THE EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN FECAL *ESCHERICHIA COLI* ISOLATES OF FEEDLOT CATTLE IN WESTERN CANADA

A Thesis Submitted to the

College of Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

in the Department of Large Animal Clinical Sciences

Western College of Veterinary Medicine

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Sylvia Lee Checkley

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work or, in their absence, by the Head of the Department of Large Animal Clinical Sciences or the Dean of the Western College of Veterinary Medicine. It is understood that any copying of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Large Animal Clinical Sciences
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, Saskatchewan
Canada, S7N 5B4

ABSTRACT

Prevalence of antimicrobial resistance in fecal *Escherichia coli* isolates from feedlot cattle was characterized. Tetracycline resistance in isolates from newly weaned, auction market derived calves on arrival at the feedlot in a clinical trial was 9.8% and resistance to three or more antimicrobials was 2.1% compared to 17.6 % and 5.9% in a cohort study. The prevalence of tetracycline resistance at 78.3% and resistance to three or more antimicrobials at 52.5 % in isolates from spring calves submitted to a regional diagnostic laboratory were higher than those found on arrival at the feedlot. Of isolates from composite feedyard pen samples late in the feeding period, 39.4% were tetracycline resistant and 7.6% were resistant to three or more antimicrobials, somewhat higher than on arrival. Use of oxytetracycline in the feed for disease prophylaxis and the metaphylactic use of long-acting injectable oxytetracycline were associated with increased proportions of cattle with one or more resistant *E. coli* isolates early in the feeding period, while the use of individual animal treatments was not. The proportion of animals with one or more tetracycline resistant *E. coli* isolates was not different between the control, metaphylactic treatment and prophylactic treatment groups preslaughter; however, there were significantly more resistant animals in all groups preslaughter than at arrival. There were also no associations found between the total volume of parenteral antimicrobials used for disease treatment in individual animals and antimicrobial resistance in the cohort study. In addition, no strong associations were found between pen-level prevalence of antimicrobial resistance antimicrobial use or other variables. There was no significant difference between the proportion of isolates per pen resistant to tetracycline, one or more, two or more antimicrobials, or three or more antimicrobials when using 20, 15, 10 or 5 isolates from composite pen-level fecal

samples. Variance for isolates resistant to three or more antimicrobials was partitioned as 12.7% at the feedyard-level and 28.7% at the pen-level. The use of diagnostic laboratory data for AMR surveillance was also discussed, and alternatives to antimicrobial treatment in the feedlot were also investigated. Overall a significant contribution to our understanding of antimicrobial resistance in feedlot cattle was achieved.

ACKNOWLEDGEMENTS

This work could not have been completed without the help and support of many people. I thank my colleagues at the University of Saskatchewan Feedlot, Pound-Maker Agventures, participating Alberta feedlots, Craig Dorin at Veterinary Agri-Health Services, the Agri-Food Laboratories Branch of Alberta Agriculture and Rural Development, the laboratory of Manuel Chirino-Trejo in the Department of Veterinary Microbiology, and the feedlot group under John McKinnon in the Department of Animal Science. I also wish to thank the Saskatchewan Agriculture Development Fund, Alberta Agriculture and Rural Development, and the British Columbia Cattlemen's Association for funding support. Most of all, I wish to thank my graduate committee at the Western College of Veterinary Medicine. The support from my supervisor John Campbell was tremendous. He was always available as a mentor in spite of other demanding commitments. I benefited greatly from his knowledge. Cheryl Waldner shared a great knowledge of statistics, epidemiology, and a joy in trying new statistical techniques. Eugene Janzen was 'all things beef'. His viewpoint and ideas helped guide me on this path. Manuel Chirino-Trejo, Trisha Dowling, and Terry Carruthers were knowledgeable, helpful, and supportive of my work. My journey started on the backroads of north-central Alberta and has taken me through halls of higher learning and introduced new ways of thinking. I sincerely thank fellow graduate students (especially Sheryl Gow), coworkers, mentors, and peers in the veterinary, animal health and epidemiology communities who encouraged, supported, and challenged me along the way. I value every step of the journey. Love to my family and thanks to you all for help making my dream possible!

DEDICATION

For my father and the rest of my family and friends who cheered me on,
for my mentors and peers who encouraged, supported and challenged me,
and especially for Morgan Lee,

‘When you get the choice to sit it out or dance,
…… I hope you dance’
by LeeAnn Womack

ORIGINAL CONTRIBUTION

My contribution to the intellectual property for the studies described in this dissertation was multifaceted. I coordinated and wrote the literature review. The metaphylaxis study had already been designed when I initiated this postgraduate work; however, I was a major collaborator on the study design and preparation of funding proposals for the passive surveillance study, Alberta feedlot study, cohort study, and *Fusobacterium necrophorum* vaccination study. I helped process and treat cattle involved in the metaphylaxis study and the cohort / *F. necrophorum* vaccination study. I was responsible for sample collection, sample submission, and data entry in the metaphylaxis study, Alberta feedlot study, cohort study, and *F. necrophorum* vaccination study. I searched archives and coordinated the photocopying and entry of medical information for the collation of passive surveillance study. I analyzed the data and prepared the scientific papers for all five studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AABP	American Association of Bovine Practitioners
ACKSSuT	Ampicillin, Chloramphenicol, Kanamycin, Streptomycin, Sulphamethoxazole, Tetracycline
ACSSuT	Ampicillin, Chloramphenicol, Streptomycin, Sulphamethoxazole, Tetracycline
$ADD_{Feedlot}$	Animal Daily Dose for a feedlot animal
ADG	Average Daily Gain
AKSSuT	Ampicillin, Kanamycin, Streptomycin, Sulphamethoxazole, Tetracycline
ALF	Ad Libitum Forage
AHI	Animal Health Institute
AMC	Amoxicillin/Clavulanic Acid
AMK	Amikacin
AMP	Ampicillin
AMPp	Ampicillin phenotype
AMR	Antimicrobial resistance
AMU	Antimicrobial use
AHI	Animal Health Institute
AUC	Area under the curve
BIC	Beef Information Centre
BC	British Columbia
BRD	Bovine Respiratory Disease
BSE	Bovine Spongiform Encephalopathy

BW	Body Weight
Cmax	Maximum serum concentration
CAST	Council for Agricultural Science and Technology
CAHI	Canadian Animal Health Institute
CBEF	Canadian Beef Export Federation
CCA	Canadian Cattlemen's Association
CCAR	Canadian Council on Antibiotic Resistance
CDC	Centers for Disease Control and Prevention
CEP	Cephalothin
CDN	Canadian
CHL	Chloramphenicol
CI	Confidence Interval
CIP	Ciprofloxacin
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CLSI	Clinical and Laboratory Standards Institute
CRO	Ceftriaxone
CVMA	Canadian Veterinary Medical Association
DDD	Defined Daily Dose
DIF	Days in feed
DIN	Drug Identification Number
DOF	Days on feed
DOT	Days on trial

DPS	Days Preslaughter
E. coli	*Escherichia coli*
ENR	Enrofloxacin
ESBL	Extended-spectrum beta-lactamase
EU	European Union
F. necrophorum	*Fusobacterium necrophorum*
FOX	Cefoxitin
TIO	Ceftiofur
GEN	Gentamicin
GEE	Generalized estimating equations
GOC	Government of Canada
HC	Health Canada
IOM	Institute of Medicine
JETACAR	Joint Expert Technical Advisory Committee on Antimicrobial Resistance
KAN	Kanamycin
LFG	Limit Fed Grain
MDR	Multi-drug resistance
MIC	Minimum inhibitory concentration
MPC	Mutant prevention concentration
MRSA	Methacilline Resistant Staphylococcus aureus
MSW	Mutant Selection Window
MUMS	Minor Uses/Minor Species

NAHMS	National Animal Health Monitoring System
NAL	Nalidixic Acid
NARMS	National Antimicrobial Resistance Monitoring System
NCCLS	Previous name of CLSI
NRC	National Research Council
NTSEC	Non-Type Specific *Escherichia coli*
OFFS	On-Farm Food Safety
OIE	World Organization for Animal Health (previously Office International des Epizooties)
OR	Odds ratio
PDD	Prescribed Daily Dose
PHAC	Public Health Agency of Canada
QA	Quality Assurance
SC	Subcutaneously
SDR	Specific-drug-resistance
SMX	Sulphamethoxazole
SMXp	Sulphamethoxazole phenotype
STR	Streptomycin
SXT	Trimethoprim/Sulphamethoxazole
TCY	Tetraycline
TCYp	Tetracycline phenotype
TMP	Trimethoprim
TMPSSS	Trimethoprim/Sulfanilamide

USDA	United States Department of Agriculture
VDD	Veterinary Drug Directorate
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
WVA	World Veterinary Association

CHAPTER 1
INTRODUCTION

1.1 Background

Antimicrobial resistance (AMR) has been perceived as an important component of both animal health and food safety in the international community and specifically by countries that import beef (Khachatourians, 1998; McGeer, 1998; Angulo et al., 2004). The potential presence of antimicrobial resistant bacteria is another food safety concern that is increasingly important in public perception of beef and other food products (Khachatourians, 1998; Conly, 2002). However, the full impact of antimicrobial resistant organisms on food safety is still unknown (Conly, 2002). Concerns about antimicrobial use (AMU) in animals causing human health concerns were first voiced in the United Kingdom in 1969 (Swann, 1969). Many other reports have echoed this same sentiment (Apley, 1998; Conly, 2002; Lipsitch et al., 2002; Smith et al., 2002; Singer et al., 2003).

In Canada, most calves are born in the spring, cow-calf pairs summer on pasture, and calves are weaned in the fall when cattle are brought in for winter feeding. Roughly 40% of the Western Canadian annual weaned male calf crop is placed directly in feedlots onto high-grain finishing diets for roughly 200 days. Another 40% of weaned calves are put on some type of backgrounding diet in a commercial feedlot or elsewhere for at least a few months before changing to a finishing diet. The other roughly 20% of calves are backgrounded over winter and put back out to grass for a second grazing

season before finishing (Calvin Booker, Feedlot Health Management Services, 2008, personal communication). Those animals are then sold to feedlots as yearlings. Heifers not retained as replacements make up a portion of the cattle on feed; heifer retention rates for replacements vary depending on the prevailing market conditions (Canfax and Gracey, 2002).

Feedlot studies are therefore of prime interest for AMU and AMR research, because cattle on feed are closest to the consumer in the beef production cycle. There is a prevailing assumption that many antimicrobials are used in intensive or high-yield animal agriculture (Khachatourians, 1998). The Canadian beef industry has traditionally exported as much as 70-80% of live beef produced (BIC, 2002), and is cognizant of issues surrounding AMU and AMR. They have been proactive in providing best management practices on AMU through industry led initiatives such as "Quality Starts Here / Verified Beef Production" and guidelines for prudent use of antimicrobials (CCA, 2006; CCA and BIC, 2006). The Canadian Veterinary Medical Association (CVMA) and other veterinary associations have presented position statements that on prudent antimicrobial use in animals (CVMA, 1998). More information is needed on AMU and AMR in the feedlot so information is available to use in risk assessments and form science-based recommendations concerning AMU.

1.2 Goals of the thesis

There are many gaps in our understanding of the epidemiology of AMU and AMR. Humans depend on antimicrobials to treat and/or prevent disease in people and animals; antimicrobials also have less common uses, such as aquaculture and apiculture and orchard management (VDD, 2002). Resistance to antimicrobials is widespread in

bacterial populations of animal origin (Stabler et al., 1982; GOC, 2005; Bartoloni et al., 2006; Funk et al., 2006). Antimicrobial resistance is associated with antimicrobial use (Stabler et al., 1982; Dunlop et al., 1998; Berge et al., 2006; Funk et al., 2006) but animal populations that have never received antimicrobials carry resistant bacteria (Caprioli et al., 1991; Dolejska et al., 2007). Genes for antimicrobial resistance are reportedly found in bacteria frozen in glaciers and are thousands of years old (Dancer et al., 1997). Scientists have suggested that AMU in animal agriculture was not carried out in a prudent fashion although their assumptions are largely unreferenced (Khachatourians, 1998). Therefore, it seemed important to better understand this issue as it related to the feedlot industry and provide evidence to direct future control strategies, rather than exercising the precautionary principle (Hurd et al., 2004).

This thesis was designed to better understand antimicrobial resistance in feedlot cattle and to determine whether some of the common uses of antimicrobials in high-risk groups of feedlot cattle were associated with antimicrobial resistance in fecal *Escherichia. Coli* isolates. The specific objectives of this thesis were to:

1. Determine the prevalence and describe patterns of antimicrobial resistance in fecal *E, coli* isolates from:
    a. newly-weaned, auction-market-derived calves on arrival at the feedlot,
    b. composite feedyard pen samples for calves late in the feeding period,
    c. spring calves through diagnostic laboratory data.
2. Examine associations between:

a. metaphylactic (injectable) and prophylactic (feed) antimicrobial use in groups of cattle and the presence of antimicrobial resistant fecal *E. coli* over time,
b. the total volume of parenteral antimicrobials used for disease treatment and changes in antimicrobial resistance, during the feeding period,
c. feedyard demographic characteristics, pen demographic characteristics and pen-level antimicrobial use on the pen-level prevalence of antimicrobial resistance.

3. Discuss the use of diagnostic laboratory data as a method of passive surveillance for antimicrobial resistance in Western Canada.
4. Determine the number of isolates necessary for establishing resistance levels in feedyard pens.
5. Evaluate the effectiveness of a *F. necrophorum* vaccine (Fusogard, Novartis Animal Health Canada, Mississauga, Ontario) as an alternative to antimicrobial use in feedlot cattle not treated with a prophylactic feed antimicrobial by comparing the prevalence of these conditions in the vaccinated cattle and the unvaccinated (control) cattle, for the prevention of:
    a. liver abscesses
    b. footrot.

1.3 References

1. Angulo FJ, Nunnery JA, Bair HD. Antimicrobial resistance in zoonotic enteric pathogens. Rev Sci Tech Off Int Epiz 2004;23 (2):485-496.

2. Apley M. Does antimicrobial use in animals affect human health? The Bovine Proceedings 1998;31:9-12.

3. Bartoloni A, Pallecchi L, Benedetti M, Fernandez C, Vallejos Y et al. Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. Emerg Infect Dis 2006;12:907-913.

4. Beef Information Centre (BIC). Canada's Beef Industry Fast Facts 2002. Available from http://www.beefinfo.org/pdf/CBIFF_P.pdf accessed December 30, 2007.

5. Berge ACB, Moore DA, Sischo WM. Field trial evaluating the influence of prophylactic and therapeutic antimicrobial administration on antimicrobial resistance in fecal *Escherichia coli* in dairy calves. Appl Environ Microbiol 2006;72:3872-3878.

6. Canadian Cattlemen's Association (CCA). Quality Starts Here / Verified Beef Production Producer Manual, 2006. Available from http://www.verifiedbeef.org/producer_resources.htm accessed December 30, 2007.

7. Canadian Cattlemen's Association (CCA), Beef Information Centre (BIC). Antibiotics in the Beef Cattle Industry, 2006. Available from http://www.cattle.ca/producer/Factsheets/issue%20factsheets.htm accessed December 30, 2007.

8. Canadian Veterinary Medical Association (CVMA). General Position Statements: Antimicrobial Use in Animals, October 1998. Available from http://canadianveterinarians.net/ShowText.aspx?ResourceID=60 accessed December 30, 2007.

9. Canfax, Gracey C. The cattle cycle. Calgary: Canadian Cattlemen's Association, 2002.

10. Caprioli A, Donelli G, Falbo V, Passi C, Pagano A, Mantovani A. Antimicrobial resistance and production of toxins in *Escherichia coli* strains from wild ruminants and the alpine marmot. J Wildl Dis 1991;27:324-327.

11. Conly J. Antimicrobial resistance in Canada. Can Med Assoc J 2002;221:268-272.

12. Dancer SJ, Shears P, Platt DJ. Isolation and characterization of coliforms from glacial ice and water in Canada's high arctic. J Appl Microbiol 1997;82:597-609.

13. Dolejska M, Cizek A, Literak I. High prevalence of antimicrobial-resistant genes and integrons in *Escherichia coli* isolates from Black-headed Gulls in the Czech Republic. J Appl Microbiol 2007;103:11-19.

14. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998;34:283-305.

15. Funk JA, LeJeune JT, Wittum TE, Rajala-Schultz PJ. The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. Microbial Drug Resistance 2006;12:210-218.

16. Government of Canada (GOC). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph, ON: Public Health Agency of Canada, 2007. http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html accessed December 30, 2007.

17. Hurd HS, Doores S, Hayes D, Mathew A, Maurer J, Silley P, Singer RS, Jones RN. Public health consequences of macrolide use in food animals: A deterministic risk assessment. J Food Prot 2004;67:980-992.

18. Khachatourians GC. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can Med Assoc J 1998;159:1129-1136.

19. Lipsitch M, Singer RS, Levin BR. Antibiotics in agriculture: When is it time to close the barn door? Proc Natl Acad Sci 2002;99(9):5752-5754.

20. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? Can Med Assoc J 1998;159:119-1120.

21. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. The Lancet Infectious Diseases 2003;3(1):47-51.

22. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci 2002;99(9):6434-6439.

23. Stabler SL, Fagerberg DJ, Quarles CL. Effects of oral and injectable tetracyclines on bacterial drug resistance in feedlot cattle. Am J Vet Res 1982;43:1763-1766.

24. Swann MM. Use of antibiotics in animal husbandry and veterinary medicine. UK Joint Committee. London: HM Stationary Office, 1969.

25. Veterinary Drug Directorate (VDD), Health Canada. Uses of antimicrobials in food animals in Canada: Impact on resistance and human health, 2002. Available from http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed December 30, 2007.

CHAPTER 2
LITERATURE REVIEW

This review is intended to summarize the major literature related to the epidemiology of antimicrobial resistance in feedlot cattle. Gray literature is included, in addition to peer-reviewed scientific papers. This review focuses on AMR in feedlot cattle Subsections include a general background on antimicrobials and resistance, a historical perspective on AMR, AMU, other research on AMR, study design issues in research, and surveillance. Each subsection focuses on the relevant papers that form the basis for our understanding of the epidemiology of AMR as it pertains to feedlot cattle. Selected examples from research in other species are used where appropriate.

2.1 Background on antimicrobial resistance

2.1.1 What are antimicrobials? and other definitions

An antimicrobial can be defined as a substance "of natural, semisynthetic, or synthetic origin that kills or inhibits the growth of a microorganism but causes little or no damage to the host" (Giguere, 2006a). Antimicrobial is a broader term than antibiotic, which is defined as "a low molecular weight substance produced by a microorganism that at low concentration inhibits or kills other microorganisms" (Giguere, 2006a).

In this thesis, the word antimicrobial will be used exclusively as it includes all antibiotics, while the term antibiotic does not include all antimicrobials. Antimicrobials

can also be subclassified with respect to the type of microorganism they affect (Guardabassi and Courvalin, 2006). This thesis is focused on those with antibacterial activity. Antimicrobials are recent developments in the history of man. Penicillin was first discovered in 1929 but antimicrobials didn't become broadly available until after World War II (Giguere, 2006a).

AMR can be defined different ways depending on what criteria are used for classification. In microbiology, a bacterial strain is resistant if it "grows in the presence of higher concentration of the (antimicrobial) compared with phylogenetically related strains" (Guardabassi and Courvalin, 2006). Resistance of bacteria to antimicrobial treatment is often suspected when the patient doesn't respond to the treatment as expected. Clinically, "a strain is considered resistant when it survives antimicrobial therapy" (Guardabassi and Courvalin, 2006). Clinical resistance is affected by many factors in the living host including host immunity, dosage, mode of administration, and infection site dynamics (Guardabassi and Courvalin, 2006). Various other discipline-related definitions exist for AMR (Acar and Rostel, 2001).

AMR can be measured and described in the laboratory quantitatively using dilution susceptibility tests such as agar dilution, broth microdilution, or broth macrodilution (Walker, 2006). Test results generated are minimum inhibitory concentrations (MIC), defined as the "lowest (antimicrobial) concentration that completely inhibits growth of the bacterial isolate" being tested (Guardabassi and Courvalin, 2006). AMR can be measured and described in the laboratory qualitatively using disk diffusion or concentration gradient tests, where the test results are a zone of inhibition (Walker, 2006). The zone of inhibition is inversely correlated with the antimicrobial

concentration needed for bacterial inhibition (Walker, 2006). For results of these tests to be useful, both types of antimicrobial susceptibility tests must be carried out following standardized methods, which in North America are set out by the Clinical and Laboratory Standards Institute (CLSI) (Ginocchio, 2002; Walker, 2006). Both quantitative and qualitative test results are interpreted using the breakpoints for the bacteria. A breakpoint is the point "at which an organism is considered to be susceptible, intermediate, or resistant" (Walker, 2006). Breakpoints can differ between countries, over time and by purpose (Wikler and Ambrose, 2005). Epidemiologic or microbiologic breakpoints, used in surveillance, are calculated based on comparison of the MIC distribution with that of a wild-type population (Guardabassi and Courvalin, 2006). Epidemiologic breakpoints, also called epidemiologic cut-off values, are used to identify emerging resistance in populations of microorganisms (Bywater et al., 2006; Wikler and Ambrose, 2005). Clinical breakpoints, used for clinical applications, take into account in vivo factors such as bacterial distribution, pharmacodynamics, pharmacokinetics, and the results of clinical trials (Guardabassi and Courvalin, 2006; Walker, 2006). For example, clinical breakpoints exist for most of the common antimicrobials used for the treatment of Bovine Respiratory Disease (BRD) in cattle (Apley and Coetzee, 2006). Clinical breakpoints, the only breakpoints calculated by CLSI, are sometimes called antimicrobial susceptibility test interpretive categories (Wikler and Ambrose, 2005). The European Committee on Antimicrobial Susceptibility Testing (EUCAST), of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), also has established epidemiological breakpoints (Wikler and Ambrose, 2005).

2.1.2 How do antimicrobials produce an antimicrobial effect?

Antimicrobials selectively target differences between bacterial and eukaryotic cells so as to inhibit bacterial growth without harming the host (Tenover, 2006). The main methods by which antimicrobial agents act on microorganisms are through inhibition of cell wall synthesis (beta-lactams, bacitracin, glycopeptides), inhibition of protein synthesis (tetracyclines, aminoglycosides, macrolides, phenicols, lincosamides, streptogramins), inhibition of nucleic acid synthesis or function (nitroimidazoles, nitrofurans, quinolones and rifamycins), inhibition of folic acid synthesis (sulfonamides and trimethoprim), or damage to cell membrane function (polymyxins) (Giguere, 2006a).

There are three phases of cell wall synthesis that antimicrobials can affect. These three phases are the cytoplasmic, membrane-associated, and extracytoplasmic phases. The phosphoenolpyruvate analogue, fosfomycin, affects the cytoplasmic phase by inhibiting binding of phosphoenolpyruvate, an enzyme important in the synthesis of peptidoglycan units in the cytoplasm (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). The polypeptide, bacitracin, affects the membrane-associated phase by forming a complex with the lipid carrier of the peptidoglycan units (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). The extracytoplasmic phase, where the peptide and glycan strands are cross-linked, is targeted by beta-lactams (penicillins and cephalosporins) that bind the penicillin-binding proteins inhibiting transpeptidation, by glycopeptides (vancomycin and avoparcin) that bind the pentapeptide precursor preventing transpeptidation, and by the glycophospholipid (bambermycins) that inhibit synthesis of

cell-wall peptidoglycan through different mechanisms (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). Polymyxins damage cell membrane function by increasing cell permeabililty (Guardabassi and Courvalin, 2006; Giguere et al., 2006b).

Protein synthesis is inhibited by a number of antimicrobials (lincosamides, pleuromutilins, streptogramins, macrolides, chloramphenicol/florfenicol, and the oxazolidinones) through binding of the 50S ribosomal subunit causing inhibition of translation (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). Aminoglycosides and tetracyclines exert their effect by binding the 30S ribosomal subunit and subsequently inhibit translation (Guardabassi and Courvalin, 2006; Giguere et al., 2006b).

Antibacterials also inhibit nucleic acid synthesis. Quinolones affect DNA synthesis through inhibition of the enzymes, topoisomerase II (DNA gyrase) and topoisomerase IV (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). Rifamycins inhibit bacterial RNA polymerase thereby inhibiting mRNA synthesis (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). Nitrofurans and nitroimidazoles directly break strands of DNA (Guardabassi and Courvalin, 2006; Giguere et al., 2006b). Sulfonamides and trimethoprim indirectly inhibit nucleic acid synthesis through inhibition of folic acid synthesis (Guardabassi and Courvalin, 2006; Giguere et al., 2006b).

Antimicrobials can also work in synergy, where the effect of using the two antimicrobials together is greater than the sum of the independent contributions of each antimicrobial (Giguere, 2006a). An example of antimicrobial synergy, commonly used in feedlot cattle, is trimethoprim/sulfonamide combinations that inhibit sequential steps

in metabolism of folic acid (Guardabassi and Courvalin, 2006; Giguere, 2006a). Antagonism or mere additive effects may be found with some antimicrobial combinations; this varies with the antimicrobial class as well as the strain of the microorganism (Giguere, 2006a).

There are other principles involved in the processes by which antimicrobials exert an inhibitory effect on microorganisms. Pharmacokinetics (PK) explain how the antimicrobial is processed by the animal, including absorption, distribution, metabolism and elimination (Martinez et al., 2006; Rybak, 2006). Pharmacodynamics (PD) explain the antimicrobial's effect on the bacterium (Martinez et al., 2006; Rybak, 2006). The killing mechanism of antimicrobials can be described in two categories: concentration dependent (fluoroquinolones, aminoglycosides), or time dependent (β-lactams, macrolides, trimethoprim/sulfonamides, and tetracyclines) (Martinez et al., 2006; Rybak, 2006). The effectiveness of antimicrobials can be measured by calculating PK/PD parameters such as ratios of the maximum antimicrobial serum concentration to its MIC (Cmax/MIC) (aminoglycosides), the ratio of the area under the (plasma concentration of the antimicrobial versus time) curve to its MIC (AUC/MIC) (fluoroquinolones, glycopeptides, lincosamides, tetracyclines), or duration of time that the plasma concentration of the antimicrobial was higher than its MIC (T>MIC) (β-lactams, macrolides, trimethoprim, sulfonamides, oxazolidinones) (Martinez et al., 2006; Rybak, 2006).

2.1.3 How does resistance occur?

AMR can be intrinsic or acquired. Intrinsic resistance means that a phylogenetic group or species of bacteria is inherently resistant to a specific antimicrobial class due

to their physical characteristics (Boerlin and White, 2006; Giguere, 2006a). For example, the outer cell membrane of gram-negative bacteria is impermeable to glycopeptides like avoparcin (Schwarz et al., 2006; Giguere, 2006a). Intrinsic resistance can be described as "insensitivity" (Guardabassi and Courvalin, 2006). This type of resistance is unrelated to AMU.

Bacteria that would normally have been susceptible to an antimicrobial agent can acquire genetic material that confers resistance to one or more antimicrobials. Gordon discusses the idea, originally voiced by Rene Dubos, that "bacteria are not simply foes to be vanquished, but a part of the natural world, capable of making deft adaptations to the drugs we use to fight them" (Gordon, 1998). This can occur through mutation (endogenous resistance) or through horizontal gene transfer (exogenous resistance) (Guardabassi and Courvalin, 2006).

Mutations are spontaneous but some genes mutate more frequently than others, and mutation rates vary between strains and species of bacteria. A common mutation would be one that occurs in 10^{-8} to 10^{-10} cells in a generation, such as the mutation leading to streptomycin, nalidixic acid and rifampin resistance (Guardabassi and Courvalin, 2006). Resistance to fluoroquinolones occurs through stepwise mutations (Guardabassi and Courvalin, 2006). Some multidrug resistant strains of bacteria have mutation rates that are 100-1000 times higher than those of similar bacteria. This could be associated with a more rapid development of AMR (Boerlin and White, 2006; Giraud et al., 2002; Guardabassi and Courvalin, 2006). A resistance mutation will then be transmitted vertically within the bacterial strain.

Resistance can be acquired through transformation, transduction or conjugation. Transformation, often responsible for penicillin resistance in *Streptococcus pneumoniae*, is acquisition and recombination of naked DNA picked up from the environment (Boerlin and White, 2006). Transduction is the transfer of DNA between bacteria by bacteriophages (Guardabassi and Courvalin, 2006). Transformation and transduction only occur between closely related bacteria; therefore, conjugation is likely more important in the spread of resistance genes (Boerlin and White, 2006; Guardabassi and Courvalin, 2006). Conjugation is the transfer of extra-chromosomal genetic material, such as plasmids, transposons and integrons, between donor and recipient bacteria (Boerlin and White, 2006; Guardabassi and Courvalin, 2006). Plasmids are nonessential self-replicating genetic elements, which are not part of the chromosome (Boerlin and White, 2006; Schwarz et al., 2006). Plasmids may also carry genes for resistance to disinfectants, heavy metals, virulence, or metabolic properties (Schwarz et al., 2006). Within a bacterial cell, plasmids may replicate themselves, integrate with other plasmids into a new larger plasmid, or integrate in part or in whole into the chromosome (Schwarz et al., 2006). Transposons are genetic elements that cannot replicate themselves; to replicate they must integrate either with the bacterial chromosome or a plasmid via insertion sequences (Boerlin and White, 2006; Schwarz et al., 2006). Composite and complex transposons carry multiple AMR genes (Boerlin and White, 2006; Schwarz et al., 2006). Some transposons move off the bacterial chromosome and undergo conjugation (Boerlin and White, 2006). Integrons, often part of composite transposons, are DNA elements carrying gene cassettes (Boerlin and White, 2006; Schwarz et al., 2006). Gene cassettes are very small and carry only a

recombination site and a gene, usually for AMR; they cannot replicate or transpose (Schwarz et al., 2006). Multiple gene cassettes are often found on integrons (Schwarz et al., 2006). Integrons have been found associated with extended-spectrum beta-lactamases (ESBL) plasmids (Fluit and Schmitz, 1999). Resistance genes can also move back and forth between plasmids and along the bacterial chromosome allowing rapid reassembly of units of multiple resistance genes (Boerlin and White, 2006; McDermott et al., 2003). Virulence factors, tolerance to heavy metals, quaternary ammonium compounds, and triclosan resistance may also be associated with multidrug resistant bacterial phenotypes (Bruins et al., 2001; Ishikawa et al., 2002; Sidhu et al., 2002; Woods, 2006; Yazdankhah et al., 2006).

2.1.4 Mechanisms of Resistance

The main mechanisms of resistance are enzymatic inactivation of the antimicrobial, target modification, active efflux and reduced antimicrobial uptake (Table 2.1). Enzymatic inactivation is clinically important for the beta-lactams, where the beta-lactam ring is hydrolyzed, and the aminoglycosides, where an acetyl group, phosphoryl group or nucleotide is transferred to the antimicrobial (Guardabassi and Courvalin, 2006). Antimicrobial target modification, due to horizontal acquisition of genetic material, causes glycopeptide resistant enterococci (GRE) and methicillin resistant *Staphylococcus aureus* (MRSA), both clinically important (Guardabassi and Courvalin, 2006). GRE acquire *vanA* or *vanB* genes and change the peptidoglycan structure in the cell wall through a complicated process, resulting in a cell wall with a low affinity for glycopeptides (Guardabassi and Courvalin, 2006). MRSA acquire the *mecA* gene that

creates a new cell wall transpeptidase (penicillin binding protein) with a low affinity for methicillin, which then competes with the competent transpeptidase (Guardabassi and Courvalin, 2006). Active efflux is a process by which cellular byproducts, antimicrobials and other foreign substances are pumped out of cells; bacteria can have specific-drug-resistance pumps (SDR) or multiple-drug-resistance (MDR) pumps (Guardabassi and Courvalin, 2006). SDR are the important mechanism resulting in high levels of resistance to tetracyclines, macrolides, lincosamides, streptogramins and phenicols (Guardabassi and Courvalin, 2006). There are different types of MDR with different substrate preferences (Guardabassi and Courvalin, 2006; Van Bambeke et al., 2000). There are also numerous superfamilies of efflux pumps, including MAR (Multi Antimicrobial Resistance) and SMR (Small Multidrug Resistance) (Van Bambeke et al., 2000). Resistance in gram-negative organisms also occurs when less antimicrobial is transported into the bacterium due to mutations causing changes in the porins of the cell membrane (Guardabassi and Courvalin, 2006). Target protection and drug trapping, less important mechanisms of resistance clinically, are also mentioned in Table 2.1.

More than one mechanism may occur in a microorganism that conveys resistance to one or more antimicrobials (Acar and Rostel, 2001; Guardabassi and Courvalin, 2006). *S. aureus* often carries resistance to tetracycline through two different mechanisms: *tetK*, active efflux, and *tetM* or *tetO*, target protection. This leads to additive resistance, where the overall increase in resistance is the sum of the increase due to each individual mechanism (Guardabassi and Courvalin, 2006). In comparison, synergistic resistance occurs when the overall resistance is much larger than the sum of the components; for

example, fluoroquinolone resistance due to multiple mutations of topoisomerase II and topoisomerase IV (Guardabassi and Courvalin, 2006).

Cross-resistance occurs when resistance to one antimicrobial is associated with resistance to one or more other antimicrobials from the same or differing antimicrobial class (Guardabassi and Courvalin, 2006). An example of cross-resistance is the mutation that causes methylation of an adenine in the 50S rRNA affecting the overlapping sites of action of macrolides, lincosamides, and streptogramins and conferring resistance to them (Guardabassi and Courvalin, 2006). Coresistance refers to multiple genes or mutations, each leading to resistance to a different antimicrobial, found in the same bacteria (Acar and Rostel, 2001; Guardabassi and Courvalin, 2006). An important example of coresistance occurred in the EU associated with the ban of avoparcin use as a growth promotant (Wegener, 2003). The prevalence of vancomycin resistant enterococci (VRE) was thought to be associated with avoparcin use but the prevalence of VRE in swine didn't substantially decrease subsequent to the avoparcin ban like it did in broilers (Wegener, 2003; WHO, 2003). Most of the swine-associated VRE were also macrolide resistant. Macrolide use was not initially banned in swine, but after it was, the prevalence of VRE in swine also decreased (Bager et al., 1999; Guardabassi and Courvalin, 2006; Wegener, 2003). This was explained by a plasmid carrying resistance to both macrolides and glycopeptides causing coselection of resistance (Aarestrup, 2000). Coresistance is the reason for persistent resistance to chloramphenicol, long after it was banned in many countries (Harada et al., 2006).

2.1.5 How *Escherichia coli* develop resistance

As this work concentrates on AMR in fecal non-type specific *E. coli* (NTSEC), a specific discussion on how AMR may develop in NTSEC follows. It is important to remember, that specific mechanisms and modes of dissemination within specific species of bacteria are evolving all the time. NTSEC have numerous mechanisms of AMR that can occur together which "shows the versatility of this pathogen and validates the study of the emergence and dissemination of antibiotic resistance in *E.coli"* (Webber and Piddock, 2005). NTSEC is a useful sentinel organism in animals, particularly cattle, as it is the most common contaminant at slaughter and ubiquitous in animals and the environment (Fluckey et al., 2007; Stopforth et al., 2006).

The primary mechanism of β-lactam resistance in *E. coli*, and other gram negative bacteria, is through β-lactamases (Babic et al., 2006; von Baum and Marre, 2005). There are different types of β-lactamases: broad spectrum, extended-spectrum, and AmpC β-lactamases, and carbapenemases (Paterson, 2006). Extended spectrum beta-lactamase (ESBL) producing enterobacteriaceae are of great public health concern, as they are associated with nosocomial and community acquired multidrug resistant infections (Paterson, 2006). In 2003, 5.8% of *E. coli,* isolated from ICU patients in the USA was nonsusceptible to 3rd generation cephalosporins, as were 31.1 % of *Enterobacter spp* (Paterson, 2006). Some ESBLs through mutations of the TEM and SHV genes inactivate many β -lactams including 3rd generation cephalosporins, and have caused significant nosocomial outbreaks in humans (Schwarz et al., 2006; von Baum and Marre, 2005). They have been widely disseminated on mobile genetic elements and are increasingly found in the community (Babic et al., 2006; Schwarz et

al., 2006). Plasmids that carry the genes for the ESBLs, conveying resistance to 3rd generation cephalosporins, often also carry genes for aminoglycoside, sulfonamide and sometimes fluoroquinolone resistance (Paterson, 2006; von Baum and Marre, 2005). AmpC β-lactamases convey resistance to 3rd and 4th generation cephalosporins, cephamycin, clavulanate and other β-lactamases (Paterson, 2006). A plasmid mediated AmpC β-lactamase, CMY-2, has been associated with resistance in *Salmonella* and is of significant concern (Paterson, 2006). The class 1 beta-lactamase mediated by the *ampC* genes through target modification and active efflux, are found on both chromosomes and plasmids (Schwarz et al., 2006; von Baum and Marre, 2005). Ceftiofur is the only 3rd generation cephalosporin licensed, in North America, for use in cattle; however, surveillance for ESBL and AmpC β-lactamases seems prudent.

The primary mechanisms mediating tetracycline resistance in *E. coli* are active efflux and target protection by ribosomal protection proteins, acquired through horizontal gene transfer (Schwarz et al., 2006; von Baum and Marre, 2005). Gram negative bacteria important in veterinary medicine have disseminated the genes, *tet*(A), *tet*(B), *tet*(C), *tet*(D), and *tet*(H), involved in the energy-dependent active efflux process (Schwarz et al., 2006). Multidrug transporters have been reported, that increase transport of tetracyclines as well as other compounds (Schwarz et al., 2006). The *tet*(M) gene is important in target protection (Schwarz et al., 2006).

The primary mechanisms mediating fluoroquinolone resistance in *E. coli* are multiple mutations of topoisomerase II (DNA gyrase) and topoisomerase IV and active efflux (von Baum and Marre, 2005). The combination of these two mechanisms, with first step mutations conferring low-level fluoroquinolone resistance, followed by

horizontal acquisition of a multidrug efflux pump, and then a second step mutation to fluoroquinolones altogether results in high-level fluoroquinolone and other AMR (Schwarz et al., 2006). The mutations alone don't result in high-level resistance but plasmid mediated fluoroquinolone resistance has been reported recently (Paterson, 2006; Schwarz et al., 2006). This horizontally acquired fluoroquinolone resistance is mediated through competitive binding with topoisomerase II (Paterson, 2006).

The primary mechanism mediating aminoglycoside resistance in *E. coli* is enzymatic modification of the antimicrobial. Numerous enzymes exist with varying substrates (Schwarz et al., 2005; von Baum and Marre, 2005). These genes are usually carried on mobile genetic elements (Schwarz et al., 2005).

The primary mechanism mediating resistance to lincosamides and streptogramins in *E. coli* is target modification (Schwarz et al., 2005). These classes of antimicrobials are grouped together to discuss resistance mechanisms because their binding sites on the 50S ribosomal subunit overlap (Schwarz et al., 2005). Target modification is largely through methylation, and expression of these genes is inducible (Schwarz et al., 2005). Macrolides have a similar action to lincosamides and streptogramins but gram-negative bacteria have been considered intrinsically resistant to macrolides because of their slow uptake and subsequent active efflux; this may actually be due to genetic resistance (McDermott et al., 2003; Schwarz et al., 2006).

The primary mechanism mediating resistance to trimethoprim and sulfonamides in *E. coli* is replacement of sensitive enzymes by resistant ones (Schwarz et al., 2005). These are also often recombined into mobile genetic elements (Schwarz et al., 2005).

The primary mechanism of chloramphenicol resistance in *E. coli* is enzymatic modification of the antimicrobial by acetylation which is mostly plasmid mediated (Schwarz et al., 2005). Florfenicol resistance is associated with membrane-associated exporter proteins that promote active efflux of both chloramphenicol and florfenicol (Schwarz et al., 2005).

Multidrug resistance in *E. coli* is also often conveyed by Class 1 integrons. They often carry genes for quaternary ammonium compounds and sulfonamides resistance as well as resistance to beta-lactams, streptomycin-spectinomycin and trimethoprim (von Baum and Marre, 2005). Some strains of *E. coli* have a high mutator frequency associated with a problem in the methyl-directed mismatch repair system. This can lead to increased emergence of resistant strains in some environments, depending on the fitness costs (von Baum and Marre, 2005). Bacteria that acquire AMR may be less able to compete in other ways. This is called a fitness cost (Cohen and Murray, 2004). In addition, bacteria with a deficient methyl-directed mismatch repair system have shown increased horizontal genetic transfer with other species (von Baum and Marre, 2005). Plasmid mediated multidrug resistance has been shown to transfer horizontally between coliforms in humans and cattle on a farm and in other situations (Kruse and Sorum, 1994; Oppegaard et al., 2001).

2.1.6 The epidemiology of antimicrobial resistance

More information is still needed to understand the extent to which the use of antimicrobials in animal agriculture is related to AMR in human infections (Bywater, 2005; Conly, 2002; Khachatourians, 1998; McGeer, 1998). Antimicrobial resistant

mechanisms likely developed naturally in antibiotic producing organisms, and were passed vertically between bacteria (Guardabassi and Courvalin, 2006; Schwarz et al., 2006). AMR has been found in bacteria, estimated to be thousands of years old, isolated from arctic glaciers (Dancer et al., 1997). Processes within bacteria developed to add these resistance genes to mobile genetic elements, plasmids, transposons and integrons, and allowed horizontal transfer (Schwarz et al., 2006). Selective pressure of low concentrations of antimicrobials and other factors greatly enhanced horizontal spread of AMR (Guardabassi and Courvalin, 2006; Schwarz et al., 2006). Under selection pressure, susceptible bacteria die and the resistant bacteria will flourish (Guardabassi and Courvalin, 2006). Heat stress and cold stress have been associated with higher shedding of resistant bacteria, apparently from the upper gastrointestinal tract (Moro et al., 1998; Moro et al., 2000). Resistant bacteria can reproduce rapidly creating a large clone of resistant bacteria with no competition. If a fitness advantage, co-resistance, or cross-resistance is associated with the resistant clone, it may continue to flourish after the selective pressure has been removed (Austin et al., 1999; Levin et al., 1997; Rozen et al., 2007; Singer et al., 2006). The degree of dissemination of the antimicrobial resistant genes in a farm environment may be as or more important than the selective pressure (Lejeune and Christie, 2004; Davis, 2002). Dissemination in the environment could be substantial, as AMR to two or more antimicrobials was found in various gram-negative bacteria isolated from river samples in the USA; resistance plasmids were found in 40% of these (Ash et al., 2002). Selective pressure may be exerted by antimicrobials or other substances such as heavy metals, quaternary ammonium compounds, and triclosan that might share a similar mechanism of resistance (Bruins et

al., 2001; Ishikawa et al., 2002; Yazdankhah et al., 2006). In some cases, the bacteria must be in prolonged contact with a sub-inhibitory concentration of an antimicrobial with a resistant bacterial population surviving (Acar and Rostel, 2001). In addition, some of the selective pressure from AMU on microorganisms associated with AMR is caused by AMU in human medicine, some of which is inappropriate (Fishman, 2006).

AMR has been recovered from cloacal *E. coli* from Black-headed Gulls in the Czech Republic and from Canada Geese in the USA, fecal *E. coli* from wild deer and marmots in Italy, and multiple bacterial species isolated from the external surfaces and intestinal tracts of cockroaches in Taiwan, from enterococci of houseflies, and in high prevalence in children from low-income communities (Bartoloni et al., 2006; Caprioli et al., 1991; Cole et al., 2005; Dolejska et al., 2007; Macovei et al., 2006; Pai et al., 2005). A study by De Graef et al. suggests that levels of AMR in fecal indicator organism differ between privately owned and kennel raised dogs (De Graef et al., 2004). Methods of dissemination of AMR bacteria and genes are not clear. Concerns have been stated that many species of wild animals that "undergo extreme migration patterns" could be involved in dissemination of AMR (O'Rourke, 2003). Relatively high prevalence of acquired resistance was found in NTSEC from people in a remote Bolivian community (Bartoloni et al., 2004). This resistance appeared associated with clonal expansion and horizontal recombination of genetic material, possibly associated with infrequent trips by members of the community to major population centres (Pallecchi et al., 2007). Fecal waste from livestock production is also typically spread on the land, and human waste also enters the environment, spreading AMR genes in the environment (Jindal et al., 2006; VDD, 2002). NTSEC was isolated from roughly 50% of cattle feed samples

on two feedlots in Colorado. Feed could be a vector of AMR genes if a resistant strain was present in the feed (Dargatz et al., 2005).

These observations serve to underline the extremely complex ecological issues involved in the epidemiology of AMR. All of these factors increase the AMR genes in bacterial pathogens, commensals, and the environment that can be picked up and modified as necessary. Ecology is "the study of how living systems interact with each other and their nonliving environment" (Summers, 2002). Many interrelated pathways intertwine that may be involved in the establishment, dissemination and general ecology of AMR as captured originally by Linton and updated by VDD (Figure 2.1) (Linton, 1977a; Linton, 1982; Linton, 1986). Host factors (appropriate use, hygiene, exposure) and factors involving the bacterial strain, the other microbial flora of the host and environment may be involved in strain-specific dynamic colonization and transmission to a human (Foxman, 2007; Larson, 2007).

Molecular techniques have added to our understanding the epidemiology of AMR. More studies are needed to better understand the dynamics of bacterial populations and genetic determinants in AMR (Boerlin, 2004). Genetic methods are useful for organisms when the phenotype may not reflect the genotype due to low-level or heterogeneous expression of genes or bacteria that are difficult to culture (Cockerill, 1999).

The volume of antimicrobials consumed is a surrogate measure for the amount and extent of antimicrobial distribution in the ecosystem including humans, animals and the environment (Acar and Rostel, 2001). PK /PD parameters are usually used to describe clinical outcomes in antimicrobial therapy (Rybak, 2006). It has been proposed that the

development of AMR in microorganisms treated with antimicrobials can be explained and predicted using pharmacokinetic/pharmacodynamic principles; this has been investigated with fluoroquinolones (Blondeau et al., 2004; Rybak, 2006). The mutant selection window (MSW) refers to the zone between the MIC and the mutant prevention concentration (MPC) where susceptible bacteria are killed and resistant bacteria flourish (Rybak, 2006). Time in MSW may also be important; if the dose was above the MPC throughout the dosing period then there was no change in the number of resistant bacteria recovered over time (Rybak, 2006). This is similar to maintaining a specific AUC/MIC ratio that suppresses or minimizes development of resistant bacteria (Rybak, 2006). A mathematical model of the population dynamics during AMU suggested that resistance is unlikely to emerge in a population of bacteria treated with antimicrobials if there are no resistant phenotypes present at the start of treatment and if the net decrease in bacteria during treatment is comparable to the rate of cell division; time to emergence can be predicted in various situations (Lipsitch and Levin, 1997).

2.2 A historical perspective on antimicrobial resistance

Concerns about the use of antimicrobials in animals, and recommendations for prudent use were first expressed many years ago (Swann, 1969) and echoed again later (Apley, 1998; Lipsitch et al., 2002; Smith et al., 2002, Singer et al., 2003); however, scientific evidence to answer many questions is still currently lacking (Conly, 2002). Many governmental and non-governmental committees have evaluated and made recommendations on the issues concerning AMR; some of the main ones will be reviewed here.

The earliest well-known report on concerns related to the use of antimicrobial in animals was "The Report to Parliament from the Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine" known as the Swann Report (Swann, 1969). This report concluded that "there were certainly instances in which antibiotics had been used in the past in ways which can now be regarded as unwise" for example the "present practice of giving antibiotics below therapeutic levels to 'stressed' animals (is) indefensible from a bacteriological standpoint" (Swann, 1969). The report went on to state that the committee members "are convinced that such practice will encourage the emergence of resistant bacterial populations and that both human and animal life may be exposed to unnecessary hazard as a result" (Swann, 1969). This report made a number of recommendations about the use and availability of drugs. Suggestions were also made for further epidemiologic studies on the important infectious diseases and management factors. A specific recommendation of note was to reclassify antimicrobials into prescription and non-prescription feed products, and to ban nutritional uses (NRC, 1999). The degree of implementation of these recommendations is not clear (NRC, 1999).

The Swann Report was followed by reports from the National Research Council (NRC) in 1980, the Council for Agricultural Science and Technology (CAST) in 1981, and the Institute of Medicine (IOM) in 1989. The 1980 report by NRC, "The effects on human health of subtherapeutic use of antimicrobials in animal feeds" suggested that subtherapeutic AMU and human health were still not clearly associated and more research was necessary (NRC, 1999). The 1981 CAST report called "Antibiotics in animal feeds" indicated that therapeutic use of AMU in people was associated with

AMR in those humans so the banning of subtherapeutic AMU in livestock was not was not going to solve the AMR problem (NRC, 1999). The 1989 IOM report "Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feeds" demonstrated increased prevalence of AMR isolates in livestock associated with subtherapeutic as well as therapeutic antimicrobial treatment on-farm, but no direct evidence that AMU in livestock poses a human health risk (NRC, 1999).

A number of well-known reports, involving issues about AMU in livestock and AMR in animals and humans were produced in parallel to developments in AMR in the EU. Throughout the 1980s and 1990s, the precautionary principle (Hurd et al., 2004) was exercised by various European countries to ban the use of feed antimicrobials in livestock based on heightened concerns about VRE in humans and livestock (van den Bogaard et al., 2000). Recognition of antimicrobial resistant phenotypes of specific veterinary-related microorganisms such as MRSA and multidrug resistant (MDR) *Salmonella* were also a concern (Boerlin and White, 2006).

The use of avoparcin, a glycopeptide, as a growth promoter in animals was first thought to be associated with the high level of community-acquired VRE in people in 1994/1995 (Bates et al., 1994; Klare et al., 1995; van den Bogaard et al., 2000; Wegener, 2003; WHO, 2003). The use of avoparcin as a growth promoter in animals had already been banned in Sweden since 1986 (Wierup, 2001). Denmark banned avoparcin in 1995. Further evidence of an association between avoparcin use and intestinal VRE carriage in humans prompted the entire European Union (EU) to ban avoparcin in 1997 (Bager et al., 1997; Wegener et al., 1999; Wegener, 2003; WHO, 2003). In 1997, WHO prepared the report "The medical impact of the use of

antimicrobials in food animals" which recommended terminating all AMU for growth promotion (NRC, 1999).

In 1998, Denmark banned the use of virginiamycin due to concerns about co-resistance with quinupristin/dalfopristin which are important in human medicine (Wegener, 2003; WHO, 2003). Danish farmers also voluntarily stopped using antimicrobial growth promoters in cattle, poultry and finisher pigs in 1998 (WHO, 2003). In 1998, the OIE reported to the OIE Regional Commission for Europe on existing efforts and capacities within the OIE to deal with AMR (Acar and Rostel, 2001). The OIE recommended harmonization of surveillance and laboratory methodologies and the use of risk analysis when establishing public health (Acar and Rostel, 2001). This report also led to the formation of the *ad hoc* group of experts on antimicrobial resistance in 1999 that has worked towards development of OIE standards for AMR (Acar and Rostel, 2001; Dehaumont, 2004).

In 1999, the EU banned tylosin, spiramycin, bacitracin and virginiamycin, all growth promotants from classes of antimicrobials important in human medicine; the EU banned olaquindox and carbadox due to human toxicity concerns; and Danish farmers also voluntarily stopped using growth promotants in all pigs (Anonymous, 1998; WHO, 2003). The NRC published "The use of drugs in food animals: Benefits and Risks", an analysis of the issues concerning AMU in food animals and disease in people (NRC, 1999). Recommendations from this work included multidisciplinary science-based decision and policy-making (NRC, 1999). The European Commission issued a report, "Opinion of the steering committee on antimicrobial resistance" (EC, 1999). Key recommendations from this report were emphasis of prudent use of antimicrobials,

infection prevention and AMR containment, assessment of the efficacy of interventions, and pursuing alternatives to AMU. Also in 1999, the report, "The use of antibiotics in food producing animals: antibiotic resistant bacteria in animals and humans" was issued by the Joint Expert Technical Advisory Committee on Antibiotic Resistance commissioned by the Government of Australia (JETACAR, 1999). This report highlighted 22 recommendations in five categories: regulation of prudent use, surveillance, infection prevention as an alternative to AMU, education, and research into AMU and its alternatives. This report provided the impetus for a national AMR/AMU strategy in Australia.

In 2001, the WHO, acting on the precautionary principle, made a blanket recommendation in the "WHO Global Strategy for Containment of Antimicrobial Resistance" that the use of growth promotant antimicrobials in animal feed should be discontinued (WHO, 2001a). Other recommendations were targeted at decreasing the emergence and dissemination of resistant organisms (WHO, 2001a). This led to a joint consultative process on non-human use of antimicrobial and antimicrobials during 2003 and 2004 (WHO, 2007a). During both 2005 and 2007, WHO convened Expert Meetings on Critically Important Antimicrobials for Human Medicine (WHO, 2007a) resulting in development of criteria for categorization of antimicrobials important in human medicine as well as a list of these antimicrobials, and recommendation on the use of antimicrobials not used in human medicine (WHO, 2007a). All antimicrobial growth promotants were subsequently banned in the EU in 2006 (EC, 2005).

In Sweden, there were no production effects associated with the ban of antimicrobial growth promoters in slaughter pigs, specialized beef and turkeys (Wierup, 2001).

Between 1986 and 1999, overall use of antimicrobials decreased by 55%, although initial increases in therapeutic AMU were seen in broiler chickens and weaned pigs (Wierup, 2001). These initial production losses were no longer evident as time went by and progressive producers adjusted to the new management systems; however, some less progressive pig farmers never recovered to the pre-ban production benchmarks (Wierup, 2001).

In Denmark between 1994 and 2001, the overall use and duration of use of antimicrobials decreased and there was a significant decrease in the prevalence of VRE in poultry almost immediately (van den Bogaard et al., 2000; Wegener et al., 1999; Wegener, 2003; WHO, 2003). A decrease in the prevalence of VRE in pigs was not seen until the macrolide tylosin was voluntarily banned in 1998 due to coselection of streptogramin and macrolide resistance through plasmid associated horizontally acquired resistance (Aarestrup, 2000; Aarestrup et al., 2001; Wegener, 2003). Between 1995 and 2001, therapeutic use in poultry was not affected by the growth promotant ban; therapeutic use of some antimicrobials (tetracyclines, penicillins, macrolides) in pigs increased and others (cephalosporins, fluoroquinolones) stayed the same (WHO, 2003). The number of antimicrobial treatments for diarrhea in newly weaned pigs and to a lesser extent finisher pigs was significantly increased after the ban in Denmark (WHO, 2003). In broiler chickens and finisher pigs, the negative production effect of the ban was small and would be partly offset by the decreased cost of antimicrobials (Wegener, 2003; WHO, 2003). There were some production losses in weaned pigs (Wegener, 2003).

Intestinal VRE prevalence in humans and in poultry meat in Europe decreased after the growth promoter bans (van den Bogaard et al., 2000; Wegener et al., 1999). It is interesting that VRE in people in the USA is a hospital-acquired problem not a community-acquired resistance like in the EU (Wegener et al., 1999). This is likely because avoparcin has never been used in animal production in North America but vancomycin has been used in human medicine (Wegener et al., 1999; VDD, 2002).

In Canada, action pertaining to AMR started in 1997 when Health Canada (HC) brought key stakeholders together for a conference called "Controlling Antimicrobial Resistance: An Integrated Action Plan for Canadians" (VDD, 2005a). This conference acknowledged issues and called for an integrated action plan for Canadians (VDD, 2005a). The Canadian Committee on Antibiotic Resistance (CCAR) subsequently took a lead role in Canada toward coordinating "a focused national approach to resistance issues" (CCAR, 2004). In 1999, HC and the Ontario Ministry of Agriculture, Food and Rural Affairs furthered discussions for a national strategy through the first conference on "Agriculture's role in Managing Antimicrobial Resistance" leading to the formation of the "Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health" (VDD, 2005a). In 2002, HC produced a report on "Animal Uses of Antimicrobial Agents and Impact on Resistance and Human Health" (VDD, 2002). This report had 38 recommendations for Canadians. The top six recommendations were

1) Antimicrobials used to treat and control disease should be available by prescription only.
2) Develop a policy for extra-label antimicrobial use.

3) All antimicrobials used in food animals must have a Drug Identification Number (DIN).
4) Stop the sale and use of antimicrobials not registered by HC.
5) Conduct risk assessments on antimicrobials used for growth promotion and phase out any that are of an unacceptable risk.
6) Conduct ongoing national surveillance for AMR associated with food animal production.

Consultations on the report led to HC initiatives on extra-label drug use, active pharmaceutical ingredients, a regulatory framework of unapproved drugs, and risk management of AMR associated with AMU in food animal production (VDD, 2005a).

2.3 Transfer of antimicrobial resistance from animals to humans

As previously mentioned, the ecology of AMU and AMR is extremely complex, as is the association between AMU in livestock and possible impacts on human health (Linton, 1977a; VDD, 2002). When livestock are treated with antimicrobials, AMU in livestock puts selection pressure on bacterial populations in the host that under certain circumstances may allow resistant bacterial populations to proliferate; this may increase the proportion of resistance bacteria available to colonize a new host or increase the proportion of AMR genes available for transfer to other bacteria (Aly et al., 1970; Linton, 1977a). There are two main pathways by which there could be a negative impact on human health.

In one pathway, zoonotic pathogens in livestock may develop cross-resistance or co-resistance to antimicrobials used in people after the livestock are treated with

antimicrobials (Angulo et al., 2004; Schwarz et al., 2006). The zoonotic pathogen could then infect people, primarily through the food supply but occasionally through direct contact of at risk groups, and cause disease that is difficult to treat due to AMR (Angulo et al., 2004; VDD, 2002). The other pathway is through commensal organisms, such as NTSEC and enterococci, carrying and spreading resistance determinants through the food, or possibly via the environment, to humans (Angulo et al., 2004; Hurd et al., 2004). Commensals can transiently colonize humans and disseminate resistance determinants, through horizontal transfer, to the normal flora of the host that may persist if there is no fitness cost of resistance to the pathogen (Cohen and Murray, 2004; Foxman, 2007; Sorensen et al., 2001). This was presumably the case with VRE in animals and humans in Europe. Resistance determinants are then disseminated into the environment in a broader fashion and can be transferred to pathogenic bacteria again causing disease that is difficult to treat (VDD, 2002). Other intermediate steps may also occur, such as improper meat handling and cross contamination (Hurd et al., 2004).

The problems in human health might include treatment failure, possibly even in an unrelated drug class, additional costs associated with the use of secondary and tertiary antimicrobials, as well as increasing AMR amongst pathogens causing more human infections that are difficult to treat (Hurd et al., 2004). The prevalence of AMR in commensal organism may be considered "an indicator of the selective pressure" of AMU and is the resistance potential for pathogens (Angulo et al., 2004).

There are many individual scientific reports that fit the description of the first pathway, often involving *Salmonella* spp. (Holmberg et al., 1984a; Holmberg et al., 1984b; Olsen et al., 2004; Ryan et al., 1987; Spika et al., 1987). One of these was an

enormous outbreak, involving over 16,000 confirmed cases associated with pasteurized milk contaminated with MDR *S.* Typhimurium (Ryan et al., 1987). The burden of disease in humans from salmonellosis, which is mostly food-borne, is difficult to quantify as people report as few as 10% of food-borne cases of illness (McDermott, 2006). In Canada (2005) and the USA (2004), approximately 20% of human *S.* Typhimurium isolates were resistant to five or more antimicrobials (CDC, 2007). *Salmonella* spp.*,* in general, acquires AMR through chromosomal mutation or horizontal transmission of genetic material and therefore multi-drug resistance is a significant problem (McDermott, 2006). One of the most prevalent MDR strains, *Salmonella enterica* serovar Typhimurium definitive phage type (DT)104, was first recognized in England in 1984 in a human isolate; it was subsequently widely disseminated worldwide (Angulo et al., 2004; Glynn et al., 1998: McDermott, 2006).

The main DT104 reservoirs appear to be cattle and poultry (McDermott, 2006). MDR DT104 carries resistance to five antimicrobials: ampicillin, chloramphenicol (including florfenicol), streptomycin, sulfonamides, and tetracyclines; this phenotype is known as ACSSuT (Angulo et al., 2004; McDermott, 2006). The genetic material encoding this resistance phenotype is usually clustered on chromosome in association with two integrons and two gene cassettes (McDermott, 2006). These resistant genes are also found on plasmids along with genetic material conveying resistance to other antimicrobials (McDermott, 2006).

MDR *Salmonella* are difficult to treat. Fluoroquinolones (ciprofloxacin) and 3rd generation cephalosporins (ceftriaxone) are clinically important for treatment of MDR infections in humans; therefore, surveillance of changes in resistance to these

antimicrobials is of high clinically relevance (CDC, 2007; GOC, 2007). Nalidixic acid resistance precedes fluoroquinolone resistance and is associated with decreased susceptibility to ciprofloxacin; ceftiofur resistance is associated with decreased ceftriaxone susceptibility (CDC, 2007).

In the USA in 2004, nalidixic acid and ceftiofur resistance in non-Typhi *Salmonella* increased to 2.6% and 3.4% respectively (CDC, 2007). In Canada, nalidixic acid and ceftiofur resistance in *S*. Typhimurium increased to 2.9% and 4.3% respectively (GOC, 2007). Ceftiofur resistance is often associated with the ESBL phenotype (CDC, 2007).

The opportunity for transmission of non-disease causing bacteria between animals and humans exists. A study from the Netherlands found differences in patterns antimicrobial susceptibility of fecal NTSEC isolates from veterinary practitioners specializing in different species (Bongers et al., 1995). A study from France demonstrated a significant association between pig farming and the presence of MRSA (Aubry–Damon et al., 2004). Antimicrobial resistant bacteria have also been recovered from retail meats (Schroeder et al., 2003; Schroeder et al., 2004; White et al., 2001a). MRSA were first identified in the 1950s, shortly after the introduction of methicillin; MRSA were originally a nosocomial problem with community acquired strains becoming problems in the 1980s (Aarestrup and Schwarz, 2006; Weese et al., 2005b). Only over the last ten years, different MRSA clones have been identified in animals with probable human to animal contact; animals could act as a reservoir for human disease (Aarestrup and Schwarz, 2006; Morris et al., 2006; Weese et al., 2005a; Weese et al., 2005b; Weese et al., 2006). There are also reports of a swine associated MRSA strain that is a risk for human populations and swine health (de Neeling et al., 2007;

Khanna et al., 2008; van Loo et al., 2007). In Ontario, 45% of 20 pig farms tested and 25% of pigs were colonized with this MRSA strain. Over half of these farms had an associated person colonized with MRSA, while none of the negative farms did (Khanna et al., 2008). Some researchers have suggested that there may be an animal reservoir of multidrug resistant *E. coli* that cause urinary tract infections in women (Magnes et al., 2007; Ramchandani et al., 2005). Mathematical models have been created that suggest agricultural AMU can have a greater effect on human health than hospital AMU, but this depends on which assumptions are used (Smith et al., 2005). The strongest evidence showing the potential of AMU in livestock to cause human health issues is the situation with VRE in Europe, described elsewhere in this review. This occurred via the second pathway described above. The OIE Ad hoc group of experts in antimicrobial resistance has prepared risk assessment methodology to assess the effect of AMU in livestock on public health for use in the future (Vose et al., 2001).

2.4 Antimicrobial use

2.4.1 Antimicrobial use in humans

In people, the majority of antimicrobials are used for individual treatment of disease, although drugs are given for disease prophylaxis occasionally (Guardabassi and Courvalin, 2006). AMR in humans causes serious complications in disease treatment and increases health care costs (Conly, 2002). Health care costs, in Canada attributable to AMR, were estimated at $200 million in 2002 (Conly, 2002). However, the economic impact of AMR on all the stakeholders in not yet well understood (McGowan, 2001). Antimicrobials considered to be critically important in human medicine include some of

the aminoglycosides (e.g. amikacin, gentamicin, streptomycin), 3rd and 4th generation cephalosporins, glycopeptides, glycylcyclines, macrolides, penicillins (natural, aminopenicillins and antipseudomonal), quinolones, and streptogramins (WHO, 2007a). Antimicrobials considered to be of high importance in human medicine include some of the aminoglycosides (e.g. neomycin, spectinomycin), chloramphenicol, 1st and 2nd generation cephalosporins, antistaphylococcal penicillins (e.g. cloxacillin, dicloxacillin, nafcillin), polymyxins, sulfonamides, and tetracyclines (WHO, 2007a). Appropriate antimicrobial stewardship is considered a crucial part of prevention and control of AMR in human hospitals, along with hand hygiene, education, shorter hospital stays, and staffing issues (Fishman, 2006; Henderson 2006). A program focused on good antimicrobial stewardship at an American hospital was credited with improving the appropriateness of AMU, and was associated with higher cure rates, lower failure rates, and decreased health-care costs in that hospital (Fishman, 2006). Inappropriate AMU in the community is common and is being addressed in human medicine (Larson, 2007). Canada had one of the highest prescription rates for human oral antimicrobial prescriptions compared to other industrialized countries (Conly, 2002). Numbers of prescriptions have declined since 1997 when the Canadian action plan for controlling antimicrobial resistance was introduced (Conly, 2002). A study comparing AMU in British Columbia (BC) to that in Europe suggested that AMU in BC was higher than that in Northern European countries and the BC consumption of newer antimicrobials like fluoroquinolones, newer macrolides (e.g. clarithromycin, azithromycin) and cephalosporins was also higher than Denmark (Patrick et al., 2004). This was measured using the WHO standardized measurement called defined daily dose (DDD), a specific

term that "corresponds to the assumed average maintenance dose per day for its main indication of the drug in adults" (Monnet et al., 2004). This consumption rate is a potential concern in Canada as these antimicrobials are important in human medicine.

2.4.2 Antimicrobial use in animals

AMU varies greatly between different species of animals and at different production stages or classes of the same species; other factors include region, environmental conditions and management style (Burkgren and Vogel, 2006). In addition, AMU and associated regulations in animal agriculture differ greatly between countries (Guardabassi and Courvalin, 2006). For example, avoparcin, used extensively in Europe, was never used in North America (van den Bogaard, 2000).

In general, antimicrobials are used in livestock for three reasons: treatment of disease, prevention of disease, and growth promotion (Phillips et al., 2004; VDD, 2002). Treatment can be of the individual animals or of the group of animals. It is not always clear how to classify AMU in to one of these three categories; antimicrobials in the feed might appear to have a growth promoter effect through prevention or treatment of subclinical disease (VDD, 2002). Ionophores are commonly included in animal rations to prevent coccidiosis but they are known to have growth promotant effects as well. This is an important consideration if restrictions on the use of growth promotants are considered. Therapeutic uses of tylosin and the ionophores were not banned in the EU (Shyrock et al., 2006). The use of subtherapeutic antimicrobials is controversial as this could select for resistance. However, avoparcin, used as a feed antimicrobial in Europe for what was commonly considered a subtherapeutic use, inhibited the

susceptible bacteria and altered gut flora in treated pigs (Wegener et al., 1999; Jensen, 1998).

The OIE has created guidelines for monitoring of AMU in livestock, methodology to supply data for risk assessment and surveillance (Nicholls et al., 2001). However, it is difficult to get accurate estimates of AMU in many countries (Aarestrup, 2005; VDD, 2002). For instance, in Canada, there is both federal and provincial regulation over the sale of veterinary drugs; but there is also a complex distribution web which includes veterinarians, lay outlets, emergency drug releases, own-use imports, and active pharmaceutical ingredients (APIs) (VDD, 2002). A recent summary of AMU data in British Columbia Canada "highlights how information deficits on veterinary drug use complicate the development of an evidence-based policy framework for combating antimicrobial resistance" (Fraser et al., 2004).

A broad estimate of total antimicrobials used in 1998 in the USA were 17.8 million pounds; 83% of this was for prevention and treatment of disease, not growth promotion (VDD, 2002). Estimates of AMU in Canada in 2001-2003 were broken down by the class of antimicrobial, showing an overall decline of 29% over the three years attributed to the economic downturn associated with BSE and the implementation of quality assurance programs in some species (CAHI, 2005). In contrast to the North American situation, good usage estimates are available from European countries (Aarestrup, 2005). There is some specific AMU information available from research studies. In 1991, antimicrobials were used commonly in the Ontario swine industry, 86% of pig producers used antimicrobials in the starter ration, while 29% fed antimicrobials to finisher pigs (Dunlop et al., 1998a). Of the operations that used antimicrobials in the

finisher ration, only 20% used them for growth promotion, the rest of the farms used the antimicrobials for disease treatment, prevention or control (Dunlop et al., 1998a). Overall, 95% of pigs were treated with antimicrobials between weaning and slaughter, and 50% of pigs were treated during the finishing period (Dunlop et al., 1998a). Antimicrobial use, measure by a garbage can audit, on 34 swine farms in Ontario suggested that treatment records by producers underestimated actual AMU by 34% (Dunlop et al., 1998b). In 2000, a survey suggested slightly higher antimicrobial use in Alberta, with 91% of Alberta pig farmers using antimicrobials in the grower ration and 80% during the feeder period (Rajic et al., 2006). Administration of antimicrobials through the water was uncommon, used on 21% of grower operations and 18% of finisher operations (Rajic et al., 2006). Injectable antimicrobials were given to pigs on 71% of grower operations and 56% of finisher operations (Rajic et al., 2006). Similar AMU data from the National Animal Health Monitoring Survey in the USA suggests that 89% of swine farms gave antimicrobials in the feed, 31% of swine farms gave antimicrobials in the water, and 65% gave antimicrobials by injection to at least some of the pigs in that group for any reason (USDA, 2002). Work from a previous study had suggested that large and intermediate sized operations were more likely to use feed antimicrobials than small operations (less than 50 sows), as were producers under regular veterinary care (Dewey et al., 1997).

AMU data is not only difficult to collect, but there are also challenges in interpretation (Singer et al., 2006). There are issues concerning clustering of the data at various levels: farm, veterinary practice, or watershed (Singer et al., 2006). AMU data is often aggregated at a very high level, which could introduce ecologic bias into

analyses (Singer et al., 2006). Different antimicrobials are formulated differently and used at different doses. The AHI and CAHI estimates above were by mass; breaking these estimates down by antimicrobial class, like CAHI, helps remove some of this confusion. A standardization technique called animal defined doses (ADD_{Animal}) is based on that of Defined Daily Dose (DDD) used in the human literature (Austin et al., 1997; Austin et al., 1999; Baquero, 1996; Grave et al., 2006). In humans, DDD is a specific term that "corresponds to the assumed average maintenance dose per day for its main indication of the drug in adults" (Monnet et al., 2004). The measurement, ADD_{Animal}, can be used to describe the average dose, for its main indication, for a defined animal of a specific age-group or type of animal, e.g. $ADD_{Feedlot}$ or $ADD_{Broiler}$, (Jensen et al., 2004).

In general, the use of a global variable such as $ADD_{Feedlot}$ also has some drawbacks. Total weight of active ingredient used might be preferable for some analyses. No international ADDs have been established for animal drugs, and approved medications and approved doses of medications may vary between countries, so care must be taken in interpretation of these numbers (Grave et al., 2006). There are concerns about how well DDDs correspond, at the national level, to the number of antimicrobial prescriptions and the number of patients treated with antimicrobial agents (Grave et al., 2006). ADD could be described differently by different jurisdictions, reflecting differing uses of antimicrobials and differing production systems; this makes the term relevant for each country. ADD could also have several different interpretations so international definition and copyright of terms is desirable, as occurred with DDD (WHO, 2007b). "DDD is an international unit of measurement linked to the ATC

classification system for human medicines, with international values for the ATC codes assigned by the WHO International Working Group for Drug Statistics Methodology" (WHO, 2007b). The WHO Collaborating Centre for Drug Statistics Methodology is the international source of information on this topic. The ATCvet Working Group of WHO is developing methods to look at animal AMU data and more international dialogue is required in this process (WHO, 2007b). VETSTAT has done a lot of work in this area (Jensen et al., 2004). When this process is complete, WHO will call this international unit DDD_{Animal} (WHO, 2007b).

Quality Assurance (QA) programs have forged the path to prudent use in animal production. The World Veterinary Association developed policy on the prudent use of antimicrobials in 1999 (WVA, 1999). These 10 principles describe antimicrobials as "health management tools" and include statements on QA programs, principles for use, indicate the antimicrobials should be used under veterinary supervision, recommend surveillance of AMR, and use of alternatives to antimicrobials (WVA, 1999). In Canada, the aquaculture, chicken, dairy, egg, pork, turkey and veal all established QA programs, led by industry; these are know as On-Farm Food Safety (OFFS) programs (CAHI, 2007). One of the principles of most, if not all, QA programs is that AMU should follow approved label instructions and extra-label use (different dose or indication than approved) should only occur if other options are limited (WVA, 1999). There are species of livestock that have few or no approved antimicrobials with which to treat them when they are ill. This includes some common livestock species, like sheep and goats, as well as less common species like bees and zoo animals (VDD,

2005b). New regulations for Minor Uses / Minor Species (MUMS) are being looked at Canada and the USA (USDA, 2007; VDD, 2005b).

In spite of these problems, the availability of various forms of AMU data for future risk assessments would enhance "validity, credibility, and usefulness" of these models (McEwen and Singer, 2006b). It has been suggested that the field of pharmaco-epidemiology, "the application of epidemiological reasoning, methods and knowledge to the study of the uses and effects (beneficial and adverse) of drugs within a large consumer population", needs to be developed in veterinary medicine, along with pharmaco-economics, to better understand antimicrobial consumption and relationships with AMR (Chauvin et al., 2002).

2.4.3 Antimicrobial use in feedlot cattle

The focus of this thesis is AMR in feedlot cattle so a more detailed discussion of AMU specifically in feedlot cattle follows. AMU in the feedlot is of higher concern than AMU at other stages in the production cycle of cattle because of the proximity with respect to slaughter and perceived high use of antimicrobials during intensive animal production (Khachatourians, 1998). In Canada, the beef industry and the veterinarians that serve them are aware of these AMR issues and have developed prudent use guidelines for antimicrobials (CCA, 2006; CCA and BIC, 2006; CVMA, 1998).

In western Canada, antimicrobials are typically used in fed cattle production for a number of purposes including metaphylaxis, prophylaxis, and individual treatment of sick animals (Radostits, 2001; Booker et al., 1999). Metaphylaxis is defined by

Radostits as “the mass medication of individual animals with an antimicrobial at a strategic time in order to prevent the onset of clinical disease” (Radostits, 2001). Metaphylaxis is commonly used in western Canada in groups of cattle that are at high risk of developing the syndrome of acute undifferentiated bovine respiratory disease (BRD) shortly after arrival at the feedlot (Smith et al., 2001). BRD, in western Canada, has an early clinical presentation of severe depression and fever with no clinical signs attributable to a body system other than the respiratory tract; as the disease progresses, respiratory signs are recognizable (Radostits et al., 2007). Before prevention and control strategies, BRD was one of the most significant feedlot diseases with morbidity of 15-45% and mortality of 1-5% (Kelly and Janzen, 1986). Treatment early in the course of the disease is more effective but early recognition of sick animals is difficult, so metaphylaxis protocols were developed (Radostits et al., 2007). The most common pathogenic component cause is *Mannheimia hemolytica* biotype A serotype1 (Radostits et al., 2007). The pathogens that alone or jointly combine with other risk factors cause disease include *M. hemolytica*, *Pasteurella multocida*, *Histophilus somni, Mycoplasma bovis*, bovine herpesvirus 1, bovine viral diarrhea virus, bovine respiratory syncytial virus and parainfluenza 3 virus (Radostits et al., 2007; Smith et al., 2001). The other risk factors that increase stress and lower immunity are well described in the literature and include younger age or lighter weight calves, movement through auction markets (commingling), transportation, insufficient immunity to pathogens, environmental stressors such as temperature fluctuations, fall of the year where there is a huge number of high risk calves arriving at feedlots greatly increasing the workload, transportation, and lack of familiarity with feed bunks and waterers (Kelly and Janzen, 1986; Radostits

et al., 2007; Ribble et al., 1998; Smith, 2001). Injectable metaphylaxis in high risk groups of feedlot calves has economic and animal health benefits (Galyean et al., 1995; Harland et al., 1991; Morck et al., 1993; Schumann et al., 1990; Van Donkersgoed, 1992; Wittum et al., 1996). In western Canada, 20-50% of feedlot placements are treated with injectable metaphylaxis on arrival; the decision to treat a group of calves with individual metaphylactic injections of antimicrobials is based on the risk profile of that group of calves (Calvin Booker, Personal Communication, Feedlot Health Management Services, 2007; Radostits, 2001). The NAHMS 1999 Feedlot report described injectable metaphylaxis use on 10% of cattle placed and in 27% of small feedlot operations and 81% large feedlot operations in the United States of America (USDA, 2000). This adds credence to the position that AMU in feedlots is targeted to high risk groups. The USA feeds proportionately less high risk calves than Canada hence the lower proportion of animals treated with metaphylaxis.

Feed antimicrobials are used early in the feeding period to treat and prevent BRD and histophilosis (Radostits et al., 2007). Feed antimicrobials for prophylaxis in high risk groups of animals reduced BRD morbidity and improved average daily gain and feed conversion (Gallo and Berg, 1995). Feed antimicrobials are also commonly used throughout the feeding period for treatment of liver abscesses. Feed or water antimicrobials were used by 85% of small operations and 78% of large operations in the USA in 1998 (USDA, 2000).

Liver abscesses are common in feedlot cattle (VanDonkergoed et al., 2001). Fermentation of carbohydrates, found in the typical high grain diet fed in western Canada, produces lactic acid that decreases ruminal pH causing rumenitis.

Fusobacterium necrophorum colonizes necrotic lesions on the ruminal surface and emboli from these lesions seed liver abscesses via the hepatic portal venous system (Aiello and Mays, 1998). The control of liver abscesses is therefore an important economic concern in feedlot cattle. Beef quality audits in Canada have evaluated economic losses due to various quality defects including liver condemnation, downgrades at slaughter, and other quality parameters which in 1995 cost the industry approximately $5.31 CDN per head (Van Donkersgoed et al., 1997). The presence of severe liver abscesses cost the industry $50-$60 CDN per head not including losses of production and feed efficiency (Van Donkersgoed et al., 1997). Extensive education programs for producers were instigated to decrease losses to the beef industry. In 1998/1999, losses in the Canadian industry due to liver quality defects were estimated at $ 2.66 per head (Van Donkersgoed et al., 2001). Antimicrobials in the feed are effective at reducing the prevalence of liver abscesses at slaughter (Brown et al., 1975; Brown et al., 1973; Vogel and Saudert, 1994).

2.4.4 Alternatives to antimicrobial use

Management options should be utilized to eliminate mass use of antimicrobials whenever possible. The concept of herd health or herd medicine has been touted as an important management tool (Radostits, 2001). Herd health is an overall planned animal health and production management program developed for an individual operation by the producer and herd veterinarian (Radostits, 2001). Biosecurity is part of herd health and is considered to be a major factor in maintaining optimal health of a pig herd (Baker, 2006). It is important that producers and veterinarians are motivated to make

appropriate changes to maintain health status if the use of growth promotants or in-feed antimicrobials is banned. In Europe, swine and poultry operations did initially experience some negative consequences due to the ban but less progressive swine operations never recovered after the changes (Wegener, 2003; Wierup, 2001).

Vaccines have been used to decrease disease and therefore decrease antimicrobial use in both human and veterinary medicine. High rates of penicillin–resistant *S. pneumoniae* in humans in eight states, causing invasive pneumococcal infections, significantly decreased by 87% between 1999 and 2004 after introduction of a pneumococcal conjugate vaccine for children (Kyaw et al., 2006). Decreases were most substantial in young children and older adults (Kyaw et al., 2006). The overall burden of disease is decreased in the population including significant decreases in transmission to siblings and adults (Kyaw et al., 2006; Whitney and Klugman, 2004). In addition, using surveillance data from Atlanta, the incidence of macrolide-resistant pneumococcal infection was shown to decrease significantly after introduction of the vaccine (Stephens et al., 2005). Conclusions were that "vaccines can be a powerful strategy for reducing antibiotic resistance in a community" (Stephens et al., 2005).

Various products have been used in different species and management systems instead of antimicrobials. There are thousands of medicinal plants that possess antibacterial activity, including cranberry, tea tree oil and green tea (Mahady, 2005). St. John's wort shows activity against MRSA (Mahady, 2005). *Peltophorum africanum* (Fabaceae) showed substantial antibacterial activity in experimental studies (Bizimenyera et al., 2005). Medicinal plants have in some cases been used for hundreds if not thousands of years; however, as medicinal plants are tested and standardized,

surveillance for antimicrobial resistance will also be important. Solutions of potassium hydroxide combined with lauric acid have been shown to significantly reduce microbial contamination on poultry skin during poultry processing (Hinton and Ingram, 2006). Maggot secretions appear to have activity against Gram-positive bacteria (Thomas et al., 1999). Other possible alternatives to AMU include bacteriophages, cell wall hydrolases, and antimicrobial peptides (Parisien et al., 2008). A strain of *Lactobacillus rhamnosus* has shown potential for probiotic use in neonatal calves to prevention and control of neonatal calf scours (Ewaschuk et al., 2004). Ruminal bacteria produce bacteriocins, which are small peptides that inhibit Gram-positive bacteria (Russell and Mantovani, 2002). One of these bacteriocins, nisin, has a similar effect on rumen ecology as monensin (Russell and Mantovani, 2002). Nisin and nisin-like bacteriocins may be able to replace ionophores, but this effect needs to be further explored (Russell and Mantovani, 2002).

There are also several products that decrease the emergence of resistant bacteria subsequent to antimicrobial therapy. Consumption of cheese containing probiotics during treatment with amoxicillin-clavulanic acid was shown to decrease emergence of amoxicillin-resistant enterococci (Bertrand et al., 2006). Inhibition of mutation during antimicrobial treatment might also decrease the emergence of AMR (Cirz et al., 2005). Bacteria may induce proteins that cause mutation when they are under stress (Cirz et al., 2005). *E. coli*, after damage from ciprofloxacin use, were unable to induce resistance mutations after interference with the activity of protease LexA (Cirz et al., 2005).

2.5 Other research concerning antimicrobial resistance and antimicrobial use

2.5.1 Other species

AMU in the feed or water has been associated with AMR in various species (Dunlop et al., 1998c; Finlayson et al., 1973; Funk et al., 2006; Howe et al., 1976). In pigs, antimicrobial medication of feed was associated with increased risk of AMR to seven of eight antimicrobials tested (Dunlop et al., 1998c). There was no association of increased risk of gentamicin resistance with the use of in-feed antimicrobials; gentamicin was the only antimicrobial tested for resistance in that study which was not used for medicating the ration of any class of pigs (Dunlop et al., 1998c). The only individual pig treatments associated with increased AMR were gentamicin treatment associated with increased gentamicin resistance (Dunlop et al., 1998c). It was suggested this effect was due to AMR associated with the feed medication overshadowing any association with individual animal treatment (Dunlop et al., 1998c). There was a marked reduction in sensitive coliforms excreted when swine were fed chlortetracycline in the feed (Finlayson et al., 1973). Subtherapeutic chlortetracycline was associated with resistance to two or three antimicrobials in swine (Funk et al., 2006). Funk et al. also noted that the variance distribution between farm, pen and pig levels depended on the AMR phenotype examined, but farm-level variance was uniformly small (Funk et al., 2006). Short-term (24 hours) exposure to tetracycline in the water was associated with a significant increase in NTSEC resistant to tetracycline (Howe et al., 1976). Trials by Langlois et al. found persistence of high levels of AMR in NTSEC up to 126 months after antimicrobials had been withdrawn from the feed (Langlois et al., 1978; Langlois et al., 1988).

Other work has examined AMR in conventional and antimicrobial-free pig production where the odds of resistance were much higher on conventional than other farms for all antimicrobials except tetracycline, although specific MDR strains occurred on antimicrobial free farms (Gebreyes et al., 2006). A cross-sectional study within an integrated multi-site farrow-to-plate operation found little evidence of association between fecal NTSEC AMR phenotypes in swine and those of swine workers or abattoir workers (Scott et al., 2005). A large Danish disease investigation involved two separate outbreaks of colibacillosis in five flocks; an enrofloxacin-resistant *E. coli* strain which was shown to be transmitted vertically from a healthy parent flock into hatcheries (Petersen et al., 2006).

2.5.2 Cattle

Few studies have fully explored associations between AMU and AMR in feedlot cattle. Direct comparisons of results between different species or different sectors of cattle production are difficult as AMU and management norms are extremely different (Radostits, 2001). A recent American feedyard study analyzed MIC profiles from NTSEC from individual and pooled fecal samples from the rectum as well as individual and pooled fecal samples from the pen floor; they found that 66% of NTSEC isolates from individual samples, 55% of isolates from pools of five samples, and 70% of isolates in pools of ten samples from pen floors were sensitive to all antimicrobials tested (Wagner et al., 2002). Tetracycline resistance was reported in 28.9-42.5% of isolates, 7.8-12.8% of isolates were classified as streptomycin-resistant, and 20.0-31.3% of isolates were classified as sulphamethoxazole-resistant from either individual or

pooled pen floor samples; 10.0-12.9% of all isolates were classified as resistant to three or more antimicrobials and 0.0-2.3% of isolates were classified as resistant to six or more antimicrobials from either individual or pooled pen floor samples (Wagner et al., 2002). The Wagner study found a maximum of 15 different AMR phenotypes in the pooled samples and a total of 40 different AMR phenotypes across all sampling methods; they found more phenotypes in individual samples from both the rectum and the pen floor than in pooled samples (Wagner et al., 2002). In the Wagner study, all isolates were susceptible to amikacin, ceftriaxone, and gentamicin, with only one isolate resistant to ciprofloxacin and between 0.0-1.7% of isolates resistant to nalidixic acid from either individual or pen-floor samples (Wagner et al., 2002). In an American study of pasture fecal samples collected from newly weaned calves, 13 to 17% of fecal samples contained one or more tetracycline-resistant NTSEC isolates (Huston et al., 2003).

Several surveys of NTSEC isolated from beef carcasses in Canadian slaughter plants have been undertaken. In one study, out of 2653 isolates, 68% of isolates were sensitive to the 18 antimicrobials tested, which included the 16 used in the current trial (Van Donkersgoed et al., 2003). Resistance was found to tetracycline in 25% of isolates, to sulfamethoxazole in 9% of isolates, and to streptomycin in 7 % of isolates. Five percent of isolates were classified as resistant to three or more antimicrobials. The second abattoir study was much smaller but found 56% of 284 total isolates, collected at different points during carcass processing, sensitive to all antimicrobials tested and 44% resistant to one or more antimicrobials (Aslam and Service, 2006).

In cattle, associations between AMR and feed antimicrobials have been described (Berge et al., 2006; Inglis et al., 2005; Inglis et al., 2006; Stabler et al., 1982). The field trial by Berge et al. involved calves less than four weeks of age in a calf rearing facility, where AMU in the milk replacer selected for highly resistant NTSEC (Berge et al., 2006). The clinical trial by Inglis et al., in a research feedlot, suggested that subtherapeutic administration of tetracycline alone or in combination with sulfamethazine selected for AMR in *Campylobacter* species (Inglis et al., 2005). This study also isolated a higher proportion of tetracycline-resistant *Campylobacter* spp. from animals on a backgrounding (high-forage) diet as compared to a finishing (high-grain) diet (Inglis et al., 2005). A second trial found that tetracycline and erythromycin resistance of NTSEC increased over time in feedlot cattle feed chlortetracycline, doxycycline, and oxytetracycline in the feed and treated metaphylactically on arrival at the feedlot with oxytetracycline (Inglis et al., 2006). A study of growth performance and bacterial shedding in feedlot cattle treated with growth promotants found only 5.9% of fecal NTSEC isolates overall with AMR (Lefebvre et al., 2006). The clinical trial by Stabler et al. was carried out many years ago in a research feedlot; they found that tetracycline-resistant NTSEC became more prevalent in heifers fed therapeutic and subtherapeutic doses of chlortetracycline (Stabler et al., 1982).

In other studies, there has been no or a much less pronounced association between injectable antimicrobials and AMR, in the absence of feed antimicrobials (Berge et al., 2006; Berge et al., 2005a; Berge et al., 2005b; Lowrance et al., 2007; Stabler et al., 1982). In the field trial by Berge et al., individual antimicrobial therapy was associated with a transient increase in isolation of increasingly multiple resistant fecal NTSEC in

calves in a calf grower facility (Berge et al., 2006). In another trial by Berge et al., AMR patterns in NTSEC of feedlot steers were assessed after a single approved dose of florfenicol (a long-acting antimicrobial); a transitory change with isolation of increasingly multiple resistant fecal NTSEC was seen (Berge et al., 2005a). This trial also had complex interactions in the statistical analysis including that between treatment and time (Berge et al., 2005a). A third trial by Berge et al., found a transient increase in the odds of calves being increasing multiply resistant within five days of individual antimicrobial treatment (Berge et al., 2005b). The cohort study by Lowrance et al. found that treatment with ceftiofur crystalline-free acid was associated with a transiently increased proportion of multi-drug resistant fecal NTSEC; this normalized 13-15 days post-injection (Lowrance et al., 2007). Ceftiofur resistance was linked to ACSSuT phenotype through coresistance (Lowrance et al., 2007). There was also a transient effect of AMU of injectable oxytetracycline (three daily approved doses) on tetracycline resistance in fecal NTSEC from feedlot cattle in another clinical trial (Stabler et al., 1982). A Canadian study found associations between AMR in fecal NTSEC isolates from bulls at a test station and individual animal AMU when feed antimicrobials were also used (O'Connor et al., 2002). The use of injectable oxytetracycline in individual cattle receiving chlortetracycline in the feed was associated only with increased prevalence of resistance to chloramphenicol and sulfisoxazole in fecal NTSEC isolates (O'Connor et al., 2002).

Studies have examined AMR in NTSEC from meat of animals raised conventionally and that labeled as "raised without antibiotics" (LeJeune and Christie, 2004). Differences were few in this study, with conclusions that factors other than the use of

subtherapeutic antimicrobials may contribute to AMR in ground beef (LeJeune and Christie, 2004). Fecal NTSEC isolated from conventionally reared dairy cows had significantly higher resistance to seven of seventeen antimicrobials tested, than those raised organically after adjustment for age (Sato et al., 2005).

AMR can spread horizontally between animals. Antimicrobial resistant NTSEC isolates rapidly colonized neonatal dairy calves (Donaldson et al., 2006; Hoyle et al., 2004; Hoyle et al., 2005). In the study by Donaldson et al., the highest prevalence of multidrug resistance was in two week-old calves, and then the prevalence declined; colonization of new-born calves by MDR NTSEC occurred shortly after birth (Donaldson et al., 2006). In the first study by Hoyle et al., calves acquired AMR rapidly after birth, associated with age and housing (Hoyle et al., 2004). In the second study, a distinct antimicrobial resistant strain spread through a cohort of beef calves over 22 weeks, associated with time and environment but not AMU (Hoyle et al. 2005). However, other studies have not described widespread horizontal dissemination of AMR across all pens and all treatment groups (Berge et al., 2006; Inglis et al., 2005; Stevenson et al., 2003). Other studies have found that younger calves have a higher prevalence of AMR (Berge et al., 2005b; Martel and Coudert, 1993). Another study examined molecular aspects of AMR on a mixed farm and found a reservoir of resistant strains that persisted over 28 months and transmitted between livestock species (Hoyle et al., 2004); 10 different genotypic groups were found in all (Hoyle et al., 2006).

2.6 Research issues

2.6.1 Why *Escherichia coli*?

NTSEC was chosen as the indicator commensal organism in this study because it is easy to isolate from all animals and is one of the major carcass contaminants at slaughter (Stopforth et al., 2006). It is representative of Gram-negative bacteria. Monitoring AMR of this commensal gives a measure of selection pressure on the microflora in that animal and allows comparison and contrast of AMR from different species (McEwen et al., 2006a). NTSEC was considered a potential reservoir of AMR genes that could transfer AMR to other zoonotic or commensal organisms that might cause disease in cattle or people (Blake et al., 2003; Hart et al., 2006; Linton et al., 1977b; Winokur et al., 2001). We do not yet have a good understanding of how well AMR in NTSEC corresponds with that of *Salmonella* or another pathogen within the same animal or population (McEwen et al., 2006a).

2.6.2 Laboratory Methodology

Susceptibility testing can be done by two main methods. Disc diffusion is a qualitative breakpoint test whereas dilution is more quantitative (White et al., 2001b). Disc diffusion tests are performed using discs that are impregnated with specific concentrations of antimicrobials (White et al., 2001b). They are placed in the culture media with the bacteria under test (White et al., 2001b). A gradient of inhibition around the antimicrobial disc occurs, called the zone of inhibition which is inversely correlated with the MIC (White et al., 2001b). Disc diffusion is an inexpensive technique but dilution methods are considered more accurate and reproducible (McEwen et al.,

2006a). In micro or macro broth dilution and agar dilution methods, bacteria are inoculated onto antimicrobial impregnated media of different dilutions and the lowest concentration of the antimicrobial that inhibits growth of the bacteria, the MIC, is identified (White et al., 2001b). Antimicrobial breakpoints, procedures and interpretation should follow the CLSI standards (CLSI, 2006; NCCLS, 2004). Potential challenges with laboratory analysis include variation in methods between and within laboratories, different breakpoints used by different jurisdictions, and the terminology used to describe susceptibility (Bywater, 2000). In addition, during laboratory analysis, NTSEC may lose resistant plasmids over time (Inoue, 1997).

The number of isolates, randomly chosen from each sample to get a representative description of the fecal NTSEC isolate patterns in individual animals or pens, needs to be determined. Previous work has suggested that one fecal isolate may not be representative of the intestinal flora of an animal (Bywater, 2000). It is difficult to predict where clustering will have the greatest effect within a study, but using three to four isolates per animals was found to adequately represent herd prevalence (Villarroel et al., 2006).

Other issues to consider in the laboratory when developing a study are sample processing and susceptibility testing. When using pooled samples, the method of sample homogenization must adequately disperse organisms throughout the sample before swabs are taken for bacterial culture. Cannon and Nicholls looked at the effect of sample weight and adequate homogenization of samples to ensure bacteria are adequately dispersed from any clusters within the feces (Cannon and Nicholls, 2002). Hedges et al. suggested that choosing ten colony samples per individual fecal specimen

would determine the number of 'majority' (>10% prevalence) *E. coli* O-serotypes but varied in ability to identify 'minority' serotypes present in an individual animal fecal sample compared to a 100 colony analysis (Hedges et al., 1977). The 10 colony analysis also performed better than the theoretical expectation in identification of major serotypes, probably due to inadequate random sampling of isolates from plates based on colonial morphology (Hedges et al., 1977).

2.6.3 Study design and sampling

Issues with study design involve generalizability, timing of samples, and collection of samples. To answer the types of questions described in the introduction, using commercial feedlots along with their management norms as opposed to experimental situations that show associations between AMU and AMR but are not clearly representative of what might occur in a western Canadian feedlot. The feedlots themselves would need to be representative in design of larger, commercial feedlots in western Canada insofar as side-by-side, open-air pens, with dirt floors and 20% porosity fencing. Waterer allocation and treatment order would also need to be considered as this could play a role in transmission of bacteria between animals. Management norms such as treatment protocols, use of feed antimicrobials, and standardized computer record systems are also important.

Samples are collected during the feeding period, to look at associations with AMU. In some studies it is important to ensure samples were collected during the last 24 hours prior to shipping for slaughter, to be more representative of bacteria at the end stage of production that might ultimately affect the consumer.

Sample collection is a concern. Fecal samples from the floor of feedlot pens appear to be fairly reliable estimates of AMR in fecal NTSEC isolates from individual animals (Wagner et al., 2002). Pooled samples have shown to yield good estimates of pen-level prevalence of AMR in other studies, when the prevalence of the AMR is greater than 2% (Dunlop et al., 1999; Wagner et al., 2002). Fecal sample collection from the pen floor has been used previously for AMR determination (Wagner et al., 2002; Dargatz et al., 2002; Galland et al., 2001) and some studies have used also pooled these samples (Wagner et al., 2002; Dargatz et al., 2002). In one study, individual animal isolates, pools of five samples and pools of ten samples were examined (Wagner et al., 2002). One set of samples was derived from the individual animals, and another set from the pen floor (Wagner et al., 2002). AMR patterns between the pooled samples and the individual animal samples did not differ significantly when the prevalence of AMR to an antimicrobial was >2% (Wagner et al., 2002). Results from another current study that estimated the prevalence of *E. coli* 0157 from fecal pats using from one to five samples/pat, suggested that sampling only one site per pat underestimated the true prevalence of the pathogen in the fecal pat (Echeverry et al. 2005).

2.6.4 Analysis

Data can be described by MICs which allows for trend analysis over time (McEwen et al., 2006a). MIC data can be aggregated by breakpoints into susceptible and resistant, but this loses some of the information in the data (McEwen et al., 2006a; Wagner et al., 2003a). When looking at literature, one must also be clear whether intermediate susceptibility stands alone or is grouped with susceptible or resistant. It is also

important to examine variance structure of data. The data can be set up in a multilevel model with hierarchical levels specified (Dohoo et al., 2003; Goldstein, 2003). In the study by Wagner et al., variation explained at the pen-level was at most 6.6%, there was one group of antimicrobials with a low prevalence (1 to 3%) where 78-89% of the variation explained at the fecal sample level and the remaining 9-17% of variation explained at the within sample level (Wagner et al., 2002). The second group of antimicrobials with a higher prevalence (8-40%) had only 34-48% of variation explained at the fecal sample level and 49-64% of variation explained at the within fecal sample level (Wagner et al., 2002). A study in Canadian pig barns found, in a three level hierarchical model looking tetracycline resistance in fecal NTSEC from pigs in rooms in pens, a very large between-pig, within-pen variance component (97.5%) with a small between pen, within room component (2.5%) and no between-room component (Dunlop et al., 1999). A Norwegian pig study found that most of the diversity between isolates was related to sample time and not to the number of isolates from an individual sample except for ampicillin in sows, where the within-sow variation was higher than the between-sow variation (Brun et al., 2002).

Various methods have been used to model associations between AMR and potential risk factors. The hierarchical structure of the data and other causes of clustering must be considered in the analysis (Dohoo et al., 2003; Wagner et al., 2003a). An interesting approach was taken by Berge et al. where cluster analysis was first used to group isolates with similar resistance patterns, then ranked resistance clusters were used as the outcome in a cumulative logistic regression model using generalized estimating equations (GEE) (Berge et al., 2005a; Berge et al., 2005b; Berge et al., 2006). AMR

phenotypes with resistant to more than one antimicrobial must be examined (D'Agata et al., 2006); this has been done by a variety of methods including factor analysis (Wagner et al., 2003a; Wagner et al., 2003b). It has been suggested that analyses based on the changes in resistance proportions, could lead to bias in estimates (Schwaber et al., 2004). Short-term repeatability of AMR measurements was found for antimicrobials for which the prevalence in greater than two percent; this is a critical assumption for interpretation of data (Wagner et al., 2003c).

2.7 Surveillance

AMR is a major public health threat leading to concerns about the use of antimicrobials in human medicine, veterinary medicine and other forms of agriculture (Caprioli et al., 2000). Caprioli et al. describe numerous reasons for monitoring AMU and AMR in animals. The information will help practitioners choose appropriate treatment protocols. It will also help epidemiologists understand prevalence and rate of change of susceptibility of various antimicrobials establish baselines, do risk assessments, and evaluate interventions. Surveillance includes the ability to respond after a health event has been detected (Aarestrup et al., 1998). Some would like to see multinational surveillance that is standardized with quality control, responds to emerging resistance concerns, compares and contrasts AMR between jurisdictions, gives feedback to clinicians, and provide data for risk analysis (Bywater, 2000; Monnet, 2000; WHO, 2001b). Surveillance should include clinical and non-clinical samples (WHO, 2001b), but different regions need to take into account management norms, AMU typical of the region, and previous AMR patterns in the region. Sampling for

AMR surveillance should also minimize bias and be correlated with AMU data (Bywater, 2000). Problems with surveillance include sampling issues (representativeness), lack of history on samples especially with respect to previous AMU and age of animal (affects AMR), lack of bacterial speciation (affects interpretation), and environmental temperature and stress (affects intestinal flora (Bywater, 2000). Two of the most important characteristics of AMR surveillance systems are first, the ability to monitor trends and detect changes in susceptibility and second, to communicate the information in a timely fashion (Bax et al., 2001).

Many surveillance systems for AMR and AMU have been developed worldwide. In 1995, the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP) was created, coinciding with the ban of avoparcin, providing a means of determining the effectiveness of the ban (Aarestrup et al., 1998; Bager, 2000; McEwen and Fedorka-Cray, 2002). This system includes indicator bacteria, zoonotic bacteria, and animal pathogens; the objectives are to monitor AMR, AMU and associations between them (Aarestrup et al., 1998; McEwen and Fedorka-Cray, 2002). AMU data in DANMAP was based on total annual sales from pharmaceutical companies without species or other demographic information (Stege et al., 2003). In 2000, VETSTAT was created to optimize AMU in production animals, specifically by monitoring AMU in food animals, providing information to veterinary practitioners, providing transparency in compliance with legislation, and providing data for research (Stege et al., 2003). VETSTAT receives electronic data from pharmacies, veterinarians, and feed mills, including information on species, age and diagnosis (Stege et al., 2003). In 1996, the National Antimicrobial Resistance Monitoring System-Enteric Bacteria (NARMS) was

developed in the USA to monitor AMR in enteric bacteria, identify emerging resistance trends, and provide AMR information to clinicians (McEwen and Fedorka-Cray, 2002). NARMS has animal, human, and retail meat arms; however, there are no AMU data associated with NARMS (Nunnery et al., 2006). A number of other countries (Australia, France, Japan, UK) have developed passive or active AMR surveillance systems (Anonymous, 2004; Jordan, 2003; Martel and Coudert, 1993; Martel et al., 2000; Nel et al., 2004; Stephens, 2003).

Efforts have been made to evaluate existing surveillance systems with goals of improvement and standardization (Jones and Masterton, 2001; Wray and Gnanou, 2000). The International Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study in human medicine and other groups have explored methods to detect emerging resistance patterns (Jones et al., 2000; Moser et al., 1999; Schrag et al., 2002).

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was developed in response recommendations made in the 2002 national report (GOC, 2007; VDD, 2002). CIPARS includes active abattoir and retail AMR surveillance for poultry, swine and beef, as well as on farm AMR and AMU surveillance in swine and feedlot cattle (GOC, 2007). Passive AMR surveillance is done for animal and human clinical *Salmonella* isolates (GOC, 2007). CIPARS is developing capacity for AMU surveillance in animals and humans (GOC, 2007).

2.8 Conclusions

A number of questions remain unanswered. What is the prevalence of resistant bacteria in calves on arrival at the feedlot and how does this change over time? Do feed

antimicrobials fed under common western Canadian feedlot management practices contribute to AMR preslaughter? Does metaphylaxis, as commonly used on arrival in Western Canada, contribute to AMR preslaughter? Does individual animal disease treatment during the feeding period contribute to AMR preslaughter?

Work that needs to be done in western Canada includes characterization of AMR in fecal NTSEC, from newly weaned, auction market derived calves, to various antimicrobials, on arrival at the feedlot, as an indication of 'baseline' AMR before treatment with antimicrobials at the feedlot. This has not been well characterized in feedlot cattle in western Canada and may represent AMR patterns associated with the herd of origin or auction market. Information on the prevalence of AMR in commensal fecal NTSEC isolates from calves in western Canadian feedyards at other times during the feeding period, especially preslaughter, is also currently sparse. Further data on AMR in commensal organisms are needed as this may represent the potential for transfer of AMR to zoonotic organisms within the host or horizontally between animals.

Associations between metaphylactic (injectable) and prophylactic (feed) AMU in groups of cattle and the presence of antimicrobial-resistant fecal NTSEC also need to be examined. It is important this is done in feedlots that are representative in design of larger, commercial feedlots in western Canada. Factors to be investigated include management practices and protocols involving AMU at the feedlot, pen and individual animal level. Analysis of risk factors associated with the presence of AMR in fecal NTSEC isolates late in the feeding period will perhaps suggest to potential intervention points in our current feedlot production system.

Questions have arisen when designing studies as to whether characteristics of the pen or feedlot level management practices have the most influence on AMR. Answers to this question could help direct future research towards control practices most likely to be effective. Examination of the variation found at different hierarchical levels in feedlot trials for different AMR outcomes will help solve these issues. This idea also needs to be further investigated as to how it relates to individual animal testing and pooling samples from multiple fecal pats. Further examination of the number of isolates necessary for characterizing AMR patterns in feedyard pens, from pooled and individual samples, is also required to determine the best methods to sample pens and obtain results that can be generalized to the wider population.

Alternatives to antimicrobial use in the feedlot should be examined. One instance where an alternative might be feasible in western Canadian feedlot production is for the prevention and control of liver abscesses. If an effective *F. necrophorum* vaccine was available, this might eliminate the long-term feeding of antimicrobials to prevent liver abscesses.

These gaps in knowledge are important to address as scientific evidence may help the beef industry either refute criticisms about antimicrobial use in livestock causing the development of AMR in humans and develop more prudent methods of livestock production if indicated.

2.9 References

1. Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic- and indicator bacteria isolated from different food animals in Denmark: a baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). APMIS 1998;106:745-770.

2. Aarestrup FM. Characterization of glycopeptide-resistant *Enterococcus faecium* (GRE) from broilers and pigs in Denmark: Genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides. J Clin Microbiol 2000;38(7):2774-2777.

3. Aarestrup FM, Seyfarth AM, Emborg H, Pedersen K, Hendriksen IS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother 2001;45:2054-2059.

4. Aarestrup FM. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic Clin Pharmacol Toxicol 2005;96:271-281.

5. Aarestrup FM and Schwarz S. Antimicrobial resistance in Staphylococci and Streptococci of animal origin. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press, 2006.

6. Acar J, Rostel B. Antimicrobial Resistance; an overview. Rev Sci Tech 2001;20(3):797-810.

7. Aiello SE, Mays A, ed. The Merck Veterinary Manual 8th Ed. Whitehouse Station: Merck & Co., Inc., 1998.

8. Aly R, Maibach HI, Strauss WG, Shinefield HR. Effects of a systemic antibiotic on nasal bacterial ecology in man. Appl Microbiol 1970;20:240-244.

9. Angulo FJ, Nunnery JA, Bair HD. Antimicrobial resistance in zoonotic enteric pathogens. Rev Sci Tech Off Int Epiz 2004;23 (2):485-496

10. Anonymous. EU bans four antibiotic feed additives. Vet Rec 1998;143:671.

11. Anonymous. Surveillance for antimicrobial resistance: DEFRA sets out its strategy. Vet Rec 2004;154:642-643.

12. Apley M. Does antimicrobial use in animals affect human health? The Bovine Proceedings 1998;31: 9-12.

13. Apley MD, Coetzee JF. Antimicrobial drug use in cattle. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006.

14. Ash RJ, Mauck B, Morgan M. Antibiotic resistance of gram-negative bacteria in riers, United States. Emerg Infect Dis 2002;8:713-716.

15. Aslam M, Service C. Antimicrobial resistance and genetic profiling of *Escherichia coli* from a commercial beef packing plant. J Food Prot 2006;69(7):1508-1513.

16. Aubry-Damon H, Grenet K, Sall-Ndiaye P, Che D. Cordeiro E. Bougnoux M, Rigaud E, Let Strat, Y, Lemanissier V, Armand-Lefevre L, Delzescaux D, Desencios J, Leinard M, Andremont A. Antimicrobial resistance in commensal flora of pig farmers. Emerg Infect Dis 2004;10:873-879.

17. Austin DJ, Kakehashi M, Anderson RM. Proc Roy Soc London Ser B 1997;264:1629-38.

18. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci 1999;96:1152-1156.

19. Babic M, Hujer AM, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. Drug Resist Update 2006;9:142-156.

20. Bager F, Madsen M, Christensen J, Aarestrup FM. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish Poultry and pig farms. Prev Vet Med 1997;31:95-112.

21. Bager F, Aarestrup FM, Madsen M, Wegener HC. Glycopeptide resistance in Enterococcus faecium from boilers and pigs following discontinued use of avoparcin. Microb Drug resist 1999;5:53-056.

22. Bager F. DANMAP: monitoring antimicrobial resistance in Denmark. Int J Antimicrob Ag 2000;14:271-274.

23. Baker R. Health management with reduced antibiotic use-The U.S. Experience. Anim biotech 2006;17:195-205.

24. Baquero F. Trends in antibiotic resistance of respiratory pathogens: an analysis and commentary on a collaborative surveillance study. J. Antimicrob Chemother 1996;38(Suppl A): 117-132.

25. Bartoloni A, Pallecchi L, Benedetti M, Fernandez C, Vallejos Y et al. Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. Emerg Infect Dis 2006;12:907-913.

26. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemother 1994;34:507-516.

27. Bax R, Bywater R, Cornaglia G, Goossens H, Hunter P, Isham V, Jarlier V, Jones R, Phillips I, Sahm D, Senn S, Struelens M, Taylor D, White A. Surveillance of antimicrobial resistance-what, how and whither? Clin Microbiol Infect 2001;7:316-325.

28. Berge ACB, Epperson WB, Pritchard RH. Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal *Escherichia coli*. Vet Res 2005a;36:723-734.

29. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005b;69:25-38.

30. Berge ACB, Moore DA, Sischo WM. Field trial evaluating the influence of prophylactic and therapeutic antimicrobial administration on antimicrobial resistance in fecal *Escherichia coli* in dairy calves. Appl Environ Microbiol 2006;72:3872-3878.

31. Bertrand X, Dufour V, Millon L, Beuvier E, Gbaguidi-Haore H, Piarroux R, Vuitton DA, Talon D. Effect of cheese consumption on emergence of antimicrobial resistance in the intestinal microflora induced by a short course of amoxicillin-clavulanic acid. J Appl Microbiol 2007;102:1052-1059.

32. Bizimenyera SE, Swan GE, Chikoto H, Eloff JN. Rationale for using *Peltophorum africanum* (Fabaceae) extracts in veterinary medicine. J S Afr Vet Assoc 2005;76:54-58.

33. Blake DP, Hillman K, Fenlon DR, Low JC. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J Appl Microbiol 2003;95:428-436.

34. Blondeau JM, Hansen G, Metzler K. Hedlin P. J Chemother 2004;16 (Suppl 3):1-19.

35. Boerlin P. Molecular epidemiology of antimicrobial resistance in veterinary medicine: where do we go? Anim Health Res Rev 2004;5:95-102.

36. Boerlin P, White DG. Antimicrobial resistance and its epidemiology. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006.

37. Bongers JH, Franssen F, Elbers ARW, Tielen MJM. Antimicrobial resistance of *Escherichia coli* isolates from the faecal flora of veterinarians with different professional specialties. Vet Quarterly 1995; 17:146-149.

38. Booker CW, Guichon PT, Schunicht OC, Wildman BK, Jim GK. Economic impact of antimicrobial use in feedlots. Bov Pract 1999;32:111-112.

39. Brown H, Elliston NG, McAskill JW, Muenster OA, Tonkinson LV. Tylosin phosphate (TP) and tylosin urea adduct (TUA) for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1973;37(5):1085-10921

40. Brown H, Bing RF, Grueter HP, McAskill JW, O'Cooley CO, Rathmacher RP. Tylosin and chlortetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1975; 40(2):207-213.

41. Bruins MR, Kapil S, Oehme FW. Plasmid and chromosomal basis of tolerance to cadmium and resistance to antibiotics in normal bovine duodenal bacterial flora. Vet Human Toxicol 2001;43:129-133.

42. Brun E, Holstad G, Kruse H, Jarp J. Within-sample and between-sample variation of antimicrobial resistance in fecal *Escherichia coli* isolates from pigs. Microb Drug Resist 2002;8:385-391.

43. Burkgren T, Vogel L. Stakeholder position paper: Food animal Veterinarian. Prev Vet Med 2006;73:177-179.

44. Bywater RJ. Sense and nonsense in surveillance programs. Acta vet scand 2000; Suppl 93:119-127.

45. Bywater RJ. Identification and surveillance of antimicrobial resistance dissemination in animal production. Poultry Science 2005;84:644-648.

46. Bywater R, Silley P, Simjee S. Letter to the Editor, Antimicrobial breakpoints-Definitions and conflicting requirements. Vet Microbiol 2006;118:158-159.

47. Canadian Animal Health Institute (CAHI). 2001-2003 kilograms of active antimicrobials distribution report. Inforum 2005;9(1). http://www.cahi-icsa.ca/comm-inforum.php?y=2005 accessed December 30, 2007.

48. Canadian Animal Health Institute (CAHI). Quality assurance backgrounder. http://www.cahi-icsa.ca/comm-factsheets-food.php#Assurance accessed December 30, 2007.

49. Canadian Cattlemen' Association. Quality Starts Here / Verified Beef Production, Producer Manual, 2006. http://www.verifiedbeef.org/producer_resources.htm accessed December 30, 2007.

50. Canadian Cattlemen's Association and Beef Information Centre. Antibiotics in the Beef Cattle Industry, 2006. http://www.cattle.ca/factsheets/Antibiotics.pdf accessed December 30, 2007.

51. Canadian Committee for Antibiotic Resistance (CCAR). National action plan to address antibiotic resistance, 2004. www.ccar-ccra.com/english/pdfs/Action%20Plan.Sept2004.pdf accessed December 30, 2007.

52. Canadian Veterinary Medical Association. CVMA General Position Statements: Antimicrobial Use in Animals, October 1998. http://canadianveterinarians.net/ShowText.aspx?ResourceID=60 accessed December 30, 2007.

53. Cannon RM, Nicholls TJ. Relationship between sample weight, homogeneity, and sensitivity of fecal culture for Salmonella enterica. J Vet Diagn Invest 2002;14:;60-02.

54. Caprioli A, Donelli G, Falbo V, Passi C, Pagano A. and Mantovani A. Antimicrobial resistance and production of toxins in *Escherichia coli* strains from wild ruminants and the alpine marmot. J Wildl Dis 1991;27(2):324-327.

55. Caprioli A, Busani L, Martel JL, Helmuth R. Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int J Antimicrob Ag 2000;14:295-301.

56. CDC. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2004. Atlanta, Georgia: U.S. Department of Health and Human Services, CDC, 2007. http://www.cdc.gov/narms/ accessed December 30, 2007

57. Chauvin C, Madec F. Guittet M, Sanders P. Pharmaco-epidemiology and – economics should be developed more extensively in veterinary medicine. J vet Pharmacol Therap 2002;25:455-459.

58. Cirz RT, Chin JK Andes DR, de Crecy-Lagard V, Craig WA, Romesberg FE. Inhibition of mutation and combating the evolution of antibiotic resistance. PLoS Biol 3:e176.

59. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. (Use for Humans). CLSI document M100-S16, 2006.

60. Cockerill FR 3rd. Genetic methods for assessing antimicrobial resistance. Antimicrob Agents chemother. 1999;43:199-212.

61. Cohen T, Murray M. Modeling epidemics of multidrug-resistant *M. tuberculosis* of heterogeneous fitness. Nat Med 2004;10:1117-1121.

62. Cole D, Drum DGV, Stallknecht DDE, White DG, Lee MD, Ayers S, Sobsey M, Maurer JJ. Free-living Canada geese and antimicrobial resistance. Emerg Infect Dis 2005;11:935-938.

63. Conly, J. Antimicrobial resistance in Canada. Can Med Assoc J 2002;167 (8):885-91.

64. D'Agata EMC, Cataldo MA, Cauda R, Tacconelli E. The importance of addressing multidrug resistance not assuming single-drug resistance in case-control studies. Infect Control Hosp Epidemiol 2006;27:670-674.

65. Dancer SJ, Shears P, Platt DJ. Isolation and characterization of coliforms from glacial ice and water in Canada's high arctic. J Appl Microbiol 1997;82:597-609.

66. Dargatz DA, Fedorka-Cray PJ, Ladely SR, Ferris KE, Green AL, Headrick ML. Antimicrobial Susceptibility patterns of Salmonella isolates from cattle in feedlots. J Am Vet Med Assoc 2002;221:268-72.

67. Davis MA, Hancock DD, Besser TE. Multiresistant clones of *Salmonella enterica*: The importance of dissemination. J Lab Clin Med 2002; 140:135-141.

68. De Graef EM, Decostere A, Devriese LA, Haesebrouck F. Antibiotic Resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb Drug Resist 2004;10:65-69.

69. Dehaumont P. OIE International standards on antimicrobial resistance. J Vet Med B 2004;51:411-414.

70. de Neeling JA, van den Broek MJM, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WDC, Boshuizen HC, van de Guissen AW, Van Duijkeren E, Huijsdens XW. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol 2007;122:366-372.

71. Dewey CE, Cox BD, Straw BE, Bush EJ, Hurd HS. Associations between off-label feed additives and farm size, veterinary consultant use, and animal age. Prev Vet Med 1997;31:329-331.

72. Dohoo IR, Tillard E, Stryhn H, Faye B. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Prev Vet Med* 2001;1592:1-18.

73. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown: AVC Inc., 2003.

74. Dolejska M, Cizek A, Literak I. High prevalence of antimicrobial-resistant genes and integrons in *Escherichia coli* isolates from Black-headed Gulls in the Czech Republic. J Appl Microbiol 2007;103:11-19.

75. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. Appl Environ Microbiol 2006;72:3940-3948.

76. Dunlop RH, McEwen SA, Meek AH, Friendship RM, Clarke RC, Black WD. Antimicrobial drug use and related management practices among Ontario swine producers. Can Vet J 1998a; 39: 87-96.

77. Dunlop RH, McEwen SA, Meek AH, Black WD, Clarke RC, Friendship RM. Individual and group antimicrobial usage rates on 34 farrow-to-finish swine farms in Ontario, Canada. Prev Vet Med 1998b; 34: 247-264.

78. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998c;34:283-305.

79. Dunlop RH, McEwen SA, Meek AH, Friendship RM, Black WD, Clarke RC. Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs. Epidemiol Infect 1999;122:485-596.

80. Echeverry A, Loneragan GH, Wagner BA, Brashears MM. Effect of intensity of fecal pat sampling on estimates of *Escherichia coli* O157 prevalence. Am J Vet Res 2005;66(12):2023-2027.

81. European Commission (EC). Opinion of the scientific steering committee on antimicrobial resistance, 1999. http://ec.europa.eu/food/fs/sc/ssc/out50_en.pdf accessed December 30, 2007.

82. European Commission (EC). Ban on antibiotics as growth promoters in animal feed enters into effect, 2005. http://ec.europa.eu/health/ph/others/antimicrob_resist/am_02_en.pdf accessed December 30, 2007.

83. Ewaschuk JB, Naylor JM, Chirino-Trejo M, Zello GA. *Lactobacillus rhamnosus* strain GG is a potential probiotic for calves. Can J Vet Res 2004;68:249-253.

84. Finlayson M, Barnum DA. The effect of chlortetracycline feed additive on the antibiotic resistance of fecal coliforms of weaned pigs subjected to experimental Salmonella infection. Can J Comp Med 1973;37;63-69.

85. Fishman N. Antimicrobial stewardship. Am J Infect Control 2006;34(5):S55-S63.

86. Fluckey WM, Loneragan GH, Warner R, Brashears MM. Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides and carcasses. J Food Prot 2007;70:551-556.

87. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. Eur J Clin Microbiol Infect Dis 1999;18:761-770.

88. Foxman B. Contributions of molecular epidemiology to the understanding of infectious disease transmission, pathogenesis, and evolution. Ann Epidemiol 2007;17:148-156.

89. Fraser E, Stephen C, Bowie WR, Wetzstein M. Availability and estimates of veterinary antimicrobial use in British Columbia. Can Vet J 2004;45:309-311.

90. Funk JA, LeJeune JT, Wittum TE, Rajala-Schultz PJ. The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. Microbial Drug Resistance 2006;12:210-218.

91. Galland JC, Hyatt DR, Crupper SS, Acheson DW. Prevalence, antibiotic susceptibility, ad diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl Environ Microbiol 2001;67(4):1619-27.

92. Gallo GF, Berg JL. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. Can Vet J 1995:36: 223-229.

93. Galyean ML, Gunter SA, Malcolm-Callis KJ. Effects of arrival medication with tilmicosin phosphate on health and performance of newly received beef cattle. J Anim Sci 1995;73:1219-121256.

94. Gebreyes WA, Thakur S, Morrow WEM. Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant Salmonella in antimicrobial-free and conventional pig production. J Food Protec 2006;69:743-748.

95. Giguere S. Antimicrobial drug action and interaction: An introduction. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006a.

96. Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006b.

97. Ginocchio CC. Role of NCCLS in antimicrobial susceptibility testing and monitoring. Am J Health-Sys Pharm 2002;59:S7-S11.

98. Giraud A, Matic I, Radman M, Fons M, Taddei F. Mutator bacteria as a risk factor in treatment of infectious diseases. Antimicrob Agents Chemother 2002;46:863-865.

99. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. New Engl J Med 1998;338:1333-1338.

100. Goldstein H. Multilevel Statistical Models 3rd Edition. London: Arnold 2003.

101. Gordon SM. Antimicrobial resistance: An ecological approach to a growing threat. Cleve Clin J Med 1998;65:232-236.

102. Government of Canada (GOC). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph, ON: Public Health Agency of Canada, 2007. http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html accessed December 30, 2007.

103. Government of Canada (GOC). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) [homepage on the internet] last updated July 23, 2007. http://www.phac-aspc.gc.ca/cipars-picra/about_e.html accessed December 30, 2007.

104. Grave K, Jensen VF, McEwen S, Kruse H. Monitoring of antimicrobial drug usage in animals: methods and applications. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006.

105. Guardabassi L, Courvalin P. Antimicrobial resistance in bacteria of animal origin. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006.

106. Harada K, Asai T, Kojima A, Ishihara K, Takahashi T. Role of coresistance in the development of resistance to chloramphenicol in *Escherichia coli* isolated from sick cattle and pigs Am J Vet Res 2006;67:230-235.

107. Harland RJ, Jim GK, Guichon PT, Townsend HGG, Janzen ED. Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves. Can Vet J 1991;32: 163-168.

108. Hart WS, Heuzenroeder MW, Barton MD. A study of the transfer of tetracycline resistance genes between *Escherichia coli* in the intestinal tract of a mouse and a chicken model. J Vet Med B 2006;53:333-340.

109. Hedges AJ, Howe K, Linton AH. Statistical considerations in the sampling of *Escherichia coli* from intestinal sources for serotyping. J Appl Microbiol 1977;43:271-280.

110. Henderson DK. Managing methicillin-resistant staphylococci: A paradigm for preventing nosocomial transmission of resistant organisms. Am J Infect Control 2006;34(5):S46-S54.

111. Hinton A, Ingram KD. Antimicrobial activity of potassium hydroxide and lauric acid against microorganisms associated with poultry processing. J Food Prot 2006;69:1611-1615.

112. Holmberg SD, Osterholm MT, Senger KA, Cohen ML. Drug-resistant Salmonella from animals fed antimicrobials. N Engl J Med 1984;311:617-622.

113. Holmberg SD, Wells JG, Cohen ML. Animal-to-man transmission of antimicrobial-resistant *Salmonella*: Investigations of US outbreaks, 1971-1983. Science 1984:225:833-835.

114. Howe K, Linton AH. The effect of tetracycline on the coliform gut flora of broiler chickens with special reference to antibiotic resistance and 0-Serotypes of *Escherichia coli*. J Appl Bacteriol 1976;41;453-464.

115. Hoyle DV, Knight HI, Shaw DJ, Hillman K, Pearce MC, Low JC, Gunn GJ, Woolhouse MEJ. Acquisition and epidemiology of antibiotic-resistant *Escherichia coli* in a cohort of newborn calves. J Antimicrob Chemother 2004;53:867-871.

116. Hoyle DV, Yates CM, Chase-Topping ME et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71 (11):6680-6688.

117. Hoyle DV, Davison HC, Knight HI, Yates CM, Dobay O, Gunn GJ, Amyes SGB, Woolhouse MEJ. Molecular characterization of bovine faecal *Escherichia coli* show persistence of defined ampicillin resistant strains and the presence of class 1 integrons on an organic beef farm. Vet Microbiol 2006;115: 250-257.

118. Hurd HS, Doores S, Hayes D, Mathew A, Maurer J, Silley P, Singer RS, Jones RN. Public health consequences of macrolide use in food animals: A deterministic risk assessment. J Food Prot 2004;67:980-992.

119. Huston CL, Bailey RH, Best TF, Huston JE, Evans RR. Antimicrobial resistance of enteric *E. coli* in beef cattle treated with antibiotics. The Proc Am Assoc Bov Pract 2003;36:156-7.

120. Inglis GD, McAllister TA, Busz HW, Yanke LJ, Morck DW, Olson ME, Read RR. Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in Campylobacter jejuni and Campylobacter hyointestinalis. Appl Environ Microbiol 2005;71;3872-3881.

121. Inglis GD, Morck DW, McAllister TA, Entz T, Olson ME, Yanke LJ, Read RR. Temporal prevalence of antimcirobial resistance in Campylobacter spp. from beef cattle in Alberta feedlots. Appl Environ Microbiol 2006;72(6):4088-4095.

122. Inoue Y. Spontaneous loss of antibiotic–resistant plasmids transferred to *Escherichia coli* in experimental chronic bladder infection. Int J Urol 1997;4:285-288.

123. Ishikawa S, Matsumura Y, Yoshizako F, Tsuchido T. Characterization of a cationic surfactant-resistant mutant isolated spontaneously from *Escherichia coli*. J Appl Microbiol 2002;92:261-268.

124. Jensen BB. The impact of feed additives on the microbial ecology of young pigs. Journal of Animal and Feed Sciences 1998;7:45-64.

125. Jensen VF, Jacobsen E, Bager F. Veterinary antimicrobial usage statistics based on standardized measures of dosage. Prev Vet Med 2004;64:201-215.

126. Jindal A, Kocherginskaya S, Mehboob A, Robert M, Mackie RI, Raskin L, Zilles JL. Antimicrobial use and resistance in swine waste treatment systems Appl Environ Microbiol 2006;72:7813-7820.

127. Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). The use of antibiotics in food producing animals: antibiotic resistant bacteria in animals and humans. Commonwealth of Australia, 1999.

128. Jones RN, MYSTIC Advisory Board. Detection of emerging resistance patterns within longitudinal surveillance sytems: data senesitivity and microbial susceptibility. J Antimicrob Chemother 2000;46:Topic T2, 1-8.

129. Jones RN, Masterton R. Determining the value of antimicrobial surveillance programs. Diagn Microbiol Infect Dis 2001;41:171-175.

130. Jordan D. Surveillance for antibiotic resistant *Escherichia coli* in food animals. Commun Dis Intell 2003;27 Suppl:S117-S120.

131. Kelly AP, Janzen ED. A review of morbidity and mortality rates and disease occurrence in North American feedlot cattle. Can Vet J 1986; 27: 496-500.

132. Khachatourians, GC. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can Med Assoc J 1998; 159:1129-1136.

133. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Vet Microbiol 2008;(In Press).

134. Klare I, Heier H, Claus H, Reissbrodt R, Witte W. vanA-mediated high –level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiology Letters 1995;125:165-172.

135. Kruse H, Sorum H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Appl Environ Microbiol 1994;60:4015-4021.

136. Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, Thomas AR, Harrison LH, Bennett NM, Farley MM, Facklam RR, Jorgensen JH, Besser J, Zell ER, Schuchat A, Whitney CG. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. N Engl J Med 2006;354:1455-1463.

137. Langlois BE, Cromwell GL, Hays VW. Influence of chlortetracycline in swine feed on reproductive performance an on incidence and persistence of antibiotic resistant enteric bacteria. J Anim Sci 1978;46:1369-1382.

138. Langlois BE, Dawson KA, Leak I, Aaron DK. Antimicrobial resistance of fecal coliforms from pigs in a herd not exposed to antimicrobial agents for 126 months. Vet Microbiol 1988;18:147-153.

139. Larson E. Community factors in the development of antibiotic resistance. Annu Rev Public Health 2007;28:2.1-2.13.

140. Lefebvre B, Malouin F, Roy G, Giguere K, Diarra M. Growth performance and shedding of some pathogenic bacteria in feedlot cattle treated with different growth-promoting agents. J Food Protect 2006;69:1256-1264.

141. Lejeune JT, Christie NP. Microbiological Quality of ground beef from conventionally-reared cattle and "Raised without Antibiotics" label claims. J Food Protec 2004;67:1433-1437.

142. Levin BR, Lipsitch M, Perrot V, Schrag S, Antia R, Simonsen L, Walker NM, Stewart FM. The population genetics of antibiotic resistance. Clin Infect Dis 1997;24(Suppl 1):S9-S16.

143. Linton AH. Antibiotic resistance: The present situation reviewed. Vet Rec 1977a;100:354-360.

144. Linton AH, Howe K, Bennett PM, Richmond MH, Whiteside EJ. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. J Appl Microbiol 1977b;43:465-469.

145. Linton AH. Antibiotic resistance in veterinary practice. Vet Record, In practice 1982;14 (1):11-13.

146. Linton AH. Flow of resistance genes in the environment and from animals to man. J of Antimicrob Chemother 1986;18 Suppl C; 189-197.

147. Lipsitch M, Levin BR. The population dynamics of antimicrobial chemotherapy. Antimicrob Agents Chemother 1997;41:363-373.

148. Lipsitch M, Singer RS, Levin BR. Antibiotics in agriculture: When is it time to close the barn door? Proc Natl Acad Sci 2002;99 (9):5752-4.

149. Lowrance TC, Loneragan GH, Kunze DJ, Platt TM, Ives SE, Scott HM, Norby B, Echeverry A, Brashears MM. Changes in antimicrobial susceptibility in a population of *Escherichia coli* isolated from feedlot cattle administered ceftiofur crystalline-free acid. Am J Vet Res 2007;68:501-507.

150. Macovei L, Zurek L. Ecology of antibiotic resistance genes: characterization of enterococci from houseflies collected in food settings. Appl Environ Microbiol 2006;72:4028-4035.

151. Magnes MR, Smith SP, Lau BJ, Nuval CJ, Eisenberg JNS, Dietrich PS, Rile LW. Retail meat consumption and the acquisition of antimicrobial resistant *Escherichia col*i causing urinary tract infections: A case –control study. Foodborne pathogens and disease 2007;4:419-431.

152. Mahady GB. Medicinal plants for the prevention and treatment of bacterial infections. Curr Pham Des 2005; 11:2405-2427.

153. Martel JL, Coudert M. Bacterial Resistance monitoring in animals: the French national experiences of surveillance schemes. Vet Micobiol 1993;35:321-38.

154. Martel JL, Tardy F. Brisabois A, Lailler R, Coudert M, Chaslus-Dancla E. The French antibiotic resistance monitoring programs. Int J Antimicrob Ag 2000;14:275-283.

155. Martinez M, Toutain PL, Walker RD. The pharmacokinetic - pharmacodynamic (PK/PD) relationship of antimicrobial agents. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006.

156. McDermott PF, Walker RD, White DG. Antimicrobials: Modes of action and mechanisms of resistance. Int J of Toxicol 2003;22:135-143.

157. McDermott PF. Antimicrobial resistance in nontyphoidal *Salmonellae*. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006.

158. McEwen SA, Fedorka-Cray PJ. Antimicrobial use and resistance in animals. Clin Inf Dis 2002:34(Suppl 3):S93-S106.

159. McEwen SA, Aarestrup FM, Jordan D. Monitoring of antimicrobial resistance in animals: Principles and practices. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press, 2006a.

160. McEwen SA, Singer RS. Stakeholder position paper: The need for antimicrobial use data for risk assessment. Prev Vet Med 2006b;73:169-176.

161. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? Can Med Assoc J 1998; 159: 1119-1120.

162. McGowan JE. Economic impact of antimicrobial resistance. Emerg Infect Dis 2001;7:286-292.

163. Monnet DL. Toward multinational antimicrobial resistance surveillance systems in Europe. Int J Antimicrob Ag 2000;15:91-101.

164. Monnet DL, Molstad S, Cars O. Defined daily doses of antimicrobial reflect antimicrobial prescriptions in ambulatory care. J Antimicrob Chemother 2004;53(6): 1109-1111.

165. Morck DW, Merrill JK, Thorlakson BE, Olson ME, Tonkinson LV, Costerton JW. Prophylactic efficacy of tilmicosin for bovine respiratory tract disease. J Am Vet Med Assoc 1993;202:273-277.

166. Moro MH, Beran GW, Hoffman LJ, Griffith RW. Effects of cold stress on the antimicrobial drug resistance of *Escherichia coli* of the intestinal flora of swine. Letters in Appl Microbiol 1998;27:251-254.

167. Moro MH, Beran GW, Griffith RW, Hoffman LJ. Effects of heat stress on the antimicrobial drug resistance of *Escherichia coli* of the intestinal flora of swine. J Appl Microbiol 2000;88:836-844.

168. Morris DO, Mauldin EA, O'Shea K, Shofer FS, Rankin SC. Clinical, microbiological and molecular characterization of methicillin-resistant *Staphylococcus aureus* infections of cats. Am J Vet Res 2006; 67:1421-1425.

169. Moser SA, Jones WT, Brossette SE. Application of data mining to intensive care unit microbiologic data. Emerg Infect Dis 1999;5:454-457.

170. National Research Council. The use of drugs in food animals: Benefits and risks. National Academy Press, Washington DC, 1999.

171. NCCLS. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolates from Animals; Information Supplement M32-S1, 2004.

172. Nel H, Van Vuuren M, Swan GE. Towards the establishment and standardization of a veterinary antimicrobial resistance surveillance and monitoring programme in South Africa. Onderstepoort J Vet 2004;71:239-245.

173. Nicholls T, Acar J, Anthony F, Franklin A, Gupta R, Tamura Y, Thompson S, Threlfall EJ, Vose D, van Vuuren M, White DG, Wegener HC, Costarrica ML. Antimicrobial resistance: monitoring the quantities of antimicrobials used in animal husbandry. Rev sci tech Off int Epiz 2001;20:841-847.

174. Nunnery J, Angulo FJ, Tollefson L. Public health policy. Prev Vet Med 2006;73:191-195.

175. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res 2002;66:145-50.

176. Olsen SJ, Ying M, Davis MF, Deasy M, Holland B, Iampietro L, Baysinger CM, Sassano F, Polk LD, Gormley B, Hung MJ, Pilot K, Orsini M, Van Duyne S, Rankin S, Genese C, Bresnitz EA, Smucker J, Moll M, Sobel J. Multidrug-resistant *Salmonella* Typhimurium infection from milk contaminated after pasteurization. Emerg Infect Dis 2004;10:932-935.

177. Oppegaard H, Steinum TM, Wasteson Y. Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. Appl Environm Microbial 2001;67:3732-3734.

178. O'Rourke K. Antimicrobial resistance in wildlife: it's making a bigger splash than you think. J Am Vet Med Assoc 2003;223:756-757.

179. Pai HH, Chen WC, Peng CF. Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). Acta Tropica 2005;93:259-265.

180. Pallecchi L, Luccheti C, Bartoloni A, Bartalesi F, Mantella A, Gamboa H, Carattoli A, Paradisi F, Rossolini GM. Population structure and resistance genes in antibiotic-resistant bacteria from a remote community with minimal antibiotic exposure. Antimicrob Agents Chemother 2007;51:1179-1184

181. Parisien A, Allain B, Zhang J, Mandevill R, Lan CQ. Noel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicoribal peptides. J Appl Microbiol 2008;104:1-13.

182. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control 2006;34:S20-S28.

183. Patrick DM, Marra F, Hutchinson J, Monnet DL, Ng H, Bowie WR. Per capita antibiotic consumption: How does a North American jurisdiction compare with Europe? Clin Infect Dis 2004;39:11-17.

184. Petersen A, Christensen JP, Kuhnert P, Bisgaard M, Olsen JE. Vertical transmission of a fluoroquinolone-resistant *Escherichia coli* within an integrated boiler operation. Vet Microbiol 2006;116:120-128.

185. Phillips I, Casewell M, Cox T, De Groot B, Friis C, Jones R, Nightingale C. Preston R, Waddell J. Does the use of antibiotics in food animals pose a risk to human health? J Antimicrob Chemotherap 2004;53:28-52.

186. Radostits OM. Principles of health management of food-producing animals. In Radostits OM Ed. Herd Health: Food Animal Production Medicine, 3rd Edition. Philadelphia: WB Saunders Company, 2001.

187. Radostits OM, Gay CC, Hinchcliff DW, Constable PD. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats, 10th Edition. Edinburgh; New York: Elsevier Saunders Company, 2007.

188. Rajic A, Reid-Smith R, Deckert AE, Dewey CE, McEwen SA. Reported antibiotic use in 90 swine farms in Alberta. Can Vet J 2006:446-452.

189. Ramchandani M, Manges AR, DebRoy C, Smith SP, Johnson JR, Riley LW. Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli.* Clin Infect Dis 2005;40:251-257.

190. Ribble CS, Meek AH, Shoukri MM, Guichon PT, Jim GK. Risk factors associated with fatal fibrinous pneumonia (shipping fever) in feedlot calves. Proc Am Assoc Bov Pract 1998;31:104-109.

191. Rozen DE, McGee L, Levin BR, Klugman KP. Fitness costs of fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2007;51:412-416.

192. Russell JB, Mantovani HC. The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. J Mol Microbiol Biotechnol 2002;4:347-355.

193. Ryan CA, Nickels MK, Hargrett-Bean NT, Potter ME, Endo T, Mayer L, Langkop CW, Gibson C, McDonald RC, Kenney RT, Puhr ND, McDonnelll PJ, Martin RJ, Cohen ML, Blake PA. Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. J Am Med Assoc 1987;258;3269-3274.

194. Rybak MJ. Pharmacodynamics: Relation to antimicrobial resistance. Am J Infect Control 2006;34:S38-S45.

195. Sato K, Bartlett PC, Saeed MA. Antimicrobial susceptibility of *Escherichia coli* isolates from dairy farms using organic versus conventional production methods. J Am Vet Med Assoc 2005;226:589-594.

196. Schumann FJ, Janzen ED, McKinnon JJ. Prophylactic tilmicosin medication of feedlot calves at arrival. Can Vet J 1990;31:285-288.

197. Schrag SJ, Zell ER, Schuchat A, Whitney CG. Sentinel surveillance: A reliable way to track antibiotic resistance in communities? Emerg Infect Dis 2002;8:496-502.

198. Schroeder CM, White DG, Ge B, Zhang Y, McDermott PF, Ayers S, Zhao S, Meng J. Isolation of antimicrobial-resistant *Escherichia coli* for retail meats purchased in Greater Washington , DC, USA. Int J Food Microbiol 2003;83:197-302.

199. Schroeder CM, White DG, Meng J. Retail meat and poultry as a reservoir of antimicrobial-resistant *Escherichia coli*. Food Microbiol 2004;21:249-255.

200. Schwaber MJ, De-Medina t, Carmeli Y. Epidemiological interpretation of antibiotic resistance studies-what are we missing? Nature Reviews Microbiology 2004;2:979-983.

201. Schwarz S, Cloeckaert A, Roberts MC. Mechanisms and spread of bacterial resistance to antimicrobial agents. In: Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006.

202. Scott HM, Campbell LD, Harvey RB, Bischoff KM, Alali WQ, Barling KS, Anderson RC. Patterns of antimicrobial resistance among commensal *Escherichia coli* isolated from integrated multi-site housing and worker cohorts of humans and swine. Foodborne Path Dis 2005;2:24-37.

203. Shryock TR, Page SW. Growth promotion uses of antimicrobial agents. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006.

204. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. The Lancet Infectious Diseases 2003;3(1):47-51.

205. Singer RS, Reid-Smith R, Sischo WM. Stakeholder position paper: Epidemiological perspectives on antibiotic use in animals. Prev Vet Med 2006;72:153-161.

206. Sidhu MS, Sorum H, Holck A. Resistance to quaternary ammonium compounds in food-related bacteria. Microb Drug Resist 2002;8:393-399.

207. Smith RA, Stokka GL, Radostits OM, Griffin DD. Health and production management in beef feedlots. In Radostits OM, ed. Herd Health: Food Animal Production Medicine 3rd Ed. Philadelphia: WB Saunders, 2001: 600-612.

208. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci 2002;99(9):6434-6439.

209. Smith DL, Dushoff J, Morris JG. Agricultural antibiotics and human health. PLoS Med 2005;2:e232.

210. Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. N Engl J Med 2001;345:1161-1166.

211. Spika JS, Waterman SH, Hoo GW, St. Louis ME, Pacer RE, James SM, Bissett ML, Mayer LW, Chiu JY, Hall B, Greene K, Potter ME, Cohen ML, Blake PA. Chloramphenicol-resistant *Salmonella Newport* traced through hamburger to dairy farms. A major persisting source of human salmonellosis in California. N Engl J Med 1987;316:565-570.

212. Stabler SL, Fagerberg DJ, Quarles CL. Effects of oral and injectable tetracyclines on bacterial drug resistance in feedlot cattle. Am J Vet Res 1982;43:1763-1766.

213. Stege H, Bager F, Jacobsen E, Thougaard A. VETSTAT-the Danish system for surveillance of the veterinary use of drugs for production animals. Prev Vet Med 2003;57:105-115.

214. Stephens CP. Surveillance for antibiotic resistance in veterinary pathogens from the perspective of a regional diagnostic laboratory. Commun Dis Intell 2003;27 Suppl:S127-S131.

215. Stephens DS, Zughaier SM, Whitney CG, Baughman WS, Barker L, Gay K, Jackson D, Orenstein WA, Arnold K, Sc,huchat A, Farley MM, Georgia Emerging Infections Program. Incidence of macrolide resistance in *Streptococcus pneumoniae* after introduction of the pneumococcal conjugate vaccine: population-based assessment. Lancet 2005;365:855-863.

216. Stevenson SML, McAllister TA, Selinger LB, Yanke LJ, Olson ME, Morck DW, Read RR. Transfer of a rifampicin-resistant *Escherichia coli* strain among feedlot cattle. J Appl Microbiol 2003;95:398-410.

217. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69 (6):1456-1459.

218. Summers AO. Generally overlooked fundamentals of bacterial genetics and ecology. Clin Infect Dis 2002;34:S85-S92.

219. Swann MM. Use of antibiotics in animal husbandry and veterinary medicine. UK Joint Committee. London: HM Stationary Office, 1969.

220. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Infect Control 2006;34(5):S3-S10.

221. Thomas S, Andrews AM, Hay NP, Bourgoise S. The antimicrobial activity of maggot secretions: results of a preliminary study. J Tissue Viability 1999;9:127-132.

222. United States Department of Agriculture (USDA) 2000. Part III: Health Management and Biosecurity in U.S. Feedlots, 1999. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N336.1200,1999.

223. United States Department of Agriculture (USDA) 2002. Part II: Reference of Swine Health and Health Management in the United States, 2000. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N355.0202,2000.

224. United States Department of Agriculture, Food and Drug Administration (USDA). [Homepage on the Internet] Animal Drugs for Minor Uses and Minor Species. http://www.fda.gov/cvm/minortoc.htm accessed December 30, 2007.

225. Van Bambeke F, Balzi E, Tulkens PM. Antibiotic efflux pumps. Biochemical Pharmacology 2000;60:457-470.

226. van den Bogaard AE, Bruinsma N, Stobberingh EE. The effect of banning avoparcin on VRE carriage in the Netherlands. J Antimicrob Chemother 2000;46:146-148.

227. Van Donkersgoed J. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. Can Vet J 1992;33:786-795.

228. Van Donkersgoed J, Jewison G, Mann M, Cherry B, Altwasser B, Lower R, Wiggins K, Dejonge R, Thorlakson B, Moss E, Mills C, Grogan H. Canadian beef quality audit. Can Vet J 1997: 38 217-225.

229. Van Donkersgoed J, Jewison G, Bygrove S, Gillis K, Malchow D, McLeod G. Canadian beef quality audit 1998-99. Can Vet J 2001: 42 121-126.

230. Van Donkersgoed J, Manninen K, Potter A, McEwen S. Bohaychuk V, Klashinsky, S, Deckert A, Irwin R. Antimicrobial susceptibility of hazard analysis critical control point *Escherichia coli* isolates from federally inspected beef processing plants in Alberta, Saskatchewan and Ontario. Can Vet J 2003;44:723-728.]

231. van Loo I, Huijsdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Beaujean D, Voss A, Kluytmans J. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis 2007;13:1834-1839.

232. Villarroel A, Morley PS, Wittum TE, Bolte DS. Use of a simulation model to evaluate sampling strategies for characterization of antimicrobial resistance in non-type-specific *Escherichia coli* isolated form dairy cows. Am J Vet Res 2006;67:951-956.

233. Vogel GJ, Saudert SB. The influence of Tylan on liver abscess control and animal performance-A 40 trial summary. J. Anim. Sci Suppl 1 1994: 72, 293.

234. Veterinary Drug Directorate, Health Canada. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health, 2002. http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed December 30, 2007.

235. Veterinary Drug Directorate, Health Canada. Current thinking on risk management measures to address antimicrobial resistance associated with the use of antimicrobial agents in food-producing animals, 2005a. http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed December 30, 2007.

236. Veterinary Drug Directorate, Health Canada. Health Protection Legislative Renewal Stakeholder Meeting on Veterinary Health Products , Ottawa, Ontario, 2005b. http://www.hc-sc.gc.ca/dhp-mps/consultation/vet/consultations/past-anterieures/protection/hplr_feedback_rep-rap_retroaction_e.html accessed December 30, 2007.

237. von Baum H, Marre R. Antimicrobial resistance of *Escherichia coli* and therapeutic implications. Int J Med Microbiol 2005;295:503-511.

238. Vose D, Acar J, Anthony F, Franklin A, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, van Vuuren M, White DG, Wegener HC, Costarrica ML. Antimicrobial resistance: risk analysis methodology for the potential impact on public health of antimicrobial resistant bacteria of animal origin. Rev sci tech Off int Epiz 2001;20:811-827.

239. Wagner BA, Dargatz DA, Salman MD, Morley PS, Wittum TE, Keefe TJ. Comparisons of sampling techniques for measuring the antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. Am J Vet Res 2002;63 (12): 1662-1670.

240. Wagner BA, Dargatz DA, Morley PS, Keefe TJ, Salman MD. Analysis methods for evaluating bacterial antimicrobial resistance outcomes. Am J Vet Res 2003a;64 (12): 1570-1579.

241. Wagner BA, Salman MD, Dargatz DA, Morley PS, Wittum TE, Keefe TJ. Factor analysis of minimum-inhibitory concentrations for *Escherichia coli* isolated from feedlot cattle to model relationships among antimicrobial-resistance outcomes. Prev Vet Med 2003b;57: 127-139.

242. Wagner B, Morley PS, Dargatz DA, Wittum TE, Keefe TJ, Salman MD. Short-term repeatability of measurements of antimicrobial susceptibility of *Escherichia coli* isolated form feces of feedlot cattle. J Vet Diagn Invest 2003c;15:535-542.

243. Walker RD. Antimicrobial susceptibility testing methods and interpretation of results. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing, 2006.

244. Webber MA, Piddock LJV. Antibiotic resistance in *Escherichia coli*. In White DG, Alekshun MN, McDermott PF ed. Frontiers in antimicrobial resistance: A tribute to Stuart Levy. Washington DC: ASM Press, 2005.

245. Weese JS, Roussseau J, Traub-Dargatz JL, Willey BM, McGeer AJ, Low DE. Community –associated methicillin-resistant *Staphlyococcus aureus* in horses and humans who work with horses. J Am Vet Med Assoc 2005a;226:580-583.

246. Weese JS, Archambault M, Willey BM, Dick H, Hearn P, Kreiswirth BN, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JF, Low DE. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. Emerg Infect Dis 2005b;11:430-435.

247. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the house hold. Vet Microbiol 2006;115:148-155.

248. Wegener HC, Aarestrup FM, Jensen LB, Hammerum AM, Bager F. Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. Emerg Infect Dis 1999;5:329-335.

249. Wegener H. Ending the use of antimicrobial growth promoters in making a difference. ASM News 2003: 69:443-448.

250. White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. N Engl J Med 2001a;345:1147-1154.

251. White DG, Acar J, Anthony F, Franklin A, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, Vose D, van Vuuren M, Wegener HC, Costarrica ML. Antimicrobial resistance: standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance. Rev sci tech Off int Epiz 2001b;20:849-858.

252. Whitney CG, Klugman KP. Vaccines as tools against resistance: The example of pneumococcal conjugate vaccine. Sem Ped Infect Dis 2004;15:86-93.

253. WHO. WHO global strategy for containment of antimicrobial resistance. Geneva: World Health Organization; 2001a. http://www.who.int/drugresistance/guidance/en/index.html accessed December 30, 2007.

254. WHO. Surveillance standards for antimicrobial resistance. Geneva: World Health Organization; 2001b.

http://www.who.int/csr/resources/publications/drugresist/whocdscsrdrs20015.pdf accessed December 30, 2007.

255. WHO. Impacts of antimicrobial growth promoter termination in Denmark. WHO/CDS/CPE/ZFK/2003.1. Geneva: World Health Organization; 2003. www.who.int/salmsurv/links/gssamrgrowthreportstory/en/ accessed December 30, 2007.

256. WHO. Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to contain Antimicrobial Resistance due to Non-Human Antimicrobial Use. Report of the second WHO Expert Meeting, Copenhagen, 29-31 May 2007a. http://www.who.int/foodborne_disease/resistance/en/ accessed December 30, 2007.

257. WHO Collaborating Centre for Drug Statistics Methodology. The DDD definition and principles. [Homepage on the Internet]. Oslo, Norway: Norwegian Institute of Public Health [Updated 2007-12-10 b]. http://www.whocc.no/atcddd/ accessed December 30, 2007b.

258. Wierup M. The Swedish experience of the 1986 year ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microb Drug Resist 2001;7:183-190.

259. Wikler MA, Ambrose PG. The breakpoint. In Lorian V, ed. Antibiotics in Laboratory Medicine. Philadelphia: Lippincott, Williams and Wilkins, 2005.

260. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and Salmonella isolates form food animals and humans. Antimicrob Agents Chemother 2001;45(10):2716-2722.

261. Wittum TE, Woollen NE, Perino LJ, Littledike ET. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. J Am Vet Med Assoc 1996;209 (4): 814-818.

262. Woods CR. Antimicrobial resistance: Mechanisms and strategies. Paediatr Respir Rev 2006;7S:SI28-SI29.

263. World Veterinary Association (WVA). WVA policy on the prudent use of antimicrobials. http://www.worldvet.org/Sections-index-req-viewarticle-artid-6.html accessed December 30, 2007.

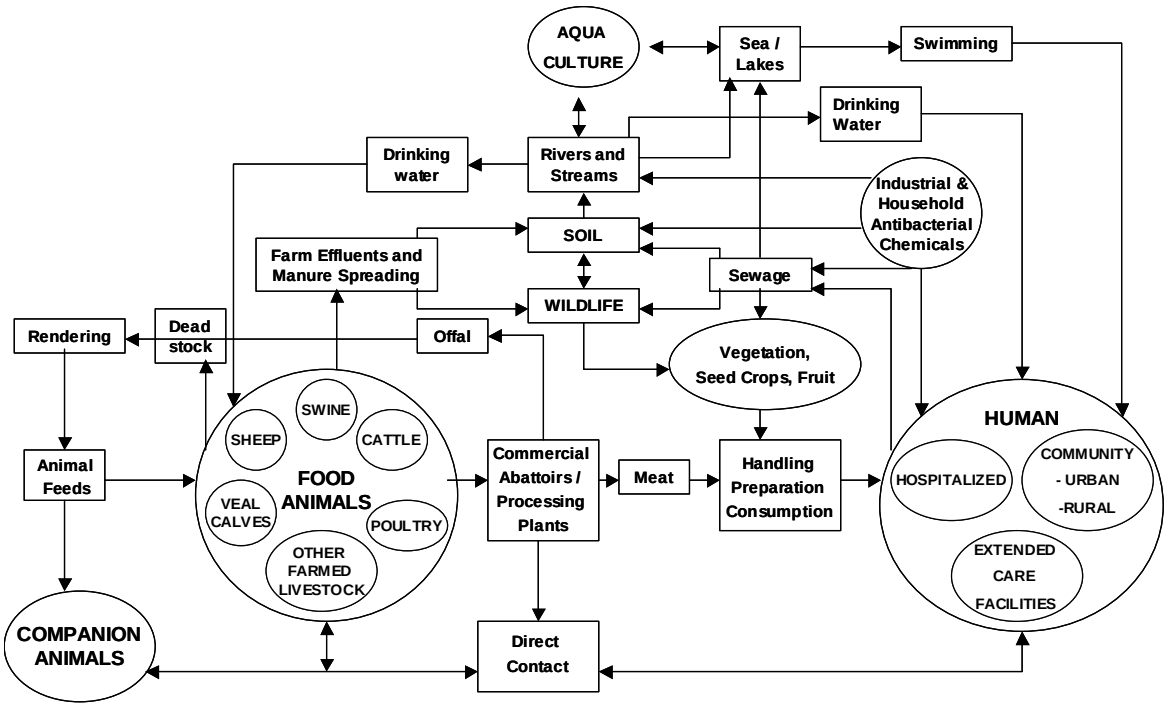
264. Wray C, Gnanou JC. Antibiotic resistance monitoring in bacteria of animal origin: analysis of antional monitoring programmes. Int J Antimicrob Ag 2000;14 :291-294.

265. Yazdankhah SP, Scheie AA, Hoiby A, Lunestad B, Heir E et al. Triclosan and antimicrobial resistance in bacteria: An overview. Microb Drug Resist 2006;12:83-90.

Table 2.1: Resistance mechanisms and the main antimicrobial class affected (based on Acar and Rostel, 2001 and Guardabassi and Courvalin, 2006).

Mechanism	**Main antimicrobial classes affected**	**Description of mechanism**
Enzymatic modification of the antimicrobial	Beta-lactams	Hydrolysis by beta-lactamases
	Aminoglycosides	Acetylation, phosphorylation, or nucleotidylation
	Phenicols (chloramphenicol)	Acetylation
Target modification or replacement	Penicillins	Homologous recombination of bacterial enzyme, antimicrobial target replacement
	Glycopeptides	Antimicrobial target replacement
	Macrolides, lincosamides and streptogramins	Methylation or mutation of ribosomal binding site
	Quinolones	Mutation of bacterial enzyme
	Sulfonamides, trimethoprim	Target replacement
Active efflux	Tetracyclines	SDR pumps
	Macrolides, lincosamides and streptogramins	SDR pumps
	Phenicols	Specific exporters
	Multi-drug resistance	MDR pumps
Reduced antimicrobial uptake	Quinolones, beta-lactams, tetracyclines, and chloramphenicol	Mutation of porins
Target protection	Tetracyclines	Ribosomal protection proteins
Antimicrobial trapping / titration	Sulfonamides, Trimethoprim Glycopeptides	Mutations causing increased antimicrobial targets leading to lowered antimicrobial concentration at the target site

Figure 2.1: The epidemiology of antimicrobial resistance (after Linton 1977a). Used with permission from the Veterinary Drug Directorate (VDD 2002)



CHAPTER THREE
A RANDOMIZED, CONTROLLED CLINICAL TRIAL EVALUATING PREVALENCE OF ANTIMICROBIAL RESISTANCE IN FECAL *E. COLI* ISOLATES AND ASSOCIATIONS WITH FEED AND METAPHYLACTIC ANTIMICROBIAL USE, IN FEEDLOT CATTLE IN WESTERN CANADA

3.1 Introduction

In western Canada, antimicrobials are typically used in fed cattle production for a number of purposes, including injectable metaphylaxis, feed prophylaxis, and individual treatment of sick animals in order to prevent or limit production losses associated with Bovine Respiratory Disease (BRD) (Booker et al., 1999; Radostits, 2001). BRD is one of the most important feedlot diseases, a multifactorial syndrome associated with many concurrent stressors, including mixing, weaning, environmental stress, and long distance transportation (Ribble et al., 1998; Radostits, 2001).

Metaphylaxis is commonly used in western Canada in groups of cattle in the fall of the year when many newly-weaned, auction-market-derived calves are placed in feedlots and are at high risk for BRD. Feed antimicrobials are used early in the feeding period to treat and prevent BRD. Injectable metaphylaxis and feed prophylaxis in high risk groups of animals reduce BRD morbidity and improve average daily gain and feed efficiency (Schumann et al., 1990; Harland et al., 1991; Van Donkersgoed, 1992; Morck et al., 1993; Gallo and Berg, 1995). Many studies have shown economic and animal health benefits from metaphylactic and prophylactic AMU in the cattle feedyard

(Harland et al., 1991; Gallo and Berg, 1995; Wittum et al., 1996; Booker et al., 1997; Jim et al., 1999; Gibb et al., 2006).

In western Canada, 20-50% of feedlot placements are treated with injectable metaphylaxis on arrival, depending on the risk profiles of the group of calves involved (Calvin Booker, Personal Communication, Feedlot Health Management Services, 2007; Radostits, 2001). The NAHMS 1999 Feedlot report described injectable metaphylaxis use by 27% of small feedlot operations and 81% of large feedlot operations in the USA (USDA, 2000). This represents 8% of cattle in small feedlot operations and 11% of cattle in large feedlot operations in the USA (USDA, 2000). Feed or water antimicrobials were used by 85% of small operations and 78% of large operations (USDA, 2000).

However, antimicrobial use (AMU) in animal agriculture is considered by many to be a major factor in the development of antimicrobial resistance (AMR) in bacteria that then caused infections in people that were difficult to treat (Khachatourians, 1998; McGeer, 1998). Concerns about the uses of antimicrobials in animals, and recommendations for prudent use were first expressed many years ago (Swann, 1969) and echoed later (Apley, 1998; Lipsitch et al., 2002; Smith et al., 2002; Singer et al., 2003). The use of prophylactic feed antimicrobials was banned in some European countries and was under scrutiny in Canada (Anonymous, 1998; Veterinary Drug Directorate, 2002), even though 'the extent to which the use of antimicrobials in the agricultural and aquaculture sectors contributes to antimicrobial resistance among bacteria affecting humans has been difficult to establish' (Conly, 2002). Antimicrobial use has been associated with antimicrobial resistance, usually on a local scale (Berge et

al., 2005a; Berge et al., 2005b; Berge et al., 2006; Dunlop et al., 1998; Finlayson et al., 1973; Funk et al., 2006; Howe et al., 1976; Inglis et al., 2005; Lowrance et al., 2007; O'Connor et al., 2002; Stabler et al., 1982), but most notably in Europe where the use of avoparcin as a growth promotant was associated with the presence of *Enterococcus faecium* resistant to vancomycin in pigs and poultry (Bager et al., 1997).

The objectives of this study were to determine the prevalence of antimicrobial resistance in fecal *Escherichia coli* isolates from newly-weaned, auction-market-derived calves on arrival at the feedlot, and to examine associations between metaphylactic (injectable) and prophylactic (feed) antimicrobial use in groups of cattle and the prevalence of antimicrobial resistant fecal *E. coli* during the feeding period.

3.2 Materials and Methods

3.2.1 Trial Facilities

The trial was conducted at the small-pen research feedlot at the University of Saskatchewan which has a one-time capacity of approximately 800 head. The calves were housed in open-air pens with dirt floors, 20% porosity fencing, and a central alley for feeding. Each pen was 286 m^2 with feed bunks 7.4 m in length. Waterers were shared between two pens.

3.2.2 Trial Animals

In fall 2000, 288 auction-market-derived, 256-353 kg, Charolais cross steers were purchased in Saskatchewan and brought to the feedlot facility at the University of Saskatchewan. The calves were allowed to adjust to the feed bunks for several days

while the entire group was accumulated. This also ensured adequate intake of the feed antimicrobial once the trial began. These calves underwent routine processing on arrival at the feedlot. Processing included vaccination with a multivalent Clostridial vaccine with *Histophilus somni* [(Fermicon 7-Somnugen™, Pfizer Canada Inc, London, Ontario) or (Ultrabac™7/Somnubac™, Pfizer Canada Inc, London, Ontario)], vaccination with a modified live vaccine for Infectious Bovine Rhinotracheitis and Parainfluenza 3 (Bovishield™ IBR-PI_3, Pfizer Canada Inc, London, Ontario), topical endectocide treatment (Ivomec® Pour-On, Merial Canada Inc., Baie D'Urfe, Quebec), eartags, and a progesterone-estradiol benzoate hormonal implant (Synovex®-S, Ayerst, Veterinary Laboratories, Guelph, Ontario). A routine open castration was performed on one calf with a retained testicle. At 90 DOT, a second vaccination with a modified live vaccine for Infectious Bovine Rhinotracheitis and Parainfluenza 3 (Bovishield™ IBR-PI_3, Pfizer Canada Inc, London, Ontario) and a second hormonal implant were given before switching the animals to a finishing diet. The feeding program consisted of grass hay, barley silage, and a barley-based concentrate. Monensin sodium 3% (Rumensin®; Elanco Animal Health, Guelph, Ontario) was fed to all cattle during the entire feeding period in the total mixed ration at 27-28 ppm DM.

All sick animals were treated for individual animal illness when necessary using routine feedlot protocols developed by the feedlot veterinarian. Animals that were identified as sick during the first 21 DOT, with a rectal temperature over 40.5^{o}C with no other identifiable cause of disease were considered to have BRD. The first line treatment was tilmicosin (Micotil®, Provel, Division Eli Lilly Canada, Guelph, ON, Canada) at the label dose of 10 mg/kg body weight (BW). Relapses were defined as

cases needing re-treatment for BRD within a two week period where further clinical signs referent to the respiratory tract were seen. The second line of treatment was florfenicol (Nuflor®, Schering-Plough Animal Health, Pointe Claire, Quebec) at the label dose of 40 mg/kg BW, and the third treatment, if necessary, was trimethoprim/sulfadoxine (Trivetrin™ Injection, Schering-Plough Animal Health, Pointe Claire, Quebec) at the label dose of 16 mg/kg BW. The other disease condition where individual animal antimicrobial treatments were required was bovine interdigital necrobacillosis (footrot). The case definition for footrot was a lame animal with a swollen foot and no other cause for the lameness. The suggested treatment protocol was ceftiofur sodium (Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, Ontario) once daily for two to three days at the approved dose. Procaine Penicillin G was also used for the treatment of footrot [(Depocillin®, Intervet Canada Ltd., Whitby, Ontario) or (Ethacillin, Pfizer Canada Inc., London, Ontario)] at a dosage of 500,000 IU per 50 kg of BW).

3.2.3 Experimental Design

Three treatment groups were compared in this trial: 1) Control, where no antimicrobials were given on arrival, 2) Feed, where oxytetracycline (Terramycin*-50 Premix, Pfizer Canada Inc., London, Canada) used at 110g/kg active ingredient fed in the starter ration at 2g/head/day for 14 days beginning at 0 DOT, and 3) Injectable, where the approved dose of 20 mg/kg body weight of long acting oxytetracycline (Liquamycin* LA-200*, Pfizer Canada Inc., London, Canada) was administered

subcutaneously at 0 DOT. The oxytetracycline premix was fed at a dose higher than the approved level but at a common dose used in western Canadian feedlots.

Pens within the feedlot were randomly assigned to one of the three treatments with adjoining pens that shared waterers assigned to the same treatment protocol. Twelve steers were randomly assigned by weight blocks into each of the 24 pens. Animals were moved through the handling facilities in a specified order: Control, Metaphylaxis (injectable), and Prophylaxis (feed).

Fecal samples were collected on arrival prior to treatment (designated as 0 DOT but taken as each truckload arrived), after injectable antimicrobial treatment was finished (7 DOT), at the end of the feed antimicrobial treatment (15 DOT), and also 35 DOT, 70 DOT, 100 DOT, 150 DOT, and preslaughter. This preslaughter sample varied between 168 and 248 DOT based on when the steers were ready for slaughter. Cattle were shipped for slaughter with a back fat measurement of eight mm on ultrasound and a maximum finish weight of 750 kg. It was thought important to ensure the last fecal sample was collected 24 hours prior to slaughter as this timing was potentially important when looking at the risk of AMR transferring to people via the food chain. The feedlot workers and the laboratory staff were blinded as to the treatment groups as well as the objectives of the study.

Fresh fecal samples were collected from the rectum of each steer. A new, plastic obstetrical glove was used for each animal. The samples were placed into clean Styrofoam cups with lids, labeled and taken to the lab within two hours.

3.2.4 Laboratory Analysis

Fresh feces were cultured overnight on MacConkey's agar. Identification of *E. coli* was confirmed by standard biochemical tests. Three individual isolates were randomly chosen for subculture in litmus milk and stored at –70^{o}C until a large group of antimicrobial susceptibilities could be performed together. The subcultures were thawed and immediately cultured on blood agar. From each blood agar plate, *E. coli* colonies were inoculated into Phosphate Buffer Sterile (PBS) to make standard solutions of 0.5 MacFarland. This solution was delivered onto Mueller-Hinton agar using the replicator technique. The MICs of seven antimicrobials were determined using the Mueller-Hinton agar dilution method. The antimicrobials tested were ampicillin (AMP), enrofloxacin (ENR), gentamicin (GEN), sulphamethoxazole (SMX), tetracycline (TCY), trimethoprim (TMP), and trimethoprim/sulfanilamide (TMP/SSS). The Mueller-Hinton plates were cultured at 37 C and antimicrobial susceptibilities were read between 18 and 24 hours. A control strain of *E. coli* ATCC 25922 was included with each plate. Antimicrobial breakpoints and interpretation were from the CLSI standards (Table 3.1) (CLSI, 2006; NCCLS, 2004). All laboratory procedures were carried out according to CLSI standards.

3.2.5 Statistical Analysis

The measurement, $ADD_{Feedlot}$, was used to quantify the number of actual individual antimicrobial treatments given at the approved dose of the antimicrobial. This does not include feed antimicrobials or routine metaphylaxis. This measurement accounted for the dosage and duration of action of the antimicrobial (Table 3.2). This concept of

$ADD_{Feedlot}$ was based on that of Defined Daily Dose (DDD) used in the human literature (Austin et al., 1997; Austin et al., 1999). Each antimicrobial treatment was described as 0, 1, 2, or 3 $ADD_{Feedlot}$ (Table 3.2), and example calculation was shown.

Example: An animal was treated with 27 cc of long acting oxytetracycline on November 9 (BW 270 kg) and 40 cc of oxytetracycline on January 19 (BW 400 kg).

20 mg/kg * 270 kg = 5400 mg, 5400 mg / (200mg/ml) = 27 ml (actual dose given)

This was a long acting treatment, equivalent to 3 $ADD_{Feedlot}$

20 mg/kg * 400 kg = 8000 mg, 8000 mg / (200mg/ml) = 40ml (actual dose given)

This was a long acting treatment, equivalent to 3 $ADD_{Feedlot.}$

The total $ADD_{Feedlot}$, actually given to this animal, was 6.

Isolates classified as either susceptible or intermediate were considered sensitive for this analysis. Resistant animals were defined as those with one or more isolates resistant to one or more antimicrobials at a specific time period. Multidrug resistant animals were defined as those with one or more isolates resistant to more than three antimicrobials at a specific time period. The hierarchical structure of the data involved 24 pens with 12 calves per pen (n=288). There were three treatment groups (control, metaphylaxis, feed antimicrobials) allocated at the pen level, with eight pens per treatment. Fecal samples were collected at the animal level, as were antimicrobial treatments given to animals for disease treatment. There were three isolates analyzed per animal, for a total of 864 isolates per test day, and 6912 isolates total over eight biologically important but unevenly spaced sampling days.

Descriptive statistics were calculated using commercial software. Baseline differences in resistance to the five antimicrobials on arrival between the three treatment groups were assessed using logistic regression. Five arrival models were built, one for each binary outcome (SPSS for Windows 15.0.0, SPSS Inc., Chicago, USA), for BRD, footrot, and resistance to the five antimicrobials (AMP, SMX, TCY, TMP/SSS and TMP). No adjustments for clustering were done at arrival as no information was available on the purchase lots of the animals. Exact confidence intervals for animal-level prevalence estimates were calculated (PEPI v 4, Sagebrush Press, Salt Lake City, USA) (Abramson, 2001).

A logistic regression model using generalized estimating equations was used to examine treatment effects in this trial, with adjustment for repeated measurements within animal using an autoregressive correlation structure (Proc Genmod, SAS for Windows v. 9.1, SAS Institute, Cary, USA). Binary AMR outcomes evaluated were resistance to AMP, SMX and TCY, at the animal-level, as these were the most prevalent resistance outcomes in this trial and in the literature (GOC, 2007). The first variables examined were treatment group (Injectable, Feed or Control) and sample time (Time 1-8) following the design of the clinical trial. Unconditional associations were first examined between each outcome and each potential risk factor. A multivariable model was built for each outcome if risk factors were identified as potentially significant through unconditional associations ($P \leq 0.20$).

The effect of individual animal antimicrobial treatments on the proportion of resistant isolates preslaughter was examined using a marginal logistic regression model using generalized estimating equations, while adjusting for clustering by pen using an

exchangeable correlation structure (Proc Genmod, SAS for Windows v. 9.1, SAS Institute, Cary, USA). Individual animal antimicrobial treatments used (Treated, Treated in last 100 DOT, $ADD_{Feedlot}$ Overall, $ADD_{Feedlot}$ in last 100 DOT) were assessed as covariates in the model that included group treatment (metaphylaxis or feed antimicrobials) using a forwards stepwise method. The individual animal antimicrobial use covariates were not independent from each other, so they were not evaluated in the same model. Individually, they represented slightly different aspects of AMU so they were all investigated. No risk factors were forced into the model. Risk factors excluded from the final model were checked for confounding first. First order interaction terms of biological significance were examined in the final model. The final models included risk factors and interaction terms with a significance level of $P<0.017$

A marginal logistic regression model using generalized estimating equations was also used to specifically examine the treatment and time effect in this trial between arrival and preslaughter. Adjustment for clustering by animal used an autoregressive correlation structure (Proc Genmod, SAS for Windows v. 9.1, SAS Institute, Cary, USA). A Bonferroni correction was used because there were three outcomes examined with different models. The level of significance was therefore set at $P=0.05/3=0.017$. The models were further developed as described above.

3.3 Results

The cumulative incidence of bovine respiratory disease (BRD) morbidity was 19% (54/288) during this trial. In the control, feed, and injectable groups, the incidence was 22.9%, 16.7%, and 13.5% respectively. These values were not significantly different at

the pen level (*P*=0.27). The incidence of footrot morbidity overall was 23% (67/288). The incidence was 27.1%, 20.8%, and 21.9% in the control feed and injectable groups respectively. These values were also not significantly different at the pen level (p=0.50). The average treatment DOT was 148 days. Samples could not be collected from two animals on arrival, four animals at D7, three animals at D15, five animals at D35, three animals at D70, four animals at D105, five animals at D154, and 15 animals preslaughter. The overall proportion of mortality for this trial was 2.8% (8/288). The deaths/euthanasias were attributable to acute bloat (3/8), a trichobezoar (1/8), *Histophilus somni* (1/8), chronic pneumonia (2/8) and a fractured leg (1/8). Five, two and one animals withdrew from the feed, control, and injectable groups respectively.

3.3.1 Arrival

There were no isolates in this trial with resistance to GEN or ENR. On arrival at the feedlot, prevalence of fecal *E. coli* isolates from calves with no resistance to any of the antimicrobials tested was 88.9% (Table 3.3). At the animal level this was 81.1% (Table 3.4). The most prevalent antimicrobial to which resistance was found at the isolate level was tetracycline at 6.0%. At the animal-level, 9.5 % of animals had at least one isolate that was tetracycline resistant. Resistance to three or more antimicrobials was found in 2.1% of isolates and 3.2% of animals. Resistance to the five antimicrobials did not vary significantly among the treatments groups on arrival (*P*>0.13).

3.3.2 AMU associations with AMR

This clinical trial looked at antimicrobial interventions in a research feedlot under commercial feedlot management conditions. During the trial, some of the animals developed disease conditions that had to be treated with antimicrobials on an individual animal basis. In this cohort of animals, there were 100/288 animals (35%) treated on an individual animal basis over the course of the feeding period. The median $ADD_{Feedlot}$ for individual animal treatments was 0 (range, 0-13.2) (Table 3.5). There were 57/276 individual animals (21%) treated in the last 100 days before slaughter, mostly for footrot (Table 3.6).

The proportion of animals with at least one of the three isolates resistant to AMP, SMX, or TCY changed over time (Table 3.7). The associations between treatment and AMR for TCY and SMX also varied with time on feed (Figures 3.1-3.3). At Day 15, an animal was 115 times (P<0.0001; 95% CI: 34.5-386.5) more likely to have one or more fecal *E. coli* isolates resistant to TCY than at Day 0, when oxytetracycline was used in the feed. At Day 15, an animal was 3.7 times (P<0.0001; 95% CI: 2.0-6.9) more likely to have one or more fecal *E. coli* isolates resistant to TCY than at Day 0, when injectable oxytetracycline was used metaphylactically on arrival. At Day 15, an animal was 2.7 times (P=0.009; 95% CI: 1.3-5.9) more likely have one or more fecal *E. coli* isolates resistant to TCY than at Day 0, in the control group. The same trend was present at Day 154 where animals were 16.7, 6.9 and 7.9 times more likely to have one or more fecal *E. coli* isolates resistant to TCY than at Day 0 in the feed, injectable and control groups respectively [(P<0.0001; 95% CI: 8.5-33.0), (P<0.0001; 95% CI: 3.3-14.3), (P<0.0001; 95% CI: 3.8-12.6)].

In addition, at Day 15, an animal was 17.4 times (P<0.0001; 95% CI: 8.6-35.0) more likely to have one or more fecal *E. coli* isolates resistant to SMX than at Day 0, when oxytetracycline was used in the feed. At Day 15, an animal was 2.8 times (P=0.04; 95% CI: 1.1-7.5) more likely to have one or more fecal *E. coli* isolates resistant to TCY than at Day 0, when injectable oxytetracycline was used metaphylactically on arrival. There was no significant difference in the control group. The same trend was present at Day 154, as with TCY resistance, where animals were 7.4, 7.0 and 5.9 times more likely to have one or more fecal *E. coli* isolates resistant to SMX than at Day 0 in the feed, injectable and control groups respectively [(P<0.0001; 95% CI: 3.6-15.1), (P<0.0001; 95% CI: 2.7-18.2), (P=0.0003; 95% CI: 2.2-15.4)].

The final model for AMP contained only treatment group and sample time, but no interaction term. Feed oxytetracycline was associated with a significantly higher proportion of animals with one or more fecal *E. coli* isolates resistant to AMP compared to the control group, while adjusting for sample time. The odds that an animal in the feed prophylaxis group would be resistant to AMP were 2.2 times higher than the odds that an animal in the control group would have one or more fecal *E. coli* isolates resistant to AMP (P=0.0001; 95% CI: 1.5 – 3.2), while adjusting for sample time. The odds that an animal in the injectable oxytetracycline metaphylaxis were not significantly different from the control group (P=0.02), while adjusting for time. AMP resistance on days 7, 15, 154 and preslaughter was significantly higher from Day 0 after adjusting for treatment group (P=0.006). No measures of individual animal antimicrobial treatment (Treated, Treated in last 100 DOT, $ADD_{Feedlot}$ Overall,

$ADD_{Feedlot}$ in last 100 DOT) were associated with a higher proportion of *E. coli* isolates resistant to TCY, SMX or AMP preslaughter.

Models built to compare only arrival and preslaughter results for TCY, SMX and AMP had only one significant variable time. The proportion of animals with one or more *E. coli* isolates resistant to tetracycline was not different between the treatment groups preslaughter ($P>0.14$). The odds of an animal having at least one isolate resistant to TCY preslaughter were 6.4 times higher than at arrival ($P<0.0001$; 95% CI: 4.3-9.6). The odds of an animal having at least one isolate resistant to SMX preslaughter were 4.2 times higher than at arrival ($P<0.0001$; 95% CI: 2.6-7.0). The odds of an animal having at least one isolate resistant to AMP preslaughter were 3.8 times higher than at arrival ($P=0.0003$; 95% CI: 1.8-8.0).

3.4 Discussion

E. coli was chosen as the indicator commensal organism in this study because was easy to isolate from all animals and is an important carcass contaminant at slaughter (Stopforth et al., 2006). *E. coli* is a potential reservoir of resistance genes that could transfer resistance to other zoonotic or commensal organisms that might cause disease in cattle or people (Blake et al., 2003; Linton et al., 1977; Winokur et al., 2001). Because of this, a fecal sample was collected during the 24 hours prior to shipping for slaughter, to be representative of bacteria at the stage of production that might ultimately affect the consumer. Three isolates were randomly chosen from each animal to represent the fecal *E. coli* isolate patterns in individual animals. Porosity fencing and waterer allocation helped prevent fecal contamination between pens assigned different

treatments. During laboratory analysis, no further passage of isolates occurred in vitro, beyond that described above, that might have contributed to loss of plasmids coding for AMR.

One of the objectives of this trial was to characterize AMR in fecal *E. coli*, from newly weaned, auction market derived calves on arrival at the feedlot, as an indication of 'baseline' resistance. This has not been well characterized in feedlot cattle in western Canada and could represent resistance patterns and AMU from the herd of origin or auction market. On arrival, calves had relatively low proportions of resistant fecal *E. coli* isolates. In a western Canadian study with similar methodologies, the proportion of animals, on arrival, with one or more isolates resistant to TCY was similar (17.6%), but the proportion of animals with one or more isolates resistant to SMX (44.4%) and AMP (20.3%) was higher than in this study, perhaps due to differences in the farm of origin, previous AMU, and other history of the calves (Checkley et al., 2008).

Direct comparisons between this and other studies can not be made due to differences in methodology and analysis so further comparisons are only meant in general terms. A recent study in western Canada analyzing *E. coli* isolates from calves pre-weaning had a similar adjusted isolate-level prevalence of AMP at 1.6% TCY at 5.0 % and SMX at 4.0% (Gow et al., 2008). The proportions of isolates resistant to TCY and SMX (9.8% and 4.7% respectively) in this study were lower than those found in a study of individual and pooled fecal *E. coli* samples from feedlot pens and individual feedlot animals (28.1-31.8 % and 16.9-25.7%) in the USA, possibly because those samples were taken later in the feeding period (Wagner et al., 2002). In this study, the proportion of animals with one or more TCY resistant *E. coli* isolates (16.4%) was

similar to that from an American study of pasture fecal samples collected from newly weaned calves where 13 to 17% of fecal samples contained one or more *E. coli* isolate resistant to TCY (Huston et al. 2003).

At the time of this trial, GEN was only licensed for intrauterine use, ENR was not licensed for use in cattle in Canada, and no resistance was found to them at any time period during this study, or in another western Canadian study (Checkley et al., 2008). A feedlot study in the USA had only one isolate resistant to ciprofloxacin, a drug from the same family as ENR (Wagner et al., 2002). These antimicrobial classes were important in human medicine so they were evaluated, even though they were not commonly used, as cross resistance and co-resistance with other related and unrelated antimicrobials can occur (Guardabassi, 2006).

Few studies have fully explored associations between AMU and AMR in feedlot cattle. Direct comparisons of results between studies of different species and different sectors of cattle production were not made as AMU and management factors are extremely different and this has a potential effect on the results (Radostits, 2001). This study showed that the use of oxytetracycline in the feed was associated with an increased proportion of animals with one or more fecal *E. coli* isolates resistant to TCY and SMX. This was not surprising as it is well known that the use of feed or water antimicrobials was associated with the development of AMR in other species (Dunlop et al., 1998; Finlayson et al., 1973; Funk et al., 2006; Howe et al., 1976). Other studies in cattle have also found associations between AMR and feed antimicrobials (Berge et al., 2006; Inglis et al., 2005; Stabler et al., 1982). The field trial by Berge et al. involved calves less than four weeks of age in a calf rearing facility, where AMU in the milk

replacer selected for highly resistant *E. coli*. The clinical trial by Inglis et al., in a research feedlot, suggested that subtherapeutic administration of tetracycline alone or in combination with sulfamethazine selected for AMR in *Campylobacter* species. The dose of chlortetracycline in the Inglis trial was lower than the dose used in the current trial, where oxytetracycline was used. Similar to the current trial, the Inglis trial found no association between antimicrobials administered in the feed and AMP resistance. Although carried out many years ago, the clinical trial by Stabler et al. reported that TCY resistance increased in heifers fed therapeutic and subtherapeutic doses of chlortetracycline in a research feedlot. Again similar to our trial, the authors found no association between in feed AMU and AMP resistance (Stabler et al., 1982).

In this study, the mass use of injectable oxytetracycline on arrival at the feedlot was associated with an increased proportion of animals with one or more isolates resistant to TCY and SMX, but not as strong as the association with the use of feed antimicrobials. In other studies, there has been no or a much less pronounced association between injectable antimicrobials and AMR than with feed antimicrobials and AMR (Berge et al., 2006; Berge et al., 2005a; Lowrance et al., 2007; Stabler et al., 1982). In the field trial by Berge et al., individual antimicrobial therapy was associated with a transient increase in AMR in fecal *E. coli* of calves in a calf grower facility (Berge et al., 2006). In another trial by Berge et al., AMR patterns in *E. coli* of feedlot steers were assessed after a single approved dose of florfenicol (a long-acting antimicrobial); a transitory shift to multiple resistant fecal *E. coli* was seen (Berge et al., 2005a). This second trial by Berge et al. also had complex interactions in the statistical analysis including that between treatment and time, as seen in this study.

The cohort study by Lowrance et al. found that and injection of ceftiofur crystalline-free acid was associated with a transiently increased proportion of multi-drug resistant fecal *E. coli* (Lowrance et al., 2007). There was also a transient effect of injectable oxytetracycline use (three daily approved doses) on tetracycline resistance in fecal *E. coli* from feedlot cattle in the Stabler clinical trial (Stabler et al., 1982). Findings from this trial can also be loosely compared with those from another Canadian study where associations between AMR in fecal *E. coli* isolates from bulls at a test station and individual AMU were found when feed antimicrobials were also used (O'Connor et al., 2002). In the study by O'Connor, the use of injectable oxytetracycline in individual cattle receiving chlortetracycline in the feed was associated only with increased prevalence of resistance to chloramphenicol and sulfisoxazole in fecal *E. coli* isolates (O'Connor et al., 2002).

An interesting increase in the proportion of animals with one or more resistant isolates was noticed in all treatment groups later in the feeding period (Figure 3.1, Figure 3.2, Figure 3.3). This later increase in the proportion of animals with one or more resistant isolates was not limited to the animals that originally received mass treatment with feed antimicrobials. Several explanatory hypotheses were considered. Of the 100 antimicrobial treatments given during the feeding period, 57 (57%) were given during the last 100 days on feed. Most of these treatments were for footrot, the incidence of which was perceived by feedlot staff to be higher than average. These late individual animal antimicrobial treatments could have contributed to the later increase in the proportion of animals with one or more resistant isolates, but associations with individual animal antimicrobial treatments late in the feeding period were not

significant. Although not significant, perhaps these treatments did play a role in the increased proportion of resistant organisms either in combination with other selective forces suggested below. Or, the study might also have been lacking in power to see this association as it was not designed for this purpose.

A second potential explanation for this increase is the change in ration during the feeding period. Another feedlot study isolated a higher proportion of TCY resistant *Campylobacter* spp. from animals on a finishing (high-grain) diet than from animals on a backgrounding (high-forage) diet (Inglis et al., 2005; Alexander et al., 2008). The secondary increase in our trial also occurred after the animals were switched onto a finishing (high-grain) diet. The change in diet may create a selective advantage for some resistant bacteria perhaps through a decrease in the pH of the rumen or perhaps feed could have been a vector of AMR genes (Dargatz et al., 2005).

A third mechanism for increasing resistance is also possible. Mobile genetic elements could be shared among the different treatment groups over time (Aarestrup, 2006). Early increases in proportions of animals with one or more resistant isolates were seen in animals following mass treatment with feed antimicrobials. This was likely due to clonal proliferation of resistant *E. coli* strains related to the selective pressure from AMU; AMU kills susceptible bacterial strains (Aly et al., 1970) and allowed resistant strains to flourish (Lowrance et al., 2007). The normal flora (based on AMR phenotype) was re-established, to some extent, after the AMU pressure decreased, similar to the Lowrance study (Lowrance et al., 2007). It seemed possible that some of the resistant isolates had a bacterial fitness advantage which was spread horizontally across the feedyard through mobile genetic elements through the processes of conjugation and

transformation, eventhough attempts were made to limit horizontal spread between treatment groups. Horizontal spread has been suggested in the literature; antimicrobial resistant *E. coli* isolates rapidly colonized neonatal dairy calves (Donaldson et al., 2006; Hoyle et al., 2005). In the study by Donaldson et al., the highest prevalence of multidrug resistance was in two week-old calves, and then the prevalence declined. In the study by Hoyle et al., a distinct antimicrobial resistant strain spread through a cohort of beef calves over 22 weeks, associated with time and environment but not AMU. However, other studies have not described this type of widespread horizontal spread of resistance across all pens and all treatment groups (Stevenson et al., 2003; Inglis et al., 2005).

This study demonstrated that calves arrived at the feedlot with a relatively low prevalence of AMR in commensal *E. coli* isolates from the feces. Use of feed antimicrobials for disease prophylaxis in groups of calves was associated with pronounced increases in proportions of cattle with one or more TCY or SMX resistant *E. coli* isolates early in the feeding period. The metaphylactic use of long-acting injectable oxytetracycline was also significantly associated with increased proportions of cattle with one or more resistant *E. coli* isolates during the feeding period. Individual animal AMU was not a significant risk factor in these associations. The proportion of animals with one or more *E. coli* isolates resistant to tetracycline was not different between the treatment groups preslaughter; however, there were significantly more animals with tetracycline resistance in one or more isolates of *E. coli* preslaughter than at arrival.

3.5 Acknowledgements

The authors thank the Saskatchewan Agricultural Development Fund for their support of this project. We also thank the staff of the University of Saskatchewan Beef Cattle Research Unit for their care of the cattle and help with the project, and Fei Huang and Jaime Gerun for technical support in the laboratory.

3.6 References

1. Aarestrup FM. The origin, evolution and local and global dissemination of antimicrobial resistance. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006

2. Abramson JH, Gahlinger PM. Computer programs for epidemiologists: PEPI version 4.0. Sagebrush Press, Salt Lake City, USA, 2001.

3. Alexander TW, Yanke LG, Topp E, Olson ME, Read RR, Morck DW, McAllister TA. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Appl Environ Microbiol 2008;74:4405-4416.

4. Aly R, Maibach HI, Strauss WG, Shinefield HR. Effects of a systemic antibiotic on nasal bacterial ecology in man. Appl Microbiol 1970;20:240-244.

5. Anonymous. EU bans four antibiotic feed additives. Vet Rec 1998;143:165.

6. Apley, M. Does antimicrobial use in animals affect human health? *The Bovine Proceedings* 1998;31:9-12.

7. Austin DJ, Kakehashi M, Anderson RM. *Proc Roy Soc London Ser B* 1997;264:1629-38.

8. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci* 1999;95:1152-1156.

9. Bager F, Madsen M, Christensen J, Aarestrup FM. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish Poultry and pig farms. Prev Vet Med 1997;31:95-112.

10. Berge ACB, Epperson WB, Pritchard RH. Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal *Escherichia coli.* Vet Res 2005A;36:723-734.

11. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005B;69:25-38.

12. Berge ACB, Moore DA, Sischo WM. Field trial evaluating the influence of prophylactic and therapeutic antimicrobial administration on antimicrobial resistance in fecal *Escherichia coli* in dairy calves. Appl Environ Microbiol 2006;72:3872—3878.

13. Blake DP, Hillman K, Fenlon DR, Low JC. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J Appl Microbiol 2003;95:428-436.

14. Booker CW, Jim GK, Guichon PT, Schunicht OC, Thorlakson BE, Lockwood PW. Evaluation of florfenicol for the treatment of undifferentiated fever in feedlot calves in western Canada. *Can Vet J* 1997;38:555-560.

15. Booker CW, Guichon PT, Schunicht OC, Wildman BK, Jim GK. Economic impact of antimicrobial use in feedlots. Bovine Proceedings 1999;32:111-112.

16. Checkley SL, Campbell JR, Chirino-Trejo M, Janzen ED, McKinnon JJ. Antimicrobial resistance in generic fecal *Escherichia coli* obtained from beef cattle on arrival at the feedlot and prior to slaughter, and associations with volume of total individual cattle antimicrobial treatemetns in one western Canadian feedlot. Can J Vet Res 2008:72; (In Press).

17. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. (Use for Humans). CLSI document M100-S16, 2006.

18. Conly J. Antimicrobial resistance in Canada. *CMAJ* 2002:167 (8):885-91.

19. Dargatz DA, Fedorka-Cray PJ, Ladely SR, Ferris KE, Green AL, Headrick ML. Antimicrobial Susceptibility patterns of Salmonella isolates from cattle in feedlots. J Am Vet Med Assoc 2002;221:268-72.

20. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. Appl Environ Microbiol 2006;72:3940-3948.

21. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. *Prev Vet Med* 1998;34:283-305.

22. Finlayson M, Barnum DA. The effect of chlortetracycline feed additive on the antibiotic resistance of fecal coliforms of weaned pigs subjected to experimental *Salmonella* infection. Can J Comp Med 1973;37;63-69.

23. Funk JA, LeJeune JT, Wittum TE, Rajala-Schultz PJ. The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. Microbial Drug Resistance 2006;12:210-218.

24. Gallo GF, Berg JL. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. *Can Vet J* 1995:36: 223-229.

25. Gibb DJ, Schwartzkopf-Genswein KS, McAllister TA, Genswein BMA, Streeter M. Effect of sub-therapeutic antibiotics and auction exposure on health, performance, and feeding behavior of weaned calves. Can J Anim Sci 2006:86:457-460.

26. Government of Canada (GOC). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph, ON: Public Health Agency of Canada, 2007. http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html accessed December 30, 2007.

27. Gow SP, Waldner CL, Rajic A, McFall ME, Reid-Smith R. Prevalence of antimicrobial resistance in fecal generic *Escherichia coli* isolated in western Canadian cow-calf herds. Part 1-Beef calves. Can J Vet Res 2008:72; (In Press).

28. Guardabassi L, Courvalin P. Antimicrobial resistance in Bacteria of Animal Origin. In Aarestrup FM, ed. Antimicrobial resistance in bacteria of animal origin. Washington: ASM Press 2006.

29. Harland RJ, Jim GK, Guichon PT, Townsend HGG, Janzen ED. Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves. *Can Vet J* 1991;32: 163-168.

30. Howe K, Linton AH. The effect of tetracycline on the coliform gut flora of broiler chickens with special reference to antibiotic resistance and 0-Serotypes of *Escherichia coli*. J Appl Bacteriol 1976;41;453-464.

31. Hoyle DV, Yates CM, Chase-Topping ME et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71 (11):6680-6688

32. Huston CL, Bailey RH, Best TF, Huston JE, Evans RR. Antimicrobial resistance of enteric *E. coli* in beef cattle treated with antibiotics. The AABP Proceedings 2003;36:156-7.

33. Inglis GD, McAllister TA, Busz HW, Yanke LJ, Morck DW, Olson ME, Read RR. Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter hyointestinalis*. Appl Environ Microbiol 2005;71;3872-3881.

34. Jim GK, Booker CW, Guichon PT, Schunicht OC, Wildman BK, Johnson JC, Lockwood PW. A comparison of florfenicol and tilmicosin for the treatment of undifferentiated fever in feedlot calves in western Canada. *Can Vet J* 1999;40: 179.

35. Khachatourians, GC. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can Med Assoc J 1998;159:1129-1136.

36. Linton AH, Howe K, Bennett PM, Richmond MH, Whiteside EJ. The colonization of the human gut by antibiotic resistant *Escherichia coli* from chickens. J Appl Microbiol 1977;43:465-469.

37. Lipsitch M, Singer RS, Levin BR. Antibiotics in agriculture: When is it time to close the barn door? *Proc Natl Acad Sci* 2002;99 (9):5752-4.

38. Lowrance TC, Loneragan GH, Kunze DJ, Platt TM, Ives SE, Scott HM, Norby B, Echeverry A, Brashears MM. Changes in antimicrobial susceptibility in a population of *Escherichia coli* isolated from feedlot cattle administered ceftiofur crystalline-free acid. Am J Vet Res 2007;68:501-507.

39. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? Can Med Assoc J 1998;159:1119-1120.

40. Morck DW, Merrill JK, Thorlakson BE, Olson ME, Tonkinson LV, Costerton JW. Prophylactic efficacy of tilmicosin for bovine respiratory tract disease. J Am Vet Med Assoc 1993;202:273-277.

41. NCCLS. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolates from Animals; Information Supplement M32-S1, 2004.

42. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res 2002;66:145-50

43. Radostits OM. Herd Health: Food Animal Production Medicine, 3rd Edition. Philadelphia: WB Saunders Company, 2001.

44. Ribble CS, Meek AH, Shoukri MM, Guichon PT, Jim GK. Risk factors associated with fatal fibrinous pneumonia (shipping fever) in feedlot calves. Proc Am Assoc Bov Pract 1998;31:104-109.

45. Schumann FJ, Janzen ED, McKinnon JJ. Prophylactic tilmicosin medication of feedlot calves at arrival. Can Vet J 1990;31:285-288.

46. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. *The Lancet Infectious Diseases* 2003;3(1):47-51.

47. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc Natl Acad Sci* 2002;99 (9):6434-9.

48. Stabler SL, Fagerberg DJ, Quarles CL. Effects of oral and injectable tetracyclines on bacterial drug resistance in feedlot cattle. Am J Vet Res 1982;43:1763-1766.

49. Stevenson SML, McAllister TA, Selinger LB, Yanke LJ, Olson ME, Morck DW, Read RR. Transfer of a rifampicin-resistant *Escherichia coli* strain among feedlot cattle. *J Appl Microbiol* 2003;95:398-410.

50. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69 (6):1456-1459.

51. Swann MM. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. *Her Majesty's Stationary Office,* London, 1969.

52. USDA 2000. Part III: Health Management and Biosecurity in U.S. Feedlots, 1999.

53. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N336.1200,1999.

54. Van Donkersgoed J. Meta-analysis of field trials of antimicrobial mass medication for prophylaxis of bovine respiratory disease in feedlot cattle. Can Vet J 1992;33:786-795.

55. Veterinary Drug Directorate, Health Canada. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health, 2002. http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed Sept. 27, 2007.

56. Wagner BA, Dargatz DA, Salman MD, Morley PS, Wittum TE, Keefe TJ. Comparisons of sampling techniques for measuring the antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. *Am J Vet Res* 2002;63 (12): 1662-1670.

57. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates form food animals and humans. Antimicrob Agents Chemother 2001;45(10):2716-2722.

58. Wittum TE, Woollen NE, Perino LJ, Littledike ET. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. *J Am Vet Med Assoc* 1996;209 (4): 814-818.

Table 3.1: Concentration range and breakpoints of antimicrobials tested (in µg/ml.)

Antimicrobial	Breakpoint for Resistant	Concentration Range Measured
Ampicillin	≥32	**<4, 4, 8, 16, 32, >32**
Enrofloxacin	≥2	**< 0.5, 0.5, 1, 2, 4, >4**
Gentamicin	≥16	**< 4, 4, 8, 16, 32, >32**
Sulphamethoxazole	≥512	**<125, 125, 256, 512, >512**
Tetracycline	≥16	**<2, 2, 4, 8, 16, 32, >32**
Trimethoprim	≥16	**<4, 4, 8, 16, 32, >32**
Trimethoprim/ Sulfanilamide	**≥4/76**	**<1/19, 1/19, 2/38, 4/76, 8/152, >8/152**

Table 3.2: Antimicrobials used and Animal Defined Dose for a feedlot animal ($ADD_{Feedlot}$) equivalent

Antimicrobials used in study (Concentration)	**Dose equivalent**	**$ADD_{Feedlot}$**
Ceftiofur 50 mg/mL Intramuscular injection (Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, ON, Canada)	1mg/kg BW[a]	1
Florfenicol 300mg/mL Subcutaneous injection (Nuflor, Schering-Plough Animal Health, Pointe Claire, QC, Canada)	40 mg/kg BW	4
Penicillin G procaine 300mg/mL[b] Intramuscular injection (Depocillin®, Intervet Canada Ltd., Whitby, ON, Canada)	33mg/kg BW	1.6
Penicillin G procaine 300mg/mL Intramuscular injection (Ethacilin®, Rogar/.STB, London, ON, Canada)	33mg/kg BW	1.6
Sulphamethazine 15 g/bolus Oral Administration (Sulphamethazine bolus, Professional Veterinary Laboratories, Winnipeg, MB, Canada)	1 bolus/80 kg BW	1
Tilmicosin 300 mg/mL Subcutaneous injection (Micotil, Provel, Guelph, ON, Canada)	10 mg/kg BW	3
Trimethoprim 40 mg/mL / Sulfadoxine (200 mg/mL) Intramuscular injection (Trivetrin™ Injection, Schering-Plough Animal Health, Pointe Claire, QC, Canada)	16 mg/kg BW	1

[a]Body Weight (BW)

[b]No longer available in Canada.

Table 3.3: Isolate-level prevalence of resistance to specific antimicrobials on arrival at the feedlot

Antimicrobial Resistance[a]	Count positive out of 858 (Prevalence as percent)	Exact 95% Confidence Interval (as percent)
AMP	16 (1.9)	1.1 – 3.0
ENR	0 (0.0)	na[a]
GEN	0 (0.0)	na
SMX	40 (4.7)	3.4 – 6.3
TCY	84 (9.8)	7.9 -12.0
TMP/SSS	10 (1.2)	0.6 – 2.1
TMP	11 (1.3)	0.6 – 2.3
AMPp	1 (0.1)	0.0– 0.6
SMXp	9 (1.0)	0.5 – 2.0
TCYp	51 (5.9)	4.5 - 7.7
AMP TCY	1 (0.1)	0.0 - 0.6
AMP TMP	1 (0.1)	0.0 - 0.6
TCY SMX	14 (1.6)	0.9 – 2.7
AMP TCY SMX	8 (0.9)	0.4 – 1.8
AMP TCY TMP/SSS TMP	1 (0.1)	0.0 - 0.6
TCY SMX TMP/SSS TMP	5 (0.6)	0.2 – 1.4
AMP TCY SMX TMP/SSS TMP	4 (0.5)	0.1 – 1.2
No Resistance	763 (88.9)	86.6 – 90.9
Resistance to 1 or more antimicrobials	95 (11.1)	9.1 – 13.4
Resistance to 3 or more antimicrobials	18 (2.1)	1.2 - 3.3

[a]AMP=ampicillin, ENR=enrofloxacin, GEN=gentamicin, SMX=sulphamethoxazole, TCY=tetracycline, TMPSSS=trimethoprim/sulfanilamide, TMP=trimethoprim, AMPp=ampicillin phenotype, SMXp=sulphamethoxazole phenotype, TCYp=tetracycline phenotype

Table 3.4: Animal-level[a] prevalence of resistance to specific antimicrobials on arrival at the feedlot

Antimicrobial Resistance[b]	Count positive out of 286 (Prevalence as percent)	95% Confidence Interval (as percent)
AMP	10 (3.5)	2.0 – 6.0
ENR	0 (0.0)	na
GEN	0 (0.0)	na
SMX	23 (8.0)	5.1-12.4
TCY	47 (16.4)	12.0-22.0
TMP/SSS	5 (1.7)	0.8-3.8
TMP	6 (2.1)	1.0-4.2
No Resistance	232 (81.1)	74.9-86.1
Resistance to 1 or more antimicrobials	54 (18.9)	74.9-86.1
Resistance to 3 or more antimicrobials	9 (3.1)	1.9-5.2

[a]At least one of the three isolates had this phenotype

[b]AMP=ampicillin, ENR=enrofloxacin, GEN=gentamicin, SMX=sulphamethoxazole, TCY=tetracycline, TMP/SSS=trimethoprim/sulfanilamide, TMP=trimethoprim

Table 3.5: Animal Defined Dose for a feedlot animal (ADDFeedlot) used during the feeding period

$ADD_{Feedlot}$	**Animal Count**	**Total $ADD_{Feedlot}$**
0	188	0
1	38	38
1.6	29	46.4
2	3	6
3	2	6
3.2	5	16
4	1	4
4.2	1	4.2
4.6	13	59.8
4.8	1	4.8
6.2	3	18.6
6.4	1	6.4
8	1	8
11.8	1	11.8
13.2	1	13.2
Total	**288**	**243.2**

Table 3.6: Animal Defined Dose for a feedlot animal ($ADD_{Feedlot}$) used during the last 100 days of the feeding period

$ADD_{Feedlot}$	**Animal Count**	**Total $ADD_{Feedlot}$**
0	219	0
1	5	5
1.6	39	62.4
2	1	2
3.2	7	22.4
4.2	1	4.2
4.8	1	4.8
6.2	1	6.2
6.4	1	6.4
8.8	1	8.8
Missing Animals	12	
Total	**288**	**122.2**

Table 3.7: Antimicrobial resistance found at animal level[a] at each time period (n=2257).

Antimicrobial Resistance[b]	Treatment Group	Arrival	Day 7	Day 15	Day 35	Day 70	Day 105	Day 154	Pre-slaughter
AMP	**Feed**	2/96	14/95	15/94	4/92	7/92	7/92	19/92	14/84
	Injectable	6/94	7/95	23/95	5/94	8/96	3/95	12/94	9/94
	Control	2/96	4/94	5/96	1/96	4/96	8/96	9/96	10/94
SMX	**Feed**	11/96	70/95	65/94	42/92	34/92	45/92	45/92	24/84
	Injectable	6/94	13/95	14/94	14/96	23/95	30/94	23/94	6/94
	Control	6/96	6/94	10/96	7/96	7/96	16/96	27/96	26/94
TCY	**Feed**	20/96	87/95	91/94	65/92	58/92	62/92	75/92	54/84
	Injectable	15/94	28/95	39/95	27/94	32/96	35/95	53/94	47/94
	Control	12/96	17/94	27/96	9/96	21/96	40/96	51/96	51/94

[a]At least one of the three isolates had this phenotype

[b]AMP=ampicillin, ENR=enrofloxacin, GEN=gentamicin, SMX=sulphamethoxazole, TCY=tetracycline, TMP/SSS=trimethoprim/sulfanilamide, TMP=trimethoprim

Figure 3.1: Proportion of animals with one or more isolates resistant to Tetracycline (TCY) described over time using a line graph to approximate the trend between points.

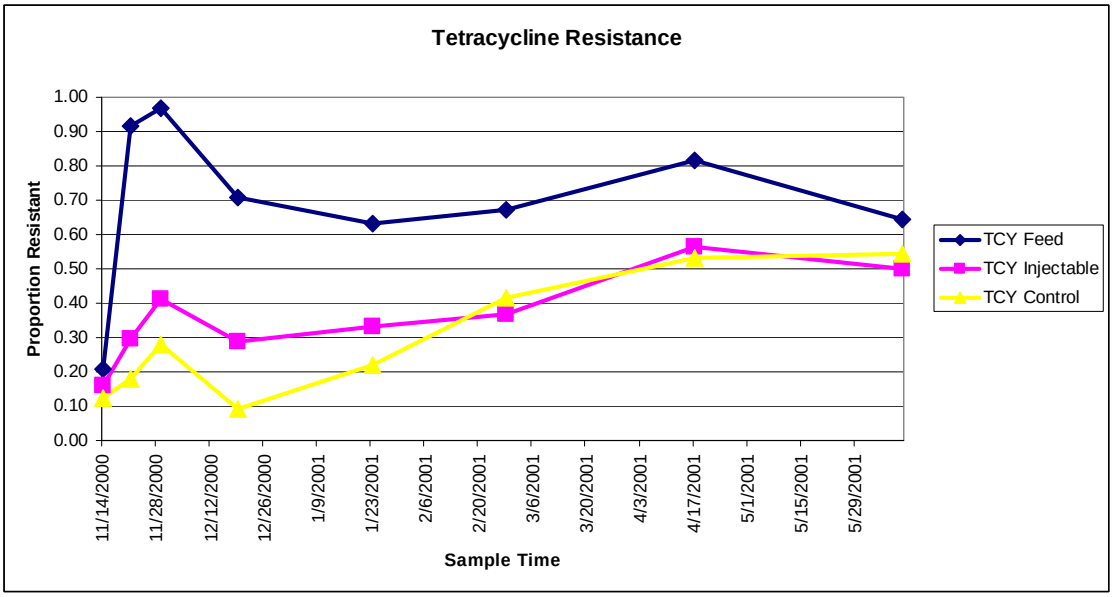


Figure 3.2: Proportion of animals with one or more isolates resistant to Sulphamethoxazole (SMX) described over time using a line graph to approximate the trend between points.

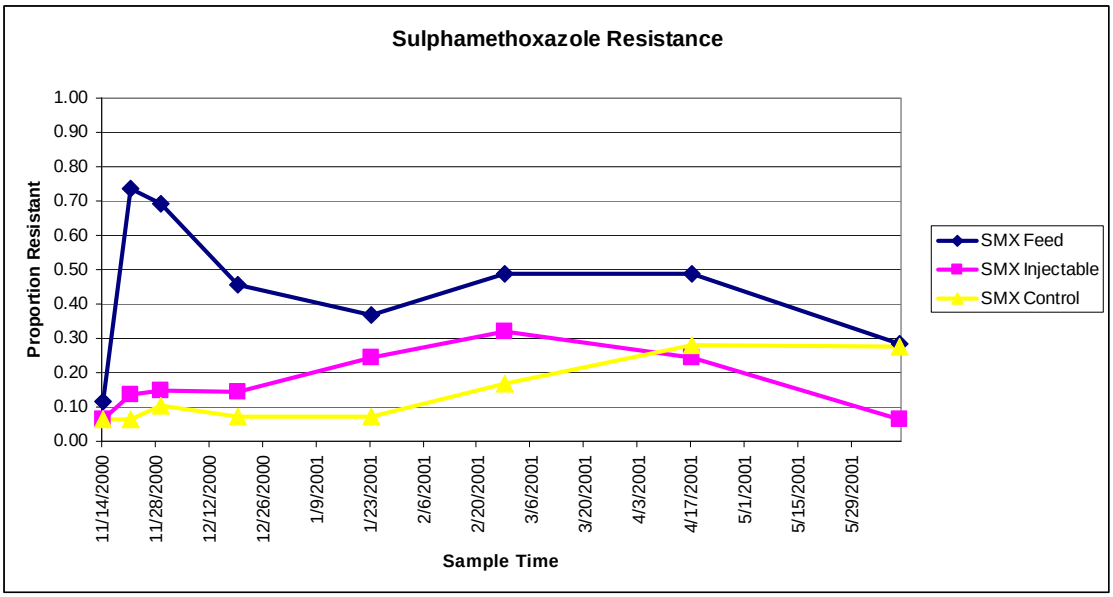
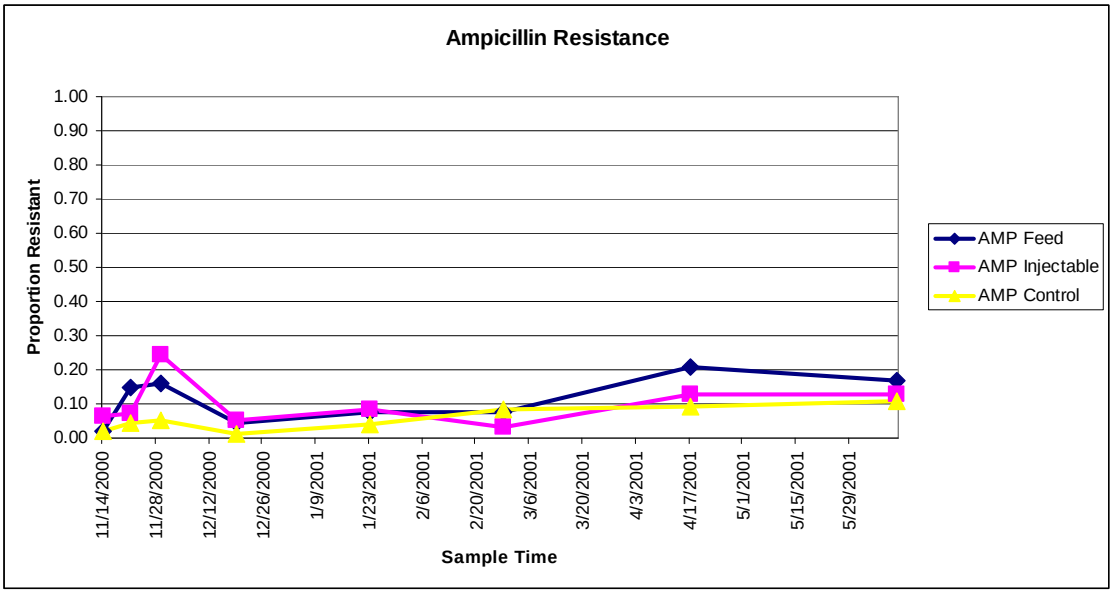


Figure 3.3: Proportion of animals with one or more isolates resistant to Ampicillin (AMP) described over time using a line graph to approximate the trend between points.



CHAPTER 4
A RETROSPECTIVE DIAGNOSTIC LABORATORY SURVEY OF ANTIMICROBIAL RESISTANCE IN FECAL *ESCHERICHIA COLI* ISOLATED FROM SPRING CALVES IN WESTERN CANADA

4.1 Introduction

Animal health surveillance is the continuous monitoring of the health status of a population over time where directed action against disease is applied if indicators of disease exceed a specific threshold (Salman, 2003). Surveillance is important for many reasons including international trade, identification of emerging disease or changes in animal health trends over time, and providing direction for research (McNab, 2007; Salman, 2003). Marketplace issues such as food safety and quality assurance issues including the potential presence of antimicrobial resistant bacteria has become an important factor in the profitability of beef production and in the global competitiveness of the industry (Acar and Rostel, 2001; Conly, 2002). Many countries have already embarked on various types of surveillance program for antimicrobial resistance (Bager, 2000; VDD, 2002). Data from diagnostic laboratories may be used in an ongoing fashion in surveillance systems (Teutsch, 2000; Trevino, 2000).

In Canada, most beef calves are born in the spring, summered on pasture, and placed in feedlots in the fall (Mathison, 1993; Chenoweth and Sanderson, 2001). One of the common conditions spring calves received antimicrobial treatment for was calf diarrhea; in the USA, 2.4% of calves less than three weeks of age, and 1.7% of calves over three weeks of age were reported with diarrhea (USDA, 1997). Most antimicrobial

treatments were administered to calves when they were less than two months of age (Gow et. al., 2008). Calf diarrhea is a condition for which fecal samples are often sent to the lab, making summary, and interpretation of existing data possible.

The objectives of this study were first, to describe antimicrobial resistance (AMR) patterns in fecal *E. coli* isolates from spring calves in western Canada and to evaluate the use of diagnostic laboratory data as a method of passive surveillance for AMR in Western Canada.

4.2 Materials and Methods

A database of voluntary diagnostic submissions from a diagnostic laboratory that served all of Western Canada was searched for cases fitting a specific case definition. Individual files were reviewed to ensure that they fit within the case definition for the project. The case definition was antimicrobial susceptibility results for fecal *E. coli* from spring beef calves between January and June of each year. Cases would consist of calves with various types of neonatal calf diarrhea. Exact confidence intervals for case-level descriptives were calculated (PEPI v 4, Sagebrush Press, Salt Lake City, USA).

Routine methodology at the laboratory included susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller Hinton agar plates according to CLSI standards for ampicillin, ampicillin/sulbactam, ceftiofur, florfenicol, gentamicin, penicillin, tetracycline, tilmicosin, and trimethoprim/sulphamethoxazole. A change in the routine antimicrobials tested for occurred in 2000 when the laboratory stopped testing for cephalothin, enrofloxacin and neomycin, and started using erythromycin. Results were reported by the laboratory as susceptible, intermediate or resistant.

Isolates classified as either susceptible or intermediate were considered susceptible for this analysis. Descriptive and analytic statistics were performed using commercial software (SPSS for Windows 15.0, SPSS Inc., Chicago, USA). Generalized estimating equations (GEE) were used to adjust AMR prevalence estimates for clustering (at the case-level) (PROC GENMOD, SAS for Windows version. 9.1, SAS Institute Inc., Cary, North Carolina). Population-averaged prevalence estimates and 95% confidence intervals were then calculated using the formula $1/(1 + \exp(-\beta))$ (Dohoo et al., 2003).

4.3 Results

There were a total of 505 cases included in the analysis from over five years (1999-2003). Samples were obtained from clinical cases sent in by private veterinary practices, or from animals treated in the Veterinary Teaching Hospital at the Western College of Veterinary Medicine. Ninety-one cases fitting the case definition were excluded due to incomplete data. Only cases with susceptibility results to the nine antimicrobials used across all five years were included. A total of 540 isolates were obtained from the 505 cases, with a second *E. coli* isolate from 30 of the cases, a third *E. coli* isolate from four of the cases, and a fourth *E. coli* isolate from one of the cases. There were 470/505 (93.1%; 95% CI: 90.5%-95.1%) cases with a province of residence indicated. The majority of known samples, 73.8% (347/470; 95% CI: 69.6%-77.7%), were from Saskatchewan, 16.4% (77/470; 95% CI: 13.2%-20.0%) from Alberta, 6.8% (32/470; 95% CI: 4.7%-9.5%) from Manitoba, and 3.0% (14/470; 95% CI: 1.6%-4.9%) from British Columbia. The majority of samples, 64.0% (323/505; 95% CI: 59.6%-68.2%), were from feces, 15.1% (76/505; 95% CI: 12.0%-18.5%) from fecal swabs, and

21.0% (106/505; 95% CI: 17.5%-24.8%) from tissues. Of all cases, 21.2% (107/505; 95% CI: 17.7%-25.0%) did not have an age described or it was in vague terms such as 'months', 'one week plus', or 'calves'. This is quite possibly because the samples were pooled or meant to represent a group of calves. Age of cases ranged between 0.5 and 150 days, with a median of 10 days and interquartile range of 9.

Of all antimicrobials, the predominant resistance was to penicillin where 100% of isolates were resistant across all years studied, and secondly to tilmicosin with 97.2% of isolates resistant overall, ranging between 88.9-98.9% across the five years. *E. coli* is intrinsically resistant to penicillin and tilmicosin, so these antimicrobials were not used in subsequent analysis (Boerlin and White, 2006). Of the other seven antimicrobials tested, tetracycline resistance was predominant with 78.3% of isolates resistant overall, ranging between 68.9-83.5% across the five years, ampicillin resistance was second highest with 50.3% of isolates resistant overall, ranging between 44.2-56.8% across the five years, and trimethoprim/sulphamethoxazole resistance was third highest with 48.4% of isolates resistant overall, ranging between 34.8-59.5% across the five years. Resistance to one or more of the seven antimicrobials was found in 80.8% of isolates overall, ranging between 72.1-85.0% across the five years. Resistance to three or more of the seven antimicrobials was found in 46.0% of isolates resistant overall, ranging between 32.6-57.4% across the five years. Resistance to other antimicrobials was lower (Table 4.1).

4.4 Discussion

E. coli was chosen as the indicator organism in this study because it was a commensal bacterium in cattle that was ubiquitous, easy to culture, and one of the major

carcass contaminants at slaughter (Stopforth et al., 2006). *Escherichia coli* were considered a potential reservoir of resistance genes that could transfer resistance to other zoonotic or commensal organisms that might cause disease in cattle or people (Donaldson et. al., 2006; Hoyle et. al., 2005, Linton et. al., 1985; Winokur et. al, 2001).

Prevalence of multidrug resistance and of resistance to individual antimicrobials in this study was similar to those found in another western Canadian study where the proportion of calves resistant to one or more antimicrobial was 48.8% (Gow et al., 2008). In this study, overall, tetracycline resistance at 78.3%, ampicillin resistance at 50.3%, and trimethoprim/sulphamethoxazole at 48.4% were higher than in the study by Gow at 46.4%, 22.7%, and 16.3% respectively (Gow et al., 2008). Calves also arrived at a western Canadian feedlot with lower proportions of resistant bacterial isolates than found in this survey: 5.9% of cattle had at least one isolate that was resistant to three or more antimicrobials out of the 7 antimicrobials tested, sulphamethoxazole resistance was observed in 44.4% of isolates, ampicillin resistant was observed in 20.3% of isolates, and tetracycline resistance was observed in 17.7% of cattle (Checkley et. al., 2008). The proportion of tetracycline resistant *E. coli* isolates, in this study, was also higher than that found in newly weaned calves on pasture in an American study where 13 to 17% of fecal samples contained *E. coli* resistant to tetracycline in at least one of the five isolates per fecal sample (Huston et al., 2003). The AMR observed in fecal *E. coli* on arrival at the feedlot represented the antimicrobial resistant strains of bacteria that the calves were carrying prior to antimicrobial treatment at the feedlot, and may be related to resistance patterns and AMU in the individual animals as well as more broadly in their herd of origin. Other factors, not related to antimicrobial use, from the

herd of origin may play a role in the expansion of populations of antimicrobial resistant organisms (Hoyle et al. 2005; Khachatryan et al., 2006; Hoyle et al., 2004; Hinton, 1983).

Although exact comparisons can not made due to differences in the studies, the proportions of resistant isolates were higher in the samples from the diagnostic laboratory compared to the study by Gow. This could be explained by differences in sampling methods and populations sampled. Animals in this diagnostic laboratory study were not randomly sampled and represent the sickest calves that were probably treated with antimicrobials, potentially creating bias. Unfortunately, antimicrobial use could not be analyzed in this study due to inadequate reporting of this information. In addition to sampling biases discussed above, differences in proportions of resistant isolates between this pre-weaning study and other studies carried out post-weaning could include time from antimicrobial treatment and the age of the animals sampled. Most antimicrobial treatments for calves were given in the springtime; therefore, bacterial populations associated with antimicrobial use in the spring had four or more months to readjust to equilibrium, in the absence of antimicrobial use, before the post-weaning samples were taken. As well, in general, younger animals tended to have higher levels of resistant microorganisms (Donaldson et al., 2006; Berge et al., 2005; Martel and Coudert, 1993).

The use of these diagnostic laboratory data alone as a source of passively acquired surveillance data was questionable; other researchers have come to similar conclusions (Canton, 2005). Resistance profiles suggested higher levels of resistant *E. coli* than did studies using other sampling methods (Gow et al., 2008). Bias existed as to what

samples are sent in for further testing, affecting generalizability of results. It has also been suggested that the sensitivity of a voluntary, user-pay diagnostic system for cow-calf producers would likely be low (Clift et al., 2006). Aggregation and analysis of diagnostic laboratory data was challenging as in previous studies due to incomplete demographic data; 91 potential cases were not used due to incomplete basic data. Further analysis was not possible on the existing data due to lack of more detailed demographic information. Inconsistencies in the way owner/farm names are submitted, leading to single farms being counted more than once, and incomplete demographic data are reasons why general recommendations have been made that each clinic submitting samples to the diagnostic laboratory have an assigned person cognizant of these issues fill out forms in a standard format (McNab, 2007). Differences in methodology existed between diagnostic and surveillance systems where, in a survey of veterinary diagnostic laboratories in the United States, 88% of laboratories used the Kirby-Bauer disk diffusion test compared to national AMR surveillance systems which use microbroth dilution methods (BEAR, 2008; GOC, 2007; Brooks et al. 2003). Diagnostic laboratory data often does not include the same antimicrobials as those used in surveillance as they are not of importance to the practitioner. In this study, it was therefore impossible to assess the prevalence of resistance phenotypes of high importance in human health such as ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphamethoxazole and tetracycline), AKSSuT (ampicillin, kanamycin, streptomycin, sulphamethoxazole and tetracycline), and ACKSSuT (ampicillin, chloramphenicol, kanamycin, streptomycin, sulphamethoxazole and tetracycline) due to the susceptibility profiles available.

The use of diagnostic laboratory data along with surveillance data from other sources in an overarching surveillance system was thought to be of value and has been used in this way in public health jurisdictions (Teutsch, 2000). Testing for a broader range of antimicrobials, using an antibiogram more consistent with those used for surveillance and including antimicrobials important in human medicine, would increase the value of these diagnostic laboratory data for surveillance. However, this panel would need to be useful for practitioners by including antimicrobials consistent with current, regional antimicrobial use. The use of current diagnostic laboratory data to create regional antibiograms on drug susceptibilities for some diseases could be useful to practitioners (El-Azizi, 2005; Karlowsky, 2002). However, when evaluating resistance of fecal *E. coli* in diarrheic calves, it is questionable how well the *E. coli* isolated in the feces represents that of the small intestine, or the pathogenic process at all (Constable, 2004; Bywater, 2000). Overall, with carefully planned antibiograms, the use of diagnostic laboratory data for surveillance purposes could add important information to larger overarching surveillance systems for emerging antimicrobial resistance by representing cases which are important enough to veterinarians and producers to be submitted to the diagnostic laboratory for further testing (McNab, 2007).

4.5 Acknowledgements

The authors would like to thank Brian Buydens for support in searching the Prairie Diagnostic Services database, as well as Marie Legault, Kathryn Rosel and Clarissa Snyder for help with data entry and verification.

4.6 References

1. Acar J, Rostel B. Antimicrobial Resistance; an overview. Rev Sci Tech 2001;20(3):797-810.

2. Bager F. DANMAP: monitoring antimicrobial resistance in Denmark. Int J Antimicrob Ag 2000;14:271-274.

3. Bacterial Epidemiology and Antimicrobial Resistance Research Unit Web Site (BEAR) [homepage on the Internet]. Athens: United States Department of Agriculture c2008. [updated 2008 February 7]. Available from http://ars.usda.gov/Main/docs.htm?docid=6750&page=2 accessed March 21, 2008.

4. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005;69:25-38.

5. Boerlin P, White DG. Antimicrobial resistance and its epidemiology. In: Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM ed. Antimicrobial therapy in veterinary medicine 4th Edition. Ames: Blackwell Publishing 2006.

6. Brooks MB, Morley PS, Dargatz DA, Hyatt DR, Salman MD, Akey BL. Survey of antimicrobial susceptibility testing practices of veterinary diagnostic laboratories in the United States. J Am Vet Med Assoc 2003;222:168-173.

7. Bywater RJ. Sense and nonsense in surveillance programs. Acta vet scand 2000; Suppl 93:119-127.

8. Canton R. Role of the microbiology laboratory in infectious disease surveillance, alert and response. Clin Microbiol Infect 2005;11(Suppl. 1):3-8.

9. Checkley SL, Campbell JR, Chirino-Trejo M, Janzen ED, McKinnon JJ. Antimicrobial resistance in generic fecal *Escherichia coli* obtained from beef cattle on arrival at the feedlot and prior to slaughter, and associations with volume of total individual cattle antimicrobial treatments in one western Canadian feedlot. Can J Vet Res 2008;72: (In Press).

10. Chenoweth PJ, Sanderson MW. Health and production management in beef cattle breeding herds. In Radostits OM ed. Herd Health: Food Animal Production Medicine, 3rd Edition. Philadelphia: WB Saunders Company, 2001.

11. Clift KH, Weaver J, Frazer JL. Rebuilding a passive surveillance program. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics, 2006. Available at www.sciquest.org.nz accessed March 21, 2008.

12. Conly, J. Antimicrobial resistance in Canada. *CMAJ* 2002;167 (8):885-91.

13. Constable PD. Antimicrobial use in the treatment of calf diarrhea. J Vet Intern Med 2004;18:8-17.

14. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown: AVC Inc., 2003.

15. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. App Environ Epidem 2006;72;3940–3948.

16. El-Azizi M, Mushtaq A, Drake C, Lawhorn J, Barenfanger J, Verhulst S, Khardori N. Evaluating antibiograms to monitor drug resistance. Emerg Infect Dis 2005;11:1301-1302.

17. Government of Canada (GOC). Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph, ON: Public Health Agency of Canada, 2007. http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html accessed March 22, 2008.

18. Gow SP, Waldner CL, Rajic A, McFall ME, Reid-Smith R. Prevalence of antimicrobial resistance in fecal generic *Escherichia coli* isolated in western Canadian cow-calf herds. Part 1-Beef calves. Can J Vet Res 2008:72; (In Press).

19. Hinton M. Antibacterial drug resistance among *Escherichia coli* isolated from calves fed on a milk substitute diet. Vet Rec 1983;112:567-568.

20. Hoyle DV, Knight HI, Shaw DJ, Hillman K, Pearce MC, Low JC, Gunn GJ, Woolhouse MEJ. Acquisition and epidemiology of antibiotic-resistant *Escherichia coli* in a cohort of newborn calves. J Antimicrob Chemother 2004;53:867-871.

21. Hoyle DV, Yates CM, Chase-Topping ME, et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71:6680–6688.

22. Huston CL, Bailey RH, Best TF, Huston JE, Evans RR. Antimicrobial resistance of enteric *E. coli* in beef cattle treated with antibiotics. The AABP Proceedings 2003;36:156–157.

23. Karlowsky JA, Sahm DF. Antibiotic resistance – is resistance detected by surveillance relevant to predicting resistance in the clinical setting? Curr Opin Pharmacol 2002;2:1-6.

24. Khachatryan AR, Hancock DD, Besser TE, Call DR. Antimicrobial drug resistance genes do not convey a secondary fitness advantage to calf –adapted *Escherichia coli*. Appl Environ Microbiol 2006;72:443-448.

25. Linton AH. Antibiotic resistance in bacteria associated with animals and their importance to man. J Antimicrob Chemother 1985;15:385–386.

26. Martel JL, Coudert M. Bacterial Resistance monitoring in animals: the French national experiences of surveillance schemes. Vet Micobiol 1993;35:321-38.

27. Mathison GW. The Beef Industry. In Martin J, Hudson RJ, Young BA ed. Animal production in Canada. Edmonton; University of Alberta, Faculty of Extension, 1993.

28. McNab B, Fairles J, McEwen B. Accurate and complete submissions data are important contributions to surveillance. Ceptor 2007;15(3):4.

29. Salman MD. Surveillance and monitoring systems for animal health programs and disease surveys. In Salman MD ed. Animal disease surveillance and survey systems. Ames: Iowa State Press, 2003.

30. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69:1456–1459.

31. Teutsch SM. Considerations in planning a surveillance system. In: Teutsch SM, Churchill RE ed. Principles and practice of public health surveillance. Oxford: Oxford University Press 2000, 21.

32. Trevino, S. Antibiotic resistance monitoring: A laboratory perspective. Mil Med 2000;165(Suppl. 2):40-42.

33. United States Department of Agriculture (USDA) 1997. Part II: Reference of 1997 Beef Cow-Calf Health and Health Management Practices, 1997. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N238-797,1997.

34. Veterinary Drug Directorate (VDD), Health Canada. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health, 2002. http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed March 22, 2008.

35. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp ID, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates form food animals and humans. Antimicrob Agents Chemother 2001;45:2716–2722

Table 4.1: Proportion of isolates resistant to given antimicrobials by year

	All Years (%) (N=540) [95% CI]	1999 (N=45) (%) [95% CI]	2000 (N=52) (%) [95% CI]	2001 (N=122) (%) [95% CI]	2002 (N= (%) [95%
Ampicillin	50.3 (270) [46.0-54.6]	56.8 (25) [42.0-70.5]	44.2 (25) [32.9-56.2]	46.8 (57) [37.9-56.0]	44.4 (6 [36.3-52
Ampicillin/Sulbactam	27.4 (146) [23.7-31.4]	22.4 (10) [12.5-37.0]	17.6 (9) [9.4-30.4]	18.3 (22) [12.3-26.3]	31.0 (4 [24.0-39
Ceftiofur	3.9 (21) [2.6-5.9]	2.2 (1) [0.3-14.2]	3.8 (2) [1.0-14.6]	2.5 (3) [0.8-7.3]	3.5 (5 [1.6-8.
Florfenicol	26.0 (139) [22.4-30.0]	6.7 (3) [2.2-18.8]	8.7 (6) [3.3-21.0]	16.3 (19) [10.8-24.0]	27.8 (4 [21.0-35
Gentamicin	2.8 (15) [1.7-4.7]	4.5 (2) [1.1-16.2]	2.0 (1) [0.3-12.7]	2.5 (3) [0.8-7.4]	3.1 (4 [1.2-7.
Penicillin	100.0 (540)	100.0 (45)	100.0 (52)	100.0 (122)	100.0 (1
Tetracycline	78.3 (423) [74.6-81.6]	79.8 (36) [65.5-89.2]	68.9 (36) [55.9-79.38]	73.3 (90) [64.4-80.7]	79.8 (1 [72.0-85
Tilmicosin	97.2 (525) [95.4-98.3]	88.9 (40) [75.9-95.3]	96.0 (49) [85.4-99.0]	98.4 (120) [93.7-99.6]	97.9 (1 [93.6-99
Trimethoprim / Sulphamethoxazole	48.4 (258) [44.1-52.7]	50.0 (23) [35.7-64.4]	34.8 (17) [22.5-49.5]	46.9 (57) [38.1-55.8]	39.4 (5 [31.5-47
MDR ≥ 1*	80.8 (436) [77.2-83.9]	82.1 (37) [68.0-90.8]	72.1 (37) [59.2-82.1]	76.9 (95) [68.2-83.8]	82.1 (1 [74.6-87
MDR ≥ 2*	62.3 (335) [58.0-66.4]	66.2 (28) [51.2-78.5]	54.6 (29) [41.3-67.4]	59.6 (74) [50.5-68.1]	53.1 (7 [44.5-61
MDR ≥ 3*	46.0 (246) [41.8-50.4]	40.7 (18) [27.4-55.6]	32.6 (16) [20.7-47.3]	42.5 (52) [33.8-51.6]	41.7(5 [33.8-50

* Of the seven antimicrobials including Ampicillin, Ampicillin/Sulbactam, Ceftiofur, Gentamicin, Penicillin, Tetracycline, Trimethoprim/Sulphamethoxazole.

CHAPTER 5
ANTIMICROBIAL RESISTANCE IN GENERIC FECAL *ESCHERICHIA COLI* OBTAINED FROM BEEF CATTLE ON ARRIVAL AND PRIOR TO SLAUGHTER, AND ASSOCIATIONS WITH VOLUME OF TOTAL INDIVIDUAL CATTLE ANTIMICROBIAL TREATMENTS IN ONE WESTERN CANADIAN FEEDLOT

5.1 Introduction

Antimicrobial resistance (AMR) has been perceived as an important component of both animal health and food safety in the international community and specifically by countries that import beef (Khachatourians, 1998; McGeer, 1998; OIE, 2003; Angulo et al., 2004). Health Canada has examined the use of antimicrobials in agriculture (VDD, 2002); however, the pathways by which antimicrobial use (AMU) in cattle could affect human health were not understood (Linton 1982; McKellar, 1998; VDD, 2002). More information was also needed about the extent to which the use of antimicrobials in animal agriculture was related to AMR in human infections (Conly, 2002; VDD, 2002; Bywater, 2005). In Canada, the beef industry and the veterinarians serving that industry were aware of these issues and thus developed prudent use guidelines for antimicrobials (CCA and BIC, 2006; Crandall and Van Donkersgoed, 2000; CVMA, 2001). The objectives of this study were to examine AMR patterns of fecal *Escherichia coli* isolates of auction market derived, newly weaned calves on arrival at the feedlot, and then evaluate the associations between the total volume of parenteral antimicrobials used for disease treatment and changes in antimicrobial resistance, during the feeding period.

5.2 Materials and Methods

The University Committee on Animal Care and Supply approved this study and the guidelines of the Canadian Council on Animal Care were followed. This study was carried out at the same time as another trial described previously (Checkley et al., 2005).

In the fall of 2001, backgrounding of 447 newly weaned, auction market derived steers was carried out at the University of Saskatchewan Research Feedlot (one time capacity 800 head) with 12 adjacent pens of 37 or 38 steers. The steers were crossbred beef calves with an average weight of 249 kg (s = 17 kg). They were divided into weight groups and randomly allocated at processing to create pens of approximately equal weights. The routine vaccination, parasiticide, and implant strategy was previously described (Checkley et al., 2005). A metaphylactic antimicrobial injection of long-acting oxytetracycline (Liquamycin LA-200; Pfizer Canada Animal Health Group, Kirkland, Quebec), 1 mL/10 kg bodyweight (BW), SC, was given to each animal with a body temperature < 41^{o}C. The rest of the cattle received tilmicosin (Micotil; Provel, Division Eli Lilly Canada, Guelph, Ontario), 1 mL/30 kg BW, SC, for disease treatment. Booster vaccinations and the 2nd hormonal implant were given at 94 days on feed (DOF), 18 d before the steers moved to the finishing feedyard. The steers were finished in two adjacent pens in a large commercial feedlot with a one time capacity of approximately 30 000 head. These cattle remained in their respective study groups until slaughter. During the study, all of the aforementioned animal health products were used according to label instructions and detailed individual animal records were maintained. Both feedlots were typical of those found in western Canada, with dirt floors, shared waterers, and a central alley for feeding. Feedlot staff checked all cattle daily for signs

of disease. Any cattle that appeared depressed, gaunt, or distinctly different from their penmates were pulled and treated according to the standard treatment protocols in use at the feedlot. Cattle were weighed before treatments, and all medications were used at the approved dose per kg body weight. The feedlot staff was blinded as to the allocation of the treatment groups and to the specific objectives of the study. Steers were also individually weighed at the feedlot within 24 h of slaughter at 245 to 260 DOF.

Pens were randomly entered into one of two feeding programs, with different diet compositions and feeding methods, used at the University feedlot during the backgrounding period (13). Monensin sodium 3% (Rumensin; Elanco Animal Health, Guelph, Ontario) was fed to all cattle during the backgrounding period in the total mixed ration at 27–28 ppm DM. All cattle were fed a high grain diet ad libitum with decoquinate at a dose of 0.5 mg/kg BW (Deccox 6% Premix; Alpharma, Mississauga, Ontario) during the finishing period.

Calculations of sample size were performed for the two main objectives (Win Episcope v2; University of Edinburgh, Edinburgh, Scotland). Fecal samples from 150 steers were sufficient to characterize AMR in as few as 5% to 10% of cattle with 80% power and 95% confidence. Sample size for the cohort study depended on the total number of treated cattle during the study, estimated between 15% and 45%, based on previously reported bovine respiratory disease (BRD) treatment rates (Kelly and Janzen, 1986). Fifty cattle per group, based on 80% power and 95% confidence, were needed to detect a significant relative risk of 1.5 to 2.

On arrival, fecal samples were collected from the rectums of all cattle using a new obstetrical glove for each animal. The samples were placed into individual, clean foam

cups with lids, labeled, and then transported to the laboratory for direct storage at –80^{o}C. One hundred and fifty of the 447 mixed-breed steers were chosen randomly for characterization of AMR in fecal *E. coli* isolates on arrival. At the end of the study, a random sample of cattle that were treated with antimicrobials for disease during the feeding period, and a sample of those that were not treated, were chosen for inclusion in the cohort study. The arrival samples from these cattle were then retrieved from the frozen, stored samples and sent for laboratory analysis. A 2nd fecal sample was collected from the cohort of cattle within 24 h of slaughter. All preslaughter fecal samples were collected in the same manner as the arrival samples, and transported to the laboratory for culture the same day.

5.2.1 Laboratory analysis

The feces were thawed (arrival samples only) and cultured overnight on MacConkey's agar. Identification of *E. coli* was confirmed by standard biochemical tests. Three individual colonies from each animal, with the characteristic phenotype of *E. coli,* were chosen randomly for subculture on blood agar. From each blood agar plate, *E. coli* were inoculated into sterile phosphate-buffered saline (PBS) to make standard solutions of 0.5 MacFarland. This solution was delivered onto Mueller-Hinton agar using the replicator technique. The minimum inhibitory concentrations (MICs) of seven antimicrobials were determined using the agar dilution method. The antimicrobials tested were ampicillin (AMP), enrofloxacin (ENR), tetracycline (TCY), gentamycin (GEN), sulphamethoxazole (SMX), trimethoprim/sulfanilamide (TMP/SSS), and trimethoprim (TMP). The Mueller-Hinton plates were cultured at 37°C and antimicrobial susceptibilities were read between 18 and 24 h. A control strain of *E.*

coli ATCC 25922 was included with each plate. Antimicrobial breakpoints and interpretation were from the CLSI standards (NCCLS, 2004; CLSI, 2006) (Table 5.1). All laboratory procedures were carried out according to CLSI standards.

5.2.2 Statistical analysis

Data were entered into a commercial spreadsheet and descriptive statistics were generated (SPSS v. 15.0.0 for Windows; SPSS, Chicago, USA). An animal was considered resistant if it had at least one resistant isolate. Exact confidence intervals for animal-level prevalence estimates were calculated (PEPI v 4; Sagebrush Press, Salt Lake City, USA)(Abramson and Gahlinger, 2001). The pen allocation changed after 112 DOF from 12 pens to two pens. No adjustment for clustering was used as there was no clustered pen structure representative of the entire feeding period.

The measurement, $ADD_{Feedlot}$, was used to quantify the number of actual antimicrobial treatments given at the approved dose of the antimicrobial. This measurement accounted for the dosage and duration of action of the antimicrobial (Table 5.2). The concept of $ADD_{Feedlot}$ was based on that of defined daily dose (DDD) used in human studies (Austin et al., 1997; Baquero, 1998; Austin et al., 1999; Grave et al., 2006). Each antimicrobial treatment was described as 0, 1, 2, or 3 $ADD_{Feedlot}$ (Table 5.2). An example calculation follows:

Example: An animal was treated with 27 cc of long acting oxytetracycline on November 9 (BW 270 kg) and 40 cc of oxytetracycline on January 19 (BW 400 kg).

20 mg/kg × 270 kg = 5400 mg, 5400 mg/(200 mg/mL) = 27 mL

(actual dose given)

This was a long-acting treatment, equivalent to 3 $ADD_{Feedlot}$

20 mg/kg × 400 kg = 8000 mg, 8000 mg/(200mg/mL) = 40 mL

(actual dose given)

This was a long-acting treatment, equivalent to 3 $ADD_{Feedlot.}$

The total $ADD_{Feedlot}$ actually given to this animal, therefore, was 6.

Two dichotomous outcomes were examined: conversion to TCY resistance and conversion to AMP resistance. Resistance conversion was defined as an animal having more resistant isolates present preslaughter than at arrival. These outcomes were chosen because TCY is the antimicrobial class most commonly used in animals (Guardabassi and Courvalin, 2006), and AMP resistance was thought to be associated with TCY resistance in previous feedlot work.

Possible explanatory variables included Total $ADD_{Feedlot}$ (continuous), diet (dichotomous), antimicrobial treatment (dichotomous), conversion to AMP resistance for the outcome TCY resistance (dichotomous), and conversion to TCY resistance (dichotomous) as an explanatory variable for the outcome AMP resistance. Total $ADD_{Feedlot}$ was the sum of the actual doses received by the animal; this was a quantitative way to assess this potential association. Diet was examined to ensure the concurrent trial had no effect on the outcomes of this study. Antimicrobial treatment

was a dichotomous variable representing whether the animal was treated or not. Tetracycline and AMP resistances were thought to be associated in previous feedlot studies, so AMP conversion was examined as an explanatory variable for the outcome TCY resistance, and TCY conversion was examined as a variable for AMP resistance.

There were no biological reasons to force any variables into the model. First, explanatory variables were each screened individually using unconditional logistic regression (SPSS v. 15.0.0 for Windows, SPSS). Variables with a statistical association at a level $P < 0.15$ were considered for entry into a multivariable model. When no variables were found to be significant through unconditional associations, the variables were also screened for entry into a multivariable model using a backwards stepwise approach. This was done because the effect of an important explanatory variable could be masked in the unconditional association due to uncontrolled confounding. The liberal *P*-value, $P < 0.15$, for entry into the model, was also used for this reason. A value of $P < 0.05$ was considered statistically significant and was necessary for a variable to stay in the model.

5.3 Results

5.3.1 Missing data

Three of the total 150 arrival samples had only two *E. coli* isolates cultured, and another three had only one isolate cultured. An extra three arrival samples were chosen randomly to be part of the study. This gave a total of 450 isolates from 153 cattle on arrival and represented an *E. coli* recovery rate of three isolates per animal from 96.1% of animals.

Fifty treated and 50 untreated cattle were chosen to be in the cohort analysis; five substitutes were also chosen if needed. Of the 55 treated cattle, 13 were excluded from the analysis; eight lost their individual identification, and five died or were euthanized during the study due to chronic disease conditions. Of the 55 untreated cattle, two were excluded because they lost their individual identification preslaughter. The three extra, untreated cattle were left in the analysis. Overall, 42 treated cattle and 53 untreated cattle (n = 95) cattle were included in the cohort analysis.

5.3.2 Arrival

The most frequent antimicrobials for which resistance was observed were SMX, AMP, and TCY where, of all cattle, 44.4% (95% CI: 36.4% to 52.7%), 20.3% (95% CI: 14.2% to 27.5%), and 17.7% (95% CI: 12.0% to 24.6%), respectively had at least one resistant isolate (Table 5.3). All isolates from an animal obtained from 36.6% (95% CI: 29.0% to 44.8%) of all cattle sampled (n = 153), were susceptible to all antimicrobials, while 5.9% (95% CI: 2.7% to 10.9%) of cattle had at least one isolate that was resistant to three or more antimicrobials out of the seven antimicrobials tested (Table 5.3).

5.3.3 Antimicrobial use

Sixty-six of the 447 cattle (14.8%) were given antimicrobials for disease treatment during the feeding period. Of the 447 cattle, 420 (94.0%) received a metaphylactic injection of long-acting oxytetracycline (Pfizer Canada Animal Health Group) on arrival. The other 27 (6.0%) were treated with tilmicosin (Division Eli Lilly Canada) on arrival due to undifferentiated fever or other early symptoms of disease.

Overall, amongst the 42 treated cattle used in the analysis, there was a total of 133 $ADD_{Feedlot}$ antimicrobials used for disease treatment; only one $ADD_{Feedlot}$ was

administered during the last 100 d immediately prior to slaughter. There were 81 $ADD_{Feedlot}$ of tilmicosin (Micotil; Provel, Division Eli Lilly Canada) given to 26 cattle, 49 $ADD_{Feedlot}$ of ceftiofur (Excenel Sterile Powder; Pharmacia Animal Health, Orangeville, Ontario) given to 20 cattle, and 3 $ADD_{Feedlot}$ of oxytetracycline (Pfizer Canada Animal Health Group) given to one animal. Some cattle received treatment with more than one antimicrobial.

Conversion of cattle from nonresistant to resistant between arrival and preslaughter was described for each antimicrobial. Resistance conversion was stratified by the number of treated (ADD ≥ 1) and untreated cattle (ADD = 0) (Table 5.4), and by the number of $ADD_{Feedlot}$ per animal (Table 5.5). Tetracycline and AMP had the highest levels of resistance conversion during the study at 72/95 (75.8%, 95% CI: 65.9% to 84.0%) and 46/95 (48.4%, 95% CI: 38.0% to 58.9%) cattle, respectively (Table 5.4). The SMX conversion occurred in 11/95 (11.6%, 95% CI: 5.9% to 19.8%) cattle (Table 5.4). Overall, 34 of the 72 cattle (47.2%, 95% CI: 35.3% to 59.3%) that showed TCY conversion were treated for disease during the feeding period with a total of 107 $ADD_{Feedlot}$ (between two and nine $ADD_{Feedlot}$ each). Thirty-eight of these 72 cattle (52.8%, 95% CI: 40.7% to 64.7%) that showed TCY conversion during the feeding period had no antimicrobial treatments during the feeding period, but all received oxytetracycline for metaphylaxis on arrival at the feedlot (Table 5.5). Similarly, 24 of the 46 cattle with positive AMP conversion (52.2%, 95% CI: 36.9% to 67.1%) were treated with a total of 70 $ADD_{Feedlot}$ during the feeding period and 22 (47.8%, 95% CI: 32.9% to 63.1%) were not, other than metaphylaxis (Table 5.5).

5.3.4 Associations between antimicrobial use and antimicrobial resistance

No statistically significant associations were found between the outcomes and explanatory variables described (Table 5.6); therefore, no multivariable analyses were undertaken.

5.4 Discussion

Escherichia coli was chosen as the indicator organism in this study because it was a commensal bacterium in cattle that was ubiquitous, easy to culture, and one of the major carcass contaminants at slaughter (Stopforth et al., 2006). *E. coli* was considered a potential reservoir of resistance genes that could transfer resistance to other zoonotic or commensal organisms that might cause disease in cattle or people (Linton, 1985; Winokur et al., 2001; Hoyle et al., 2005; Donaldson et al., 2006). It was therefore judged important to ensure that the last fecal sample was collected during the last 24 h prior to shipping for slaughter, to be representative of bacteria at the stage of production that might ultimately affect the consumer.

There were few published studies on AMU and AMR in feedlot cattle. Direct comparison of results between species or even between different stages in the production cycle was not appropriate as AMU and management norms were extremely different (Radostits, 2001). In general, calves arrive at the feedlot with relatively low proportions of resistant bacterial isolates. In 2005, surveillance of generic *E. coli* isolates from colon samples at Canadian abattoirs suggested that the prevalence of isolates resistant to more than one antimicrobials was about 27% in beef cattle, 85% in swine, and 77% in chickens (PHAC, 2005). Numbers in this study suggested a slightly higher prevalence, but only because it was calculated for animals not isolates, where an

animal was considered resistant if it had more than one resistant isolate. Even in comparable studies in feedlot cattle, differences may exist between methodologies for sample collection, culture, and determination of antimicrobial susceptibilities, analysis and presentation of results due to differing underlying purposes or interests (McEwen et al., 2006a). The use of veterinary specific antimicrobial susceptibility methods and standards from the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing, in this study and in the Canadian and American national surveillance systems, narrows some of these differences. However, it is important to keep in mind that CLSI methods and interpretive criteria were developed for approved indications of the antimicrobial for specific pathogens, but have been applied to surveillance of commensal bacteria (PHAC, 2005; Watts and Lindeman, 2006).

The AMR profiles of generic *E. coli* isolated from cattle feces on arrival at the feedlot in western Canada have not been previously characterized. Similarly, baseline levels of resistant bacteria in the feces of newly weaned, auction market derived calves on arrival at the feedlot in Western Canada are not well documented. The AMR in fecal *E. coli* on arrival at the feedlot represents the antimicrobial resistant strains of bacteria that the calves were carrying prior to antimicrobial treatment at the feedlot, and may be related to resistance patterns and AMU in their herd of origin. In this study, there was no resistance found to ENR (a fluoroquinolone) and GEN (an aminoglycoside), both of which represented drug classes used in human medicine. At the time of this study, ENR was not licensed in cattle and GEN was only liscensed for intrauterine use in cattle in Canada. They were evaluated due to concerns about cross-resistance and coresistance, mechanisms by which resistance to one antimicrobial may be associated with resistance

to another, related or unrelated, antimicrobial (Guardabassi and Courvalin, 2006). Enrofloxacin (ENR) has now been licensed for use in cattle for the treatment of bovine respiratory disease. Another feedlot study in the USA also found no resistance to GEN or ciprofloxacin, a fluoroquinolone like ENR (Wagner et al., 2002). The proportion of TCY resistant *E. coli* isolates was similar to that found in newly weaned calves on pasture in an American study where 13 to 17% of fecal samples contained *E. coli* resistant to TCY in at least one of the five isolates per fecal sample (Huston et al., 2003).

This study examined individual animal antimicrobial usage in the absence of feed antimicrobials other than coccidiostats. The use of feed antimicrobials for disease prophylaxis and treatment was a common practice in feedlots in western Canada, and elsewhere in North America; growth promotion was not always the primary objective of this use (Booker et al., 1999; USDA, 1999). There were no published studies on the quantities of antimicrobials used in western Canadian feedlots, but regional feedlot consultants describe their use of feed antimicrobials, other than ionophores, as primarily for disease prophylaxis (Booker et al., 1999). In western Canadian feedlots, use of antimicrobials by approved instructions was the operating norm (Booker et al., 1999). Coccidiostats, including ionophores, are antimicrobials but they have not been used in human medicine and the importance of these agents with respect to AMR remains unclear.

There were concerns that DDD in humans do not represent the prescribed daily dose (PDD) in humans at the national level (Monnet et al., 2004; Grave et al., 2006). This

was not a valid concern for the use of $ADD_{Feedlot}$ in this study where each $ADD_{Feedlot}$ was calculated for the actual antimicrobial dose received.

Recent studies in western Canada examined associations between AMR and AMU in *Campylobacter* spp. (Inglis et al., 2005; Inglis et al., 2006). In one of the studies, the authors suggest that the increased proportion of cattle with tetracycline resistant isolates of *Campylobacter* spp. preslaughter might be associated with the use of oxytetracycline or chlortetracycline in the feed (Inglis et al., 2006). Other attempts to evaluate the association between individual animal AMU and AMR have been confounded by the concurrent use of antimicrobials in the feed (Dunlop et al., 1998; O'Connor et al., 2002). Therefore, the opportunity to evaluate associations in this study, in the absence of feed antimicrobials, such as oxytetracycline or tylosin, was considered important.

In the cohort of cattle examined, there were treated and untreated cattle with no resistant isolates on arrival, but more than one resistant isolate preslaughter. There were also cattle that had at least one resistant isolate on arrival but none preslaughter. No associations were found in this study between individual animal AMU and AMR. This was somewhat different than findings from other studies where associations were found between AMR in fecal *E. coli* isolates in pigs and cattle and individual AMU when feed antimicrobials were also used (Dunlop et al., 1998; O'Connor et al., 2002). In one study, individual animal use of GEN was associated with the farm-level prevalence of GEN resistance in fecal *E. coli* isolates in pigs (Dunlop et al., 1998). In another study, the use of injectable oxytetracycline in individual cattle receiving chlortetracycline in the feed was associated with increased prevalence of resistance to chloramphenicol and sulfisoxazole in fecal *E. coli* isolates (O'Connor et al., 2002). In both above cases, the

associations were found with antimicrobials (GEN, chloramphenicol and sulfisoxazole) not used for mass medication of cattle in the feed. It was suggested that an association of AMR with feed medication might obscure the relationship between individual animal antimicrobial treatment and AMR if this relationship did indeed exist (Dunlop et al., 1998; O'Connor et al., 2002). Studies in pre-weaned dairy calves have shown associations between AMR and AMU. In the one study, calves treated within five days prior to sampling were more likely to have multiple drug resistant fecal *E. coli* than those not treated during that time frame (Berge et al., 2005). This may indicate that individual antimicrobial treatment has a transient effect that would no longer be present in the current study, at the time immediately preslaughter when the 2nd sample was taken. Only one animal was treated in the 100 d immediately preslaughter. Again, the focus of this study was the change in AMR over the feeding period to understand the effect of antimicrobial agents over that time. It should also be noted that AMR does occur in the absence of AMU pressure (Donaldson et al., 2006; Hoyle et al., 2006).

A potential weakness in this study was that metaphylactic antimicrobial injections were given to all cattle; there were several reasons for this. The goal of the study was evaluation of associations between the volume of individual animal AMU and AMR. In western Canada, metaphylactic usage of antimicrobials was estimated at 20% to 50% of calves on arrival at the feedlot across all groups, based on the risk profiles of each group of cattle on arrival (Radostits, 2001). Metaphylaxis use in this study was representative of commercial feedlot management; only one AMU variable was removed, the in-feed use of oxytetracycline or tylosin. It is likely that metaphylaxis was associated with AMR in this study; considerable TCY and AMP conversion occurred in both groups in

the cohort study, not associated with the individual animal antimicrobial disease treatments. The volume of individual animal antimicrobial doses was a logical way to evaluate antimicrobial use quantified beyond that used for metaphylaxis. Using the variable Total $ADD_{Feedlot}$, not $ADD_{Feedlot}$ calculated for specific antimicrobials, could have masked associations between AMU of specific antimicrobials and AMR (Dohoo et al., 2003). This study was not designed examine these specific associations.

In conclusion, calves arrived at the feedlot with relatively low numbers of resistant bacteria compared with other species (PHAC, 2005). Basic description of AMU in one group of calves in a western Canadian commercial feedlot was given. Understanding of resistance patterns existing on arrival at the feedlot as well as AMU during the feeding period was essential to understanding the changing patterns of antimicrobial resistant isolates at the feedlot and interpreting resistance patterns preslaughter. No association was found between total AMU for individual animal disease treatment and AMR in fecal *E. coli* isolates. This study allowed a unique opportunity to evaluate these associations in cattle that were not fed antimicrobials, other than a coccidiostat, during the entire feeding period. The results suggest that metaphylaxis and AMR patterns should be evaluated further, and that the evaluation of conversion to resistance during the feeding period is important when assessing associations of AMR attributable to AMU during the feeding period.

5.5 Acknowledgments

The authors thank the management and staff of the University of Saskatchewan Feedlot and Pound-Maker Agventures for their cooperation during this study. The

Saskatchewan Agriculture Development Fund and Novartis Animal Health Canada are also thanked for their support of this study.

5.6 References

1. Abramson JH, Gahlinger PM. Computer programs for epidemiologists: PEPI version 4.0. Salt Lake City, Utah: Sagebrush Pr, 2001.

2. Angulo FJ, Nunnery JA, Bair HD. Antimicrobial resistance in zoonotic enteric pathogens. Rev Sci Tech 2004;23:485–496.

3. Austin DJ, Kakehashi M, Anderson RM. The transmission dynamics of antibiotic-resistant bacteria: The relationship between resistance in commensal organisms and antibiotic consumption. Proc Biol Sci 1997;264:1629–1638.

4. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA 1999;96:1152–1156.

5. Baquero F. Trends in antibiotic resistance of respiratory pathogens: An analysis and commentary on a collaborative surveillance study. J. Antimicrob Chemother 1998;38(Suppl A):117–132.

6. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005;69:25–38.

7. Booker CW, Guichon PT, Schunicht OC, Wildman BK, Jim GK. Economic impact of antimicrobial use in feedlots. Bovine Proceedings 1999;32:111–112.

8. Bywater RJ. Identification and surveillance of antimicrobial resistance dissemination in animal production. Poultry Science 2005;84:644–648.

9. Canadian Cattlemen's Association and Beef Information Centre (2006). Antibiotics in the Beef Cattle Industry, 2006. [factsheet on the Internet]. Available from http://www.cattle.ca/factsheets/Antibiotics.pdf Last accessed March 25, 2007.

10. Canadian Veterinary Medical Association. Prudent use guidelines, 2001. [homepage on the Interenet] Available from http://canadianveterinarians.net/ShowText.aspx?ResourceID=86 Last accessed October 7, 2007.

11. Checkley SL, Janzen ED, Campbell JR, McKinnon JJ. Efficacy of vaccination against *Fusobacterium necrophorum* infection for control of liver abscesses and footrot in feedlot cattle in western Canada. Can Vet J 2005;46:1002–1007.

12. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; 16th Informational Supplement. (Use for Humans). CLSI document M100-S16, 2006.

13. Conly J. Antimicrobial resistance in Canada. CMAJ 2002;167:885–891.

14. Crandall J, Van Donkersgoed J. Canadian Cattlemen Quality Starts Here Recommended Operating Procedures for Feedlot Animal Health. Calgary: Canadian Cattlemen's Association, 2000.

15. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown: AVC Inc., University of Prince Edward Island, 2003.

16. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. App Environ Epidem 2006;72;3940–3948.

17. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998;34:283–305.

18. Grave K, Jensen VF, McEwen S, Kruse H. Monitoring of antimicrobial drug usage in animals: Methods and applications. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006;380–385.

19. Guardabassi L, Courvalin P. Antimicrobial resistance in bacteria of animal origin. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington, DC: ASM Pr, 2006, 5, 7.

20. Hoyle DV, Yates CM, Chase-Topping ME, et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71:6680–6688.

21. Hoyle DV, Davison HC, Knight HI, et al. Molecular characterisation of bovine faecal *Escherichia coli* show persistence of defined ampicillin resistant strains and the presence of class 1 integrons on an organic beef farm. Vet Microbiol 2006;115:250–257.

22. Huston CL, Bailey RH, Best TF, Huston JE, Evans RR. Antimicrobial resistance of enteric *E. coli* in beef cattle treated with antibiotics. The AABP Proceedings 2003;36:156–157.

23. Inglis GD, McAllister TA, Busz HW, et al. Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter hyointestinalis.* Appl Environ Microbiol 2005;71:3872–3881.

24. Inglis GD, Morck DW, McAllister TA, et al. Temporal prevalence of antimicrobial resistance in *Campylobacter* spp. from beef cattle in Alberta feedlots. Appl Environ Microbiol 2006;72:4088–4095.

25. Kelly AP, Janzen ED. A review of morbidity and mortality rates and disease occurrence in North American feedlot cattle. Can Vet J 1986;27:496–500.

26. Khachatourians, GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. CMAJ 1998;159:1129–1136.

27. Linton AH. Antibiotic resistance in veterinary practice. Vet Record, In practice 1982;14:11–13.

28. Linton AH. Antibiotic resistance in bacteria associated with animals and their importance to man. J Antimicrob Chemother 1985;15:385–386.

29. McEwen SA, Aarestrup FM, Jordan D. Monitoring of antimicrobial resistance in animals: Principles and practices. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006, 398-399.

30. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? CMAJ 1998;159:1119–1120.

31. McKellar QA. Antimicrobial resistance: A veterinary perspective. BMJ 1998;317:610–611.

32. Monnet DL, Molstad S, Cars O. Defined daily doses of antimicrobial reflect antimicrobial prescriptions in ambulatory care. J Antimicrob Chemother 2004;53:1109–1111.

33. NCCLS. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolates from Animals; Information Supplement M32-S1, 2004.

34. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res 2002;66:145–150.

35. OIE, Paris, France. OIE International Standards on Antimicrobial Resistance, 2003. [homepage on the Internet] Available from http://www.oie.int/eng/publicat/ouvrages/A_119.htm Last accessed Jan 14, 2008.

36. Public Health Agency of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005 - Final Report, 2005. [homepage on the Internet]. Available from http://www.phac-aspc.gc.ca/cipars-picra/2005_e.html#sum. Last accessed October 7, 2007.

37. Radostits OM. Control of infectious diseases of food-producing animals. In: Radostits OM, ed. Herd Health: Food Animal Production Medicine, 3rd ed. Philadelphia: WB Saunders, 2001:151.

38. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69:1456–1459.

39. USDA. Part III: Health Management and Biosecurity in US Feedlots, 1999. USDA:APHIS:VS: CEAH, National Animal Health Monitoring System. Fort Collins, CO. #N336.1200.

40. Veterinary Drug Directorate, Health Canada. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health. 2002. [homepage on the Internet]. Available from http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html Last accessed Sept. 27, 2007.

41. Wagner BA, Dargatz DA, Salman MD, Morley PS, Wittum TE, Keefe TJ. Comparison of sampling techniques for measuring the antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. Am J Vet Res 2002;63:1662–1670.

42. Watts JL, Lindeman CJ. Antimicrobial susceptibility testing of bacteria of veterinary origin. In: Aarestrup FM, ed. Antimicrobial Resistance in Bacteria of Animal Origin. Washington DC: ASM Pr, 2006, 30, 33-34.

43. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp ID, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates form food animals and humans. Antimicrob Agents Chemother 2001;45:2716–2722.

Table 5.1: Concentration range and breakpoints of antimicrobials tested.

Antimicrobial[a]	Breakpoint for resistance (μg/mL)	Concentration range measured (μg/mL)
AMP	≥ 32	< 4, 4, 8, 16, 32, > 32
ENR	≥ 2	< 0.5, 0.5, 1, 2, 4, > 4
GEN	≥ 16	< 4, 4, 8, 16, 32, > 32
SMX	≥ 512	< 125, 125, 256, 512, > 512
TCY	≥ 16	< 2, 2, 4, 8, 16, 32, > 32
TMP/SSS	≥ 4/76	< 1/19, 1/19, 2/38, 4/76, 8/152, > 8/152
TMP	≥ 16	< 4, 4, 8, 16, 32, > 32

[a]AMP—Ampicillin; ENR—Enrofloxacin; GEN—Gentamicin; SMX—Sulphamethoxazole; TCY—Tetracycline; TMP/SSS=Trimethoprim/sulfanilamide; TMP—Trimethoprim

Table 5.2: Antimicrobials used in this study and Animal Defined Dose ($ADD_{Feedlot}$) equivalent for a feedlot animal.

Antimicrobials used in study—concentration	Dose equivalent (mg/kg BW)[a]	$ADD_{Feedlot}$ equivalent
Ceftiofur—50 mg/mL; intramuscular injection (Excenel Sterile Powder; Pharmacia Animal Health, Orangeville, Ontario)	1	1
Oxytetracycline—200 mg/mL; intramuscular injection (Liquamycin × LA-200; Pfizer Canada Animal Health Group, Kirkland, Quebec)	20	3
Tilmicosin—300 mg/mL; subcutaneous injection (Micotil; Provel, Division Eli Lilly Canada, Guelph, Ontario)	10	3

[a]Actual body weight of the animal at the time of treatment.

Table 5.3: Antimicrobial resistance in fecal *Escherichia coli* isolates from steers on arrival at the feedlot

Antimicrobial resistance	Count resistant (% resistant) *n* = 153	Exact lower confidence limit (%)	Exact upper confidence limit (%)
Resistance to antimicrobials[a]			
AMP[b] resistance	31 (20.3)	14.2	27.5
SMX resistance	68 (44.4)	36.4	52.7
TCY resistance	27 (17.6)	12.0	24.6
TMP/SSS resistance	7 (4.6)	1.9	9.2
TMP resistance	1 (0.7)	0.02	3.6
Multidrug Resistance[c]			
Resistant to 0 antimicrobials	56 (36.6)	29.0	44.8
Resistant to 1 antimicrobial	71 (46.4)	38.3	54.6
Resistant to ≥ 1antimicrobials	97 (63.4)	55.2	71.0
Resistant to ≥ 2 antimicrobials	17 (11.1)	6.6	17.2
Resistant to ≥ 3 antimicrobials	9 (5.9)	2.7	10.9

[a]An animal was considered "resistant" if 1 or more isolate was resistant to the antimicrobial.

[b]AMP—Ampicillin, SMX—Sulphamethoxazole, TCY—Tetracycline, TMP/SSS—Trimethoprim/sulfanilamide, TMP—Trimethoprim.

[c]The number of antimicrobials to which an animal was resistant, was characterized by the isolate with resistance to the most number of antimicrobials.

Table 5.4: Antimicrobial treatments stratified by whether animals converted to a resistant status to specific antimicrobials during the feeding period.

Antimicrobial resistance to:	Treatment during feeding period	Resistance conversion[b]	No conversion	Total cattle
AMP[a]	No	22	31	53
	Yes	24	18	42
	Total	46	49	95
TCY	No	38	15	53
	Yes	34	8	42
	Total	72	23	95
SMX	No	6	47	53
	Yes	5	37	42
	Total	11	84	95
TMP/SSS	No	2	51	53
	Yes	0	42	42
	Total	2	93	95
TMP	No	3	50	53
	Yes	0	42	42
	Total	3	92	95

[a]AMP—Ampicillin; SMX—Sulphamethoxazole; TCY—Tetracycline; TMP/SSS—Trimethoprim/sulfanilamide; TMP—Trimethoprim.

[b]Resistance conversion was defined as an animal having more resistant isolates present preslaughter than at arrival.

Table 5.5: Number of $ADD_{Feedlot}$ per animal level stratified by whether animals converted to a resistant status to specific antimicrobials during the feeding period.

Change in antimicrobial resistance pattern	$ADD_{Feedlot}$ total						Grand total
	0	2	3	5	6	9	
No TCY[a] conversion	15	1	6	0	1	0	23
TCY conversion	38	8	22	2	1	1	72
No AMP conversion	31	4	10	2	1	1	49
AMP conversion	22	5	18	0	1	0	46
No SMX conversion	47	6	27	2	1	1	84
SMX conversion	6	3	1	0	1	0	11
No TMP/SSS conversion	51	9	28	2	2	1	93
TMP/SSS conversion	2	0	0	0	0	0	2
No TMP conversion	50	9	28	2	2	1	92
TMP conversion	3	0	0	0	0	0	3
Grand total	53	9	28	2	2	1	95

[a]AMP—Ampicillin; SMX—Sulphamethoxazole; TCY—Tetracycline; TMP/SSS—Trimethoprim/sulfanilamide; TMP—Trimethoprim.

Table 5.6: Univariate associations for antimicrobial resistance in cattle that had an increase in resistant isolates to tetracycline preslaughter.

Variable	Variable (categorical)[a]	β	SE	*P*
TCY[b]	Treatment group	-0.517	0.497	0.298
	Diet	-0.374	0.482	0.439
	Total $ADD_{Feedlot}$ (overall)	--	--	0.494
	AMP conversion	-0.497	0.488	0.308
AMP	Treatment group	-0.631	0.418	0.132
	Diet	0.128	0.411	0.756
	Total $ADD_{Feedlot}$ (overall)	--	--	0.319
	TCY conversion	0.497	0.488	0.308

[a]β—Beta coefficient; SE—standard error of Beta coefficient; *P*—probability value

[b]AMP—Ampicillin; TCY—Tetracycline.

CHAPTER 6
ANTIMICROBIAL RESISTANCE IN FECAL *E. COLI* ISOLATES FROM COMMERCIAL FEEDYARDS IN ALBERTA AND THE EFFECT OF INDIVIDUAL ANTIMICROBIAL USE AND PEN CHARACTERISTICS

6.1 Introduction:

The potential for resistant strains of bacteria from livestock to affect people is perceived to be a human health concern (Acar et al., 2001; Conly, 2002). Therefore, the cattle industry has made promotion of prudent use of antimicrobials in cattle a priority (CVMA, 1998; Crandall et al., 2000; CCA and BIC, 2006). Antimicrobials are used in cattle production for a number of purposes including metaphylaxis in newly-arrived high-risk calves, disease prophylaxis, and individual treatment of sick animals. Many studies have shown economic and animal health benefits from metaphylactic and prophylactic antimicrobial use in feedlot cattle (Gallo and Berg, 1995; Wittum et al., 1996; Booker et al., 1997; Jim et al., 1999). The World Health Organization, acting on the precautionary principle, made a blanket recommendation that the use of growth promotant antimicrobials in animal feed be discontinued (WHO, 2002). This concern that the use of antimicrobials in animals could cause antimicrobial resistance that might negatively affect human health was first voiced many decades ago (Swann, 1969) and is debated today (Apley, 1998; Conly, 2002; Lipsitch et al., 2002; Smith et al., 2002; Singer et al., 2003). More information is needed about the extent to which the use of antimicrobials in animal agriculture is related to development of antimicrobial resistance in human infections (Conly, 2002).

Information on the prevalence of antimicrobial resistance in commensal fecal *E. coli* isolates from calves in feedyards late in the feeding period in Western Canada is currently sparse. Further data on antimicrobial resistance in commensal organisms are needed as this may represent the potential for transfer of antimicrobial resistance to zoonotic organisms within the host or horizontally between animals (Austin et al., 1997; Stevenson et al., 2003). Questions have also arisen when designing studies as to whether characteristics of the pen or feedlot level management practices have the most influence on antimicrobial resistance. Answers to this question could help direct future research towards control practices most likely to be effective. Further examination of the number of isolates necessary for establishing resistance levels in feedyard pens is also required to determine the best methods to sample pens and obtain results that can be generalized to the wider population (Dunlop et al., 1999; Wagner et al., 2003; Echeverry et al., 2005). Analysis of risk factors associated with the presence of antimicrobial resistance in fecal *E. coli* isolates late in the feeding period will perhaps indicate intervention points in our current feedlot production system.

The objectives of this study were to determine the prevalence of antimicrobial resistance in non-pathogenic fecal coliforms from composite feedyard pen samples for calves late in the feeding period; to determine the number of isolates necessary for establishing resistance levels in feedyard pens; and to evaluate the effect of feedyard demographic characteristics, pen demographic characteristics and pen-level antimicrobial use on the prevalence of antimicrobial resistance.

6.2 Materials and Methods

This project was a cross-sectional study of pens of cattle from southern Alberta commercial feedyards. Four feedyards from one veterinary practice, with standardized computer record systems, were identified and asked to participate. All feedyards consisted of side-by-side, open-air pens, with dirt floors and 20% porosity fencing. Information on feedyard demographics, pen characteristics, and individual animal treatments was collected. All pens were treated with feed antimicrobials, as is the norm in commercial feedyard production. Information was collected about the type of antimicrobials used and duration of treatment.

The unit of interest in this study was the feedyard pen. Composite fecal samples and animal health information were collected at the pen-level. Stratified random sampling was used to proportionally choose pens from each feedyard within each class of animal (yearling, fall-placed calf, winter-placed calf) from all available pens within 90 days of slaughter. A total of 25 out of 77 potential pens were chosen. Four of these pens, in feedyard 1, had been sorted and contained animals from more than one lot. Composite fecal samples were collected by taking approximately 20 grams of feces from each of 30 fresh fecal pats selected while walking a preset pattern around each pen. These pooled samples were refrigerated and transported fresh to the laboratory for analysis within a maximum time period of 48 hours.

6.2.1 Laboratory Analysis

Thirty pooled fecal samples weighing approximately 600 grams were collected in stomacher bags (Tekmar Stomacher Model 400, Tekmar Company, Cincinnati, Ohio, USA) and transported to the laboratory on ice. Two hundred mL of sterile saline was

added to each bag and mixed. The feces were then divided evenly into two bags and another 100 mL of saline was added to each bag. Samples were homogenized in the stomacher for 20 seconds. Sterile swabs were used to obtain a representation of the sample from both bags and inoculated onto MacConkey agar plates for a total of four plates per sample. These were incubated at 35°C for 18-24 hours. Five colonies that morphologically appeared as *E. coli* were chosen randomly from each plate and cultured onto blood agar and MacConkey agar plates. There were a total of 20 isolates per pen. Colonies taken from blood agar were verified as *E. coli* by EC Medium with MUG and IMViC. These same isolates were subcultured onto blood agar plates for resistance testing with CMV7CNCD panels (Sensititre®, TREK Diagnostic Systems, Inc., Cleveland, USA). This was carried out by making a 0.5 McFarland standard solution in 5 mL of demineralized water. Ten µL of this solution was transferred into 11 mL of Mueller-Hinton broth with TES and mixed. Fifty µL of broth was inoculated into each well in the 96 well panel using the autoinoculator and incubated for 18-24 hours at 35°C. Plates were read using a semi automated system (Sensitouch®, TREK diagnostic Systems, Inc., Cleveland, USA). Minimum inhibitory concentrations (MIC) of 16 antimicrobials were determined (Table 6.9). Breakpoints and subsequent interpretations of isolates as sensitive, intermediate or resistant, for all antimicrobials used except streptomycin, were taken from the Clinical and Laboratory Standards Institute (CLSI) guidelines see Table 6.9 (NCCLS, 2004; CLSI, 2006). Human breakpoints were used where animal breakpoints were not established. The breakpoint for streptomycin of ≥ 64µg/mL, used by the National Animal Resistance Monitoring System (NARMS), was also used in this study (USDA, 2004). This panel included

antimicrobials used in humans and livestock and followed the composition of NARMS panels (USDA, 2004).

6.2.2 Statistical Analysis

Isolates classified as either susceptible or intermediate were considered sensitive for this analysis. Descriptive and analytic statistics were performed using commercial software (SPSS for Windows 11.5.0, SPSS Inc., Chicago, USA). Generalized estimating equations (GEE) were used to adjust prevalence estimates for clustering (at the pen-level) (PROC GENMOD, SAS for Windows version. 9.1, SAS Institute Inc., Cary, North Carolina). Population-averaged prevalence estimates and 95% confidence intervals were then calculated using the formula $1/(1 + \exp(-\beta))$ (Dohoo et al., 2003).

The proportion of resistant isolates when 5, 10, or 15 of the isolates were chosen using randomly generated numbers from the 20 observed isolates and the four proportions were compared using the Kruskal-Wallis Test.

Variance components were partitioned in a three-level null binomial model for the outcomes resistance to three or more antimicrobials (multi-drug resistance) and resistance to tetracycline (MLwiN Release 2.02, Centre for Multilevel Modelling, Institute of Education, London, United Kingdom). A restricted iterative generalized least squares method (second order, penalized quasilikelihood) was used for estimation in this computer program. Models were checked for the presence of extra-binomial variation. Extra-binomial parameters in the range of 0.9 to 1.0 were reset at 1.0 (binomial variation). The proportion of level-1 variance was assumed to be $\pi^2/3$ or 3.29, the variance of the standard logistic distribution (Dohoo et al., 2003; Goldstein, 2003).

A logistic regression model using generalized estimating equations was used to examine the association between AMR and potential explanatory variables (PROC GENMOD, SAS for Windows version 9.1, SAS Institute Inc., Cary, North Carolina). Two binary outcomes were evaluated; these were resistance to three or more antimicrobials (multi-drug resistance) and resistance to tetracycline. Categorical risk factors explored for both outcomes were feedyard, and sex of the animal. Continuous risk factors explored for both outcomes were number of animals in pen, projected DOF (days between arrival date and projected slaughter date), Animal Defined Doses for a feedlot animal ($ADD_{Feedlot}$) of antimicrobials used at the pen-level, and $ADD_{Feedlot}$ used at the pen-level in the last 100 days before the sampling was carried out. The linearity of continuous risk factors was assessed by graphing log odds of the outcome against the categories of the risk factors divided at the quartiles. Residuals were also assessed. The measurement, $ADD_{Feedlot}$, was created to describe the number of antimicrobial treatments received per pen at the approved dose of the antimicrobial in feedlot management systems typical in western Canada. This measurement accounted for the dosage and duration of action of the antimicrobial (Table 6.1). The concept of $ADD_{Feedlot}$ is based on that of Defined Daily Dose (DDD) used in the human literature (Baquero, 1996; Austin et al., 1997; Austin et al., 1999; Grave et al., 2006). Antimicrobial use information was not available at one of the four feedyards. Collinearity between risk factors was assessed using Spearman's rank correlation coefficients. When risk factors were collinear, only unconditional associations were examined. Projected DOF was a linear variable chosen to represent several factors in the analysis that were highly collinear. This included the categorical variables

metaphylaxis treatment, chlortetracycline used in feed, and class of cattle. Longer projected DOF occur in fall placed calves that are treated with chlortetracycline in the feed and receive metaphylactic antimicrobial injections on arrival at the feedlot.

6.3 Results

6.3.1 Feedyard and Pen Demographics

Feedyard capacity, number of animals per pen, and medications used vary greatly between feedyards (Table 6.2). Differences also existed in factors related to the individual pen (Table 6.3).

6.3.2 Prevalence of Antimicrobial Resistance

No *E. coli* isolates showed resistance to amikacin, ceftriaxone, ciprofloxacin, gentamicin, or nalidixic acid during this study. Low levels of resistance were found to most antimicrobials tested (Table 6.4). With adjustment for clustering, 55.4% of all isolates were not resistant to any antimicrobials tested, and another 25.4% of all isolates were resistant to only one antimicrobial. The predominant antimicrobial resistance was to tetracycline with 39.4% of all isolates being classified as resistant. Resistance to streptomycin was present in 13.4% of all isolates and resistance to sulphamethoxazole was evident in 13.0%. There was some evidence of multi-drug resistance (Table 6.5) and resistance to three or more antimicrobials was found in 7.6% of all isolates. One isolate was resistant to ten antimicrobials (amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulphamethoxazole, tetracycline, and trimethoprim/sulfamethoxazole). There were 22 different resistance

phenotypes represented in this study and 12 of them were associated with resistance to three or more antimicrobials (Table 6.6).

6.3.3 Number of isolates needed for determination of pen resistance status

There was no significant difference between the proportion of isolates resistant to one or more antimicrobials, two or more antimicrobials, or three or more antimicrobials, nor in the proportion tetracycline resistant isolates per pen when using 20, 15, 10 or 5 isolates for interpreting the pen-level resistance.

6.3.4 Evaluation of factors associated with the antimicrobial resistance

In a 3-level null model summarizing resistance to three or more antimicrobials 12.7% of the total variation in resistance was accounted for at the feedyard level [v_{0k}=0.713 (se=0.993)] and 28.7% at the pen level [μ_{0jk}=1.614 (se=1.023)]. The feedyard accounted for 19.3% of total variation in tetracycline resistance [v_{0k}=0.842 (se=0.658)], pen accounted for 5.1% [μ_{0jk}=0.220 (se=0.145)], and the remaining 75.6% of total variation was within the pen (sample) level.

The number of animals in pen (OR=0.90, 95% CI: 0.84-0.97, *P*=0.01) was unconditionally associated with the occurrence of tetracycline resistance (Table 6.8). Other possible associations of interest include projected days on feed (OR=1.05, 95% CI: 1.00-1.10, *P*=0.12) and Animal Defined Doses of antimicrobials in a feedlot animals (OR=0.98, 95% CI: 0.96-0.99, *P*=0.06) unconditionally associated with resistance to three or more antimicrobials (Table 6.7) and Animal Defined Doses of antimicrobials in a feedlot animals (OR=0.95, 95% CI: 0.91-0.99, *P*=0.06) unconditionally associated with the occurrence of tetracycline resistance.

6.4 Discussion

The overall prevalence of antimicrobial resistance in this study is comparable with results from a recent American feedyard study (Wagner et al., 2003). This study examined antimicrobial resistance in commensal *E. coli* collected from individual and pooled fecal samples from the rectum or the pen floor using the same MIC breakpoints as our study. Overall prevalence of resistance to tetracycline, streptomycin, and sulphamethoxazole were similar between the studies as was the prevalence of isolates sensitive to all antimicrobials tested. The Wagner study showed slightly higher prevalence of isolates resistant to three or more antimicrobials or resistant to six or more antimicrobials than our study. Our study found 22 different resistance phenotypes in the *E. coli* sampled whereas the Wagner study found a maximum of 15 different resistance phenotypes in the pooled samples and a total of 40 different resistance phenotypes across all sampling methods (Wagner et al., 2003). This could indicate a difference in the prevalence of resistance phenotypes in different geographical locations or perhaps the multiple isolates cultured from pooled samples in our study maximized the resistance phenotypes found. Also of note, no *E. coli* isolates in our study showed resistance to amikacin, ceftriaxone, ciprofloxacin, gentamicin, or nalidixic acid whereas, in the Wagner study, one isolate was resistant to ciprofloxacin and between 0.0-1.7% of isolates were resistant to nalidixic acid from either individual or pen-floor samples.

Surveys of generic *E. coli* isolated from beef carcasses in Canadian slaughter plants found similar levels of antimicrobial resistance to the current study. In one study, out of 2653 isolates, 68% of isolates were sensitive to the 18 antimicrobials tested, which included the 16 used in the current trial (VanDonkersgoed et al., 2003). Resistance was

found to tetracycline in 25% of isolates, to sulfamethoxazole in 9% of isolates, and to streptomycin in 7% of isolates. Five percent of isolates were classified as resistance to three or more antimicrobials. The same MIC breakpoints for resistance interpretation were used in the slaughter plant study as in the current study. This information, from a much larger sample size, showed a similar distribution of resistance profiles although the proportions of isolates classified as resistant were somewhat lower than in the current study. The second abattoir study was much smaller but found 56% of 284 total isolates sensitive to all antimicrobials tested and 44% resistant to one or more antimicrobials (Aslam and Service, 2006). These samples were collected at different points during carcass processing. The susceptibility testing in the second abattoir study was also done using the same methods as the current study.

The current study provided an opportunity to address some important questions about sampling methods. Sampling issues were considered in the current study. Fecal samples from the floor of feedlot pens, as used in the current study, appear to be fairly reliable estimates of antimicrobial resistance in fecal *E. coli* isolates from individual animals (Wagner et al., 2003). Pooled samples have shown to yield good estimates of pen-level prevalences of antimicrobial resistance in other studies, when the prevalence of the resistance is greater than 2% (Dunlop et al., 1999). Fecal sample collection from the pen floor has been used previously for antimicrobial resistance determination (Galland et al., 2001; Dargatz et al., 2002; Wagner et al., 2003) and some studies have also pooled these samples (Dargatz et al., 2002; Wagner et al., 2003). The stomacher method of sample homogenization was used in the current study to disperse organisms throughout the sample before swabs were taken for bacterial culture. Researchers have

investigated the effect of sample weight and adequate homogenization of samples to ensure bacteria are adequately dispersed from any clusters within the feces (Cannon et al., 2002). It has been suggested that choosing ten colony samples per individual fecal specimen would determine the number of 'majority' (>10% prevalence) *E. coli* O-serotypes, but varied in ability to identify 'minority' serotypes present in an individual animal fecal sample compared to a 100 colony analysis (Hedges et al., 1977). The ten colony analysis also performed better than the theoretical expectation in identification of major serotypes, probably due to inadequate random sampling of isolates from plates based on colonial morphology (Hedges et al., 1977).

A recent American study also examined pen level fecal samples within feedyards (Wagner et al., 2003). In this study, individual animal isolates, pools of five samples and pools of 10 samples were examined. One set of samples was derived from the individual animals, and another set from the pen floor. Antimicrobial resistance patterns were determined for the same set of antimicrobials used in our study plus one additional. Antimicrobial resistance patterns between the pooled samples and the individual animal samples did not differ significantly when the prevalence of resistance to an antimicrobial was >2%. Results from our current study add to these findings, in that there was no additional benefit to examining 10, 15, or 20 isolates from the pooled pen fecal samples, compared to 5 isolates. However, recent results from a current study that estimated the prevalence of *E. coli* 0157 from fecal pats using from one to five samples/pat, suggested that sampling only one site per pat underestimated the true prevalence of the pathogen (Echeverry et al., 2005). This idea will need to be further investigated as to how it relates to individual animal testing and pooling samples from

multiple fecal pats. Overall, the results of the current trial are another step towards understanding optimal sampling strategies for both individual animals and pen floors.

Variance for the outcome isolates resistant to three or more antimicrobials was examined in the absence of any risk factors and was higher at the pen level (28.7%) than at the feedyard level (12.7%). Partitioning of the variance from the three-level binomial model for the outcome resistance to tetracycline revealed higher variance at the feedyard level (19.3%) than at the pen level (5.1%). One of our feedyards differed significantly from the other three with regards to tetracycline resistance. The current study included a less homogenous group of cattle than reported in a similar analysis by Wagner (Wagner et al., 2003). In that study, variation explained at the pen-level was at most 6.6%. There was one group of antimicrobials with a low prevalence (1 to 3%) where 78-89% of the variation explained at the fecal sample level and the remaining 9-17% of variation explained at the within sample level. The second group of antimicrobials with a higher prevalence (8-40%) had only 34-48% of variation explained at the fecal sample level and 49-64% of variation explained at the within fecal sample level. A study in Canadian pig barns found, in a three level hierarchical model looking tetracycline resistance in fecal *E. coli* from pigs in rooms in pens, a very large between-pig, within-pen variance component (97.5%) with a small between pen, within room component (2.5%) and no between-room component (Dunlop et al., 1999).

Outcomes chosen for analyses of risk factors included resistance to three or more antimicrobials late in the feeding period because it was thought to have animal and public health relevance and tetracycline resistance which is one of the antimicrobials most commonly used in beef feedyards. The other potential explanatory variable

examined for associations with tetracycline resistance was the number of animals in the pen. As the number of animals in the pen decreased the odds of tetracycline resistance increased. This was true in our study where the one feedlot with much higher tetracycline resistance than the other three, and also had the smallest pens. Feedlots 1, 2 and three differ significantly from Feedlot 4 when comparing the likelihood of tetracycline resistance. Differences between antimicrobial resistance profiles in the different feedyards could potentially be due to other factors not measured in this study such as source of calves, environmental pressure of antimicrobial resistance genes in the feedyard, or history of antimicrobial use in the feedyard. This is not necessarily representative of other feedlots. This and other explanatory variables should be considered in future studies. It is important to note that the management practices and individual animal medication used in this study are specific for these four feedlots and weren't meant to be generalized to all Canadian feedyards as those in the study were under the supervision of one veterinary consultant.

Projected DOF was a potential explanatory variable for resistance to three or more antimicrobials late in the feeding period, as it approached significance. This variable encompassed several AMU factors; including, class of cattle, use of chlortetracycline in the feed and use of metaphylaxis on arrival. Calves that enter the feedlot in the fall at a lighter weight have a longer projected DOF. They are also likely to have other associated risk factors for BRD such as recent weaning, younger age, mixing at the auction market prior to arrival at the feedlot, transportation, and lower immunity to *Mannheimia hemolytica* and the bovine respiratory viruses (Smith et al., 2001). In addition, large pen size, temperature fluctuations, and lack of bunk familiarity, leading

to water deprivation and energy deficits, may also play a role in the total stress on the calf. On arrival at the feedlot, groups of calves are assessed for BRD risk. The risk designation also dictates the management protocols that will then be followed during the introductory phase in the feedlot (personal communication, Calvin Booker; Feedlot Health Management Services). Groups of high-risk calves, are commonly treated with an individual antimicrobial injection for BRD metaphylaxis, treated with chlortetracycline in the feed for BRD prophylaxis, and are in a group of animals where a higher level of individual injectable antimicrobials are used to treat BRD.

Younger age of calves on arrival (longer Projected DOF) might also be associated with AMR in another way. Previous work has shown that young calves have more resistant enteric flora isolates than adults although individual animal treatment was unknown in that study (Martel and Coudert, 1993).

$ADD_{Feedlot}$ was used to quantify individual animal treatments in the feedyard. In general, the use of a global variable such as $ADD_{Feedlot}$ also has some drawbacks. Total weight of active ingredient used might be preferable for some analyses. No international ADDs have been established for animal drugs, and approved medications and approved doses of medications may vary between countries, so care must be taken in interpretation of these numbers (Grave et al., 2006). In humans, DDD is a specific term that "corresponds to the assumed average maintenance dose per day for its main indication of the drug in adults."(Monnet et al., 2004). There are concerns about how well DDDs correspond, at the national level, to the number of antimicrobial prescriptions and the number of patients treated with antimicrobial agents." (Monnet et al., 2004). In this study, the $ADD_{Feedlot}$ was used to quantify the number of weight

appropriate doses (mg/kg) used for a specific class of animal giving a surrogate for selection pressure. All animal treatments were recorded. There were no injectable antimicrobials used in an extra-label fashion during this trial.

$ADD_{Feedlot}$ was also considered as a potential explanatory variable for resistance to three or more antimicrobials late in the feeding period as well as for resistance to tetracycline, even though it was only measured in three out of four feedlots that varied greatly in antimicrobial use. The relationship between Overall $ADD_{Feedlot}$ and either multidrug resistance or tetracycline resistance where resistance increased and Overall $ADD_{Feedlot}$ decreased was not biologically plausible and will not be considered further. $ADD_{Feedlot}$ for individual antimicrobials were not analyzed separately due to sample size limitations.

The current study has similarities to other studies evaluating associations between antimicrobial resistance cand antimicrobial use. One of these studies examines the development of resistance in generic fecal *E. coli* isolates in 139 yearling bulls housed in small pens (O'Connor et al., 2002). In that study, the use of subcutaneous injectable long-acting oxytetracycline in addition to chlortetracycline in the feed was associated with an increased amount of chloramphenicol and sulfisoxazole resistance in fecal *E. coli* isolates; no effect on tetracycline resistance was found (O'Connor et al., 2002). In pigs, antimicrobial medication of feed was associated with increased risk of antimicrobial resistance to seven of eight antimicrobials tested (Dunlop et al., 1998). There was no association of increased risk of gentamicin resistance with the use of in-feed antimicrobials; gentamicin was the only antimicrobial tested for resistance in that study which was not used for medicating the ration of any class of pigs (Dunlop et al.,

1998). The only individual pig treatments associated with increased antimicrobial resistance were gentamicin treatment associated with increased gentamicin resistance (Dunlop et al., 1998). It was suggested this effect was due to resistance associated with the feed medication overshadowing any association with individual animal treatment (Dunlop et al., 1998; O'Connor et al., 2002). Another study of antimicrobial resistance in fecal isolates from veterinarians appears to be confounded by individual antimicrobial use (Bongers et al., 1995). In our study, the association between feed antimicrobials (given to a group), individual antimicrobials (given to individual animals and summarized to the pen-level) and antimicrobial resistance cannot be evaluated as all pens received antimicrobial treatment in the feed for disease prophylaxis throughout the feeding period.

Overall, this study provides some basic information on the prevalence of resistance to 16 antimicrobials in feedyard pens late in the feeding period in Western Canada. Isolates from composite fecal samples from pen floors were a satisfactory method of describing antimicrobial resistance. A three-level analysis using a logistic model found more variation between pens than between feedlots when the outcome was resistance to three or more antimicrobials, but more variation between feedlots when the outcome is tetracycline resistance. This will be applicable to future research design. A study with the primary aim of examining risk factors could have less isolates per pen while maximizing the number of pens and the number of feedlots under different management protocols. This study suggests factors to be investigated in future studies including management practices and protocols involving AMU at the feedlot, pen and individual animal level.

6.5 Acknowledgements

The authors wish to thank the participating feedyards for their interest and involvement in this study. We also thank the technical staff of the Agri-Food Laboratories Branch, Food Safety Division, Alberta Agriculture and Food.

6.6 References

1. Acar J, Rostel B. Antimicrobial Resistance; an overview. Rev Sci Tech 2001;20(3):797-810.

2. Apley, M (1998). Does antimicrobial use in animals affect human health? *The Bovine Practitioner* 1998;31: 9-12.

3. Aslam M, Service C. Antimicrobial resistance and genetic profiling of *Escherichia coli* from a commercial beef packing plant. J Food Prot 2006;69(7):1508-1513.

4. Austin DJ, Kakehashi M, Anderson RM. The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption. *Proc Roy Soc London Ser B* 1997;264:1629-38.

5. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc Natl Acad Sci* 1999;95:1152-1156.

6. Baquero F. Trends in antibiotic resistance of respiratory pathogens: an analysis and commentary on a collaborative surveillance study. *J. Antimicrob Chemother* 1996;38 (Suppl A): 117-132.

7. Bongers JH, Franssen F, Elbers ARW, Tielen MJM. Antimicrobial resistance of *Escherichia coli* isolates from the faecal flora of veterinarians with different professional specialties. *Vet Quart* 1995;17:146-159.

8. Booker CW, Jim GK, Guichon PT, Schunicht OC, Thorlakson BE, Lockwood PW. Evaluation of florfenicol for the treatment of undifferentiated fever in feedlot calves in western Canada. *Can Vet J* 1997;38: 555-560.

9. Canadian Cattlemen's Association and Beef Information Centre (2006). Antibiotics in the Beef Cattle Industry. http://www.cattle.ca/factsheets/Antibiotics.pdf

10. Canadian Veterinary Medical Association 1998. Http://canadianveterinarians.com/showText.aspx?ResourceID=60 accessed April, 14, 2008.

11. Cannon RM, Nicholls TJ. Relationship between sample weight, homogeneity, and sensitivity of fecal culture for *Salmonella enterica. J Vet Diagn Invest* 2002;14:;60-02.

12. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. (Use for Humans). CLSI document M100-S16, 2006.

13. Conly, J. Antimicrobial resistance in Canada. *CMAJ* 2002;167 (8):885-91.

14. Crandall J, Van Donkersgoed J. Canadian Cattlemen Quality Starts Here Recommended Operating Procedures for Feedlot Animal Health, 2000.

15. Dargatz DA, Fedorka-Cray PJ, Ladely SR, Ferris KE, Green AL, Headrick ML. Antimicrobial Susceptibility patterns of *Salmonella* isolates from cattle in feedlots. *J Am Vet Med Assoc* 2002;221:268-72.

16. Dohoo I, Martin W, Stryhn H. Veterinary Epidemiologic Research. Charlottetown: AVC Inc., University of Prince Edward Island, 2003.

17. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. *Prev Vet Med* 1998;34:283-305.

18. Dunlop RH, McEwen SA, Meek AH, Friendship RM, Black WD, Clarke RC. Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs. *Epidemiol Infect* 1999;122:485-596.

19. Echeverry A, Loneragan GH, Wagner BA, Brashears MM. Effect of intensity of fecal pat sampling on estimates of *Escherichia coli*)157 prevalence. Am J Vet Res 2005;66(12):2023-2027.

20. Galland JC, Hyatt DR, Crupper SS, Acheson DW. Prevalence, antibiotic susceptibility, ad diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. *Appl Environ Microbiol* 2001;67(4):1619-27.

21. Gallo GF, Berg JL. Efficacy of a feed-additive antibacterial combination for improving feedlot cattle performance and health. *Can Vet J* 1995;36: 223-229.

22. Goldstein H. Multilevel Statistical Models 3rd Edition. London, Arnold., 2003.

23. Grave K, Jensen VF, McEwen S, Kruse H. Monitoring of antimicrobial drug usage in animals: methods and applications. In Aarestrup FM, ed. *Antimicrobial resistance in bacteria of animal origin*. Washington: ASM Press 2006; 380-385.

24. Hedges AJ, Howe K, Linton AH. Statistical considerations in the sampling of *Escherichia coli* from intestinal sources for serotyping. *J Appl Microbiol* 1977;43:271-280.

25. Jim GK, Booker CW, Guichon PT, Schunicht OC, Wildman BK, Johnson JC, Lockwood PW. A comparison of florfenicol and tilmicosin for the treatment of undifferentiated fever in feedlot calves in western Canada. *Can Vet J* 1999;40: 179.

26. Lipsitch M, Singer RS, Levin BR. Antibiotics in agriculture: When is it time to close the barn door? *Proc Natl Acad Sci* 2002;99 (9):5752-4.

27. Martel JJL, Coudert M. Bacterial Resistance monitoring in animals: the French national experiences of surveillance schemes. *Vet Micobiol* 1993;35:321-38.

28. Monnet DL, Molstad S, Cars O. Defined daily doses of antimicrobial reflect antimicrobial prescriptions in ambulatory care. J Antimicrob Chemother 2004;53(6): 1109-1111.

29. NCCLS. Performance standards for Antimicrobial Disk and Dilution Susceptibility Tests for bacteria Isolates from Animals; Information Supplement M32-S1, 2004.

30. O'Connor AM, Poppe C, McEwen SA (2002). Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. *Can J Vet Res* 2002;66:145-50*.*

31. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. *The Lancet Infectious Diseases* 2003;3(1):47-51.

32. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc Natl Acad Sci* 2002;99 (9):6434-9.

33. Smith RA, Stokka GL, Radostits OM, Griffin DD. Health and production management in beef feedlots. In Radostits OM, ed. *Herd Health: Food Animal Production Medicine 3rd Ed*. Phildelphia: WB Saunders, 2001: 600-612.

34. Stevenson SML, McAllister TA, Selinger LB, Yanke LJ, Olson ME, Morck DW, Read RR. Transfer of a rifampicin-resistant *Escherichia coli* strain among feedlot cattle. *J Appl Microbiol* 2003 95:398-410.

35. Swann MM. Report of the joint committee on the use of antibiotics in animal husbandry and veterinary medicine. *Her Majesty's Stationary Office,* London, 1969.

36. United States Department of Agriculture/ Agricultural Research Service. *National Antimicrobial Resistance Monitoring System – Escherichia coli* 2004

Report, Athens, GA: http://www.ars.usda.gov/Business/docs.htm?docid=6750 (accessed Dec 28, 2006).

37. VanDonkersgoed J, Manninen K, Potter A, McEwen S. Bohaychuk V, Klashinsky, S, Deckert A, Irwin R. Antimicrobial susceptibility of hazard analysis critical control point *E scherichia coli* isolates from federally inspected beef processing plants in Alberta, Saskatchewan and Ontario. Can Vet J 2003;44:723-728.

38. Wagner BA, Dargatz DA, Salman MD, Morley PS, Wittum TE, Keefe TJ. Comparisons of sampling techniques for measuring the antimicrobial susceptibility of enteric *Escherichia coli* recovered from feedlot cattle. *Am J Vet Res* 2003;63 (12): 1662-1670.

39. Wittum TE, Woollen NE, Perino LJ, Littledike ET. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. *J Am Vet Med Assoc* 1996;209 (4): 814-818.

40. WHO. Impacts of antimicrobial growth promoter termination in Denmark. WHO/CDS/CPE/ZFK/2003.1. Geneva: World Health Organization; 2003. www.who.int/salmsurv/links/gssamrgrowthreportstory/en/ accessed December 30, 2007.

Table 6.1: Antimicrobials used and Animal Defined Dose for a feedlot animal ($ADD_{Feedlot}$) equivalent

Antimicrobials used in study (Concentration)	**Dose equivalent**	**$ADD_{Feedlot}$ equivalent (days)**
Ceftiofur 50 mg/mL Intramuscular injection (Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, ON, Canada)	1mg/kg BW[a]	1
Florfenicol 300mg/mL Subcutaneous injection (Nuflor, Schering-Plough Animal Health, Pointe Claire, QC, Canada)	40 mg/kg BW	4
Oxytetracycline 100mg/mL Intramuscular injection (Tetraject® LP, Bimeda-MTC Animal Health Inc., Lavaltrie, QC, Canada)	300 mg/45 kg BW	1
Oxytetracycline 200mg/mL Subcutaneous injection (Bio-Mycin™ 200, Boehringer Ingelheim (Canada) Ltd., Burlington, ON, Canada)	20 mg/kg BW	3
Oxytetracycline 200 mg/mL Intramuscular injection (Oxymycine LA, Wyeth Animal Health, Whitby, ON, Canada)	20 mg/kg BW	3
Oxytetracycline 200 mg/mL Subcutaneous injection (Tetradure® LA 300, Merial Canada Inc., Baie D'Urfe, QC, Canada)	20 mg/kg BW	4
Penicillin G, Procaine 300mg/mL Subcutaneous injection (Derapen® SQ/LA, Wyeth Animal Health, Guelph, ON, Canada)	20 mg/kg BW	3
Spectinomycin 100 mg/mL Subcutaneous injection (Adspec® Sterile Solution, Pharmacia Animal Health, Orangeville, ON, Canada)	10 mg/kg BW	1
Sulbactam 60mg/mL / Ampicillin 120 mg/mL Intramuscular injection	Sulbactam: 3.3 mg/kg BW	1

(Synergistin*, Pfizer Canada Inc., Kirkland, QC, Canada)	Ampicillin: 6.6 mg/kg BW	
Sulphamethazine 15 g/bolus Oral Administration (Sulphamethazine bolus, Professional Veterinary Laboratories, Winnipeg, MB, Canada)	1 bolus/80 kg BW	1
Sulphonamide combination Oral administration (Cocci Bol-O-Tab® Sr., Intervet Canada Ltd., Whitby, ON, Canada)	1 bolus / 90 kg BW	1
Tilmicosin 300 mg/mL Subcutaneous injection (Micotil, Provel, Guelph, ON, Canada)	10 mg/kg BW	3
Trimethoprim 40 mg/mL / Sulfadoxine (200 mg/mL) Intramuscular injection (Borgal®, Intervet Canada Ltd., Whitby, ON, Canada)	16 mg/kg BW	1

[a]Body Weight (BW)

Table 6.2: Demographics of the 4 feedyards in the cross sectional study, including information on antimicrobial use

	Feedyard 1	**Feedyard 2**	**Feedyard 3**	**Feedyard 4**
Number of pens	**10**	**5**	**5**	**5**
Feedyard Capacity	16 000	18 000	5000	7000
Number in Pen Median (interquartile Range)	330.5 (177.8)	273.0 (103.0)	311.0 (207.5)	100.0 (41.0)
Projected DOF[a] ***Median (interquartile Range)***	200.5 (192.8)	174.0 (77.5)	273. 0 (62.5)	299.0 (183.5)
Projected DPS[b] ***Median (interquartile Range)***	28.0 (30.0)	23.0 (59.0)	29.0 (41.5)	67.0 (58.0)
Tylosin DIF[c] ***Median (interquartile range)***	145.5 (123.0)	147.0 (67.5)	188.0 (24.0)	166.0 (134.5)
Total ADD_{Feedot}[d] Median (interquartile range)	202.0 (371.0)	116.0 (71.0)	n/a[e]	13.0 (17.0)
Tilmicosin ADD_{Feedot} ***Median (interquartile range)***	60.0 (321.0)	33.0 (21.0)	n/a	0.0 (9.0)
Florfenicol ADD_{Feedot} ***Median (interquartile range)***	7.0 (54.0)	20.0 (20.0)	n/a	0 (0.0)
Derapen ADD_{Feedot} ***Median***	4.5 (171.0)	21.0 (18.0)	n/a	0.0 (3.0)

(interquartile range)				
Excenel ADD_{Feedot} ***Median (interquartile range)***	0 (0.0)	16.0 (8.0)	n/a	1.0 (3.0)
Other ADD_{Feedot} ***Median (interquartile range)***	12.5 (69.0)	21.0 (27.0)	n/a	0.0 (5.0)
Late Tx ADD_{Feedot} ***Median (interquartile range)***	23.5 (174.0)	50.0 (23.0)	n/a	1.0 (5.0)

[a] Days on Feed (DOF)
[b] Days preslaughter (DPS)
[c] Days in Feed (DIF)
[d] Animal Defined Dose for a Feedlot Animal (ADDFeedlot)
[e] Not Available (n/a)

Table 6.3: Demographics of the 25 feedlot pens in the cross sectional study.

Feedyard	Class	Gender	Days Preslaughter	Metaphylaxis	Chlortetracycline in Feed
1 (10)[a]	Yearling (5)	Steer (4)	<30 (3)	No (5)	No (5)
			<60 (1)		
		Heifer (1)	<60 (1)		
	Fall Placed (5)	Steer (4)	<30 (3)	Yes (5)	Yes (5)
			<60 (1)		
		Heifer (1)	<90 (1)		
2 (5)	Yearling (3)	Steer (5)	<30 (2)	No (3)	No (5)
			<60 (1)		
	Winter Placed (2)		<30 (1)	Yes (2)	
			<90 (1)		
3 (5)	Fall Placed (5)	Steer (4)	<30 (3)	Yes (5)	Yes (5)
			<90 (1)		
		Heifer (1)	<60 (1)		
4 (5)	Yearling (2)	Steer (1)	<90 (1)	No (2)	No (2)
		Heifer (1)	<30 (1)		
	Fall-Placed (3)	Steer (2)	<90 (2)	Yes (3)	Yes (3)
		Heifer (1)	<30 (1)		

[a]Number of pens in category

Table 6.4: Prevalence of resistance to specific antimicrobials adjusted for clustering by pen

Resistance to specific antimicrobial	Count Positive out of 500	Population based Prevalence estimate (as percent) adjusted by pen	Lower 95% CI for population based estimate (as percent) adjusted by pen	Upper 95% CI for population based estimate (as percent) adjusted by pen
AMK[a]	0	0.00	na[1]	na
AMC	4	0.80	0.18	3.53
AMP	10	2.00	0.79	4.96
FOX	2	0.40	0.11	1.50
TIO	1	0.20	0.03	1.35
CRO	0	0.00	0.00	0.00
CEP	17	3.40	1.70	6.68
CHL	3	0.60	0.21	1.73
CIP	0	0.00	na	na
GEN	0	0.00	na	na
KAN	7	1.40	0.51	3.81
NAL	0	0.00	na	na
STR	67	13.40	9.46	18.65
SMX	65	13.00	8.52	19.34
TCY	197	39.40	31.49	47.91
SXT	4	0.80	0.33	1.96

[1]na=not applicable

[a] AMK=amikacin, AMC=amoxicillin/clavulanic acid, AMP=ampicillin, FOX=cefoxitin, TIO=ceftiofur, CRO= ceftriaxone, CEP=cephalothin, CHL=chloramphenicol, CIP=ciprofloxacin, GEN=gentamicin, KAN=kanamycin, NAL=nalidixic acid, STR=streptomycin, SMX=sulphamethoxazole, TCY=tetracycline, SXT=trimethoprim/sulphamethoxazole

Table 6.5: Prevalence of multi drug resistance adjusted for clustering by pen

Number of Antimicrobials to which the isolate is Resistant	Count Positive out of 500	Population based Prevalence estimate (as percent)	Lower 95% CI for population based estimate (as percent)	Upper 95% CI for population based estimate (as percent)
0	**277**	**55.40**	**47.51**	**63.03**
1	**126**	**25.20**	**18.98**	**32.63**
three or more	**38**	**7.60**	**4.47**	**12.64**

[1]na=not applicable

Table 6.6: Prevalence of resistance to specific phenotypes[a] adjusted for clustering by pen

Resistance to specific phenotype	Count Positive out of 500	Population based Prevalence estimate (as percent)	Lower 95% CL for population based estimate (as percent)	Upper 95% CL for population based estimate (as percent)
Resistance to only 1 antimicrobial				
AMPp	4	0.80	0.26	2.47
CEPp	3	0.60	0.21	1.73
SMXp	1	0.20	0.03	1.35
TCYp	104	21.00	14.97	28.64
STRp	14	2.80	1.03	7.40
Resistance to 2 antimicrobials				
SMX TCY	33	6.60	3.85	11.08
AMP TCY	1	0.20	0.03	1.35
CEP TCY	3	0.60	0.15	2.43
SMX SXT	2	0.40	0.11	1.50
STR TCY	20	4.00	1.98	7.90
Resistance to 3 antimicrobials				
AMC AMP CEP	1	0.20	0.03	1.35
STR SMX TCY	18	3.60	1.72	7.38
STR SMX SXT	1	0.20	0.03	1.35
CHL SMX TCY	1	0.20	0.03	1.35
AMP STR TCY	1	0.20	0.03	1.35
CEP STR TCY	4	0.80	0.18	3.53
FOX CEP TCY	1	0.20	0.03	1.35
Resistance to 4 antimicrobials				
AMC AMP CEP TCY	2	0.40	0.06	2.69
KAN STR SMX TCY	6	1.00	0.29	3.41
Resistance to 5 antimicrobials				
CEP CHL STR SMX TCY	1	0.20	0.03	1.35
CEP KAN STR SMX TCY	1	0.20	0.03	1.35
Resistance to 10 antimicrobials				
AMC AMP FOX TIO CEP CHL STR SMX TCY SXT	1	0.20	0.03	1.35
Total	**218**			

[a] AMPp=ampicillin only, CEPp=cephalothin only, STRp=streptomycin only, SMXp=sulphamethoxazole only, TCYp=tetracycline only, AMK=amikacin, AMC=amoxicillin/clavulanic acid, AMP=ampicillin, FOX=cefoxitin, TIO=ceftiofur, CRO= ceftriaxone, CEP=cephalothin, CHL=chloramphenicol, CIP=ciprofloxacin, GEN=gentamicin, KAN=kanamycin, NAL=nalidixic acid, STR=streptomycin, SMX=sulphamethoxazole, TCY=tetracycline, SXT=trimethoprim/sulphamethoxazole

Table 6.7: Univariate associations between multidrug resistance (resistance to three or more antimicrobials) and individual management and antimicrobial usage characteristics for 25 pens in 4 feedlots, adjusting for clustering by pen.

Variable	***P***	**OR**	**95% CI OR**
Feedlot	*0.16*		
Feedlot 1	0.06	0.34	0.11-1.06
Feedlot 2	0.00	0.05	0.01-0.37
Feedlot 3	0.37	0.52	0.12-2.16
Feedlot 4 (ref)			
Sex	*0.69*		
Heifer	0.67	1.27	0.42-3.79
Steer (ref)			
Number of animals in pen	0.46	0.98	0.94-1.03
Projected Days on Feed	0.12	1.04	1.00-1.10
Total $ADD_{FEEDLOT}$	0.06	0.98	0.96-0.99
Late Treatment $ADD_{FEEDLOT}$	0.22	0.97	0.93-1.02

Table 6.8: Univariate associations between tetracycline resistance and individual management and antimicrobial usage characteristics for 25 pens in 4 feedlots, adjusting for clustering by pen.

Variable	*P*	OR	95% CI OR
Feedlot	*<0.07*		
Feedlot 1	<0.01	0.14	0.07-0.29
Feedlot 2	<0.01	0.20	0.09-0.42
Feedlot 3	<0.01	0.18	0.08-0.40
Feedlot 4 (ref)			
Sex	*0.46*		
Heifer	0.43	1.40	0.60-3.29
Steer (ref)			
Number of animals in pen	0.01	0.90	0.84-0.97
Projected Days on Feed	0.31	1.05	0.96-1.16
Total $ADD_{FEEDLOT}$	0.06	0.95	0.91-0.99
Late Treatment $ADD_{FEEDLOT}$	0.41	0.96	0.86-1.07

Table 6.9: Concentration range and breakpoints of antimicrobials tested.

Antimicrobial	**Breakpoint (µg/mL)**	**Concentration Range Measured (µg/mL)**
Amikacin	≥64	≤0.5, 1, 2, 4, >4
Amoxicillin/ Clavulanic Acid	≥32/16	≤1/0.5, 2/1, 4/2, 8/4, 16/8, 32/16, >32/16
Ampicillin	≥32	≤1, 2, 4, 8, 16, 32, >32
Cefoxitin	≥32	≤0.5, 1, 2, 4, 8, 16, >16
Ceftiofur	≥8	≤0.12, 0.25, 0.5, 1, 2, 4, 8, >8
Ceftriaxone	≥64	≤0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, >64
Cephalothin	≥32	≤2, 4, 8, 16, 32>32
Chloramphenicol	≥32	≤2, 4, 8, 16, 32, >32
Ciprofloxacin	≥4	<0.015, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, >4
Gentamicin	≥16	≤0.25, 0.5, 1, 2, 4, 8, 16, >16
Kanamycin	≥64	≤8, 16, 32, 64, >64
Nalidixic Acid	≥32	≤0.5, 1, 2, 4, 8, 16, 32, >32
Streptomycin	≥64	≤32, 64, >64
Sulphamethoxazole	≥512	≤16, 32, 64, 128, 256, 512, >512
Tetracycline	≥16	≤4, 8, 16, 32, >32
Trimethoprim/ Sulphamethoxazole	≥4/76	≤0.12/2.38, 0.25/4.75, 0.5/9.5, 1/19, 2/38, 4/76, >4/76

CHAPTER 7
EFFICIACY OF VACCINATION AGAINST FUSOBACTERIUM NECROPHORUM INFECTION FOR CONTROL OF LIVER ABCESSES AND FOOTROT IN FEEDLOT CATTLE IN WESTERN CANADA

7.1 Introduction:

The control of liver abscesses is an important economic concern in feedlot cattle. Beef quality audits in Canada have evaluated economic losses due to various quality defects, including liver condemnation, downgrades at slaughter, and other quality parameters that, in 1995, cost the industry approximately $5.31 CDN per head, not including losses of production and feed efficiency (Van Donkersgoed et al., 1997). Extensive educational programs for producers were instigated to decrease losses to the beef industry. In 1998/1999, losses across the entire Canadian industry due to liver quality defects were estimated at $ 2.66 CDN per head (Van Donkersgoed et al., 1998-99).

Antimicrobials in the feed are effective at reducing the prevalence of liver abscesses at slaughter (Brown et al., 1973; Brown et al., 1975). Most trials evaluating the efficacy of vaccination against *F. necrophorum* infection on decreasing the prevalence of liver abscesses at slaughter either have been performed in combination with prophylactic feed antimicrobials or have evaluated leukotoxin-based *F. necrophorum* vaccines under experimental conditions (Saginala et al., 1996; Saginala et al., 1997; Liem et al., 1999). The feeding of antimicrobials in the livestock industry has been blamed for increases in antimicrobial resistance in humans (Khachatourians, 1998; McGeer, 1998). The use of

prophylactic feed antimicrobials is banned in some European countries and is currently under scrutiny in Canada (Anonymous, 1998; VDD, 2002). If prophylactic feed antimicrobials were no longer available to prevent liver abscesses, a vaccine that would decrease the prevalence of liver abscesses would be highly desirable. In addition, if vaccination against *F. necrophorum* infection also decreased the prevalence of footrot in the feedlot, labor and treatment costs associated with footrot, along with costs of lost productivity due to severe liver abscesses, would be decreased (Brown et al., 1973; Brown et al., 1975).

The objectives of this trial were to evaluate the effectiveness of a *F. necrophorum* vaccine (Fusogard, Novartis Animal Health Canada, Mississauga, Ontario) for the prevention, first, of liver abscesses and, second, footrot in feedlot cattle not treated with a prophylactic feed antimicrobial by comparing the prevalence of these conditions in the vaccinated cattle and the unvaccinated (control) cattle.

7.2 Materials and methods

7.2.1 Trial facilities

Backgrounding was carried out in 12 pens at the University of Saskatchewan Research Feedlot with 8 pens of 37 animals and four pens of 38 animals. The cattle were moved later to a large commercial feedlot with a capacity of approximately 30 000 head for the finishing period, where they were housed in two pens of about 222 animals. Both feedlots are typical of those found in western Canada with open air pens, dirt floors, a central alley, and 20% porosity fencing. The design of the research feedlot is a small scale representation of larger regional feedlots. Both feedlots maintained individual animal records.

7.2.2 Animals

The University Committee on Animal Care and Supply approved this trial and the guidelines of the Canadian Council on Animal Care were followed. In the fall of 2001, 447 auction market-derived steer calves arrived at the University of Saskatchewan feedlot. The steers were crossbred beef calves with an average weight of 249 kg (s = 17 kg). Routine processing was carried out at arrival; this included ear tagging for individual animal identification, weighing, vaccination against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3), bovine virus diarrhea (BVD), *Histophilus somni*, and *Mannheimia hemolytica* (Starvac 3 Plus/Somnu-Star Ph, Novartis Animal Health Canada) and vaccination against clostridial diseases (Tasvax 8, Schering-Plough Animal Health, Division of Schering Canada, Pointe-Claire, Quebec). The animals also received topical parasiticidal treatment (Dectomax Pour-On Solution, Pfizer Canada Animal Health Group, Kirkland, Quebec), 1 mL/10 kg body weight (BW), and a hormonal implant (Ralgro, Schering-Plough Animal Health). A metaphylactic injection of antimicrobial was given to each animal, based on body temperature at processing; each animal with a body temperature greater than 41 C received tilmicosin (Micotil, Provel, Division Eli Lilly Canada, Guelph, Ontario), 1 mL/30 kg BW, subcutaneously (SC), while the rest of the animals received long-acting oxytetracycline (Liquamycin LA-200, Pfizer Canada Animal Health Group), 1mL/10 kg BW, SC. Animals received a 2nd hormonal implant (Component TE-S, Elanco Animal Health, Division Eli Lilly Canada, Guelph, Ontario) and a booster vaccination against IBR and PI3 (StarVac 2, Novartis Animal Health Canada) before moving to the finishing feedyard at 112 d on feed (DOF). All of the above animal health products were used according to label

instructions. These steers remained in their respective study groups during finishing, until slaughter. Steers were individually weighed at the feedlot within 24 h before slaughter. Animals were slaughtered between 245 and 260 DOF.

7.2.3 Experimental design

Calculations for sample size were based on the two major outcomes (Win Episcope v2; University of Edinburgh, Edinburgh, Scotland). The trial had the power to show a decrease in liver abscesses of 40% or more from approximately 27% (the expected level of liver abscessation in controls), with 80% power and a 95% level of confidence. The trial also had the power to see a change of 0.09 kg or more in average daily gain (ADG) across the entire feeding period from a base value of approximately 1.5 kg/d with 80% power and a 95% level of confidence.

Outcomes measured to assess the effect of the vaccine on liver abscessation were liver scores at slaughter and the ADG. Liver scoring at slaughter followed the Elanco system where scores are as follows: 0 (no abscesses), A- (1 or two small abscesses or abscess scars), A (two to 4 well organized abscesses less than 2.5 cm in diameter), or A+ (1 or more large active abscesses with inflammation of surrounding liver tissue) (Brown et al., 1975; Brink et al., 1990; Van Donkersgoed et al., 1997). Outcomes measured for assessment of the vaccination for prevention of footrot were treatment rates for all lameness, and specifically for footrot. The feedlot staff and researchers who scored livers were blinded as to the allocation of the treatment and the specific objectives of the trial.

The 447 steers were randomly allocated by blocks into 12 feedlot pens at processing. The allocation was random within weight blocks to create approximately equal pen

weights. Two milliliters of *F. necrophorum* vaccine was given, SC, in the neck to one half of the calves chosen by random number table from each pen. A placebo injection of saline was given by the same method to all animals not receiving the vaccine. The trial was set up to analyze associations at the individual animal level; therefore, unvaccinated calves intermingled with the vaccinated calves at equal proportions within each pen. No antimicrobials for liver abscess prophylaxis were given in the feed during this trial so that comparisons could be made with a negative control group. Monensin sodium 3% (Rumensin®; Elanco Animal Health, Guelph, Ontario) was fed to all cattle during the backgrounding period in the total mixed ration at 27-28 ppm DM. Decoquinate (Deccox 6% Premix, Alpharma Canada, Mississauga, Ontario) was given to all trial animals at a dose of 0.5 mg/kg BW. A 2nd vaccination of 2 mL was given, SC, at 94 DOF, 18 d before the steers moved to the finishing feedyard (112 DOF). The on-label recommendations for the vaccine are that the 2nd vaccination should be given 21 d after the 1st injection for footrot, and 60 d after the 1st vaccination for liver abscesses. The timing of this procedure differed from the on-label recommendation of a 2nd vaccination at 60 DOF, because the timing was synchronized with the processing time when the cattle received their 2nd hormonal implant. This meets the withdrawal period of 60d stated by the manufacturer.

Two feeding programs were used at the University feedlot during the backgrounding period to produce a gain of 1.15 to 1.25 kg/d. The diet and the feeding method were different between the two groups. One half of the animals had ad libitum access to a forage-based growing diet consisting of 23.6% barley silage, 27% grass hay, and 49.25% barley based concentrate/supplement on a dry matter (DM) basis. This group

will be called the Ad Libitum Forage (ALF) group. The other group was limit-fed a grain based growing diet consisting of 8.5% barley silage, 92.5% barley based concentrate/supplement. This group will be called the Limit-Fed Grain (LFG) group. Calcium, phosphorus, and crude protein were adjusted, so that animals on either diet would have equal intakes. The feeding programs overlapped the vaccination groups in a balanced design to prevent bias. Therefore, a quarter of the animals were in each of the following treatment groups: vaccinated and LFG, vaccinated and ALF, unvaccinated and LFG, and unvaccinated and ALF. The animals remained with their original diet groups throughout the 2nd part of the feeding period, but all animals were fed a high grain diet ad libitum. The diet of the LFG group now consisted of 89.8% barley based concentrate/supplement and 10.2% forage (DM basis). The ALF group was now fed a ration consisting of 90.3% barley based concentrate/supplement and 9.7% forage (DM basis).

The case definition used for footrot was a calf with sudden onset, single-leg lameness, with no other obvious cause for the lameness; for example, no joint involvement or evidence of trauma. Feedlot workers followed a treatment protocol and completed a questionnaire for each animal treated for any lameness during the trial. The questionnaires for footrot included a lameness scale and questions meant to rule out other common causes of lameness (Greenough et al., 1997). Questions were asked about which foot was affected, fever, swelling of the foot, swollen joints, pus at the coronary band, response to treatment, and any other obvious cause of lameness. It was decided to leave all single leg lameness cases in the analysis, as long as no other obvious cause of the lameness could be identified. All lame animals were treated with

ceftiofur sodium (Excenel Sterile Powder, Pharmacia Animal Health, Division of Pharmacia & Upjohn, Orangeville, Ontario) at the label dose for two to three injections, depending on response to treatment.

7.2.4 Missing data

Four animals were lost from the trial due to injury, polyarthritis, myocarditis, or chronic bloat. Eleven other animals had tags missing at the end of the trial, so liver scores could not be matched with the appropriate animal. The feed groups were slaughtered on different days, therefore it is known which feed group these animals with missing tags came from but not which vaccination group. However, treatment data were available for these 11 animals. The liver abscess and ADG analyses included 432 animals. The footrot analysis included 443 animals.

7.2.5 Statistical analysis

Liver codes were dichotomized, combining liver abscess categories 0 and A- into the referent category and combining liver abscess categories A and A+ into the other category. Prevalences of liver abscesses in this categorization are shown in Table 7.1. This method of categorization was chosen a priori to evaluate the effect of the *F. necrophorum* vaccine on decreasing the number of A and A+ livers, as recommended in the 1997 Canadian Beef Quality Audit (Van Donkersgoed et al., 1997).

Logistic regression (SPSS v. 11.0.1 for Windows, SPSS Inc., Chicago, Illinois, USA) was used to analyze the association between *F. necrophorum* vaccination and the presence at slaughter of liver abscesses grading A or A+, while adjusting for the effect of diet. A similar method was used to analyze the association between *F. necrophorum*

vaccine and the number of footrot cases treated for the 1st time during the feeding period. Attributable rates and attributable fractions were calculated for the significant outcomes (Martin et al., 1987). The linear outcome of ADG was compared between the two vaccine groups while blocking for diet effect, with a factorial ANOVA using the Type III sum-of-squares method. Four percent shrink was calculated on the animal weights on arrival and at slaughter, for ADG calculations. The assumptions of all statistical tests were met. No adjustment for clustering was used as there was no clustered pen structure representative of the entire feeding period.

7.3 Results

7.3.1 Liver scores

The overall prevalence of liver abscess in this trial was 20%. This included all A-, A, and A+ livers. Overall, there were 3% A- livers, 1% A livers, and 16% A+ livers.

Initial exploration of the data revealed that diet was a strong predictor of the presence of A or A+ liver abscesses at slaughter, as would be expected. The odds of an animal in the LFG group having an A or A+ liver abscess score at slaughter were 5.7 times higher than the odds of an animal in the ALF group having an A or A+ liver abscess score at slaughter [(95% CI: 3.0-10.8), $P<0.0001$]. The crude association of vaccine group with the presence of A or A+ liver abscesses at slaughter was not statistically or clinically significant when both diet groups were considered together.

When the association between vaccination with *F. necrophorum* vaccine and presence of A or A+ liver abscesses at slaughter was adjusted for diet, effect modification of a large magnitude was apparent. This effect modification has clinical and biological significance; therefore, vaccine efficacy was presented separately for

each feed group. The odds that a vaccinated animal in the ALF group would develop an A or A+ liver abscess were less than 1/3 the odds that an unvaccinated animal in the ALF group would develop an A or A+ liver abscess [OR=0.3, (95% CI: 0.1 – 1.0), *P* =0.05]. Within the LFG group, there was no difference in the odds of having an A or A+ liver abscess score at slaughter between vaccinated and unvaccinated animals [OR=0.8, (95% CI=0.4 – 1.4), *P* =0.35].

In the ALF group in this trial, the presence of A and A+ liver abscesses in unvaccinated animals that may be attributed to not vaccinating against *F. necrophorum* is seven cases per 100 animals. Seventy-one percent of A and A+ liver abscesses in unvaccinated animals in the ALF group can be attributed to not vaccinating against *F. necrophorum*.

7.3.2 Footrot

The overall incidence of footrot in this trial was 6.5%. The distribution and incidence of 1st footrot treatments are presented in Table 7.2. Thirty individual cases of lameness were reported. One of these cases had a leg laceration, did not fit the case definition, and was not included in the footrot analysis. All other cases were single leg lameness where no other cause for lameness could be determined. One animal from the LFG unvaccinated group relapsed 18 d after initial treatment: the footrot relapse rate for the trial was 3.4%. Two animals (one from the LFG vaccinated group and one from the ALF unvaccinated group) were treated initially with antimicrobials for longer than three d (5 d each), due to the slow response to treatment.

The crude association of vaccine group with treatment for footrot suggests a preventive vaccine effect where the odds of a vaccinated animal are about one-half the

odds that an unvaccinated animal will be treated for footrot [OR=0.5 (95% CI: 0.2-1.1), *P*=0.07]. The effect of diet in the association between vaccination against *F. necrophorum* and treatment for footrot was also investigated. Diet alone was not a strong predictor of footrot treatment [OR=0.7 (95% CI: 0.3-1.5), *P*=0.33]. When the analysis for vaccine group and footrot treatment was adjusted for diet, effect modification was again apparent. Therefore, vaccine efficacy will be presented separately for each feed group. The odds that a vaccinated animal in the ALF group would be treated for footrot were less than one-fifth the odds that an unvaccinated animal in the ALF group would be treated for footrot [OR=0.2 (95% CI: 0.0-0.8), *P* =0.03]. Within the LFG group, there was no difference in the odds of an animal being treated for footrot between vaccinated and unvaccinated animals [OR=0.9 (95% CI: 0.3-2.3), *P* =0.76].

In this trial, the rate of footrot treatments in unvaccinated animals in the ALF group that may be attributed to not vaccinating against *F. necrophorum* is seven cases per 100 animals. Seventy-two percent of footrot treatments in unvaccinated animals in the ALF group can be attributed to not vaccinating against *F. necrophorum*.

7.3.3 Average daily gain

Average daily gain over the entire feeding period did not differ significantly between the two vaccination groups, even when adjusted for diet group, as presented in Table 7.3.

7.4 Discussion

High grain diets are associated with higher numbers and increasing severity of liver abscesses (Brown et al., 1973; Brown et al., 1975; Brink et al., 1990; Nagaraja and Chengappa, 1998). Limit feeding this type of diet under western Canadian environmental conditions has not yet been evaluated extensively (Block et al., 2001). In this trial, the association of diet in the LFG with the presence of an A or A+ liver abscess score at slaughter was strong. It appears that a challenge of limit feeding in western Canada may be managing subclinical acidosis. No strong protective effect of vaccination can be seen across the whole trial for either outcome. When the effect of vaccination was adjusted for diet group, an effect modification of vaccine by diet became apparent. Because of this effect modification, the effect of vaccination on liver abscesses and footrot was reported separately for each diet group. The protective effect of vaccination against development of severe (A or A+) liver abscesses or in decreasing footrot treatments can be seen in the ALF group.

This trial has demonstrated a different effect of the *F. necrophorum* vaccine in animals on different diets. This is a significant finding in itself and has not previously been reported. If the two diet groups had not been present, the effect of diet in the LFG group might have led to the erroneous conclusion that the vaccine has no effect. Similarly the use of only the ALF group might have led to the erroneous conclusion that the vaccine does work in the feedlot with no qualifications. The effect modification of diet on the association between the disease outcomes and vaccination is difficult to interpret, because several variables differed between the feed groups. The main difference between the two groups was in diet composition and feeding method during the backgrounding period (0-112 DOF). The LFG group had a higher grain diet and was

limit fed during the growing phase. The ALF group was fed a higher forage diet ad libitum during the growing phase. Both groups were fed similar high grain finishing rations.

The power of this trial was effectively halved by the interaction between vaccine and diet. The effect of the vaccine on severe liver abscess scores at slaughter or footrot treatments can be seen only in the ALF group, which consisted of half of the animals in the trial. Sample size was limiting when looking at the association in half of the group; however, borderline significance for the liver abscess outcome and significance for the footrot outcome were still seen. A priori power calculations suggested that 177 animals per group would be needed to show a significant difference in severe liver abscess prevalence at slaughter, from an expected 27% in controls down to 16%. This trial has 105 to 108 animals per vaccine group within the ALF group and analysis still showed borderline significance. There may also have been a herd immunity effect, especially with respect to footrot, as vaccinated and unvaccinated animals were mixed in the same pens, making it more difficult to detect a difference between the two vaccination groups.

The lack of a protective effect of the vaccine in the LFG group on the presence of liver abscess at slaughter may be due to the strong acidotic challenge of this high-grain diet. The possible acidosis associated with this diet would lead to more liver abscesses (Galyean et al., 1998; Nagaraja et al., 1998; Owens et al., 1998). Limit feeding cattle has also been associated with increased liver abscesses and increased acidosis (Loerch, 1990; Cooper et al., 1999). The effect of the LFG diet may simply overwhelm any vaccine effect. Another theory is that other bacteria or a different strain of *F.*

necrophorum may become involved in the pathogenesis of liver abscesses in the LFG group (Scanlan et al., 1983). The concentration of *F. necrophorum* in the rumen has been shown to increase with lactic acidosis (Coe et al., 1999; Nagaraja et al., 1999). The late revaccination with the vaccine may also have decreased the protective effect of the vaccine, as good immunoglobulin G levels would not be expected until after the 2nd vaccine (Tizard, 2000). This might have limited the vaccine's ability to protect against liver abscesses in the LFG group in the face of the strong diet challenge that occurred early on in the backgrounding period.

All of the footrot cases used were identified by the feedlot personnel as footrot. Bias might have resulted if a certain type of case had been excluded post hoc. Several hypotheses exist for why the vaccine did not protect against footrot in the LFG group. The strain of *F. necrophorum* might change in the groups with the high grain diet. Other bacteria like *Prevotella* spp. and *Porphyromonas* spp. might be causative agents of footrot in some animals or some situations (Olson et al., 1998). The lameness seen in animals on this type of diet might not be due to *F. necrophorum*. It is possible that the LFG diet induces an acidosis-associated chronic laminitis that manifests itself in a way that cannot be differentiated easily from footrot in the feedlot.

Since, in this trial, feed bunks were managed to maintain a specific ADG on the different diets during the backgrounding period, there was no difference in ADG between the vaccinated and unvaccinated animals even when corrected for diet group.

Results from this trial suggest that vaccination against *F. necrophorum* might have application in decreasing the prevalence of severe liver abscesses at slaughter and decreasing footrot treatments in certain diet situations. Applications for the vaccine

would include feedlot animals on backgrounding diets fed ad libitum with higher forage levels. In reality, most animals in western Canada are fed in this way, so this is an important finding. The protective effect of this vaccine appeared to be overwhelmed by the challenge of a limit-fed high grain diet. A management strategy to decrease the prevalence of liver abscesses in the feedlot without prophylactic feed antimicrobials might include vaccination and diet management during the backgrounding period.

7.5 Acknowledgements

We thank the management and staff of the University of Saskatchewan Feedlot and Pound-Maker Agventures Ltd. for their cooperation during this trial. Also, we thank Sharon Gould and Teresa Binetruy for help in processing and scoring liver abscesses during this trial, and we are also grateful to Drs. Richard Harland and Grant Royan for their input in the design of the trial and review of the manuscript.

7.6 References

1. Anonymous. EU bans four antibiotic feed additives. Vet Rec 1998;143:679.

2. Block HC, McKinnon JJ, Mustafa AF, Christensen DA. Evaluation of the 1996 NRC beef model under western Canadian environmental conditions. J Anim Sci 2001;79:267-275.

3. Brink DR, Lowry SR, Stock RA, Parrott JC. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. J Anim Sci 1990;68:1201-1207.

4. Brown H, Bing RF, Grueter HP, McAskill JW, Cooley CO, Rathmacher RP. Tylosin and chlortetracycyline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1975;40:207-213.

5. Brown H, Elliston NG, McAskill, JW, Muenster OA, Tonkinson LV. Tylosin phosphate (TP) and tylosin urea adduct (TUA) for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1973;37:1085-1091.

6. Coe ML, Nagaraja TG, Sun YD, et al. Effect of virginiamycin on ruminal fermentation in cattle during adaptation to a high concentrate diet and during an induced acidosis. J Anim Sci 1999;77:2259-2268.

7. Cooper RJ, Klopfenstein TJ, Stock RA, et al. Effects of imposed feed intake variation on acidosis and performance of finishing steers. J Anim Sci 1999;77:1093-1099.

8. Galyean ML, Eng KS. Application of research findings and summary of research needs: Bud Britton Memorial Symposium on metabolic disorders of feedlot cattle. J Anim Sci 1998;76:323-327.

9. Greenough PR, Weever AD. Lameness in Cattle. 3rd ed. Philadelphia; WB Saunders, 1997.

10. Health Canada's advisory committee on uses of antimicrobials and impact on resistance and human health. Final report on animal uses of antimicrobials and impact on resistance and human health [monograph on the internet]. Ontario: Veterinary Drug Directorate, Health Canada 2002. Available from http://www.hc-sc.gc.ca/vetdrugs-medsvet/amr_final_report_june27_tc_e.html Last accessed 04/09/05.

11. Khachatourians, GC. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can Med Assoc J 1998;159:1129-1136.

12. Liem, A, Anderson GA, Stine DL, MacGregor S, Cain DV. Control of footrot and liver abscesses with Fusogard™: A new *Fusobacterium necrophorum* bacterin for cattle. Proc 32nd Annu Conv Am Assoc Bov Pract 1999;32:262-265.

13. Loerch SC. Effects of feeding growing cattle high-concentrate diets at a restricted intake on feedlot performance. J Anim Sci 1990;68:3086-3095.

14. Martin SW, Meek AH, Willeberg P. Veterinary Epidemiology Principles and Methods. Ames: Iowa State Univ Pr, 1987.

15. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? Can Med Assoc J 1998;159:1119-1120.

16. Nagaraja, TG, Chengappa MM. Liver abscesses in feedlot cattle: a review. J Anim Sci 1998;76:287-298.

17. Nagaraja TG, Sun YU, Wallace N, Kemp KE, Parrott CJ. Effects of tylosin on concentrations of *Fusobacterium necrophorum* and fermentation products in the rumen of cattle fed a high-concentrate diet. Am J Vet Res 1999;60:1061-1065.

18. Olson ME, Morck DW. Patent Application: Bovine footrot treatment and prevention. PCT/CA1998/000823. In: Canadian Patents Database [database on the internet] Gatineau, Quebec: Canadian Intellectual Property Office c1998. Available from http://patents1.ic.gc.ca/details?patent_number=2300958&language=EN Last accessed 04/09/05.

19. Owens FN, Secrist, DS, Hill WJ, Gill DR. Acidosis in cattle: a review. J Anim Sci 1998;76:275-286.

20. Saginala S, Nagaraja TG, Lechtenberg KF, Chengappa MM, Kemp KE, Hine PM. Effect of *Fusobacterium necrophorum* leukotoxoid vaccine on susceptibility to experimentally induced liver abscesses in cattle. J Anim Sci 1997;75:1160-1166.

21. Saginala S, Nagaraja TG, Tan ZL, et al. Serum neutralizing antibody response and protection against experimentally induced liver abscesses in steers vaccinated with Fusobacterium necrophorum. Am J Vet Res 1996;57:483-488.

22. Scanlan CM, Hathcock TL. Bovine rumenitis-Liver abscess complex: A bacteriological review. Cornell Vet 1983;73:288-297.

23. Tizard IR. Veterinary Immunology: An Introduction. 6th ed. Philadelphia; WB Saunders, 2000.

24. Van Donkersgoed J, Jewison G, Bygrove S, Gillis K, Malchow D, McLeod G. Canadian beef quality audit 1998-99. Can Vet J 2001;42:121-126.

25. Van Donkersgoed J, Jewison G, Mann M, et al. Canadian beef quality audit. Can Vet J 1997;38:217-225.

Table 7.1: Liver scores stratified by vaccination and diet groups, in a study examining the effect of vaccination against *Fusobacterium necrophorum* infection on the prevalence, at slaughter, of A and A+ liver scores, while adjusting for diet during the feeding period.

Diet	Treatment	Liver scores		Total Animals	Prevalence (%)
		0 or A-	A or A+		(of A or A+)
LFG	Vaccinated	84	27	111	24.3
	Not Vaccinated	75	32	107	30.0
ALF	Vaccinated	105	3	108	2.8
	Not Vaccinated	95	10	105	9.5
Totals		359	72	431	16.7

LFG=Limit-fed grain (limit-fed a grain based growing diet consisting of 8.5% barley silage, 92.5% barley based concentrate/supplement).

ALF=Ad-libitum forage (ad libitum access to a forage-based growing diet consisting of 23.6% barley silage, 27% grass hay, and 49.25% barley based concentrate/supplement on a dry matter (DM) basis).

Table 7.2: Footrot treatments stratified by vaccination and diet groups, in a study examining the effect of vaccination against *Fusobacterium necrophorum* infection on the incidence of footrot treatments in feedlot cattle, while adjusting for diet during the feeding period.

Diet	Treatment	Footrot cases (Number Treated)	Total Animals	Incidence (% Treatments)
LFG	Vaccinated	8	112	7.1
	Not Vaccinated	8	109	7.4
ALF	Vaccinated	3	114	2.6
	Not Vaccinated	10	108	9.3
Totals		29	443	6.5

LFG=Limit-fed grain (limit-fed a grain based growing diet consisting of 8.5% barley silage, 92.5% barley based concentrate/supplement).

ALF=Ad libitum forage (ad libitum access to a forage-based growing diet consisting of 23.6% barley silage, 27% grass hay, and 49.25% barley based concentrate/supplement on a dry matter (DM) basis).

Table 7.3: Analysis of variance table presenting the results of a study examining the effect of vaccination against *Fusobacterium necrophorum* infection, on average daily gain (adjusted for 4% shrink) in feedlot cattle, while also adjusting for diet during the feeding period. The two diets used in this trial were a limit-fed grain-based diet and an ad libitum forage-based diet.

Source	Type III Sum of Squares	Degrees of Freedom	Mean Square	F	Sig
Corrected Model	.055(a)	3	.018	.766	.514
Intercept	1040.905	1	1040.905	43694.067	.000
Diet	.050	1	.050	2.103	.148
Vaccine	.002	1	.002	.102	.749
Diet * Vaccine	.002	1	.002	.074	.786
Error	10.172	427	.024		
Total	1051.830	431			
Corrected Total	10.227	430			

F= the test statistic for the F distribution
Sig = statistical significance of each factor on the dependent variable: average daily gain

CHAPTER 8
GENERAL SUMMARY AND DISCUSSION

Little work had been done describing AMR isolated from fecal *E. coli* in feedlot cattle. Baseline data description of AMR throughout the feeding period was needed. Literature review was an early step to better understand the questions that needed to be asked, further develop hypotheses and contemplate appropriate study design to address the questions at hand. Throughout this work, fecal *E. coli* was used as the indicator organism for AMR because it was a commensal bacterium in cattle that was ubiquitous, easy to culture, and one of the major carcass contaminants at slaughter (Stopforth et al., 2006). *E. colii* were considered a potential reservoir of resistance genes that could transfer resistance to other zoonotic or commensal organisms that might cause disease in cattle or people (Linton, 1985; Winokur et al., 2001; Hoyle et al., 2005; Donaldson et al., 2006).

The major objectives of this thesis were to characterize the prevalence and patterns of AMR in fecal *E. coli* isolates in auction market derived feedlot calves at different stages during the feeding period to understand AMR patterns at arrival and how AMR patterns changed during the feeding period. The major associations then evaluated were between AMR and mass uses of antimicrobials in the feed and on arrival at the feedlot, as well as between AMR and individual animal antimicrobial treatments.

The first study, described in Chapter 3, was a randomized controlled clinical trial that compared the mass use of antimicrobials in feed for disease prophylaxis, the mass use of injectable antimicrobials on arrival at the feedlot for disease metaphylaxis, and a

negative control group. Prevalence of AMR was also described at arrival and at different stages during the feeding period, under commercial feedlot conditions. Individual antimicrobial treatments for disease were quantified during this trial and associations with AMR were examined. Chapter 4 was a descriptive study of passively acquired diagnostic data, over a five-year period, on AMR in generic fecal *E. coli* from spring calves. The majority of antimicrobial treatments used in pre-weaned calves were administered in the spring of the year (Gow et. al., 2008), often for treatment of calf diarrhea (USDA, 1997). This could be compared to baseline AMR in fecal *E. coli* isolates on feedlot arrival to better understand the epidemiology of AMR during the pre-weaned period. A cohort study discussed in Chapter 5 was carried out using cattle not fed antimicrobials in the feed, other than coccidiostats. As the vast majority of feedlot cattle are fed antimicrobials for liver abscess control (Booker et al., 1999), this was a unique opportunity to assess associations between individual animal AMU and AMR in *E. coli* collected from cattle feces, in the absence of feed antimicrobials which might mask or confound results (Dunlop et al., 1998; O'Connor et al., 2002). Animals treated with antimicrobials for disease conditions during the feeding period were compared with animals that were not. A pen-level, cross-sectional, observational trial was the basis for Chapter 6. The prevalence of AMR in *E. coli* from pen-level fecal isolates in auction market derived feedlot animals was described late in the feeding period. Sampling issues were also examined; first, the number of isolates per sample, needed to describe the major AMR phenotypes, was examined. The partitioning of variance between different hierarchical levels in the feedlot was examined to better understand where to concentrate sampling resources in future studies. Chapter 7 consisted of a

vaccination trial, run concurrently to the cohort trial in Chapter 4, which examined alternatives to AMU in the feedlot. Feed antimicrobials are effective at reducing the prevalence of liver abscesses at slaughter (Brown et al., 1973; Brown et al., 1975). Most trials evaluating the efficacy of vaccination against *F. necrophorum* infection on decreasing the prevalence of liver abscesses at slaughter either have been performed in combination with prophylactic feed antimicrobials or have evaluated leukotoxin-based *F. necrophorum* vaccines under experimental conditions (Liem et al., 1999; Saginala et al., 1996; Saginala et al., 1997). The feeding of antimicrobials in the livestock industry has been blamed for increases in AMR in humans (McGeer, 1998; Khachatourians, 1998). The use of prophylactic feed antimicrobials is banned in some European countries and is currently under scrutiny in Canada (Anonymous, 1998; VDD, 2002). If prophylactic feed antimicrobials were no longer available to prevent liver abscesses, a vaccine that would decrease the prevalence of liver abscesses would be highly desirable. Evaluation of the effectiveness of a *F. necrophorum* vaccine (Fusogard®, Novartis Animal Health Canada, Mississauga, Ontario) for the prevention of liver abscesses in feedlot cattle not treated with a prophylactic feed antimicrobial was carried out by comparing the prevalence of these conditions in the vaccinated cattle and the unvaccinated (control) cattle. In addition, if vaccination against *F. necrophorum* infection also decreased the prevalence of footrot in the feedlot, labor and treatment costs associated with footrot would also be decreased.

8.1 Chapter Summaries

8.1.1 Randomized, controlled clinical trial assessing mass uses of antimicrobials and antimicrobial resistance

A randomized, controlled clinical trial was performed at a research feedlot in western Canada. Auction market derived steers (n=288) were randomly assigned into one of three treatment groups: 1) Control, where no antimicrobials were given on arrival; 2) Feed, where oxytetracycline was fed in the starter ration for 14 days beginning on Day 0; and 3) Injectable, where long acting oxytetracycline was administered subcutaneously on Day 0 of the trial. Fecal samples were collected on arrival (prior to treatment), and also on Days 7, 15, 35, 70, 100, 150, and preslaughter. Three isolates of generic *E. coli* were cultured from fresh feces. Minimal inhibitory concentrations of seven antimicrobials were determined using the Mueller-Hinton agar dilution method. Calves arrived at the feedlot with a relatively low prevalence of AMR in commensal *E. coli* isolates from feces. Use of oxytetracycline in the feed and the metaphylactic use of long-acting injectable oxytetracycline in groups of calves on arrival at the feedlot, was associated with increased proportions of cattle with one or more resistant *E. coli* isolates early in the feeding period. Individual animal treatments were not associated with increased proportions of cattle with one or more resistant *E. coli* isolates preslaughter. The proportion of animals with one or more *E. coli* isolates resistant to tetracycline was not different between the treatment groups preslaughter; however, there were significantly more animals with tetracycline resistance in one or more isolates of *E. coli* preslaughter than at arrival.

8.1.2 Diagnostic laboratory survey on antimicrobial resistance in spring calves

Diagnostic laboratory data on antimicrobial susceptibility of *E. coli* isolated from feces of spring calves were evaluated retrospectively for a five year period, 1999-2003. Overall, of the antimicrobials tested, tetracycline, ampicillin and trimethoprim/sulphamethoxazole resistance were most prevalent. Multidrug resistance was found with 46.0 % [95% CI: 41.8-50.4] of calves showing resistance to three or more antimicrobials. The use of diagnostic laboratory data for surveillance is also discussed.

8.1.3 Cohort study on individual animal antimicrobial use and antimicrobial resistance

A prospective observational study was carried out to examine antimicrobial resistance patterns of fecal *Escherichia coli* isolates of calves on arrival at the feedlot, and then evaluate the associations between the total volume of antimicrobial used for disease treatment and changes in antimicrobial resistance, during the feeding period. No macrolides or tetracyclines were administered in the feed during this study. On arrival, at the animal level, all three isolates obtained from 36.6% (95% CI: 29.0 to 44.8) of all cattle sampled (n = 153), were susceptible to all antimicrobials, while 5.9% (95% CI: 2.7 to 10.9) of cattle had at least one isolate that was resistant to three or more antimicrobials out of the seven antimicrobials tested. The most frequent antimicrobials for which resistance was observed were sulphamethoxazole, ampicillin, and tetracycline where, of all cattle, 44.4% (95% CI: 36.4 to 52.7), 20.3% (95% CI: 14.2 to 27.5), and 17.7% (95% CI: 12.0 to 24.6), respectively had at least one resistant isolate. All cattle received antimicrobial metaphylaxis on arrival at the feedlot. Antimicrobial use was

described for a cohort of 95 cattle. Antimicrobials were given to 42 of the 95 cattle during the feeding period, to treat disease. Amongst the 42 treated cattle, there were a total of 133 animal daily doses ($ADD_{Feedlot}$), where one $ADD_{Feedlot}$ represented one day of antimicrobial treatment received by a feedlot animal at the approved dose. Only one $ADD_{Feedlot}$ was given in the 100 days immediately prior to slaughter. There were no associations found between total AMU for individual animal disease treatment and AMR in fecal *E. coli* isolates. Considerable conversion to tetracycline and ampicillin resistance occurred in both groups during this study, not associated with the individual animal antimicrobial disease treatments. The results suggest that metaphylaxis and AMR patterns should be evaluated further, and that the evaluation of conversion to resistance during the feeding period is important when assessing associations of AMR attributable to AMU during the feeding period.

8.1.4 Pen-level antimicrobial resistance and the effect of antimicrobial use and pen characteristics

Overall, this study provides some basic information on the prevalence of resistance to 16 antimicrobials in feedyard pens late in the feeding period in Western Canada and is similar to prevalence information from a feedyard studies in the USA. A three-level analysis using a logistic model found the more variation between pens than between feedlots when the outcome was resistance to 3 or more antimicrobials but more variation at the feedlot level when the outcome is tetracycline resistance. This will be applicable to future research design. Examination of the number of isolates necessary for establishing resistance levels in feedyard pens agreed with other available information even using samples from different feedyards. Isolates from composite fecal

samples from pen floors are a satisfactory method of describing antimicrobial resistance in feedlot pens for resistance to common antimicrobials. Differences between variation and explanatory variables for multidrug resistance and tetracycline resistance are also interesting and of note for future study design. This suggests factors to be investigated in future studies including management practices and protocols involving AMU at the feedlot, pen and individual animal level.

8.1.5 Alternatives to antimicrobial use

A randomized and blinded field trial was carried out to evaluate the efficacy of a *F. necrophorum* bacterin for control of liver abscesses and footrot under commercial feedlot conditions in western Canada. Half of the vaccinated and half of the unvaccinated control animals had ad libitum access to a forage-based (ALF) growing diet. The other half of each group was limit-fed a grain-based (LFG) growing diet. The overall prevalence of A and A+ liver abscesses in this trial was 16.7%. A strong association was found between diet group and presence of A or A+ liver abscessation at slaughter. Diet group modified the effect of vaccination on the prevalence of liver abscesses at slaughter, and on the incidence of footrot during the feeding period. The odds that a vaccinated animal in the ALF group would have an A or A+ liver abscess at slaughter were less than 1/3 the odds that an unvaccinated animal in the same diet group would have an A or A+ liver abscess at slaughter [OR=0.3, (95% CI: 0.1 – 1.0), *P*=0.05]. The overall incidence of footrot in this trial was 6.5%. The odds that a vaccinated animal in the ALF group would be treated for footrot were less than 1/5 the odds that an unvaccinated animal in the same group would be treated for footrot [OR=0.2, (95% CI: 0.0-0.8), *P* =0.03]. Within the LFG group there were no differences

between vaccinated and unvaccinated animals in the odds of an animal being treated for footrot, or in the odds of having an A or A+ liver abscess score at slaughter.

Results from this trial suggest that vaccination against *F. necrophorum* might have application in decreasing the prevalence of severe liver abscesses at slaughter and decreasing footrot treatments in certain diet situations. Applications for the vaccine would include feedlot animals on backgrounding diets fed ad libitum with higher forage levels. In reality, most animals in western Canada are fed in this way, so this is an important finding. The protective effect of this vaccine appeared to be overwhelmed by the challenge of a limit-fed high grain diet. This vaccine may also be of use to prevent footrot in the cow-calf operation. A management strategy to decrease the prevalence of liver abscesses in the feedlot without prophylactic feed antimicrobials might include vaccination and diet management during the backgrounding period.

8.2 Conclusions

The objectives of this thesis were met. The prevalence and patterns of antimicrobial resistance in fecal *E. coli* isolates from newly-weaned, auction-market-derived calves were characterized on arrival at the feedlot. Levels of resistance in *E. coli* isolates in the cohort trial, Chapter 6, were slightly higher than those found in the clinical trial in Chapter 3. For example, the tetracycline resistance in the clinical trial was 9.8% (95% CI: 7.9-12.0) compared to 17.6 % (95% CI: 12.0-24.6) in the cohort study. Resistance to three or more antimicrobials was low in both groups of cattle, with resistance in the clinical trial at 2.1% (95% CI: 1.2-3.3) compared to 5.9% (95% CI: 2.7-10.9). Differences are representative of the AMR in fecal *E. coli* prior to antimicrobial treatment at the feedlot, and may be related to resistance patterns and AMU in the

individual animals as well as more broadly in their herd of origin. Other factors, not related to AMU, from the herd of origin may play a role the expansion of populations of antimicrobial resistant organisms (Hinton, 1983; Hoyle et al., 2004; Hoyle et al. 2005; Khachatryan et al., 2006). The prevalence and patterns of AMR from samples from spring calves submitted to a regional diagnostic laboratory were much higher than those found on arrival at the feedlot, with tetracycline resistance at 78.3% (95% CI: 74.6-81.6) and resistance to three or more antimicrobials at 46.0 % (95% CI: 41.8-50.4). This may be because most antimicrobial treatments for calves were given in the springtime; therefore, bacterial populations associated with antimicrobial use in the spring had four or more months to readjust to equilibrium, in the absence of antimicrobial use, before the post-weaning samples were taken. As well, in general, younger animals tended to have higher levels of antimicrobial resistance microorganisms (Martel and Coudert, 1993; Berge et al., 2005; Donaldson et al., 2006).

The prevalence and patterns of antimicrobial resistance in fecal *Escherichia coli* isolates from composite feedyard pen samples for calves late in the feeding period, and for spring calves through diagnostic laboratory data were characterized. The predominant antimicrobial resistance was to tetracycline where 39.4% (95% CI: 31.5-47.9) of isolates were resistant, somewhat higher than that found on arrival at the feedlot. Resistance to three or more antimicrobials was still quite low at 7.6% (95% CI: 4.5-12.6) of isolates.

Associations between AMR and AMU and other potential risk factors were explored in the clinical trial in Chapter 3, in the cohort study in Chapter 5, and in the pen-level study in Chapter 6. In the clinical trial, use of oxytetracycline in the feed and the

metaphylactic use of long-acting injectable oxytetracycline in groups of calves on arrival at the feedlot, was associated with increased proportions of cattle with one or more resistant *E. coli* isolates early in the feeding period. However, individual animal treatments were not associated with increased proportions of cattle with one or more resistant *E. coli* isolates preslaughter. In addition, the proportion of animals with one or more *E. coli* isolates resistant to tetracycline gradually increased over the feeding period. There were also no associations found between individual animal antimicrobial use, quantified as the total volume of parenteral antimicrobials used for disease treatment, and antimicrobial resistance in the cohort study, despite adequate power to detect strong associations if they existed. No strong associations were found between feedyard demographic characteristics, pen demographic characteristics and pen-level antimicrobial use on the pen-level prevalence of antimicrobial resistance. However, this study suggested factors to be investigated in future studies including management practices and protocols involving AMU at the feedlot, pen and individual animal level.

In Chapter 4, the use of diagnostic laboratory data along with surveillance data from other sources in an overarching surveillance system was thought to be of value and has been used in this way in public health jurisdictions (Teutsch, 2000). Testing for a broader range of antimicrobials, using an antibiogram more consistent with those used for surveillance and including antimicrobials important in human medicine, would increase the value of these diagnostic laboratory data for surveillance. However, this panel would need to be useful for practitioners by including antimicrobials consistent with current, regional antimicrobial use. The use of current diagnostic laboratory data to create regional antibiograms on drug susceptibilities for some diseases could be

useful to practitioners (Karlowsky, 2002; El-Azizi, 2005). However, when evaluating resistance of fecal *E. coli* in diarrheic calves, it is questionable how well the *E. coli* isolated in the feces represents that of the small intestine, or the pathogenic process at all (Bywater, 2000; Constable, 2004). Overall, with carefully planned antibiograms, the use of diagnostic laboratory data for surveillance purposes could add important information to larger overarching surveillance systems for emerging antimicrobial resistance by representing cases which are important enough to veterinarians and producers to be submitted to the diagnostic laboratory for further testing (McNab, 2007).

The number of isolates necessary for establishing resistance levels in feedyard pens was examined in Chapter 6. There was no significant difference between the proportion of isolates resistant to one or more, two or more, or three or more antimicrobials, nor in the proportion of tetracycline resistant isolates per pen when using 20, 15, 10 or 5 isolates from composite pen-level fecal samples to describe pen-level antimicrobial resistance. Variance for isolates resistant to three or more antimicrobials was partitioned as 12.7% at the feedyard-level and 28.7% at the pen-level.

An *F. necrophorum* vaccine (Fusogard, Novartis Animal Health Canada, Mississauga, Ontario) could be used as an alternative to antimicrobial use in feedlot cattle not treated with a prophylactic feed antimicrobial by comparing the prevalence of these conditions in the vaccinated cattle and the unvaccinated (control) cattle, for the prevention of liver abscesses and footrot. The clinical trial in Chapter 7 suggested that vaccination against *F. necrophorum* infection may have applications to decrease the

prevalence of severe liver abscesses at slaughter and decrease footrot treatments in certain diet situations.

Overall this body of work fulfilled its objectives and added significantly to the literature on antimicrobial resistance in feedlot cattle. Baseline resistance in cattle on arrival at the feedlot and at different times throughout the feeding period was described. Associations between AMR and AMU and other risk factors were examined. The number of isolates necessary to describe pen-level resistance was quantified, along with the variance found at different hierarchical levels in feedlot studies. This was useful for planning future feedlot studies. The use of diagnostic laboratory data for AMR surveillance was also examined, again with suggestions made for future surveillance. Lastly, vaccination with an *F. necrophorum* vaccine was evaluated for use as an alternative to antimicrobial treatment for liver abscesses and footrot.

8.3 References

1. Anonymous. EU bans four antibiotic feed additives. Vet Rec 1998;143:671.

2. Berge ACB, Atwill ER, Sischo WM. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. Prev Vet Med 2005;69:25-38.

3. Booker CW, Guichon PT, Schunicht OC, Wildman BK, Jim GK. Economic impact of antimicrobial use in feedlots. Bovine Proceedings 1999;32:111-112.

4. Brown H, Elliston NG, McAskill JW, Muenster OA, Tonkinson LV. Tylosin phosphate (TP) and tylosin urea adduct (TUA) for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1973;37(5):1085-1092.

5. Brown H, Bing RF, Grueter HP, McAskill JW, O'Cooley CO, Rathmacher RP. Tylosin and chlortetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. J Anim Sci 1975; 40(2):207-213.

6. Bywater RJ. Sense and nonsense in surveillance programs. Acta vet scand 2000; Suppl 93:119-127.

7. Constable PD. Antimicrobial use in the treatment of calf diarrhea. J Vet Intern Med 2004;18:8-17.

8. Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. App Environ Epidem 2006;72;3940–3948.

9. Dunlop RH, McEwen SA, Meek AH, Clarke RC, Black WD, Friendship RM. Associations among antimicrobial drug treatments and antimicrobial resistance of fecal *Escherichia coli* of swine on 34 farrow-to-finish farms in Ontario, Canada. Prev Vet Med 1998;34:283-305.

10. El-Azizi M, Mushtaq A, Drake C, Lawhorn J, Barenfanger J, Verhulst S, Khardori N. Evaluating antibiograms to monitor drug resistance. Emerg Infect Dis 2005;11:1301-1302.

11. Gow SP, Waldner CL, Rajic A, McFall ME, Reid-Smith R. Prevalence of antimicrobial resistance in fecal generic *Escherichia coli* isolated in western Canadian cow-calf herds. Part 1-Beef calves. Can J Vet Res 2008:72; (In Press).

12. Hinton M. Antibacterial drug resistance among *Escherichia coli* isolated from calves fed on a milk substitute diet. Vet Rec 1983;112:567-568.

13. Hoyle DV, Knight HI, Shaw DJ, Hillman K, Pearce MC, Low JC, Gunn GJ, Woolhouse MEJ. Acquisition and epidemiology of antibiotic-resistant *Escherichia coli* in a cohort of newborn calves. J Antimicrob Chemother 2004;53:867-871.

14. Hoyle DV, Yates CM, Chase-Topping ME, et al. Molecular epidemiology of antimicrobial-resistant commensal *Escherichia coli* strains in a cohort of newborn calves. Appl Environ Microbiol 2005;71:6680–6688.

15. Karlowsky JA, Sahm DF. Antibiotic resistance – is resistance detected by surveillance relevant to predicting resistance in the clinical setting? Curr Opin Pharmacol 2002;2:1-6.

16. Khachatourians GC. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can Med Assoc J 1998;159:1129-1136.

17. Khachatryan AR, Hancock DD, Besser TE, Call DR. Antimicrobial drug resistance genes do not convey a secondary fitness advantage to calf –adapted *Escherichia coli*. Appl Environ Microbiol 2006;72:443-448.

18. Liem, A, Anderson GA, Stine DL, MacGregor S, Cain DV. Control of footrot and liver abscesses with Fusogard®: A new *Fusobacterium necrophorum* bacterin for cattle. Proc 32nd Annu Conv Am Assoc Bov Pract 1999;32:262-265.

19. Linton AH. Antibiotic resistance in bacteria associated with animals and their importance to man. J Antimicrob Chemother 1985;15:385–386.

20. Martel JL, Coudert M. Bacterial Resistance monitoring in animals: the French national experiences of surveillance schemes. Vet Micobiol 1993;35:321-38.

21. McGeer AJ. Agricultural antibiotics and resistance in human pathogens: Villain or scapegoat? Can Med Assoc J 1998;159:119-1120.

22. McNab B, Fairles J, McEwen B. Accurate and complete submissions data are important contributions to surveillance. Ceptor 2007;15(3):4.

23. O'Connor AM, Poppe C, McEwen SA. Changes in the prevalence of resistant *Escherichia coli* in cattle receiving subcutaneously injectable oxytetracycline in addition to in-feed chlortetracycline compared with cattle receiving only in-feed chlortetracycline. Can J Vet Res 2002:66:145-150.

24. Saginala S, Nagaraja TG, Tan ZL, Lechtenberg KF, Chengappa MM, Kemp KE, Hine PM. Serum neutralizing antibody response and protection against experimentally induced liver abscesses in steers vaccinated with Fusobacterium necrophorum. Am J Vet Res 1996;57:483-488.

25. Saginala S, Nagaraja TG, Lechtenberg KF, Chengappa MM, Kemp KE, Hine PM. Effect of *Fusobacterium necrophorum* leukotoxoid vaccine on susceptibility to experimentally induced liver abscesses in cattle. J Anim Sci 1997;75:1160-1166.

26. Stopforth JD, Lopes M, Shultz JE, Miksch RR, Samadpour M. Microbiological status of fresh beef cuts. J Food Prot 2006;69:1456–1459.

27. Teutsch SM. Considerations in planning a surveillance system. In: Teutsch SM, Churchill RE ed. Principles and practice of public health surveillance. Oxford: Oxford University Press 2000, 21.

28. United States Department of Agriculture (USDA) 1997. Part II: Reference of 1997 Beef Cow-Calf Health and Health Management Practices, 1997. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N238-797,1997.

29. Veterinary Drug Directorate (VDD), Health Canada. Uses of antimicrobials in food animals in Canada: Impact on resistance and human health, 2002. Available from http://www.hc-sc.gc.ca/dhp-mps/pubs/vet/amr-ram_final_report-rapport_06-27_cp-pc_e.html accessed December 30, 2007.

30. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp ID, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates form food animals and humans. Antimicrob Agents Chemother 2001;45:2716–2722.