance to bovine surgery

Information derived from this IP project is not likely to significantly alter the perioperative antimicrobial use of bovine veterinarians. Those who already believe IP antimicrobial administration is beneficial can examine our data and can reasonably make the following conclusions:

- penicillin is quickly absorbed and high plasma concentrations are attained, and is therefore likely to be an effective perioperative treatment

- milk and meat residue depletion is rapid, so residue violations are unlikely

- IP administration is safe and non-irritating for the cow

Conversely, others who view this practice as irrational can rebut that efficacy data is still lacking after IP antimicrobial use, therefore it cannot be justified over conventional routes of administration. If clinical efficacy studies were performed that demonstrated a reduction in post-surgical infection rates in IP-penicillin treated cows compared to other groups, this practice may become more widely accepted. On the other hand, if our results showed a clear risk of serious adverse effects such as peritoneal inflammation or fibrin deposits, or extended milk and meat residues, the practice of IP administration would be discredited. Neither scenario occurred so it is unlikely this study will produce major changes in current IP antimicrobial practices.

5.3 Limitations of study data

The experimental data was overall quite useful, but of course in retrospect some aspects of experimental design and implementation were not optimal. Certain limitations were known *a priori* but could not be corrected due to financial or logistical constraints. Other limitations were only uncovered after *post hoc* analysis. The following discussion elaborates on limitations presented in the earlier chapters before describing future areas of research.

5.3.1 Survey data limitations

WCABP members were surveyed as we felt this group was representative of bovine practitioners across Western Canada and a central mailing list was easily accessible. The survey response rate (98/240, or 40.8%) was satisfactory. As there are approximately 885 mixed or large animal veterinarians in Western Canada (Jelinski, 2006), the 98 responses represent more than 10% of all bovine veterinarians so conclusions can be safely drawn from this sample size. However, because all respondents are from Western Canada the results cannot be generalized across Canada or North America. Specifically, the regular use of IP administration may only be a regional phenomenon. This is certainly feasible; many Western Canadian veterinarians were trained at the same institution (WCVM) by the same professors. Anecdotally, some veterinarians reported learning of IP neomycin use from WCVM faculty. As well, many veterinarians are mentored by more experienced practitioners and certain techniques (such as IP administration) could easily be "passed down the veterinary generations" within a specific region. Unfortunately, resources were not available to survey bovine veterinarians in other regions.

Bias was present in the survey design. Because surveys were anonymous, follow up was not available. No ethics approval was obtained for this survey, and some veterinarians may not have responded without knowing how the data was to be used. Practitioners who do not routinely use perioperative antimicrobials simply may have not responded, skewing the results towards those who use antimicrobials. Personal information such as graduation year or practice location was not asked to keep responses confidential. Without this data, we cannot determine if respondents were clustered by age (experience) or geography.

Survey interpretation was complicated by the rather informal survey design (see Figure 3.1 for the survey design). Further proofreading and editing may have clarified the questions. Some responses listed multiple antimicrobials commonly used at each surgical time frame (there were 3 lines available), but what percentage of cows were treated with each drug was not asked. Likewise, percentage of cows treated at each surgical time frame was estimated and true occurrence cannot be determined. Withdrawal recommendations after pre- or postoperative administration were not solicited; we assumed that veterinarians would recommend label withdrawal times but this assumption may not be correct. Finally, errors were possible when categorizing multiple drug trade names and formulations together. For example, a practitioner who responded "oxytetracycline" (OTC) may have meant a short-acting (OTC HCl) or long-acting (OTC in polyethylene glycol) product, or some combination.

5.3.2 Pharmacokinetic and residue experimental design limitations

Animal selection: All cows used in this trial were cull dairy cows. The cows were in relatively good health, were free of mastitis, ate and drank well, and were normal on physical exam. Abnormalities included lameness (Cow B) and an inflammatory leukogram (Cow C). Milk production was generally much lower than expected on a commercial dairy, 8.5 ± 3.1

kg/cow/day in this trial versus approximately 30 kg/cow/day. This difference makes

extrapolation of milk drug excretion rates to other dairy cows suspect. However, low milk

production correlates with lower drug clearance and prolonged milk residues (Whittem, 1999),

therefore the rapid penicillin excretion after IP infusion should also be applicable to high

producing dairy cows.

Analytical methodology: The protocols used in this experiment were derived from the CFIA's

validated tissue penicillin residue protocol and adapted for plasma and milk matrices.

Unfortunately, method validation was beyond the scope of this project. Method validation is

required to have full confidence in the numbers generated for either plasma or milk. The

accuracy and recovery of the methods were not determined, neither was the precision. Limit of

detection (LOD) and limit of quantification (LOQ) were estimated from the standard curves

based on signal:noise ratios but a quantitative approach using deviation of standard curve y-

intercepts would be more precise. The HP1100 HPLC apparatus used in the WCVM lab

produced consistent results, especially after the initial phase of the trial when sample preparation

difficulties were resolved. Analysis of earlier samples (cows B and C) was more problematic.

Likewise, samples should have been stored at -70°C (not -20°C) as penicillin degradation likely

occurred at the higher temperature. This is suspected because samples from cows B and C were

re-analyzed 8 months after initial tests; penicillin concentrations were roughly half the original

values. Penicillin residues in milk from Cow C could not be determined, as no Pen G (or Pen V

internal standard) peaks were detectable on the chromatogram. This occurred despite multiple

analyses at both CVDR and WCVM labs. I suspect some component of this particular cow's

milk (such as increased milk fat, protein, or somatic cell count) bound penicillin molecules;

therefore any penicillin in the milk was lost along with these components during sample preparation.

Plasma penicillin concentrations: The plasma PK experimental design worked very well overall. Knowing now the rapid IP absorption, I would incorporate extra blood samples at 0.75 and 1.5 h to further clarify the T_{max} and C_{max}. Comparisons to plasma PK parameters obtained in other studies were made in Chapter 4, but these comparisons may not be valid for a number of reasons. The study populations will not be identical. For example, penicillin clearance in steers may not be the same as in lactating dairy cows because steers do not have drug excretion into milk. Differences in housing, feed, and seasonal conditions between studies should not influence penicillin disposition but this is not known conclusively. Values obtained using one analytical method cannot be assumed equal to values obtained from other assays, as systemic bias between methods may account for differences.

Milk penicillin residues: A 12h milk sampling schedule was chosen to mimic typical dairy milking frequencies. However, because the milk penicillin residues were not detectable after only 3-5 milkings the milk residue depletion curve consisted of few data points. Taking more frequent milk samples (every 6 hours, for example) would create more data for the residue depletion curves. The problem with this approach is that it does not represent what happens on farm. If cows are milked every 12 hours, the penicillin concentration in milk will reflect excretion over that entire period. Milk produced earlier in the 12 h interval with high penicillin concentrations will be diluted by lower penicillin concentrations in milk produced later. The rate of milk production may also change over the milking interval, thus altering the penicillin concentrations. Milk penicillin concentrations from two 6 h intervals cannot be averaged to

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predict a 12 h interval concentration. Using the IDEXX SNAP ß-lactam rapid test kits on

individual milk samples allowed early yes-or-no impressions of milk residues. However, this

qualitative analysis was performed visually and milk samples taken 48 – 60 h after penicillin

infusion were difficult to assess. Investment in a semi-quantitative sample reader would provide

objective results, but the test kits are only certified for use on bulk tank milk so individual cow

milk results produced by the sample reader may not be accurate either.

Tissue penicillin residues: Tissue residue depletion curves could not be constructed from our

data, as all muscle, kidney, and liver samples taken 10 days after penicillin administration were

below the limit of detection of the HPLC assay (LOD = 2 ppb, MRL = 50 ppb). One proposal

was to euthanize each batch of 3 cows at different intervals, such as 4, 7, and 10 days post-

administration. This may have resulted in detectable penicillin residues, and possibly a residue

depletion curve. There were two problems with this approach, however. The first is that only 3

data samples would be available at each time point, which does not give a good estimate of

variability. The second problem is that the residue depletion curve would consist of a maximum

of 3 points, and possibly only one or two if the depletion was rapid. Because the project budget

was limited to nine cows, increasing the sample size was not an option. One suggestion was to

obtain muscle biopsies from each cow at multiple time points after drug administration to create

a muscle residue depletion curve. This was not feasible as 5 g of muscle tissue is required for

each assay (too large for a normal biopsy), local anesthetic infusion required for biopsy may

interfere with the chromatogram, and superficial muscle samples from one region are not

acceptable for CFIA residue analysis. We decided instead to euthanize all cows at 10 days post-

infusion because this is the label withdrawal time after intramuscular administration of the same

penicillin dose. Constructing a residue depletion curve would be helpful but not vital; the ability

to recommend a withdrawal interval with confidence was more important. Because the CgFARAD's policy is not to recommend a withdrawal interval shorter than the label withdrawal time, it is unlikely that withdrawal interval less than 10 days would be recommended anyways. It was more important that all tissue residues be below the MRL at this time point.

IP irritability/safety: Overall, IP procaine penicillin did not result in peritoneal inflammation or irritability. The blood chemistry results showed no evidence of inflammation, other than one cow with a pre-existing inflammatory process. Lesions described in previous studies (Klein, Firth et al., 1989) such as fibrin deposits and peritonitis were not observed. Significance of the consistent focal omental hemorrhages seen at necropsy cannot be determined, however. Were they a result of chemical irritation due to penicillin, a normal response to abdominal surgery, or an incidental finding in old cows? The design of this trial did not allow for statistical evaluation of IP penicillin irritability. A control group of IP saline-infused cows was considered, but would have required doubling the number of cows which was cost prohibitive. The other option was to divide the nine cows into penicillin and saline treated groups, but this would produce less penicillin data (the rationale behind the entire study). A crossover trial was also considered, whereby half the cows would receive IP penicillin and half IP saline. After a washout period of two or three weeks the treatments would be switched and the cows euthanized. If differences were noted between groups at necropsy (such as IP penicillin cows having focal omental hemorrhage, but not saline cows) then the statistical likelihood of differences between groups could be assessed. However, with such a small sample size and non-parametric results (necropsy score or area of hemorrhage) it would be difficult to find a statistically significant difference. Conversely, if both groups (penicillin and saline) had the same lesions at necropsy, the safety of IP penicillin would not be proven. Perhaps the hemorrhage in the IP saline group was due to the

previous cycle's IP penicillin administration (not the IP saline) and did not resolve after the washout period. In either case, determining if IP penicillin causes abdominal lesions would require a larger number of control animals than was feasible here.

5.4 Areas for Future Intraperitoneal Drug Research

5.4.1 Evaluation of clinical efficacy

Various investigations designed to assess the efficacy of perioperative antimicrobials in preventing or treating post-surgical infections are possible. One difficulty common to all investigations is that the generic endpoint "post-surgical infection" is a subjective definition. Surgical site (incision) infections, intra-abdominal inflammation/abscesses, or animals with high temperature or poor performance after surgery may all be classified as "post-surgical infections". Establishing an objective definition for post-surgical infections is essential. The gold standard test would be a positive bacterial culture from the infected area. This is feasible for incision site infections, but not when the infection is intra-abdominal. Ultrasound could be employed to diagnose abdominal abscesses, though bacterial culture from the abscess may not be possible. Even a positive bacteria culture does not confirm an "infection" per se as the bacteria may not be pathogenic but part of the normal flora. Any classification scheme would have to incorporate a number of observations, such as clinical findings (elevated rectal temperature, depression score, decreased appetite or milk production) and laboratory results (neutrophils/bacteria on cytology or complete blood count, decreased fibrinogen:total solids ratio).

Retrospective cohort study: Examining the WCVM large animal clinic hospital records may provide the information necessary to determine the incidence of post-surgical bovine infections.

Intraperitoneal antimicrobial use is not routine at the WCVM however, so no data is available on infection rates after IP administration. In veterinary clinics that routinely use IP antimicrobials, lack of detailed records on post-surgical infections makes comparisons between cases difficult. Even if this information was available, retrospective studies cannot prove cause-and-effect. A correlation may be found between IP antimicrobial use and rates of post-surgical infections, but due to the multitude of confounding factors causality cannot be established.

Experimental abdominal infection model: In this experiment, cows would receive an IP inoculum of a known quantity of bacteria to mimic contamination during abdominal surgery. Presence of infection would be determined at various time points after inoculation. One group of cows would receive no antimicrobials (negative control), one would receive intraoperative IP antimicrobials, and other groups would be positive controls (such as preoperative IV or postoperative IM antimicrobials). However, the specific pathogens hypothesized to cause post-surgical infections are not conclusively defined in cattle. Even if known, IP challenge with pathogenic bacteria does not mimic real-life conditions. Surgical infections may arise due to traumatic tissue handling and local necrosis, or polybacterial infection (such as fecal contamination). It would be difficult to mimic real-life operating conditions while maintaining a consistent bacterial challenge between animals. As well, because most animals undergoing simple abdominal surgery do not become infected (Klein & Firth, 1988; Haven, Wichtel et al., 1992; Desrochers, 2005), the sample size needed to establish significant differences between groups becomes prohibitive.

Prospective randomized clinical trial: As the number of bovine abdominal surgeries performed at the WCVM is limited, private bovine practitioners and their clients could be enlisted in a

prospective clinical trial to determine post-surgical infections during "normal" bovine abdominal surgery. This is extremely difficult for a number of reasons. Besides the problem with establishing a common definition of post-surgical infections (especially among multiple veterinarians and producers), no two surgeries are identical. Even among routine abdominal surgeries such as caesarian sections, some are performed quickly and easily while others are lengthy and difficult. The degree of abdominal contamination can vary drastically, especially if the cow becomes recumbent during surgery or some other breach in sterility occurs. Surgical skill varies between veterinarians, so between-surgeon rates of infection could confound the results. Although each animal undergoing abdominal surgery could be randomized into various treatment groups before surgery (perhaps by rolling a dice before each surgery), it would be impossible to blind the surgeons as to which treatment group the cow was in. Bias is therefore inevitable. Veterinarians may be more careful when performing surgery if knowing the cow will be a negative control with no antimicrobials, or careless if antimicrobials are administered. The veterinarian and producer must also agree to follow the randomization scheme and not treat the animal empirically.

It would certainly be difficult to define objective outcome assessments (post-surgical infection) and properly control bias in this trial. However, once these challenges are resolved the experimental results would unequivocally answer if IP antimicrobial use reduces post-surgical infections. Another benefit would be a current, accurate assessment of post-surgical infection rates and possible etiologies (if culture results are obtained). I hypothesize that animals treated with IP antimicrobials would have post-surgical infection rates no lower (and likely higher) than cows with preoperative IV treatment but lower than negative controls. IP use would therefore not be justified if a better alternative (preoperative IV treatment) exists. However, this is only a

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hypothesis at this time. Other questions to answer: would intraoperative IP antimicrobials result in fewer infections than postoperative IM treatment? Would all treatment groups have infection rates lower than the negative control group? Are there differences between infection rates for treatment groups depending on co-variables such as the type of surgical procedure or location of surgery?

Unfortunately, with 4 treatment groups (negative control, intraoperative IP, preoperative IV, and postoperative IM), co-variables (surgeon, surgical procedure, location of surgery, etc.), and relatively low post-surgical infection rates, a large sample size would be required. Assuming a relatively high post-surgical infection rate of 15% in the negative control group (de Kruif, van den Brand et al., 1987; Seger, Grunert et al., 1994; Desrochers, St-Jean et al., 1996; Bedard, Desrochers et al., 2001; Desrochers, 2005), and a 5% infection rate in treated animals (a 67% reduction), each group would need 159 animals for the study to demonstrate a significant difference with 95% confidence (two-tailed) and 80% power (Browner, 2001). That requires enlisting at least 636 separate surgeries in the trial, a difficult (though not impossible) number.

5.4.2 *Further survey data*

Is IP antimicrobial use only a Western Canadian phenomenon? Surveys of other jurisdictions in North America could answer this question. Specifically, the American Association of Bovine Practitioners (AABP) would provide an excellent base of large animal veterinarians. I suspect IP antimicrobial use does occur in other jurisdictions, based on anecdotal evidence from the AABP listserve. A survey of AABP members would be feasible; links to other electronic surveys have been posted on their listserve. An electronic survey might allow for follow up with respondents and non-respondents, which was not possible with the previous

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survey. As for refining the survey questions, I would keep the same general design but include

more detail.

- Asking respondents to write the percentages of abdominal surgeries they treat with specific antimicrobials may give more accurate data. For example, "What percentage of abdominal surgeries receive preoperative:"
  Procaine Penicillin   _____
  Oxytetracycline LP   _____
  Ceftiofur   _____
  Other, etc.   _____
  This question would be repeated for postoperative and IP use. Listing multiple antimicrobials ensures seldom-used antimicrobials won't be overlooked by the respondent.

- Questions regarding frequency of IP antimicrobial use should include a breakdown for specific surgeries, in case some veterinarians use the IP route for some surgeries but not others. For example, perhaps some practitioners treat all ceasarian sections with IP antimicrobials due to possible contamination from the uterus, but not displaced abomasums or umbilical hernias.

- After asking for recommended meat and milk withdrawal information, ask how these values were determined. Did the practitioner contact FARAD, find kinetic data on his/her own to base a withdrawal interval, simply extend the label time, or just make up a number?

- Because the survey would be electronic and completely confidential, personal information could be requested. Variables such as "# years in practice" and "geographic location" could be analyzed to look for significant differences in perioperative antimicrobial use.

5.4.3 *Refining analytical methods to detect penicillin*

Before pursuing further experiments requiring penicillin analysis of plasma, milk, or any

other non-tissue matrix, validation of the HPLC penicillin assay is required. Method validation

can be time-consuming and difficult, and was not deemed necessary due to the limited scope and

"pilot project" nature of this thesis. Although our plasma and milk concentrations cannot be

accepted with full confidence, they are valid as initial estimates. However, any additional

projects analyzing larger sample numbers require greater confidence in the data produced, thus method validation is mandatory.

5.4.4 Kinetic differences between IP and other routes of administration

If the concept that clinical efficacy of an antimicrobial can be predicted based on its PK/PD parameters (such as C_{max}, T_{max}, AUC, or T>MIC) is accepted, then the relative efficacies of different routes of administration for the same antimicrobial can also be predicted. It must be noted that the predictive value of these PK/PD parameters has not been conclusively determined for perioperative antimicrobial drug use. PK/PD parameters for each route of administration can be obtained using a simple crossover trial. For example, 9 cows would be divided into three groups, each group receiving procaine penicillin by a different route (such as IM, SC, and IP). Blood would be collected as per the sampling schedule in this trial. After an appropriate washout period (14 days), the cows would be treated again but with a different route until each group receives penicillin by all three routes. In this manner each cow serves as her own control, thus increasing the power of the trial to detect significant differences in plasma kinetics.

5.4.5 Complete milk and meat residue depletion studies

As addressed in the study limitations, full meat and milk penicillin residue depletion trials were not performed after IP infusion. Although this information would be interesting, it is not vital—our data demonstrates that the label meat and milk withdrawal times for IM administration should be adequate. Full residue depletion data is required if a company wishes to pursue a product label claim for IP administration, but this is highly unlikely as the target market for this procedure (veterinarians performing abdominal surgery) is too small to support the financial expenditure required.

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5.4.6 Extended irritability/safety studies

Although our trial did not demonstrate peritoneal irritation or systemic adverse effects after IP procaine penicillin administration, it could not conclusively prove the safety of this practice. As discussed earlier, proof of safety requires a prospective study using a negative control group (with IP infusions of saline). Frankly this information seems unnecessary. The cows in our trial did not exhibit any signs of peritonitis or inflammation and had normal leukograms. One could argue that the low milk production was due to abdominal discomfort and decreased appetite, but I would argue that the low production was due to old, cull cows transported to a new facility and eating a new ration.

5.4.7 Kinetics and residues of other IP antimicrobials

The survey results indicate that many other drugs other than procaine penicillin G are administered IP during bovine abdominal surgery. When reporting results of the kinetics and residue trial to veterinarians, we caution against extrapolating these results to other antimicrobials or penicillin formulations administered by IP infusion. Specifically, it must not be assumed that other drugs (such as oxytetracycline, neomycin, ceftiofur, etc.) or formulations (benzathine penicillin) will be rapidly absorbed and excreted after IP use. Nor can the residue depletion conclusions of this trial be applied to other drugs. Although IM procaine penicillin meat and milk withdrawal times can safely be recommended after IP administration, the IM label withdrawal time may not apply for IP administration of other drugs (especially if the formulation is irritating and absorption is delayed). Finally, despite concluding that IP procaine penicillin in cattle is not unsafe does not mean this conclusion is universally appropriate. I suspect cows will react similarly to IP administration of other antimicrobials as they do to procaine penicillin (so

long as the drug formulations are non-irritating), but the only way to verify this hypothesis is if similar data is produced for these products. Because procaine penicillin was the most commonly used IP antimicrobial in the survey it seemed rational to use it for our kinetic trials. Similar experiments using oxytetracycline (the 2nd most commonly used IP antimicrobial) may be warranted. Unless new data indicates a pressing need to evaluate IP use of other drugs, I would limit work to penicillin and oxytetracycline. The IP use of other antimicrobials was either too infrequent (eg. ceftiofur, trimethorpim-sulfa), or too inappropriate (eg. neomycin) to warrant further investigation at this time.

5.4.8 IP absorption studies

Although we determined the kinetics of procaine penicillin absorption after IP administration, the anatomic considerations of injecting drugs into the abdomen are not fully understood. Is the liquid absorbed by blood vessels on visceral surfaces or within the omentum, or by peritoneal vessels on the ventral abdominal wall? This question is not of terrible importance for injecting penicillin into cows, but is relevant for other species where IP administration is more common, such as rodents. Drugs absorbed by peritoneal vessels are transported directly to the heart and then to the systemic circulation, whereas drugs absorbed by the splanchnic circulation may undergo extensive first pass metabolism in the liver. Experiments could be devised to assess the exact locations of IP absorption and circulation. Animals could receive IP injections containing a dye such as India ink and euthanized at various time points to determine the exact ink location. A more suitable experiment would use IP injections of a radio-labeled drug. Serial imaging using nuclear scintigraphy would reveal the location of the compound, both within the abdomen and after absorption into the bloodstream. The logistics of

this project would need to be addressed, such as the proper concentration of radio-labeled drug required and the necessary image timing and frequency.

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