Environmental Fate of Chlortetracycline, Sulfamethazine and Tylosin Fed to Feedlot Cattle

by

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ABSTRACT

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Antimicrobials are widely used in North America's livestock industry to treat and prevent infections and also sub-therapeutically for growth promotion. Field application of livestock manure disperses antimicrobials in to the environment. Antimicrobials can disturb ecological functioning via toxicity towards bacteria and also can increase the level of antimicrobial resistance development in human and livestock pathogens.

Three main studies were conducted to: i) quantify the simulated rain runoff losses of chlortetracycline, tylosin and sulfamethazine following surface application vs. soil incorporation of beef cattle feedlot manure; ii) quantify the dissipation kinetics of these antimicrobials in a seasonally-frozen soil following field application of manure; and iii) quantify and compare the dissipation of excreted vs. fortified (that is, added to antimicrobial-free manure) antimicrobials during indoor composting. Manure was sourced from cattle (*Bos taurus*) receiving no antimicrobial (control), 44 mg chlortetracycline, 44 mg each of chlortetracycline and sulfamethazine, or 11 mg tylosin per kg^{-1} feed.

Antimicrobial concentration in simulated rain runoff following field application of manure generally reflected the corresponding concentrations in manure and decreased in the order chlortetracycline > sulfamethazine > tylosin. Mass loss (% of antimicrobial amount applied) ranged from 1.7 to 6.5% for chlortetracycline and was 4.8% for sulfamethazine and 0.24% for tylosin. Incorporation of manure into the top 10 cm of soil following broadcast application

significantly reduced the mass loss of chlortetracycline and the concentration in runoff of both chlortetracycline and sulfamethazine.

Both chlortetracycline and sulfamethazine were persistent in the seasonally-frozen Canadian prairie soil tested. The first-order dissipation half-life (DT_{50}) for chlortetracycline added along with sulfamethazine was 77 d during the growing season and 648 d during the non-growing season when the soil was frozen for an extended period. By comparison, the DT_{50} of chlortetracycline added alone did not differ significantly between the two seasons (mean DT_{50} = 121 d). Sulfamethazine was detected throughout the 10-mo monitoring period at mean concentrations of up to 16 ± 10 μg kg^{-1}. The mean tylosin concentration was ≤ 11 ± 6.6 μg kg^{-1} and gradually dissipated.

Composting dissipated 85–99% of initial concentrations of chlortetracycline, sulfamethazine, and tylosin in manure within 30 d, indicating the potential of composting to minimize the dispersal of these antimicrobials in agroecosystems.

Manure fortification with antimicrobials is commonly used in studies on the dissipation behavior of antimicrobials in manure. We tested the dissipation behaviour of fortified vs. that of excreted antimicrobials during composting. The first-order dissipation constant (*k*) was significantly greater for excreted chlortetracycline (0.29 d^{-1} - 0.54 d^{-1}) than for the fortified (0.11 d^{-1} - 0.13 d^{-1}) compound. In contrast, dissipation was significantly greater for fortified sulfamethazine (0.47 d^{-1}) and tylosin (0.31 d^{-1}) than for the excreted antimicrobials (0.08 d^{-1} for sulfamethazine and 0.07 $d^{-1}$for tylosin). Thus, the dissipation rates of fortified antimicrobials may not accurately reflect those of the excreted compounds, suggesting that caution should be exercised when interpreting results from studies using fortified antimicrobials.

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FOREWORD

This thesis is prepared in manuscript style in accordance with the Department of Soil Science, University of Manitoba guidelines. The thesis consists of four chapters. Chapter 1 is the general introduction. Chapters 2 through 4 are research chapters prepared as standalone manuscripts. Chapter 2 describes the quantification of antimicrobial runoff losses, in a simulated rain runoff study, following surface application and soil incorporation of beef cattle manure containing chlortetracycline, sulfamethazine and tylosin. Chapter 3 describes a study conducted to determine chlortetracycline, sulfamethazine and tylosin persistence in a seasonally-frozen Canadian prairie soil following soil incorporation of manure. Chapter 4 focuses on a study conducted to quantify the dissipation of the three antimicrobials during composting and also to compare antimicrobial dissipation between antimicrobials that are orally-administered and then excreted vs. those that are added (fortified) to antimicrobial-free manure. A microwave-assisted extraction method was developed to simultaneously extract chlortetracycline, sulfamethazine and tylosin from manure and manure-amended soil and was utilized in the study presented in Chapter 3. Method development work is described in detail in the Appendix. Chapter 5 is the overall synthesis of findings reported in Chapters 2 through 4. Versions of Chapters 2 and 4 have been published in the Journal of Environmental Quality, while the manuscript based on Chapter 3 has been submitted to the same journal and is currently under review.

Publication based on Chapter 2:

Amarakoon, I.D., F. Zvomuya, A.J. Cessna, D. Degenhardt, F.J. Larney, and T.A. McAllister. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. J. Environ. Qual. 43:549-557. **This**

paper was selected by the Journal of Environmental Quality for highlighting in the April 2014 issue of the CSA News Magazine.

Publication based on Chapter 4:

Amarakoon, I.D., F. Zvomuya, S. Sura, F.J. Larney, A.J. Cessna, S. Xu, and T.A. McAllister. 2015. Dissipation of antimicrobials in feedlot manure compost after oral administration vs. fortification after excretion. J. Environ. Qual. doi:10.2134/jeq2015.07.0408. **This paper was selected by the Journal of Environmental Quality in January 2016 for a website story and for a news release for the general public, and also for highlighting in the April 2016 issue of the CSA News Magazine.**

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

The discovery of penicillin in 1928 by the Scottish scientist Sir Alexander Fleming, launched the antibiotic era. Penicillin was mass produced during World War II to treat war casualties. At later stages of the war, veterinarians tested penicillin as an intra-mammary infusion to treat bovine mastitis, marking the first veterinary use of antimicrobials (Gustafson and Bowen 1997). Veterinary antimicrobial use has since increased in North America where antimicrobials are currently used to treat and prevent bacterial infections in livestock and also routinely for growth promotion, often mixed into livestock feed (Sarmah et al. 2006). Systematic record-keeping is not available for antimicrobials in North America; however, the veterinary use of antimicrobials has recently been estimated to be 11 to16 million kg yr^{-1} in the United States (Sarmah et al. 2006; Kim et al. 2011). Definitive data on the percentage of antimicrobials used sub-therapeutically in livestock is also not available, but it is estimated that 50 - 70% of total antimicrobial use in livestock is for growth promotion (Sarmah et al. 2006).

Antimicrobials are by design poorly absorbed by animals (Thiele-Bruhn 2003), reducing the risk of contamination of livestock products such as meat and milk. Thus, approximately 75% of the administered dose is excreted either as the parent compound or as metabolites, with higher or lower values reported depending on the nature of the antimicrobial, the dose, method of administration, and the breed and age of the animal (Kumar et al. 2005b; Sarmah et al. 2006; Arikan et al. 2007; Arikan et al. 2009; Chee-Sanford et al. 2009; Kim et al. 2011).

Manure is generated in large quantities in North America, with estimates of 180 million dry tons per year in the United States (Roe and Pillai 2003) and Canada (Statistics Canada 2016). Livestock manure has traditionally been used in agriculture as a fertilizer and a soil conditioner

and also as a method of livestock waste disposal, given the expansion of confined animal feeding operations. Field application of livestock manure can introduce antimicrobials into agricultural soils, along with antimicrobial resistant bacteria and antimicrobial resistant genes that can persist within the environment (Seveno et al. 2002; Boxall et al. 2004; Sarmah et al. 2006; Kemper et al. 2008).

Antimicrobial resistance in bacteria is a result of mutations in dividing cells and also in non-dividing cells under stress conditions. Bacteria further exchange acquired resistant genes via horizontal gene transfer mechanisms, transformation, conjugation, and transduction (Witte 2000; Seveno et al. 2002). Indication of antibiotic resistance development in turkey gut bacteria due to experimental feeding of streptomycin was reported as early as 1951, shortly after antimicrobials were introduced to the livestock industry (Dibner and Richards 2005). When selective pressure is imposed on bacteria by the spread of antimicrobials in the environment in the presence of antimicrobial resistant genes, this may enhance the development of antimicrobial resistance in human and livestock bacterial pathogens, rendering existing antimicrobials less effective against infections (Kummerer 2003; Ghosh and LaPara 2007; Kemper 2008; Chee-Sanford et al. 2009; Zhang et al. 2009).

Long-term exposure of antimicrobials at low concentrations may lead to antimicrobial resistance development in intestinal flora of humans and livestock (Witte 2000; Langford et al. 2003). Chronic exposure of antimicrobials to humans and livestock may occur through drinking water contamination. For example, Pruden et al. (2006) observed two tetracycline resistant genes, *tet*(W) and *tet*(O), in treated drinking water in northern Colorado, USA. Exposure via crop uptake has also been reported (Kumar et al. 2005a; Boxall et al. 2006; Pruden et al. 2006; Dolliver et al. 2007; Dolliver et al. 2007). Antimicrobials can also be directly toxic to soil

microorganisms and may therefore disturb ecosystem function (Halling-Sorensen 2001; Halling-Sorensen et al. 2002; Toth et al. 2011; Fang et al. 2014).

Antimicrobials have been in use for over eight decades, but they did not receive much attention as an environmental pollutant until the late 1990s, after which awareness has been on the rise (Sarmah et al. 2006). Society and government institutions are now concerned about the potential negative impacts of antimicrobial use in the livestock industry. However, research on these compounds is still an emerging area of science, with limited understanding of the threat these compounds may pose to human health and wellbeing and to the environment. The overall objective of the studies reported in this thesis was, therefore, to enhance our understanding of the fate of livestock fed antimicrobials under Canadian soil and climatic conditions.

1.1. Antimicrobial Compounds

The antimicrobials selected for these studies were chlortetracycline, sulfamethazine and tylosin, which are commonly used therapeutically and sub-therapeutically in North America's livestock industry and elsewhere across the globe. Chlortetracycline and sulfamethazine are used in both human and animal therapy, while tylosin is used primarily in animal therapy.

1.1.1 Chlortetracycline

Chlortetracycline belongs to the tetracycline family of antibiotics, which are naturally produced by the genus *Streptomyces*. Antibacterial activity of chlortetracycline is attained by the inhibition of protein synthesis in target bacteria (Oka et al. 2000; Halling-Sorensen et al. 2002; Sassman and Lee, 2005). Chlortetracycline consists of four ring structures partially conjugated to each other and contains carboxyamide and hydroxyl functional groups (Fig. 1.1). Its molecular

weight is 479 g mol^{-1}. Chlortetracycline is characterized as immobile with soil partition coefficient (K_d) values of 1,208 to 2,386 L kg^{-1}, an octanol-water partition coefficient (K_{ow}) of 0.4, and a water solubility of 600 mg L^{-1} (Tolls 2001; Sarmah et al. 2006). Chlortetracycline has three acid dissociation constants (pK_a), 3.3, 7.4 and 9.3, and exists as a cation (+ 0 0), zwitterions (+ – 0) and negatively charged ion (+ – –), giving cation exchange as the main mechanism of chlortetracycline sorption in soil (Sassman and Lee 2005; Sarmah et al. 2006; Pils and Laird 2007). Hydrophobic interactions become important over other mechanisms as pH increases (Sassman and Lee 2005). Chlortetracycline can form chelate complexes with divalent and trivalent metal ions such as Mg^{2+}, Ca^{2+}, Zn^{2+}, $Fe^{3+}$and Al^{3+}, and also with β-diketones and carboxyamide. It can also bind strongly to proteins and silanolic groups (Oka et al. 2000; Halling-Sorensen et al. 2002).

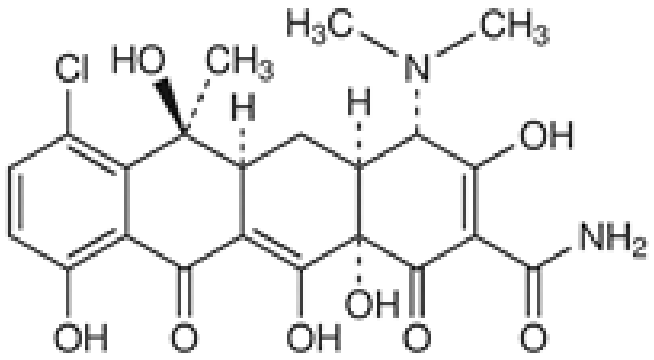


Figure 1.1: Molecular structure of chlortetracycline.
Sarmah et al. (2006) and Essington et al. (2010).

1.1.2 Sulfamethazine

Sulfamethazine belongs to the sulfonamide group of antimicrobials. Sulfonamides are synthetic antimicrobial compounds. All sulfonamides consist of a benzene ring, an amine moiety and a sulfonamide group (Fig. 1.2). Antibacterial activity is achieved by the disruption of the

folic acid metabolism cycle in target bacteria (Sarmah et al. 2006). Sulfamethazine has a distinctly different environmental behavior to chlortetracycline and tylsoin. With pK_a's of 2.65 and 7.49, sulfamethazine is predominantly charge neutral at or below neutral pH, whereas the anionic form predominates at alkaline pH. Sulfamethazine has a molecular weight of 278 g mol^{-1} and a K_{ow} of 0.7. Sulfamethazine is classified as moderately to highly mobile (Tolls 2001; Boxall et al. 2002), with a K_d of 0.6–3.2 L kg^{-1}, an organic carbon normalized partition coefficient (K_{oc}) of 82-208 L kg^{-1}, and a solubility of 1500 mg L^{-1} (Tolls 2001; Thiele-Bruhn 2003; Sarmah et al. 2006). It therefore generally shows a low tendency to sorb onto soil particles (Thiele-Bruhn, 2003; Kwon 2011). Nevertheless, sulfamethazine, like all sulfonamides, is reported to be persistent in the environment (Haller et al. 2002; Baran et al. 2006). Sulfamethazine bonds strongly with soil organic matter, forming strong hydrogen (Teixido et al. 2011) and covalent bonds (Bialk et al. 2005; Gulkowska et al. 2013). Formation of non-extractable residual fractions in manured soils has also been reported for sulfonamides (Kreuzig and Holtge 2005a; Heise et al. 2006; Forster et al. 2009).

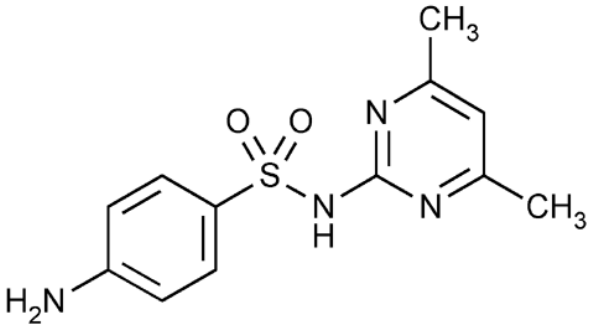


Figure 1.2: Molecular structure of sulfamethazine.
Sarmah et al. (2006) and Essington et al. (2010)

1.1.3 Tylosin

Tylosin belongs to the macrolide family of antibiotics, which are produced by the genus *Streptomyces.* All macrolide antibiotics are characterized by a large macrolide ring (Fig. 1.3). The macrolide ring is a macrocyclic lactone ring with more than ten C atoms. Tylosin acts as a protein synthesis inhibitor and is the most common macrolide used in the livestock industry. Tylosin is a mixture of tylosin A, B, C, and D, all of which contribute to its potency and biological effects (Sarmah et al. 2006). Similar to chlortetracycline, tylosin is predominantly cationic in neutral to acid soils (pK_a of 7.5) and participates largely in cation exchange processes for sorption, thus showing strong affinity for soil particles (Sassman et al. 2007; Essington et al. 2010), especially in soils with high cation exchange capacity. In addition, because of its large molecular weight (917 g mol^{-1}) and high K_{ow} (3.41), tylosin shows a strong affinity for organic matter via hydrophobic interactions (Loke et al. 2002; Clay et al. 2005; Sarmah et al. 2006; Wegst-Uhrich et al. 2014). Tylosin is low to slightly mobile (Tolls 2001), with a K_d of 37–270 L kg^{-1}, a K_{oc} of 110-831 L kg^{-1}, and a solubility of 500 mg L^{-1} (Sarmah et al. 2006).

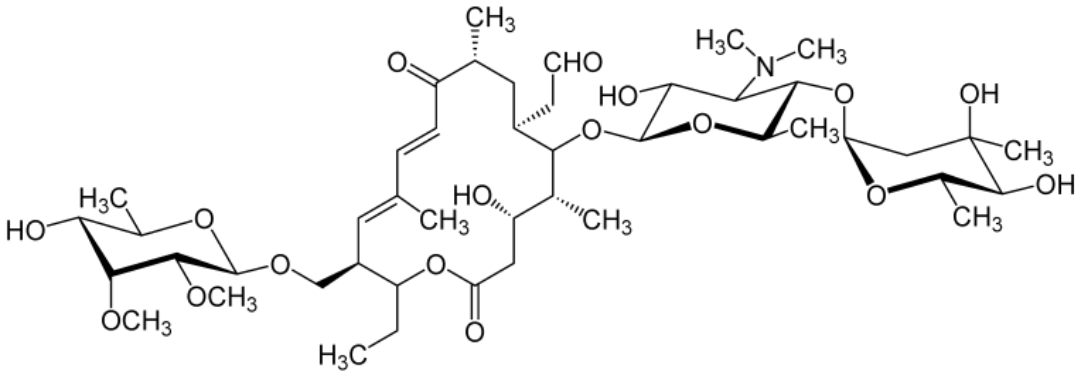


Figure 1.3: Molecular structure of tylosin A, which accounts for 80-90% of the parent compound mixture. Sarmah et al. (2006) and Essington et al. (2010)

1.2 Antimicrobial Transport

Once antimicrobial-containing manure is land applied, the antimicrobials may be subject to transport via (i) surface runoff following rainfall and snowmelt (Boxall et al. 2004; Kay et al. 2004; Stoob et al. 2007; Kemper 2008; Dolliver and Gupta 2008) and (ii) leaching (Aust et al. 2008; Kwon et al. 2011; Dolliver and Gupta 2008), contaminating soil and water resources. The first reported surface water contamination was in England, where tetracyclines, sulfonamides, and macrolides were measured at concentrations of up to 1 μg L^{-1} (Watts et al. 1982; Sarmah et al. 2006). Occurrence of chlortetracycline, sulfamethazine and tylosin in surface waters in Canada has been reported in several tributaries to the Grand River in Ontario (Lissemore et al. 2006), in streams in Alberta (Forrest et al. 2011) and Saskatchewan (Waiser et al. 2011), and in surface waters in the United States (Kolpin et al. 2002; Yang and Carlson 2003). Distinguishing between veterinary and human (i.e., municipal sewage waste and hospital waste) sources is rather difficult for antimicrobials that are used in both livestock and human therapy. Nevertheless, the presence of antimicrobials in surface water, regardless of source, represents a decrease in the water quality. However, the extent and processes of antimicrobial loss from agricultural soils amended with antimicrobial contaminated manure via runoff are not fully understood, yet this is one of the key pieces of information needed to understand and manage the environmental impact of antimicrobials that arise from their use in livestock.

Studies of antimicrobial transport in surface runoff following field application of manure have generally fallen into three broad categories, namely, those using (i) antimicrobials excreted following administration to livestock, with the manure containing antimicrobials either broadcasted or incorporated into field soils (Burkhardt et al. 2004; Dolliver and Gupta 2008; Hoese et al. 2009); (ii) antimicrobials added directly to manure prior to soil application

(Burkhardt et al. 2005; Blackwell et al. 2007) often referred to as fortification studies; and (iii) antimicrobials mixed with methanol/water in the absence of manure and sprayed directly onto soil (Davis et al. 2006; Kim et al. 2010). Category (i) studies provide the most realistic representation of antimicrobial behavior in cropland soils receiving manure applications. Dolliver and Gupta (2008) reported total chlortetracycline and tylosin losses in rainfall and snowmelt runoff of up to 5% of the amount from fall application of solid beef cattle manure and swine manure collected from animals receiving antimicrobials in their feed. Hoese et al. (2009) reported total losses in simulated surface runoff of 0.9-3.5% of applied chlortetracycline and 8.4-12% of applied tylosin following surface application of liquid swine manure.

Fortification of manure with antimicrobials in antimicrobial transport studies is more economical and more efficient than administering antimicrobials to livestock and collecting manure containing excreted antimicrobials. However, the antimicrobials in fortified manure may not accurately reflect the transport behaviour of excreted antimicrobials as fortified antimicrobials in manure usually have less contact time with manure and are not exposed to host metabolism and gut microorganisms. Antimicrobials sprayed directly onto soil, in the absence of manure, may have greater contact with soil particles, which may not be the case when they are added in a manure matrix.

Manure incorporation into the soil during field application is recommended in many jurisdictions in North America. Chlortetracycline and tylosin losses were lower when cattle manure was incorporated via chisel plow tillage compared with surface-application under no-till conditions (Dolliver and Gupta 2008). Kay et al. (2004) observed that the peak concentrations and total losses of sulfachloropyridazine (a sulfonamide) and oxytetracycline (a tetracycline) in tile drain flow were lower when soil was tilled prior to pig slurry application as compared to no-

till. Similarly, conventionally cultivated land had lower runoff volume and lower sulfonamide losses after manure slurry application compared with grassland, which was undisturbed by tillage (Kreuzig et al. 2005b).

The majority of studies quantifying antimicrobial transport in surface runoff have used liquid swine manure (Burkhardt et al. 2004; Burkhardt et al. 2005; Blackwell et al. 2007; Hoese et al. 2009), whereas there is a lack of published studies using solid cattle manure (Dolliver and Gupta 2008). Fortification and field application are more efficient with swine manure as it is mostly liquid than solid cattle manure. However, runoff estimates based on the application of swine manure slurry may not adequately represent runoff losses of antimicrobials added with solid cattle manure. Sealing of surface soil pores was blamed for an increase in the antimicrobial mass loss of sulfonamide antimicrobials, sulfadiazine and sulfathiazole, following surface application of manure slurry on grassland (Burkhardt et al. 2005), indicating that manure texture may have an effect on antimicrobial loss during surface runoff. North America is home to a large cattle industry and, therefore, it is important to assess the transport behavior of antimicrobials field applied with solid cattle manure. Chapter 2 of this thesis focuses on a study conducted to quantify antimicrobial transport in surface runoff following field application of solid beef cattle manure containing excreted chlortetracycline, sulfamethazine and tylosin. Antimicrobial losses from surface application and from field incorporation following surface application of manure were also compared.

1.3 Antimicrobial Persistence

1.3.1 Dissipation in Soil

Dissipation of antimicrobials administered to livestock can occur via host metabolism, microbial and chemical degradation inside the animal's digestive system and on the feedlot floor where manure accumulates, and in soil and water following dispersal (Sassman et al. 2007; Arikan et al. 2009; Yang et al. 2009; Liu et al. 2010; Yang et al. 2012; Srinivasan and Sarmah 2014). Soil bacteria were reported to be more involved than fungi in the process of antimicrobial degradation (Srinivasan and Sarmah 2014).

Once manure containing antimicrobials is field-applied, a fraction of the added antimicrobials gets sorbed to soil constituents. Sorption mechanisms involved in soil include cation exchange, cation bridging at clay surfaces, surface complexation, hydrogen bonding and hydrophobic bonding (Sarmah et al. 2006). Antimicrobial sorption in soil, regardless of the clay minerals or organic matter fraction of the soil, helps retain these compounds (Stoob et al. 2007), reducing the risk of antimicrobial transport offsite. However, on the negative side, sorption may also reduce bioavailability and degradation, thus increasing the persistence of the antimicrobial (Thiele-Bruhn 2003). Increased persistence may enhance crop uptake during the growing season in contaminated soils. Green onions (*Allium cepa* L.), cabbage (*Brassica oleracea* L.), and corn (*Zea mays* L.) growing in a contaminated soil had 2–17 ng chlortetracycline g^{-1} fresh weight (Kumar et al. 2005a). Corn (*Zea mays* L.), lettuce (*Lactuca sativa* L.), and potato (*Solanum tuberosum* L.) plant tissues had sulfamethazine in the range of 0.1–1.2 mg kg^{-1} dry weight (Dolliver et al. 2007). Tylosin poses lower uptake risk compared to chlortetracycline and sulfamethazine, likely due to the large molecular weight of tylosin, which limits its active and passive uptake by plant roots (Kumar et al., 2005a). Tetracyclines and sulfonamides have also

been detected in cucumbers (*Cucumis sativus* L.), tomatoes (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.) (Ahmed et al. 2015).

Antimicrobial persistence in soil also increases the chance for antimicrobial resistance development in environmental bacteria (Esiobu et al. 2002; Sengelov et al. 2003; Ghosh et al. 2007). A study by Halling-Sorensen et al. (2005) showed a ~1 – 5% increase in the tylosin- and tetracycline-resistant population in the first set of samples collected 3 d following field application of manure containing 30-50 μg kg^{-1} of these antimicrobials in a sandy and a sandy loam soils. However, antimicrobials target several genes at once and therefore mutations have to occur in multiple genes within an organism in order to develop full resistance towards a particular antimicrobial (Martinez and Baquero 2000; Seveno et al. 2002).

Thus, rapid degradation of antimicrobials is desirable; however, the antibiotic chlortetracycline is known to persist in agricultural soils with repeated manure application (Hamscher et al. 2002; Hamscher et al. 2005; Martinez-Carballo et al. 2007). Only a handful of published studies have examined antimicrobial dissipation half-lives under field conditions, which is another important aspect of antimicrobial behavior following manure application. Information on antimicrobial dissipation is needed to understand and manage the environmental impact of veterinary antimicrobials.

Laboratory microcosm studies using soil collected from long-term field plots in Ontario, Canada, showed DT_{50} values of 2.8-3.3 d for chlortetracycline, 1.3-5.3 d for sulfamethazine, and 2-10.2 d for tylosin (Topp et al. 2013). Dissipation half-lives of 23-24 d for tylosin (Sassman et al. 2007), 18.6 d for sulfamethazine (Accinelli et al. 2007), and 8 d for tylosin (Schluesener and Bester 2006) have also been reported. Laboratory microcosm incubation studies contribute significantly to the pool of knowledge. However, they are often performed under optimum

conditions for antimicrobial dissipation and may not represent the complex dissipation processes taking place under field conditions. Thus, there is a need for more field studies to quantify antimicrobial dissipation.

Dissipation half-lives of 25 d in a sandy loam and 34 d in a sandy soil have been reported for chlortetracycline, while corresponding values of 67 d in a sandy loam and 49 d in a sandy soil have been reported for tylosin under field conditions in Denmark following swine manure application (Halling-Sorensen et al. 2005). Sulfamethazine was reported to reach approximately half of its initial concentration in 42 d in a field receiving liquid swine manure in Switzerland (Stoob et al. 2006). First-order dissipation half-lives were 21 d for chlortetracycline and 6.1 d for tylosin when the antimicrobials were directly incorporated into a sand and a sandy loam in Ontario, Canada (Carlson and Mabury 2006). Dissipation half-lives were 24 d for chlortetracycline and 4.5 d for tylosin in the same study when dairy cattle manure was incorporated separately.

Many of the published field studies monitored antimicrobial dissipation in soil for periods of less than a year and only during the summer. Such short-duration studies may fail to show dissipation patterns in soil over a year or longer. Because the studies were conducted in the summer, they may also not adequately represent antimicrobial dissipation in seasonally-frozen soils, such as those in the Canadian prairies.

As discussed above, field application of manure containing excreted antimicrobials following administration to livestock would be the most representative of antimicrobial behavior in agricultural soils receiving manure. Fortified antimicrobials in manure and antimicrobials directly applied to soil may not accurately reflect the dissipation behaviour of compounds added along with manure. The use of swine manure is reported often and to our knowledge there is no

published field dissipation data for chlortetracycline, sulfamethazine, and tylosin following field application of beef cattle manure containing these antimicrobials. Therefore, the study presented in Chapter 3 of this thesis was conducted to quantify the dissipation of these antimicrobials in a seasonally-frozen Canadian prairie soil following field application of solid beef cattle manure containing the excreted antimicrobials.

1.3.2 Dissipation in Manure

If antimicrobial dissipation can occur before field application within the farm, that would be better than trying to control the spread and promote in-situ degradation after dispersal in the environment. Composting is a relatively new manure handling practice in feedlots (Larney et al. 2006) which has demonstrated a potential to enhance antimicrobial dissipation (Arikan et al. 2007; Storteboom et al. 2007; Arikan et al. 2009; Bao et al. 2009; Ramaswamy et al. 2010; Ho et al. 2013). Composting is a process of aerobic decomposition of organic material, using existing microorganisms associated with the composting substrate. The process of composting increases the temperature of manure often beyond 55 ºC for a prolonged period (Larney et al. 2006). The rise in temperature was also shown to have a major effect on the process of organic matter degradation during composting (Arikan et al. 2009). Composting reduces the mass and volume of manure, concentrating plant nutrients such as phosphorus. By doing so, composting increases the transportation distance that is economical for its disposal as compared with fresh manure (Larney et al. 2006). Composting also reduces pathogens, parasites, and the viability of weed seeds (Larney et al. 2003; Larney and Hao 2007). Thus, composted manure offers many advantages as an agricultural amendment over fresh manure. However, to date, there has been very little research on antimicrobial dissipation during composting of beef cattle manure. The

dissipation of chlortetracycline, sulfamethazine and tylosin during composting was therefore the focus of the study reported in Chapter 4 of this thesis.

Numerous studies on the dissipation of antimicrobials have involved the addition of antimicrobials to antimicrobial-free manure (Burkhardt et al. 2005; Dolliver et al. 2008; Ho et al. 2013). Such manure fortification with antimicrobials is economical and efficient compared to administering antimicrobials to livestock as it is expensive to maintain the research herds. However, the behavior of fortified antimicrobials in manure may not accurately reflect the behavior of antimicrobials that have been administered to cattle and subsequently excreted. Comparison of the dissipation behavior of antimicrobials excreted following oral administration versus those that are added to manure post-excretion has not been adequately studied and was the second focus of the study presented in Chapter 4.

1.4 Objectives

The overall objective of this thesis was to enhance the understanding of the fate of livestock fed antimicrobials under Canadian soil and climatic conditions. Specific objectives were (i) to quantify simulated rain runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin, and to compare antimicrobial losses between surface application and soil incorporation of beef cattle manure **(Chapter 2)**; (ii) to quantify the rates of chlortetracycline, sulfamethazine and tylosin dissipation in a seasonally-frozen agricultural soil following fall application of beef cattle manure containing excreted antimicrobials (**Chapter 3**); and (iii) to quantify and compare the dissipation, during indoor composting, of chlortetracycline, sulfamethazine, and tylosin excreted by beef cattle administered the three antimicrobials vs. that of the same antimicrobials added to antimicrobial-free manure **(Chapter 4)**.

1.5 Thesis Outline

This thesis is prepared in manuscript style in accordance with the Department of Soil Science, University of Manitoba guidelines. There are three standalone manuscripts (Chapters 2 through 4):

Chapter 2: Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure;

Chapter 3: Dissipation of antimicrobials in a seasonally-frozen soil following beef cattle feedlot manure application; and

Chapter 4: Dissipation of antimicrobials in feedlot manure compost following oral administration vs. fortification post-excretion.

My contribution to Chapters 2 through 4 included experimental design, conducting field sampling, laboratory analysis, data processing and statistical analysis, writing of the first draft, and incorporation of revisions suggested by co-authors of the manuscripts and members of my advisory committee. I also developed a method to simultaneously extract chlortetracycline, sulfamethazine, and tylosin from manure and manure-amended soil (Appendix). The method was employed in the study presented in Chapter 3.

1.6 References

Accinelli, C., Koskinen, W. C., Becker, J. M. and Sadowsky, M. J. 2007. Environmental fate of two sulfonamide antimicrobial agents in soil. J. Agric. Food Chem. **55:** 2677-2682.

Ahmed, M. B. M., Rajapaksha, A. U., Lim, J. E., Vu, N. T., Kim, I. S., King, H. M., Lee, S. S. and Ok, Y. S. 2015. Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. J. Agric. Food Chem. **63:** 398-405.

Arikan, O. A., Mulbry, W. and Rice, C. 2009. Management of antibiotic residues from agricultural sources: Use of composting to reduce chlortetracycline residues in beef manure from treated animals. J. Hazard. Mater. **164:** 483-489.

Arikan, O. A., Sikora, L. J., Mulbry, W., Khan, S. U. and Foster, G. D. 2007. Composting rapidly reduces levels of extractable oxytetracycline in manure from therapeutically treated beef calves. Bioresour. Technol. **98:** 169-176.

Aust, M. O., Godlinski, F., Travis, G. R., Hao X., McAllister, T. A., Leinweber, P. and Thiele-Bruhn, S. 2008. Distribution of sulfamethazine, chlortetracycline and tylosin in manure and soil of Canadian feedlots after sub-therapeutic use in cattle. Environ. Pollut. **156:** 1243-1251.

Bao, Y. Y., Zhou, Q. X., Guan, L. Z. and Wang, Y. Y. 2009. Depletion of chlortetracycline during composting of aged and spiked manures. Waste Manage. **29:** 1416-1423.

Baran, W., Sochacka, J. and Wardas, W. 2006. Toxicity and biodegradability of sulfonamides and products of their photocatalytic degradation in aqueous solutions. Chemosphere **65:** 1295-1299.

Bialk, H. M., Simpson, A. J. and Pedersen, J. A. 2005. Cross-coupling of sulfonamide antimicrobial agents with model humic constituents. Environ. Sci. Technol. **39:** 4463-4473.

Blackwell, P. A., Kay, P. and Boxall, A. B. A. 2007. The dissipation and transport of veterinary antibiotics in a sandy loam soil. Chemosphere **67:** 292-299.

Boxall, A. B. A., Blackwell, P., Cavallo, R., Kay, P. and Tolls, J. 2002. The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol. Lett. **131:** 19-28.

Boxall, A. B. A., Fogg, L. A., Blackwell, P. A., Kay, P., Pemberton, E. J. and Croxford, A. 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. **180:** 1-91.

Boxall, A. B. A., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E. and Levy, L. S. 2006. Uptake of veterinary medicines from soils into plants. J. Agric. Food Chem. **54:** 2288-2297.

Burkhardt, M., Stamm, C., Waul, C., Singer, H. and Muller, S. 2005. Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. J. Environ. Qual. **34:** 1363-1371.

Burkhardt, M., Stoob, K., Stamm, C., Singer, H. and Muller, S. 2004. Veterinary antibiotics in animal slurries - A new environmental issue in grassland research. Grassland Sci. Eur. **9:** 322-324.

Carlson, J. C. and Mabury, S. A. 2006. Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environ. Toxicol. Chem. **25:** 1-10.

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S. and Aminov, R. I. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. **38:** 1086-1108.

Clay, S., Liu, Z., Thaler, R. and Kennouche, H. 2005. Tylosin sorption to silty clay loam soils, swine manure, and sand. J. Environ. Sci. Heal. B **40:** 841-850.

Davis, J. G., Truman, C. C., Kim, S. C., Ascough, J. C. II and Carlson, K. 2006. Antibiotic transport via runoff and soil loss. J. Environ. Qual. **35:** 2250-2260.

Dibner, J. J. and Richards, J. D. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. **84:** 634-643.

Dolliver, H. and Gupta, S. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. **37:** 1227-1237.

Dolliver, H., Gupta, S. and Noll, S. 2008. Antibiotic degradation during manure composting. J. Environ. Qual. **37:** 1245-1253.

Dolliver, H., Kumar, K. and Gupta, S. 2007. Sulfamethazine uptake by plants from manure-amended soil. J. Environ. Qual. **36:** 1224-1230.

Esiobu, N., Armenta, L. and Ike, J. 2002. Antibiotic resistance in soil and water environments. Int. J. Environ. Health Res. **12:** 133-144.

Essington, M. E., Lee, J. and Seo, Y. 2010. Adsorption of Antibiotics by Montmorillonite and Kaolinite. Soil Sci. Soc. Am. J. **74:** 1577-1588.

Fang, H., Han, Y., Yin, Y., Pan, X. and Yu, Y. 2014. Variations in dissipation rate, microbial function and antibiotic resistance due to repeated introductions of manure containing sulfadiazine and chlortetracycline to soil. Chemosphere **96:** 51-56.

Forrest, F., Lorenz, K., Thompson, T., Keenliside, J., Kendall, J. and Charest, J. 2011. A scoping study of livestock antimicrobials in agricultural streams of Alberta. Can. Water Resour. J. **36:** 1-16.

Forster, M., Laabs, V., Lamshoft, M., Groeneweg, J., Zuhlke, S., Spiteller, M., Krauss, M., Kaupenjohann, M. and Amelung, W. 2009. Sequestration of manure-applied sulfadiazine residues in soils. Environ. Sci. Technol. **43:** 1824-1830.

Ghosh, S. and LaPara, T. M. 2007. The effects of sub-therapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. Isme J. **1:** 191-203.

Gulkowska, A., Sander, M., Hollender, J. and Krauss, M. 2013. Covalent binding of sulfamethazine to natural and synthetic humic acids: assessing laccase catalysis and covalent bond stability. Environ. Sci. Technol. **47:** 6916-6924.

Gustafson, R. H. and Bowen, R. E. 1997. Antibiotic use in animal agriculture. J. Appl. Microbiol. **83:** 531-541.

Haller, M. Y., Muller, S. R., McArdell, C. S., Alder, A. C. and Suter, M. J. F. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. A **952:** 111-120.

Halling-Sorensen, B. 2001. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Arch. Environ. Contam. Toxicol. **40:** 451-460.

Halling-Sorensen, B., Jacobsen, A. M., Jensen, J., Sengelov, G., Vaclavik, E. and Ingerslev, F. 2005. Dissipation and effects of chlortetracycline and tylosin in two agricultural soils: A field-scale study in Southern Denmark. Environ. Toxicol. Chem. **24:** 802-810.

Halling-Sorensen, B., Sengelov, G. and Tjornelund, J. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch. Environ. Contam. Toxicol. **42:** 263-271.

Hamscher, G., Pawelzick, H. T., Hoper, H. and Nau, H. 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environ. Toxicol. Chem. **24:** 861-868.

Hamscher, G., Sczesny, S., Hoper, H. and Nau, H. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. **74:** 1509-1518.

Heise, J., Holtge, S., Schrader, S. and Kreuzig, R. 2006. Chemical and biological characterization of non-extractable sulfonamide residues in soil. Chemosphere **65:** 2352-2357.

Ho, Y. B., Zakaria, M. P., Latif, P. A. and Saari, N. 2013. Degradation of veterinary antibiotics and hormone during broiler manure composting. Bioresour. Technol. **131:** 476-484.

Hoese, A., Clay, S. A., Clay, D. E., Oswald, J., Trooien, T., Thaler, R. and Carlson, C. G. 2009. Chlortetracycline and tylosin runoff from soils treated with antimicrobial containing manure. J. Environ. Sci. Heal. B. **44:** 371-378.

Kay, P., Blackwell, P. A. and Boxall, A. B. A. 2004. Fate of veterinary antibiotics in a macro porous tile drained clay soil. Environ. Toxicol. Chem. **23:** 1136-1144.

Kemper, N., Farber, H., Skutlarek, D. and Krieter, J. 2008. Analysis of antibiotic residues in liquid manure and leachate of dairy farms in Northern Germany. Agric. Water Manage. **95:** 1288-1292.

Kemper, N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Ind. **8:** 1-13.

Kim, K., Owens, G., Kwon, S., So, K., Lee, D. and Ok, Y. S. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut. **214:** 163-174.

Kim, S., Davis, J. G., Truman, C. C., Ascough, J. C. II and Carlson, K. 2010. Simulated rainfall study for transport of veterinary antibiotics - mass balance analysis. J. Hazard. Mater. **175:** 836-843.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. **36:** 1202-1211.

Kreuzig, R. and Holtge, S. 2005a. Investigations on the fate of sulfadiazine in manured soil: Laboratory experiments and test plot studies. Environ. Toxicol. Chem. **24:** 771-776.

Kreuzig, R., Holtge, S., Brunotte, J., Berenzen, N., Wogram, J. and Schulz, R. 2005b. Test-plot studies on runoff of sulfonamides from manured soils after sprinkler irrigation. Environ. Toxicol. Chem. **24:** 777-781.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y. and Rosen, C. J. 2005a. Antibiotic uptake by plants from soil fertilized with animal manure. J. Environ. Qual. **34:** 2082-2085.

Kumar, K., Gupta, S. C., Chander, Y. and Singh, A. K. 2005b. Antibiotic use in agriculture and its impact on the terrestrial environment. Adv. Agron. **87:** 1-54.

Kummerer, K. 2003. Significance of antibiotics in the environment. J. Antimicrob. Chemother. **52:** 317-317.

Kwon, J. 2011. Mobility of veterinary drugs in soil with application of manure compost. Bull. Environ. Contam. Toxicol. **87:** 40-44.

Langford, F. M., Weary, D. M. and Fisher, L. 2003. Antibiotic resistance in gut bacteria from dairy calves: A dose response to the level of antibiotics fed in milk. J. Dairy Sci. **86:** 3963-3966.

Larney, F. J., Yanke, L. J., Miller, J. J. and McAllister, T. A. 2003. Fate of coliform bacteria in composted beef cattle feedlot manure. J. Environ. Qual. **32:** 1508-1515.

Larney, F. J., Buckley, K. E., Hao, X. and McCaughey, W. P. 2006. Fresh, stockpiled, and composted beef cattle feedlot manure: Nutrient levels and mass balance estimates in Alberta and Manitoba. J. Environ. Qual. **35:** 1844-1854.

Larney, F. J. and Hao, X. 2007. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta, Canada. Bioresour. Technol. **98:** 3221-3227.

Lissemore, L., Hao, C. Y., Yang, P., Sibley, P. K., Mabury, S. and Solomon, K. R. 2006. An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. Chemosphere **64:** 717-729.

Liu, F., Ying, G., Yang, J., Zhou, L., Tao, R., Wang, L., Zhang, L. and Peng, P. 2010. Dissipation of sulfamethoxazole, trimethoprim and tylosin in a soil under aerobic and anoxic conditions. Environ. Chem. **7:** 370-376.

Loke, M. L., Tjornelund, J. and Halling-Sorensen, B. 2002. Determination of the distribution coefficient (log K-d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere **48:** 351-361.

Martinez, J. L. and Baquero, F. 2000. Mutation frequencies and antibiotic resistance. Antimicrobial Agents Chemother. **44:** 1771-1777.

Martinez-Carballo, E., Gonzalez-Barreiro, C., Scharf, S. and Gans, O. 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ. Pollut. **148:** 570-579.

Oka, H., Ito, Y. and Matsumoto, H. 2000. Chromatographic analysis of tetracycline antibiotics in foods. J. Chromatogr. A **882:** 109-133.

Pils, J. R. V. and Laird, D. A. 2007. Sorption of tetracycline and chlortetracycline on K- and Ca-saturated soil clays, humic substances, and clay-humic complexes. Environ. Sci. Technol. **41:** 1928-1933.

Pruden, A., Pei, R., Storteboom, H. and Carlson, K. H. 2006. Antibiotic resistance genes as emerging contaminants: Studies in Northern Colorado. Environ. Sci. Technol. **40:** 7445-7450.

Ramaswamy, J., Prasher, S. O., Patel, R. M., Hussain, S. A. and Barrington, S. F. 2010. The effect of composting on the degradation of a veterinary pharmaceutical. Bioresour. Technol. **101:** 2294-2299.

Roe, M. T. and Pillai, S. D. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. Poult. Sci. **82:** 622-626.

Sarmah, A. K., Meyer, M. T. and Boxall, A. B. A. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere **65:** 725-759.

Sassman, S. A. and Lee, L. S. 2005. Sorption of three tetracyclines by several soils: Assessing the role of pH and cation exchange. Environ. Sci. Technol. **39:** 7452-7459.

Sassman, S. A., Sarmah, A. K. and Lee, L. S. 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environ. Toxicol. Chem. **26:** 1629-1635.

Schluesener, M. P. and Bester, K. 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. Environ. Pollut. **143:** 565-571.

Seveno, N. A., Kallifidas, D., Smalla, K., van Elsas, J. D., Collard, J. M., Karagouni, A. D. and Wellington, E. M. H. 2002. Occurrence and reservoirs of antibiotic resistance genes in the environment. Rev. Med. Microbiol. **13:** 15-27.

Speltini, A., Sturini, M., Maraschi, F., Profumo, A. and Albini, A. 2011. Analytical methods for the determination of fluoroquinolones in solid environmental matrices. TrAC Trends Anal. Chem. **30:** 1337-1350.

Srinivasan, P. and Sarmah, A. K. 2014. Dissipation of sulfamethoxazole in pasture soils as affected by soil and environmental factors. Sci. Total Environ. **479:** 284-291.

Statistics Canada 2016. A geographical profile of livestock manure production in Canada [Online]. Available: http://www.statcan.gc.ca/pub/16-002-x/2008004/article/10751-eng.htm [verified 8 June 2016].

Stoob, K., Singer, H. P., Mueller, S. R., Schwarzenbach, R. P. and Stamm, C. H. 2007. Dissipation and transport of veterinary sulfonamide antibiotics after manure application to grassland in a small catchment. Environ. Sci. Technol. **41:** 7349-7355.

Stoob, K., Singer, H. P., Stettler, S., Hartmann, N., Mueller, S. R. and Stamm, C. H. 2006. Exhaustive extraction of sulfonamide antibiotics from aged agricultural soils using pressurized liquid extraction. J. Chromatogr. **1128:** 1-9.

Storteboom, H. N., Kim, S. C., Doesken, K. C., Carlson, K. H., Davis, J. G. and Pruden, A. 2007. Response of antibiotics and resistance genes to high-intensity and low-intensity manure management. J. Environ. Qual. **36:** 1695-1703.

Sura, S., Degenhardt, D., Cessna, A. J., Larney, F. J., Olson, A. F. and McAllister, T. A. 2014. Dissipation of three veterinary antimicrobials in beef cattle feedlot manure stockpiled over winter. J. Environ. Qual. **43:** 1061-1070.

Teixido, M., Pignatello, J. J., Beltran, J. L., Granados, M. and Peccia, J. 2011. Speciation of the ionizable antibiotic sulfamethazine on black carbon (biochar). Environ. Sci. Technol. **45:** 10020-10027.

Thiele-Bruhn, S. 2003. Pharmaceutical antibiotic compounds in soils - A review. J. Plant Nutr. Soil Sci. **166:** 145-167.

Tolls, J. 2001. Sorption of veterinary pharmaceuticals in soils: A review. Environ. Sci. Technol. **35:** 3397-3406.

Topp, E., Chapman, R., Devers-Lamrani, M., Hartmann, A., Marti, R., Martin-Laurent, F., Sabourin, L., Scott, A. and Sumarah, M. 2013. Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine-degrading Microbacterium sp. J. Environ. Qual. **42:** 173-178.

Toth, J. D., Feng, Y. and Dou, Z. 2011. Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. Soil Biol. Biochem. **43:** 2470-2472.

Waiser, M. J., Humphries, D., Tumber, V. and Holm, J. 2011. Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. Environ. Toxicol. Chem. **30:** 508-519.

Wegst-Uhrich, S. R., Navarro, D. A. G., Zimmerman, L. and Aga, D. S. 2014. Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. Chem. Central J. **8:** 5.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. Int. J. Antimicrob. Agents **14:** 321-325.

Yang, S. W. and Carlson, K. 2003. Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes. Water Res. **37:** 4645-4656.

Yang, J., Ying, G., Liu, S., Zhou, L., Zhao, J., Tao, R. and Peng, P. 2012. Biological degradation and microbial function effect of norfloxacin in a soil under different conditions. J. Environ. Sci. Heal. B **47:** 288-295.

Yang, J. F., Ying, G. G., Yang, L. H., Zhao, J. L., Liu, F., Tao, R., Yu, Z. Q. and Peng, P. 2009. Degradation behavior of sulfadiazine in soils under different conditions. J. Environ. Sci. Heal. B **44:** 241-248.

Zhang, X. X., Zhang, T. and Fang, H. 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. **82:** 397-414.

2. RUNOFF LOSSES OF EXCRETED CHLORTETRACYCLINE, SULFAMETHAZINE, AND TYLOSIN FROM SURFACE-APPLIED AND SOIL-INCORPORATED BEEF CATTLE FEEDLOT MANURE

2.1. Abstract

Antimicrobials in land-applied manure can move to surface waters via rain or snowmelt runoff, thus increasing their dispersion in agro-environments. This study quantified losses of excreted chlortetracycline, sulfamethazine, and tylosin in simulated rain runoff from surface-applied and soil-incorporated beef cattle (*Bos taurus* L.) feedlot manure (60 Mg ha^{-1}, wet wt.). Antimicrobial concentrations in runoff generally reflected the corresponding concentrations in the manure. Soil incorporation of manure reduced the concentrations of chlortetracycline (from 75 to 12 µg L^{-1} for CTCSMZ and from 43 to 17 µg L^{-1} for CTC) and sulfamethazine (from 3.9 to 2.6 µg L^{-1}) in runoff compared with surface application. However, there was no significant effect of manure application method on tylosin concentration (range 0.02 to 0.06 µg L^{-1}) in runoff. Mass loss as a percent of the amount applied for chlortetracycline and sulfamethazine appeared to be independent of their respective soil sorption coefficients. Mass losses of chlortetracycline were significantly reduced with soil-incorporation of manure (from 6.5 to 1.7% when applied with sulfamethazine and from 6.5 to 3.5% when applied alone). Mass losses of sulfamethazine (4.8%) and tylosin (0.24%) in runoff were not affected by manure incorporation. While our results confirm that cattle excreted antimicrobials can be removed via surface runoff after field application, the magnitudes of chlortetracycline and sulfamethazine losses were reduced by soil incorporation of manure immediately after application.

2.2. Introduction

Antimicrobials are widely used by the livestock industry in North America at therapeutic levels for treatment of infections and at sub-therapeutic levels to prevent disease and improve feed efficiency (Sarmah et al., 2006). While publicly available data on antimicrobial use in livestock are scarce, recent estimates indicate that between ~11 and 16 million kg are administered annually in the United States (Kim et al., 2011; Sarmah et al., 2006).

To be approved for use in livestock, antimicrobials must not accumulate but rapidly dissipate from edible tissues (Thiele-Bruhn, 2003). Excretion rates in feces and urine can be up to 95% or more and vary depending on the antimicrobial, the dose administered, and the species and age of the animal (Arikan et al., 2007; Chee-Sanford et al., 2009; Sarmah et al., 2006). Excretion rates for three antimicrobials commonly administered to cattle have been reported to be 65–75% for chlortetracycline, 90% for sulfamethazine, and 50–100% for tylosin (Kim et al., 2011). In addition, for those antimicrobials metabolized in the animal, metabolites that possess antimicrobial activity can also be excreted (Sarmah et al., 2006). Hence, land-application of manure as a means of recycling nutrients is a major mode of transfer of antimicrobials to the environment, which may contaminate surface water by runoff following rain or snowmelt (Boxall et al., 2004; Dolliver and Gupta, 2008; Sarmah et al., 2006).

The occurrence of antimicrobials in surface water has been reported in Canada (Forrest et al., 2011; Lissemore et al., 2006; Waiser et al., 2011), the United States (Kolpin et al., 2002; Yang and Carlson, 2003), Europe (Calamari et al., 2003; Christian et al., 2003; Hamscher et al., 2005), and Asia (Managaki et al., 2007). There is a growing concern that the introduction of antimicrobials residues and antimicrobial resistant genes into the environment could select for antimicrobial resistant bacteria, including pathogens. As a consequence, antimicrobials currently

in use may become less effective at treating bacterial infections in both humans and animals (Chee-Sanford et al., 2009; Sarmah et al., 2006). Contamination of drinking water with antimicrobial resistant genes may select for antimicrobial resistance in pathogenic bacteria in humans and livestock due to chronic exposure (Pruden et al., 2006; Xi et al., 2009).

The extent and processes of antimicrobial loss from soil surfaces via rain runoff are poorly understood. To date, studies of antimicrobial transport in surface runoff following field application have generally fallen into three broad categories, namely, those using (i) antimicrobials administered to animals and excreted in manure (Burkhardt et al., 2004; Dolliver and Gupta, 2008; Hoese et al., 2009); (ii) antimicrobials added to excreted manure and therefore not subjected to microbial conditions in the rumen (Blackwell et al., 2007; Burkhardt et al., 2005); and (iii) antimicrobials mixed with methanol/water and sprayed directly onto soil in the absence of manure (Davis et al., 2006; Kim et al., 2010). Results from the latter two categories may not fully represent the transport behaviour of antimicrobials that are administered to and excreted by animals.

The majority of studies quantifying antimicrobial transport in surface runoff have used liquid swine manure (Blackwell et al., 2007; Burkhardt et al., 2004; Burkhardt et al., 2005; Hoese et al., 2009). Fewer studies have focussed on cattle manure despite the widespread land application of non-processed (fresh) and processed (e.g., stockpiled, composted) manure from animals receiving therapeutic and sub-therapeutic doses of antimicrobials. Dolliver and Gupta (2008) quantified the rainfall and snowmelt runoff of antimicrobials following the fall application of solid beef cattle manure collected from cattle receiving antimicrobials in their feed. Maximum concentrations measured in rainfall and snowmelt runoff samples were 57.5 µg L^{-1} for monensin, 1.9 µg L^{-1} for tylosin, and below detection for chlortetracycline. The total loss

of each antimicrobial was <5% of the amount applied, with the majority of losses (99%) occurring during the non-growing season.

The province of Alberta accounts for 40% of Canada's cattle population, with a yearly feedlot cattle population of ~13.5×10^6 (Statistics Canada, 2012). Feedlot manure may contain chlortetracycline, sulfamethazine and tylosin, with the majority of feedlot cattle being administered at least one type of these antimicrobials in the feed. In western Canada, manure from the feedlot pens is removed biannually or annually and immediately land-applied (early spring or late fall) or composted (Cessna et al., 2011). Best management practices in Alberta recommend the incorporation of manure applied to cultivated land within 48 h of application (Alberta Agriculture and Food, 2007). Surface application without incorporation is permitted when manure is applied to forages or direct-seeded (no-till or zero till) crops. To date, there has been limited research under Canadian prairie conditions on the loss of antimicrobials in surface runoff from soils amended with solid manure collected from animals receiving antimicrobials in their diets. Therefore, the objectives of the present study were to (i) quantify and compare simulated rain runoff losses of chlortetracycline, sulfamethazine, and tylosin, and (ii) compare antimicrobials losses between surface application and soil incorporation of manure.

2.3. Materials and Methods

2.3.1. Study Site

The field study was conducted in the fall of 2010 at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Alberta (49°42′ N, 112°50′ W), on a site with no previous history of manure application. The soil was an Orthic Dark Brown Chernozem (Typic Haploboroll) with clay-loam texture, an organic carbon content of ~15 g kg^{-1} , a pH of 7.5 (0-15 cm depth) and a

30-yr (1981-2010) mean annual precipitation of 399 mm. The site had been cropped to spring wheat (*Triticum aestivum* L.) in the preceding growing season the same year. Wheat stubble was mowed to ground level and raked off the plot area to facilitate uniform spreading of manure. The slope at the site ranged from 0.02 to 0.05%.

2.3.2. Antimicrobial Treatments

Antimicrobials were administered to beef cattle in four pens (9 steers per pen) at the Research Centre feedlot between June and October 2010. Each pen was assigned as follows: (i) chlortetracycline (44 mg kg^{-1} feed, treatment denoted CTC) as Aureomycin-100 G (Alpharma Inc., Bridgewater, NJ, USA), (ii) chlortetracycline and sulfamethazine (each at 44 mg kg^{-1} feed; treatment denoted CTCSMZ) as Aureo S-700 G (Alpharma Inc., Bridgewater, NJ, USA), (iii) tylosin (11 mg kg^{-1} feed; treatment denoted TYL) as Tylan (Elanco Animal Health, Calgary, AB, Canada), and (iv) control (no antimicrobials). The antimicrobials were administered according to recommended practice (Canadian Animal Health Institute, 2011) in a barley (*Hordeum vulgare* L.) grain–barley silage diet typical of that used in feedlots in western Canada. Cattle were bedded as required with equal amounts of barley straw being added to all pens to ensure similar manure:bedding ratios (~4:1) across antimicrobials treatments.

2.3.3. Manure Application

The study design was a randomized complete block with a split plot treatment structure and three replications. Manure application method (that is, surface application or incorporation) was the main plot while antimicrobial treatments (that is, control, CTC, CTCSMZ, or TYL) were considered subplots. Individual subplots were 3 × 3 m.

Manure was separately collected from each treatment pen and mixed (1 min) in batches in a 0.34-m^3 capacity mortar mixer (Model No. 12SGH9, Crown Construction Equipment, Winnipeg, MB, Canada) to improve uniformity. Manure (~54 kg wet wt.) was applied to each plot using 80-L plastic bins to achieve an application rate of 60 Mg ha^{-1} (wet wt.), typical for irrigated crops in southern Alberta (Alberta Agriculture and Forestry, 2016). Garden rakes were used to break large lumps to allow even spreading across the plot area. For the incorporation application, manure was immediately worked into the 10-cm soil depth with a single pass of a disk harrow. For each treatment, three manure samples (one for each replicate) were collected prior to field application to estimate the concentration of antimicrobials in manure. Manure samples were freeze-dried (-40ºC), weighed, and stored at -40ºC until analysis.

2.3.4. Rainfall Simulation

Simulated rain was applied within 1-2 h of manure application to a randomly selected 1 × 1-m area in each plot using a portable Guelph Rainfall Simulator II (Tossell et al., 1987) set at 100 kPa pressure. The rain was applied from a height of 0.8 m at an intensity of 105 mm h^{-1}, which represented return periods of 18 to >50 yr for the Lethbridge area (Miller et al., 2006). The boundary of each 1-m^2 test area was delineated by a stainless steel frame that was hammered into the soil to a depth of ~5 cm. Simulations continued until 13 L of runoff were collected. Simulation duration ranged from 15 to 25 min, depending on individual plot surface conditions. Runoff water was channelled out of the test area via a stainless steel flume into amber glass sample bottles. Seven consecutive runoff samples (numbered 1 through 7) were collected after consistent runoff was established, with a volume of 1 L apiece for samples 1, 3, 5, and 7 and 3 L apiece for samples 2, 4, and 6 (that is, a total volume of 13 L per plot). The simulator was then turned off and the residual runoff volume from the test area collected. Based on relative volumes,

one composite 1-L sample per plot was created from the three 3-L samples and from residual volumes collected after simulations ended. Samples were stored at 4ºC for approximately 1 wk after which they were subjected to solid-phase extraction.

Analysis of the four, 1-L samples (that is, samples 1, 3, 5 and 7, which correspond to the first, fifth, nineth and thirteenth litres of runoff) from three replicate plots of a same treatment allowed the evaluation of changes in antimicrobial concentrations with time during runoff events. Total antimicrobial losses, on a mass basis, from each plot during runoff events were similarly determined from antimicrobial concentrations in the four 1-L samples (that is, samples 1, 3, 5, and 7) and in the 1-L composite sample from samples 2, 4, and 6 plus the residual runoff collected after the simulation.

2.3.5. Sample Extraction and Cleanup

2.3.5.1. Manure Samples

Antimicrobials were extracted from freeze-dried manure by pressurized liquid extraction (PLE) followed by solid-phase extraction (SPE), as previously described by Cessna et al. (2011). Briefly, freeze-dried manure subsamples (2 g) were mixed with Ottawa sand (~20 g; Fischer Scientific, Ottawa, ON, Canada) and placed into a 33-mL stainless steel PLE cell in which two glass fiber filters had been placed at the outlet. The packed cell was subjected to PLE (ASE 200, Dionex Canada Ltd., Oakville, ON, Canada) using a citric acid buffer solution (pH = 5.0) for tylosin and sulfamethazine and an 80/20 methanol:citric acid buffer (pH = 5.0) solution for chlortetracycline. Extractions were performed at 75ºC and 10.3 MPa, with a heat-up time of 5 min and a static time of 2 min. The cell was flushed with the respective extraction solvent at 60% of cell dead volume and purged for 90 s with nitrogen gas (1.03 MPa). The extract (~60 mL per

subsample from 2 extraction cycles) was diluted to 500 mL with Milli-Q water prior to SPE cleanup as described below for runoff samples.

2.3.5.2. Runoff Samples

Runoff samples were filtered through a Whatman No. 1 filter paper to remove suspended solid particles, followed by addition of citric acid buffer (~60 mL) to adjust sample pH to 5.0 prior to SPE. Samples (500 mL) were subjected to SPE using an Oasis weak cation exchange (WCX) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA, USA) stacked on top of an Oasis hydrophilic-lipophilic balance (HLB) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA, USA), both conditioned with 10 mL methanol followed by 10 mL deionized (Milli-Q) water. Samples were passed through the cartridge assembly under vacuum at a flow rate of 1 mL min^{-1}. Cartridges were rinsed with deionized water (10 mL) to remove excess salts, allowed to dry for a minute under vacuum, and maintained at -20ºC until they were transferred, 24 h prior to elution (see below), to a refrigerator set at 4ºC.

After separation of the cartridges, the HLB cartridge was eluted with 10 mL of a dichloromethane/acetone mixture (3:2, v/v) while the WCX cartridge was eluted with 10 mL methanol and then with 10 mL methanol containing 2% formic acid. The three eluates were collected into separate 10-mL Pyrex test tubes and concentrated to a volume of 1–2 mL under a gentle stream of air. The tubes were then transferred to a 30ºC water bath and the eluates were concentrated to ~50 µL under a gentle stream of nitrogen gas. Evaporation of the eluates to dryness reduced the recovery of antimicrobials and therefore the eluates were concentrated to ~50 µL. The extract residue in each test tube was taken to a final volume of 1 mL by adding deionized water and vortexing. The resulting cloudy extracts were filtered through 0.45-µm syringe filters (Waters, Milford, MA, USA), transferred into amber liquid chromatography (LC) vials, and maintained at -15ºC until analysis.

2.3.6. Sample Analysis

Sample extracts were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) using a Waters 2965 HPLC system interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA).

Freeze-dried, antimicrobial-free (control) beef cattle manure (1 g) was spiked with 50, 100, 250 and 500 ng of *iso*-chlortetracycline, sulfamethazine and tylosin, resulting in concentrations of 50, 100, 250, and 500 μg kg^{-1}. Stock solutions (100 μg L^{-1}) used for spiking were prepared in acetonitrile and subsequently diluted to 1 mg L^{-1} using mili-Q water. Recoveries were 85 ± 13% for *iso*-chlortetracycline, 102 ± 16% for sulfamethazine and 106 ± 8% for tylosin. Background corrections for antimicrobial concentrations in manure were 246 ± 99 μg kg^{-1} for *iso*-chlortetracycline, 50 ± 23 μg kg^{-1} for sulfamethazine, and 42 ± 23 μg kg^{-1} for tylosin.

Triplicate subsamples of runoff water samples collected from control plots were spiked with 100 and 500 ng of *iso*-chlortetracycline, sulfamethazine and tylosin per 500 mL, resulting in concentrations of 100 and 500 μg L^{-1} in the final 1 mL extract analyzed (or 0.2 and 1 μg L^{-1} in runoff water). Recoveries were 161 ± 70% for *iso*-chlortetracycline, 121 ± 11% for sulfamethazine and 90 ± 27% for tylosin. Background corrections for antimicrobial concentrations in runoff samples were 0.12 ± 0.03 μg L^{-1} for *iso*-chlortetracycline, 0.05 ± 0.02 μg L^{-1} for sulfamethazine and 0.05 ± 0.02 μg L^{-1} for tylosin.

2.3.6.1. Liquid Chromatography

Analyte separation was achieved using a 100-mm × 2.1-mm i.d. Waters MS Xterra C-18 stainless steel column (3.5 μm diam. packing; Waters Corp., Milford, MA, USA). Two mobile phases consisting of acetonitrile and water [10:90 (v/v) for mobile phase A and 90:10 (v/v) for mobile phase B] and each containing 0.1% formic acid were delivered at a flow rate of 200 μL

min^{-1}. The gradient elution employed 90% of phase A and 10% of phase B during the first minute followed by 100% of phase B for 9 min. At 10 min, reconditioning of the column for the next injection (at 15.0 min) was begun by switching back to 90% mobile phase A and 10% mobile phase B. Injection volumes were 20 µL. Under the chromatographic conditions described above, the retention times were 6.83 min for tylosin, 6.92 min for *iso*-chlortetracycline, 7.11 min for sulfamethazine, and 7.11 min for $^{13}C_6$-sulfamethazine, internal standard.

2.3.6.2. Mass Spectrometry

The mass spectrometer was equipped with an electrospray ionization interface set to positive ion mode. Ionization and MS-MS conditions for each compound were optimized by infusing a 0.5 mg L^{-1} solution of each antimicrobial into the ion source in a 50:50 acetonitrile: water (v/v) solution with a syringe pump at a flow rate of 10 µL min^{-1}. The parent ion for each antimicrobial (M+H) was selected for collision induced dissociation using the first quadrupole. The second quadrupole, into which argon gas was introduced, functioned as a collision cell and the third quadrupole was used to monitor the resulting major product ions.

Suitable multiple reaction monitoring (MRM) transitions were chosen from the product ion scans. Two transitions were used for chlortetracycline and sulfamethazine and three transitions for tylosin. The sum of two MRM transitions was as follows: *iso*-chlortetracycline: m/z 478.9–443.9 and m/z 478.9–461.9; sulfamethazine: m/z 279.2–155.7 and m/z 279.2–185.7; $^{13}C_6$-sulfamethazine: m/z 285.0–161.1 and m/z 285.0–186.0; and tylosin: m/z 916.3–100.1, m/z 916.3–174.0, and m/z 917.8–772.5.

Optimized instrumental settings were as follows: capillary voltage, 3.5 kV; hexapole 1 voltage, 2 V; hexapole 2 voltage and aperture voltage, 0 V; RF lens voltage, 0.5 V; source temperature, 90°C; desolvation temperature, 220°C; and multiplier voltage, 700 V. Nitrogen was used as the nebulizing, desolvation, and cone gas: nebulizer gas flow rate set to maximum flow;

desolvation gas flow rate, 487 L h^{-1}; and cone gas flow rate, 153 L h^{-1}. The inter channel delay was 0.10 s whereas dwell time, cone voltage, and collision energy were dependent on the MRM channel. Argon was used as collision gas at a pressure which increased the Pirani gauge reading to 2.83×10^{-3} mbar. Resolution was set to achieve unit mass resolution for quadrupole 1 and approximately 2 atomic mass units resolution for quadrupole 3.

2.3.7. Statistical Analysis

Data for antimicrobial concentrations in runoff (based on the four 1-L samples collected for each antimicrobial treatment) were analyzed separately for each antimicrobial using the mixed procedure (PROC MIXED) for repeated measures in SAS (SAS Institute, 2012), with cumulative volume as the repeated measures effect. Based on the Akaike Information Criterion (AIC) (Littell et al., 2006), the covariance structures used in the final mixed models were first-order heterogeneous autoregressive [ARH (1)] for chlortetracycline and first-order autoregressive [AR (1)] for sulfamethazine and tylosin.

Data for the maximum concentration of each antimicrobial in runoff were analyzed using PROC MIXED in SAS (SAS Institute, 2012). Maximum concentration of antimicrobial for each replicate was selected from the runoff samples 1, 3, 5, and 7 collected during each rainfall simulation event respective to the replicate. Antimicrobial mass loss data (product of runoff volume and antimicrobial concentration in the 1-L composite sample and the four 1-L samples listed above) were similarly analyzed using PROC MIXED following normalization to percentage loss based on the amount applied per 1-m^2 test area.

The Tukey multiple comparison procedure was used for all pairwise comparisons. Effects were considered significant if $P < 0.1$ for all comparisons.

2.4. Results

2.4.1. Antimicrobial Concentrations in Manure

Concentrations of chlortetracycline, sulfamethazine, and tylosin in manure are presented in Table 2.1, along with application rates of manure and the antimicrobials. Chlortetracycline concentration was, on average, 1.3 times greater in manure from cattle administered chlortetracycline mixed with sulfamethazine (CTCSMZ) compared to that from cattle administered chlortetracycline alone (CTC; 3954 vs. 3130 µg kg^{-1}) even though the two formulations supplied the same dietary dose of chlortetracycline. Sulfamethazine was applied at the same rate as chlortetracycline, yet its concentration in manure was 8% of chlortetracycline concentration in CTCSMZ and 11% of the chlortetracycline concentration of manure from cattle administered chlortetracycline alone.

Table 2.1: Manure antimicrobial concentrations and application rates for different antimicrobial formulations.

Antimicrobial	Formulation	Manure application	Manure water content	Manure rate (dry wt.)	Antimicrobial conc. in manure†(dry wt. basis)	Antimicrobial rate
			$g\ g^{-1}$	$kg\ m^{-2}$	$\mu g\ kg^{-1}$	$mg\ m^{-2}$
Chlortetracycline	CTC	Surface	0.55	2.7	3193	8.5
		Incorporated	0.63	2.2	3068	6.9
	CTCSMZ‡	Surface	0.52	2.9	4841	14.0
		Incorporated	0.58	2.6	4918	12.5
Sulfamethazine	CTCSMZ	Surface	0.52	2.9	301	0.9
		Incorporated	0.58	2.6	359	0.9
Tylosin	TYL	Surface	0.60	2.4	108	0.3
		Incorporated	0.65	2.1	181	0.4

† Concentrations and rates are arithmetic means of three replicates.

‡ Treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine.

2.4.2. Antimicrobial Concentrations in Runoff

2.4.2.1. Mean Antimicrobial Concentrations

Mean chlortetracycline and sulfamethazine concentrations in runoff (across all four sampling times corresponding to the first, fifth, ninth, and thirteenth liter of runoff) were significantly greater when manure was surface-applied compared with manure incorporation (Table 2.2). For chlortetracycline applied alone, the mean concentration in runoff from the surface-applied manure was 2.6 times higher than that from incorporated manure. For sulfamethazine, the concentration was 50% higher in runoff from surface-application compared with incorporation. A significant interaction between application method and runoff volume was observed for chlortetracycline from the CTCSMZ treatment and for tylosin. When manure was surface-applied, chlortetracycline concentration increased as a quadratic function of cumulative runoff volume (Fig. 2.1). By comparison, when manure was incorporated, chlortetracycline concentration did not vary significantly with cumulative runoff volume. For tylosin, runoff concentrations after cumulative volumes of 1 and 5 L were higher when manure was incorporated compared to surface application (Fig. 2.2) but were similar in the latter stages of runoff. Runoff concentrations of chlortetracycline when it was administered alone and sulfamethazine did not vary significantly with volume or time (Table 2.2).

Table 2.2: Least square mean antimicrobial concentration in runoff as affected by manure application method and runoff volume collected.

Treatment	Chlortetracycline from CTC†	Chlortetracycline from CTCSMZ‡	Sulfamethazine	Tylosin
		µg L^{-1}		
Application method				
Surface	43.3a§	75.3	3.9a	0.02
Incorporated	16.9b	12.3	2.6b	0.06
Volume (L)				
1	31.3	39.1	3.1	0.03
5	34.9	42.9	3.7	0.04
9	29.1	46.0	3.3	0.02
13	24.9	47.2	2.9	0.06
		P value		
Application method	0.07	0.0001	0.03	0.005
Volume	0.13	0.02	0.16	0.11
Application method × Volume	0.92	0.03	0.45	0.03

† Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.1$).

‡ Treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine.

§ Means in the same column followed by the same letter are not significantly different according to the Tukey-Kramer test ($P < 0.1$).

Mean separation letters are applied to the main effects only in the absence of a significant interaction. Significant interactions are plotted in Fig. 2.1 (chlortetracycline from CTCSMZ) and Fig. 2.2 (tylosin).

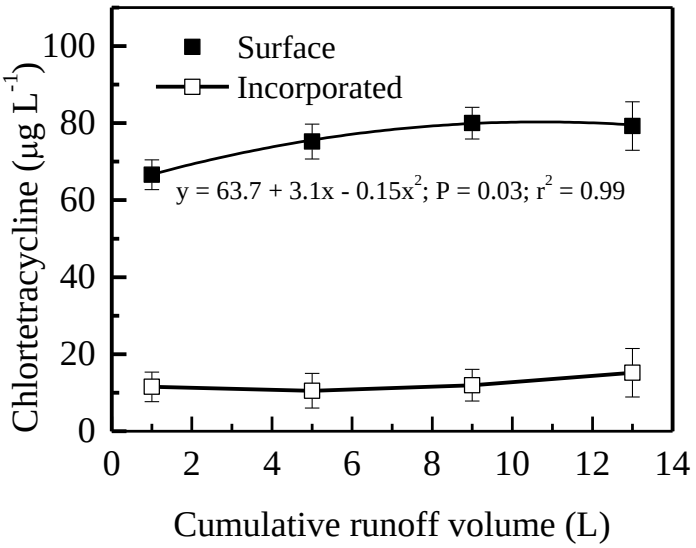


Figure 2.1: Change in concentration of chlortetracycline from the treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine treatment in runoff from surface-applied vs. incorporated feedlot cattle manure. Vertical bars represent SE.

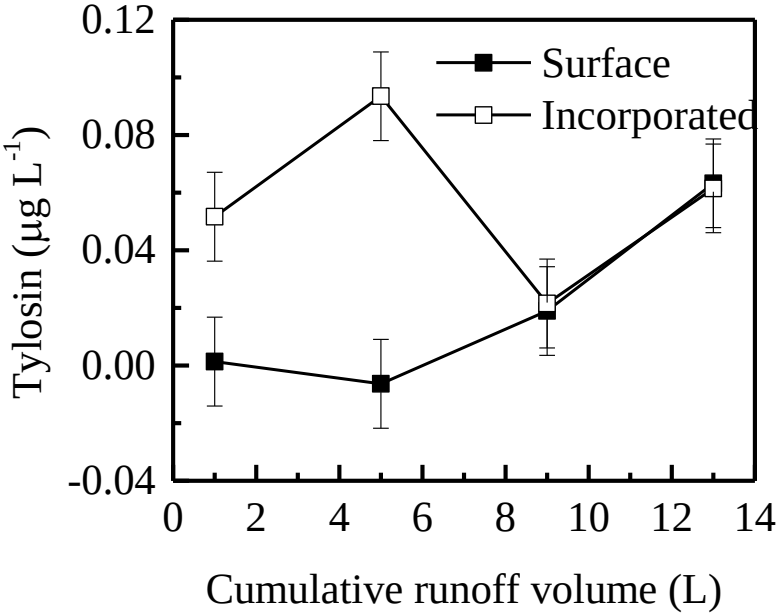


Figure 2.2: Change in dissolved tylosin concentration with runoff volume as affected by manure application method. Vertical bars represent SE.

2.4.2.2. Maximum Antimicrobial Concentrations

Maximum chlortetracycline concentration in runoff from plots receiving manure from CTCSMZ treated cattle was greater ($P < 0.0001$) when manure was surface-applied (83.2 µg L^{-1}) than when it was incorporated (17.8 µg L^{-1}). Similarly, maximum chlortetracycline concentration in runoff from surface-applied manure from cattle administered chlortetracycline (48.6 µg L^{-1}) was greater ($P = 0.003$) than that from incorporated manure (22.2 µg L^{-1}). Maximum concentrations in runoff from surface-applied manure were 4.70 µg L^{-1} for sulfamethazine and 0.06 µg L^{-1} for tylosin while that from incorporated manure were 3.03 µg L^{-1} for sulfamethazine and 0.09 µg L^{-1} for tylosin.

2.4.3. Mass Loss of Antimicrobials in Runoff

2.4.3.1. Effect of Antimicrobial

There was a significant interaction between antimicrobial treatment and manure application method for antimicrobial mass losses in runoff (Table 2.3). When manure was surface-applied (Fig. 2.3), mass losses of chlortetracycline (whether administered alone or with sulfamethazine) and sulfamethazine were significantly greater than those of tylosin. By comparison, when manure was incorporated, mass losses of chlortetracycline administered alone and sulfamethazine were significantly greater than tylosin while there was no significant difference between chlortetracycline from the CTCSMZ treatment and tylosin. Regardless of manure application method, mass losses of chlortetracycline and sulfamethazine did not differ significantly.

Table 2.3: Least square mean antimicrobial mass loss as affected by antimicrobial formulation and manure application method.

Treatment	Total loss	
	μg m^{-2}	% of added
Antimicrobial		
Chlortetracycline from CTC	590	5.42
Chlortetracycline from CTCSMZ	431	4.31
Sulfamethazine	43	4.84
tylosin	0.62	0.24
Application method		
Surface	399	4.59
Incorporated	105	2.28
		P value
Antimicrobial	-	<0.001
Application method	-	<0.001
Antimicrobial × Application method	-	0.004

† Mean separation letters are not applied because the interaction is significant. The significant interaction is plotted in Fig. 2.3. ‡ CTC, chlortetracycline treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine

2.4.3.2. Effect of Manure Application Method

Compared with surface application, incorporation of manure into the 0- to 10-cm soil layer significantly reduced chlortetracycline mass losses in runoff from 6.5% to 3.5% of applied for chlortetracycline applied alone and from 6.5% to 1.7% of applied for CTCSMZ (Fig. 2.3). Runoff losses of sulfamethazine and tylosin were not significantly affected by manure application method. Within each manure application method, there was no significant difference in chlortetracycline loss between the CTC and CTCSMZ treatments.

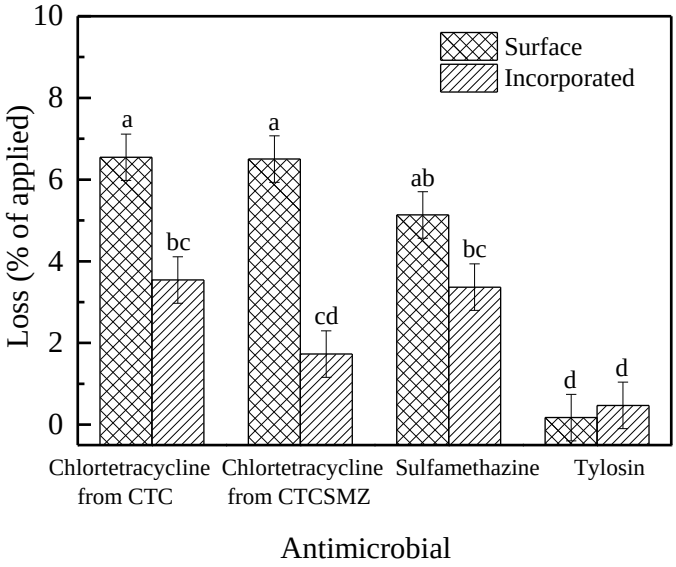


Figure 2.3: Effects of antimicrobial formulation (administered to animals) and method of manure application on runoff losses of dissolved antimicrobials. Error bars represent SE. CTC, chlortetracycline treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine.

2.5. Discussion

2.5.1. Antimicrobial Concentrations in Manure

Reasons for the high concentration of chlortetracycline in manure from CTCSMZ compared to chlortetracycline alone, despite the equal rates added to cattle feed, are not clear. However, it is possible that sulfamethazine, which was co-administered with chlortetracycline in the CTCSMZ formulation, may have altered the microbial metabolism of chlortetracycline within the intestines, resulting in a greater proportion of chlortetracycline being excreted compared to the administration of chlortetracycline alone. Additionally, after excretion of chlortetracycline, its degradation may have been suppressed by the presence of sulfamethazine, resulting in a higher concentration of chlortetracycline in manure from the CTCSMZ treatment. Cessna et al. (2011) also reported higher (although non-significant) mean chlortetracycline

concentrations (1.6 times) in manure from cattle administered CTCSMZ compared to manure from cattle receiving CTC.

The sulfamethazine concentration in manure was only 7–11% of the concentration of chlortetracycline, despite the two antimicrobials being administered to animals at the same concentration in the feed. This is consistent with observations by Cessna et al. (2011) and contradicts earlier results that indicated greater excretion rates of sulfamethazine (90% of amount administered) compared with chlortetracycline (65– 75%) (Kim et al., 2011). In the present study, cattle were in feedlot pens for 4 months (June to October) and manure was allowed to accumulate on pen floors during that period. Consequently, the manure and the excreted antimicrobials were exposed to high summer air temperatures and recurring wetting-drying cycles due to rainfall followed by wind desiccation. Thus, the lower concentration of sulfamethazine may be related to the greater persistence of chlortetracycline compared with sulfamethazine under such environmental conditions. In addition, the sorption of chlortetracycline to manure is greater than that of sulfamethazine, which is consistent with the greater water solubility of sulfamethazine (Chee-Sanford et al., 2009; Sarmah et al., 2006). Although the higher mobility of sulfamethazine due to its lower sorption coefficient (K_d value) and higher water solubility (Chee-Sanford et al., 2009) can potentially result in high runoff losses, in the present study there was not enough rainfall to cause measurable runoff losses of the antimicrobial from the pens. Also, the pens were partially roofed to protect the manure pack from direct rainfall to further reduce the potential of considerable antimicrobial runoff from manure pack. Moreover, the half-lives of tetracyclines in manure average ~100 d (Chee-Sanford et al., 2009), compared with ~30 d for sulfonamides (Boxall et al., 2004). A greater proportion of sulfamethazine was likely degraded during the period between excretion and land application of

the manure. The main metabolites of sulfamethazine are the acetylated conjugates. However, these metabolites were not quantified in this study. Nonetheless, reported concentrations of acetylated metabolites of sulfamethazine in manure, soil and similar matrices are often much smaller than those of sulfamethazine (Haller et al., 2002; Garcia-Galan et al., 2013). Acetylated metabolites of sulfamethazine can be cleaved back to the parent compound (Lertpaitoonpan, 2008).

Forster et al. (2009) reported the presence of a residual fraction of sulfadiazine in soil mixed with pig manure which they extracted with acetonitrile/water at 150°C for 15 min using a microwave extraction system (Forster et al., 2008). Part of the sulfamethazine in manure in this study may also have formed a residual fraction during the four months period (June to October) in which sulfamethazine was in contact with manure. Resistance of the residual fraction to extraction may have lowered the recovery of sulfamethazine in this study, resulting in the lower sulfamethazine concentration compared to chlortetracycline. However, the extraction procedure used in our study was similar to the method used by Forster et al. (2009) to extract the residual fraction and therefore at least part of any residual SMZ formed in manure in this study would have been accounted for.

Tylosin was present in the lowest concentration in the manure applied to the runoff plots, in part because it was administered to the cattle at 25% of the dose of chlortetracycline and sulfamethazine. The concentration of tylosin in the manure was approximately 3–6% of that of chlortetracycline from CTC. The low concentrations of tylosin in the manure may reflect relatively greater metabolism of this antimicrobial within the digestive tract or greater degradation in the manure pack in the feedlot pen (De Liguoro et al., 2003).

2.5.2. Antimicrobial Concentrations in Runoff

Runoff studies involving pesticides have shown that only pesticides present within the runoff-soil interaction zone (0- to 1-cm soil layer) are susceptible to transport in surface runoff (Ahuja et al., 1981; Spencer and Cliath, 1991; Wauchope, 1978). Generally, pesticide concentrations in surface runoff decrease as runoff proceeds due to the depletion of pesticides within the runoff-soil interaction zone and leaching below this zone (Ahuja et al., 1981). This trend of decreasing concentrations during surface runoff events has also been observed with antimicrobials (including chlortetracycline, sulfamethazine and tylosin) sprayed directly onto soil (Davis et al., 2006; Kim et al., 2010), ostensibly due to the same processes. In the present study, this pattern was not consistently evident for any of the three antimicrobials, whether the manure containing the antimicrobials was surface-applied or soil-incorporated. Rather, with the exception of chlortetracycline concentrations from the surface-applied CTCSMZ treatment (Fig. 2.1) and tylosin concentrations from incorporated TYL treatment, antimicrobial concentrations tended to remain constant and not vary significantly with runoff volume. These patterns of runoff concentrations may reflect the fact that the antimicrobials, at least within the time frame of the current study, were desorbed/leached from the manure at a relatively constant rate, whether the manure was surface-applied or soil-incorporated.

Mean chlortetracycline and sulfamethazine concentrations in runoff generally reflected their respective rates of application; however, the relationship was not clearly shown when the manure was soil-incorporated, most likely because part of the manure and antimicrobials were placed below the runoff-soil interaction zone during incorporation, thus limiting the availability of antimicrobials for runoff loss. Soil incorporation of manure significantly reduced chlortetracycline and sulfamethazine concentrations in runoff (Table 2.2), because, as mentioned

earlier, the soil incorporation placed part of the manure and antimicrobials below the runoff-soil interaction zone.

Dolliver and Gupta (2008) reported a higher maximum concentration of tylosin (1.9 µg L^{-1}), compared to the present study, and in rainfall and snowmelt runoff from agricultural land amended with cattle manure in the fall. In that study, chlortetracycline was not detected in runoff, most likely because of a lower rate of application (0.6 to 2.1 mg m^{-2}) compared to that in the present study, whereas tylosin was applied at a somewhat higher rate (0.37 to 0.57 mg m^{-2}).

Using simulated rainfall runoff studies in which antimicrobials were applied directly to soil, Davis et al. (2006) and Kim et al. (2010) demonstrate the importance of antimicrobial sorption to soil in determining the extent to which the antimicrobials are transported in runoff. The application rate (~35 mg m^{-2}) used in these studies was higher than those used in the present study (Table 1). However, the mean concentrations in the simulated rainfall runoff reported by Davis et al. (2006) and Kim et al. (2010) were substantially lower for chlortetracycline (0.04–0.09 µg L^{-1}) than those in the present study (Table 2.2) and comparable in magnitude for sulfamethazine (0.6–3.45 µg L^{-1}) and for tylosin (0.09–0.17 µg L^{-1}). Further, in the studies by Davis et al. (2006) and Kim et al. (2010) the antimicrobial concentrations in simulated rainfall runoff reflected their relative soil adsorption coefficients rather than the application rates and decreased with increasing soil adsorption coefficient in the order: sulfamethazine (0.6–3.1 L kg^{-1}; high to medium mobility) > tylosin (8–240 L kg^{-1}; low mobility to immobile) > chlortetracycline (282–2608 L kg^{-1}; classified as immobile) (Chee-Sanford et al., 2009). In contrast, when antimicrobials are applied with manure, as in the present study, it appears they may be more readily desorbed/leached from the manure into runoff and, once in solution in the runoff, the role played by sorption to soil appears to be considerably less.

2.5.3. Mass Loss of Antimicrobials

Results from the present study are consistent with those reported by Dolliver and Gupta (2008), which showed runoff losses of up to 5% of the total mass of chlortetracycline and tylosin applied with swine and beef cattle manures under chisel plow and no-till systems. Similar results have been reported when agricultural land was amended with swine manure (Hoese et al., 2009).

The role of soil incorporation of manure in reducing mass losses of chlortetracycline in runoff in this study is in general agreement with the results from Dolliver and Gupta (2008) who reported lower losses of tylosin and monensin from soil-incorporated (chisel plow tillage) manure compared with surface-applied (no-till) manure. However, the difference was significant only in one of the 3 yr. In our study, antimicrobial transport in surface runoff decreased when manure was soil-incorporated, most likely because the manure was placed below the runoff-soil interaction zone, thus rendering antimicrobial present in that component of the manure relatively protected and unavailable for transport in the runoff water. The lack of a significant application method effect on tylosin loss in the present study may be related to the low concentrations of tylosin detected in runoff relative to the variability in the concentrations.

The relative mass losses of the three antimicrobials examined in this study were inconsistent with reported relative sorptivities of the antimicrobials in soil as observed with antimicrobial concentrations in runoff. However, as noted earlier, runoff losses of sulfamethazine and tylosin were greater than that of chlortetracycline when antimicrobials were directly sprayed onto soil (Davis et al. 2006; Kim et al. 2010). Also, antimicrobial mass loss following swine manure application has been shown to decrease with increasing affinity to soil particle surfaces (Kay et al., 2005), with mass losses of 0.42% for sulfachloropyradazine, 0.07% for oxytetracycline, and negligible for tylosin. Perhaps in the present study, the antimicrobials

would have to desorb from solid cattle manure prior to transport in surface runoff; thus, runoff losses may not follow the order expected based on the soil/water partition coefficients of the antimicrobials.

As with antimicrobial concentrations, mass losses of the antimicrobials in our study were higher (Table 2.3) than those reported by Davis et al. (2006) and Kim et al. (2010) (0.004 to 0.06%) who sprayed the same antimicrobials directly onto soil. The higher losses in our study from surface-applied manure are most likely explained by much weaker sorption of these antimicrobials to cattle manure compared to soil and by the magnitude of their relative soil partition coefficients.

Leaching of antimicrobials was not monitored in this study. Leaching of antimicrobials is a possibility and the extent of antimicrobial leaching may increase with soil incorporation. A study by Aust et al. (2008) examined the leaching potential of chlortetracycline, sulfamethazine, and tylosin immediately below the same feedlot pens from which manure was sourced for the present study. Sulfamethazine was detected at concentrations of up to 72 μg kg^{-1} in random samples from the 0- to 10-cm soil layer, while chlortetracycline was detected at concentrations of up to 52 μg kg^{-1} in the 10- to 40-cm soil layer. Tylosin, on the other hand, was not detected in the soil. By comparison, Dolliver and Gupta (2008) measured up to 1.2 μg tylosin L^{-1} but no chlortetracycline in leachate from the 60-cm soil depth following field application of cattle manure containing excreted chlortetracycline and tylosin. These contrasting results indicate that leaching of antimicrobials after field application is a complex process and depends on many factors such as type and form of antimicrobial; soil physical, chemical, and biological properties; precipitation; and farm management practices.

This study confirms that antimicrobials can be lost in surface runoff if a high-intensity rainfall event occurs shortly after field-application of manure containing these antimicrobials. The movement of antimicrobials, along with other contaminants, such as plant nutrients (nitrogen and phosphorus), can adversely affect the quality of receiving waters. Freshwater aquatic life may be compromised (Halling-Sorensen, 2001; Kummerer, 2003; Sarmah et al., 2006) or the receiving waters may be rendered unfit for human or animal consumption (Pruden et al., 2006; Xi et al., 2009). Further, the residual quantities of dispersed antimicrobials in the environment may promote the development and spread of antimicrobial resistance, especially among pathogenic bacteria (Chee-Sanford et al., 2001; Chee-Sanford et al., 2009; Kemper, 2008). Antimicrobials used in the livestock industry have been recently detected in several tributaries to the Grand River in Ontario (Lissemore et al., 2006), in streams in Alberta (Forrest et al., 2011) and Saskatchewan (Waiser et al., 2011), and in wetlands in Saskatchewan (Kuchta and Cessna, 2009; Kuchta et al., 2009). The Canadian public is increasingly demanding that the amounts of antimicrobials and other agrochemicals entering water supplies be quantified and that management practices to minimize or prevent such inputs be developed. This study contributes to addressing the growing concern of the Canadian public regarding the quality of their water supplies.

2.6. Conclusions

Whether beef cattle manure was surface-applied or soil-incorporated into the 0- to 10-cm layer, excreted residues of chlortetracycline, sulfamethazine, and tylosin were available for transport in simulated rainfall runoff, indicating the potential for adverse environmental effects in receiving waters. The percentage loss of antimicrobials in surface runoff was in the order:

chlortetracycline from CTC > sulfamethazine > chlortetracycline from CTCSMZ > tylosin; thus, overall, soil sorption coefficients (K_d values) were not very useful in predicting the concentrations and mass loss of these antimicrobials in runoff when land-applied with solid beef cattle manure. Soil incorporation of manure is a recommended beneficial management practice mainly for reasons related to nutrient retention and odour reduction. Our findings show that the potential for surface water contamination by chlortetracycline and sulfamethazine can be reduced by incorporation of manure shortly after application, thereby strengthening the rationale for this practice to further reduce the risk to aquatic ecosystems.

2.7. References

Ahuja, L.R., J.D. Ross, and O.R. Lehman. 1981. A theoretical-analysis of interflow of water through surface soil horizons with implications for movement of chemicals in field runoff. Water Resour. Res. 17:65-72.

Alberta Agriculture and Food. 2007. Nutrient management planning guide [Online]. Available: http://www.agric.gov.ab.ca/flippingbook/agdex/400_28-3/html/index.html#/1/ (verified 3 Sep 2013).

Alberta Agriculture and Forestry. 2016. Agriculture: Manure application [Online]. Available: http://www.agric.gov.ab.ca (verified 8 June 2016).

Arikan, O.A., L.J. Sikora, W. Mulbry, S.U. Khan, and G.D. Foster. 2007. Composting rapidly reduces levels of extractable oxytetracycline in manure from therapeutically treated beef calves. Bioresour. Technol. 98:169-176.

Aust, M.O., F. Godlinski, G.R. Travis, Hao XiYing, T.A. McAllister, P. Leinweber, and S. Thiele-Bruhn. 2008. Distribution of sulfamethazine, chlortetracycline and tylosin in manure and soil of Canadian feedlots after sub-therapeutic use in cattle. Environ. Pollut. 156:1243-1251.

Blackwell, P.A., P. Kay, and A.B.A. Boxall. 2007. The dissipation and transport of veterinary antibiotics in a sandy loam soil. Chemosphere 67:292-299.

Boxall, A.B.A., L.A. Fogg, P.A. Blackwell, P. Kay, E.J. Pemberton, and A. Croxford. 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. 180:1-91.

Burkhardt, M., K. Stoob, C. Stamm, H. Singer, and S. Muller. 2004. Veterinary antibiotics in animal slurries - A new environmental issue in grassland research. Grassl. Sci. Eur. 9:322-324.

Burkhardt, M., C. Stamm, C. Waul, H. Singer, and S. Muller. 2005. Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. J. Environ. Qual. 34:1363-1371.

Calamari, D., E. Zuccato, S. Castiglioni, R. Bagnati, and R. Fanelli. 2003. Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environ. Sci. Technol. 37:1241-1248.

Canadian Animal Health Institute. 2011. Compendium of veterinary products, 12th ed., North American Compendiums Ltd., Hensall, Ont. Canada.

Cessna, A.J., F.J. Larney, S.L. Kuchta, X. Hao, T. Entz, E. Topp, and T.A. McAllister. 2011. Veterinary antimicrobials in feedlot manure: Dissipation during composting and effects on composting processes. J. Environ. Qual. 40:188-198.

Chee-Sanford, J.C., R.I. Aminov, I.J. Krapac, N. Garrigues-Jeanjean, and R.I. Mackie. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. Appl. Environ. Microbiol. 67:1494-1502.

Chee-Sanford, J.C., R.I. Mackie, S. Koike, I.G. Krapac, Y.F. Lin, A.C. Yannarell, S. Maxwell, and R.I. Aminov. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38:1086-1108.

Davis, J.G., C.C. Truman, S.C. Kim, J.C. Ascough II, and K. Carlson. 2006. Antibiotic transport via runoff and soil loss. J. Environ. Qual. 35:2250-2260.

De Liguoro, M., V. Cibin, F. Capolongo, B. Halling-Sorensen, and C. Montesissa. 2003. Use of oxytetracycline and tylosin in intensive calf farming: Evaluation of transfer to manure and soil. Chemosphere 52:203-212.

Dolliver, H. and S. Gupta. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. 37:1227-1237.

Forrest, F., K. Lorenz, T. Thompson, J. Keenliside, J. Kendall, and J. Charest. 2011. A scoping study of livestock antimicrobials in agricultural streams of Alberta. Can. Water Resour. J. 36:1-16.

Forster, M., V. Laabs, M. Lamshoft, J. Groeneweg, S. Zuhlke, M. Spiteller, M. Krauss, M. Kaupenjohann, and W. Amelung. 2009. Sequestration of manure-applied sulfadiazine residues in soils. Environ. Sci. Technol. 43:1824-1830.

Forster, M., V. Laabs, M. Lamshoeft, T. Puetz, and W. Amelung. 2008. Analysis of aged sulfadiazine residues in soils using microwave extraction and liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 391:1029-1038.

Haller, M.Y., S.R. Muller, C.S. McArdell, A.C. Alder, and M.J.F. Suter. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. 952:111-120.

Halling-Sorensen, B. 2001. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Arch. Environ. Contam. Toxicol. 40:451-460.

Hamscher, G., H.T. Pawelzick, H. Hoper, and H. Nau. 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environ. Toxicol. Chem. 24:861-868.

Hoese, A., S.A. Clay, D.E. Clay, J. Oswald, T. Trooien, R. Thaler and C.G. Carlson. 2009. Chlortetracycline and tylosin runoff from soils treated with antimicrobial containing manure. J. Environ. Sci. Heal. B 44:371-378.

Garcia-Galan, M.J., S. Diaz-Cruz, and D. Barcelo. 2013. Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography-electrospray-quadrupole linear ion trap mass spectrometry. J. Chromatogr. 1275:32-40.

Kay, P., P.A. Blackwell, and A.B.A. Boxall. 2005. Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land. Chemosphere 60:497-507.

Kim, S.C., J.G. Davis, C.C. Truman, J.C. Ascough II, and K. Carlson. 2010. Simulated rainfall study for transport of veterinary antibiotics-mass balance analysis. J. Hazard. Mater. 175:836-843.

Kim, K., G. Owens, S. Kwon, K. So, D. Lee, and Y.S. Ok. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut. 214:163-174.

Kolpin, D.W., E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, and H.T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environ. Sci. Technol. 36:1202-1211.

Kuchta, S.L. and A.J. Cessna. 2009. Fate of lincomycin in snowmelt runoff from manure-amended pasture. Chemosphere 76:439-446.

Kuchta, S.L., A.J. Cessna, J.A. Elliott, K.M. Peru, and J.V. Headley. 2009. Transport of lincomycin to surface and ground water from manure-amended cropland. J. Environ. Qual. 38:1719-1727.

Kummerer, K. 2003. Significance of antibiotics in the environment. J. Antimicrob. Chemoth. 52:5-7.

Lertpaitoonpan, W. 2008. Sorption, degradation, and transport of sulfamethazine in soils and manure-amended soils. Graduate Theses and Dissertations. Paper 11180. Iowa State University, Ames, Iowa.

Lissemore, L., C.Y. Hao, P. Yang, P.K. Sibley, S. Mabury, and K.R. Solomon. 2006. An exposure assessment for selected pharmaceuticals within a watershed in southern Ontario. Chemosphere 64:717-729.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 2006. SAS System for mixed models, 2nd ed. SAS Institute Inc., Cary, NC.

Managaki S, A. Murata, H. Takada, B.C. Tuyen, and N.H. Chiem. 2007. Distribution of macrolides, sulfonamides, and trimethoprim in tropical waters: Ubiquitous occurrence of veterinary antibiotics in the Mekong Delta. Environ. Sci. Technol. 41:8004-8010.

Miller, J.J., E.C.S. Olson, D.S. Chanasyk, B.W. Beasley, F.J. Larney, and B.M. Olson. 2006. Phosphorus and nitrogen in rainfall simulation runoff after fresh and composted beef cattle manure application. J. Environ. Qual. 35:1279-1290.

Pruden, A., R. Pei, H. Storteboom, and K.H. Carlson. 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40:7445-7450.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725-759.

SAS Institute. 2012. SAS/STAT User's guide version 9.3. SAS Inst., Inc., Cary, NC.

Spencer, W. and M. Cliath. 1991. Pesticide losses in surface runoff from irrigated fields. Plenum Publishing Corp, New York, NY.

Statistics Canada. 2012. Livestock and aquaculture. [Online] Available: http://www.statcan.gc.ca/tables-tableaux/sum-som/101/cst01/prim50a-eng.htm (verified 3 Feb 2013).

Thiele-Bruhn, S. 2003. Pharmaceutical antibiotic compounds in soils - A review. J. Plant Nutr. Soil Sci. 166:145-167.

Tossell, R.W., W.T. Dickinson, R.P. Rudra, and G.J. Wall. 1987. A portable rainfall simulator. Can. Agric. Eng. 29:155-162.

Waiser, M.J., D. Humphries, V. Tumber, and J. Holm. 2011. Effluent-dominated streams. Part 2: Presence and possible effects of pharmaceuticals and personal care products in Wascana creek, Saskatchewan, Canada. Environ. Toxicol. Chem. 30:508-519.

Wauchope, R.D. 1978. Pesticide content of surface-water draining from agricultural fields - review. J. Environ. Qual. 7:459-472.

Xi, C., Y. Zhang, C.F. Marrs, W. Ye, C. Simon, B. Foxman, and J. Nriagu. 2009. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl. Environ. Microbiol. 75: 5714-5718.

Yang, S.W. and K. Carlson. 2003. Evolution of antibiotic occurrence in a river through pristine, urban, and agricultural landscapes. Water Res. 37.4645-4656.

3. DISSIPATION OF ANTIMICROBIALS IN A SEASONALLY-FROZEN SOIL FOLLOWING BEEF CATTLE MANURE APPLICATION

3.1. Abstract

Land application of manure containing antimicrobials results in the dispersion of the antimicrobials in agro-ecosystems. Dissipation of excreted antimicrobials in seasonally-frozen agricultural soils has not been fully characterized under field conditions. This study investigated the field dissipation kinetics of chlortetracycline, sulfamethazine, and tylosin over a 10-mo period following fall application of manure from cattle (*Bos taurus*) administered 44 mg chlortetracycline (CTC), 44 mg each of chlortetracycline and sulfamethazine (CTCSMZ), or 11 mg tylosin (TYL) per kg feed daily. Antimicrobial concentrations in manured soil reflected the same relative concentrations in manure: chlortetracycline > sulfamethazine > tyosin. The first-order dissipation half-life (DT_{50}) for chlortetracycline from the CTCSMZ treatment was 77 d during the growing season and 648 d during the non-growing season when the soil was frozen for an extended period. By comparison, dissipation of chlortetracycline added alone (treatment CTC) did not differ significantly between the two seasons (mean DT_{50} = 121 d). During the non-growing season, chlortetracycline from the CTC ($P = 0.004$) dissipated faster than that from the CTCSMZ, indicating that the presence of sulfamethazine may have altered the dissipation of chlortetracycline. Dissipation kinetics for sulfamethazine and tylosin were not determined due to low detection in the manure-amended soil. Sulfamethazine was detected up to 16 ± 10 μg kg^{-1} throughout the 10-mo monitoring period. Tylosin concentration was ≤ 11 ± 6.6 μg kg^{-1} and gradually dissipated. Chlortetracycline was detectable 10 mo after application in the

seasonally-frozen soil, indicating a risk for residue build-up in the soil with repeated manure application and subsequent offsite contamination.

3.2. Introduction

The livestock industry in North America uses antimicrobials for the treatment and prevention of disease as well as for growth promotion. The estimated annual use in the United States is 11-16 million kg (Kim et al., 2011; Sarmah et al., 2006). Approximately 75% of antimicrobials administered to animals are excreted (CheeSanford et al., 2009), with higher or lower values reported depending on the nature of the antibiotic, the dose, the method of administration, the breed, or the age of the animal (Kim et al., 2011).

Livestock manure has been traditionally used in agriculture as a fertilizer and a soil conditioner. When livestock manure that contains excreted antimicrobials is land applied, antimicrobials are introduced into agricultural soils (Boxall et al., 2003; Sarmah et al., 2006), which may then be transported in water from surface runoff (Amarakoon et al., 2014; Davis et al., 2006; Dolliver and Gupta, 2008; Hoese et al., 2009) or via leaching (Dolliver and Gupta, 2008; Hamscher et al., 2005; Kim et al., 2010). Such events can result in surface and ground water contamination. Antimicrobials can be directly toxic to soil microorganisms (Fang et al., 2014; Halling-Sorensen et al., 2002; Toth et al., 2011), thereby altering the microbial balance within ecosystems. More importantly, antimicrobials may increase the level of antimicrobial resistance development in bacteria within the environment, and if antimicrobial resistant genes are transferred to pathogens, antimicrobials may become less effective against infections (CheeSanford et al., 2009; Ghosh and LaPara, 2007; Kemper, 2008; Kummerer, 2003). Furthermore, if antimicrobials persist in agricultural soils, they may be taken up by food and

feed crops (Dolliver et al., 2007; Kumar et al., 2005), potentially leading to chronic exposure in humans and livestock and increasing the risk of resistance development in intestinal flora (Witte, 2000).

Chlortetracycline, sulfamethazine, and tylosin are three antimicrobials commonly used in livestock production worldwide that have relevance to human health and have been reported in agricultural soils (Sarmah et al., 2006). Knowledge of the persistence of these antimicrobials in manured agricultural soils is needed to understand the environmental fate and potential environmental mobility of these compounds. However, research on the persistence of these antimicrobials in the environment is still an emerging area, with only a few studies reported to date.

A number of laboratory microcosm fortification studies have been conducted to study the dissipation of these antimicrobials in soils. Topp et al. (2013) reported dissipation half-life (DT_{50}) values of 3.3 and 2.8 d for chlortetracycline, 1.3 and 5.3 d for sulfamethzine, and 2 and 10 d for tylosin in soils collected from field plots in Ontario, Canada. Sassman et al. (2007) observed DT_{50} values of 23 – 24 d for tylosin in six midwestern United States surface soils and one sandy soil collected from Florida. Accinelli et al. (2007) reported a DT_{50} of 18.6 d for sulfamethazine in silty loam and sandy soils collected in Minnesota. Schluesener and Bester (2006) reported a DT_{50} of 8 d for tylosin in a sandy loam surface soil collected in Germany. Although such laboratory microcosm studies have merit, they are often performed in controlled environments that are optimal for antimicrobial degradation, conditions that may not be representative of the more complex dissipation processes which occur under variable field conditions.

Chlortetracycline levels in farm lands in Germany (Hamscher et al., 2002) were 5-7 µg kg^{-1} soil following two annual applications of liquid swine manure. When the swine manure was applied annually for 4 yr, chlortetracycline concentrations of 4-39 µg kg^{-1} were reported (Hamscher et al., 2005). Chlortetracycline residues have also been reported in Austrian farmland soils amended with swine manure (Martinez-Carballo et al., 2007). In Denmark, DT_{50} values of 25 d for chlortetracycline were reported in a sandy loam soil and 34 d in a sandy soil in the summer following field application of liquid swine manure (Halling-Sorensen et al., 2005). In the same study, DT_{50} values for tylosin were 67 d in the sandy loam and 49 d in the sandy soil. In Switzerland, the concentration of sulfamethazine was one half (250-400 µg kg^{-1} wet soil) the initial concentration (500–700 µg kg^{-1}) within 42 d of application in a soil amended with liquid swine manure (Stoob et al., 2006). In Ontario, Canada, Carlson and Mabury (2006) reported first-order DT_{50} values of 21 d for chlortetracycline and 6.1 d for tylosin during a 50-d field study in the summer following field application (incorporation) of the antimicrobials mixed with sand. In the same study, half-lives of 24 d for chlortetracycline and 4.5 d for tylosin were observed in plots receiving dairy manure following antimicrobial incorporation.

To date, the few field studies that have evaluated antimicrobial dissipation in manure-amended soils have been conducted during summer months. Furthermore, none of the studies have monitored antimicrobial dissipation for a year or longer to capture seasonal effects (e.g., wet-dry and freeze-thaw cycles). There is also a dearth of published studies using manure containing antimicrobials that were orally administered to livestock and excreted, which better represents actual conditions by which antimicrobials enter agricultural soils (Amarakoon et al., 2015), as opposed to directly fortifying soil or manure with antimicrobials. Moreover, there are

fewer published antimicrobial dissipation studies utilizing cattle manure compared with those using liquid swine manure, despite a large cattle industry in North America.

This study, therefore, was conducted to quantify the rates of dissipation of chlortetracycline, sulfamethazine, and tylosin in a seasonally-frozen agricultural soil following fall application of beef cattle manure containing the excreted antimicrobials.

3.3. Materials and Methods

3.3.1. Site Description

The 2-year experiment was conducted in 2010/11 and repeated on adjacent plots in 2011/12 at the Agriculture and Agri-Food Canada Research and Development Centre in Lethbridge, Alberta, Canada (49° 42′ N, 112° 50′ W). The soil was a semiarid Dark Brown Chernozem (Soil Classification Working Group, 1998) with a clay-loam texture. Organic carbon content was 15 g kg^{-1} and the pH was 7.5 in the 0–15 cm depth. The site had no history of manure application and had been continuously cropped to spring wheat (*Triticum aestivum* L.) prior to the experiment. The 30-yr (1981-2010) annual mean for air temperature was 6.4 ºC and that for precipitation was 399 mm (recorded at a weather station located ~500 m from the study site).

3.3.2. Antimicrobial Treatments Administered to Cattle

Antimicrobials were administered via feed at concentrations of 44 mg kg^{-1} feed chlortetracycline (treatment CTC), 44 mg kg^{-1} feed each of chlortetracycline and sulfamethazine (treatment CTCSMZ), and 11 mg kg^{-1} feed tylosin (treatment TYL) to 9 steers housed in a pen specific to each antimicrobial treatment (Amarakoon et al., 2014). A fourth pen of 9 steers was

used as a control as no antimicrobials were administered in the diet. In the first year (2010), the feeding period was 111 d (18 June to 7 Oct.) for all treatments. In the second year (2011), the feeding periods were 105 d (28 June to 11 Oct.) for TYL and the control and 98 d (5 July to 11 Oct.) for CTC and CTCSMZ. A manure:bedding ratio of ~4:1 was maintained in the pens by addition of barley (*Hordeum vulgare* L.) straw at regular intervals. Manure was allowed to accumulate throughout the feeding periods.

3.3.3. Plot Set Up and Manure Application

The experimental design was a randomized complete block with four antimicrobial treatments (CTC, CTCSMZ, TYL, and control, as described above) and four replications per treatment. Each of the four blocks was divided into four 5-m × 3-m plots, i.e., one for each antimicrobial treatment.

Manure was collected from each of the four treatments (pens) described above and mixed individually in a 0.34-m3–capacity mortar mixer (Model 12SGH9, Crown Construction Equipment) to improve uniformity. Average manure water content was 0.63 kg kg^{-1} in 2010 and 0.69 kg kg^{-1} in 2011. Manure was surface applied at a rate of ~90 kg wet wt. $plot^{-1}$, which corresponds to 60 Mg ha^{-1} (wet wt.), an application rate that is commonly used by irrigation farmers in southern Alberta (Alberta Agriculture and Forestry, 2016). Manure was incorporated into the 0- to 10-cm soil layer with a single pass of a disk harrow on 7-8 October 2010 and 11 October 2011. Cross contamination among antimicrobial treatments was prevented by pressure-washing the equipment between treatments as needed. Manure samples were collected from the mixed manure immediately before field application for determination of antimicrobial concentrations. Manure samples were freeze-dried, ground to pass through a 2-mm screen, and maintained at -30 ºC until antimicrobials were extracted.

3.3.4. Soil Sample Collection

Samples of manure-amended soil were collected from the 0- to 10-cm soil layer using a 5-cm diameter split core slide hammer. When soil conditions were conducive, a truck-mounted Giddings soil sampler (Giddings Machine Co.) was used during the monitoring period. Four soil cores were randomly collected from each plot and combined to make one composite sample per plot. The initial set of soil samples was taken the day after manure application in each year. During the first 30 d following manure applications, soil samples were collected weekly from the TYL treatment (as tylosin has a short DT_{50} in soil) and bi-weekly from the CTC, CTCSMZ and control treatments. Thereafter, samples were collected at ~30-d intervals. Final soil samplings occurred on 16 Aug. 2011 and 14 Aug. 2012, that is, ~10 mo after manure application. Soil samples were freeze-dried, ground (< 2 mm), and maintained at -30 ºC until antimicrobials were extracted.

3.3.5. Antimicrobial Extraction and Determination

3.3.5.1. Microwave-Assisted Extraction

Manure and manure-amended soil samples were subjected to microwave-assisted extraction (MAE) using a Mars Xpress microwave system (CEM Corporation, Matthews, NC). Freeze-dried and ground manure-amended soil or manure (1 g) was placed in 75-mL Teflon microwave digestion vessels (CEM Corporation, Matthews, NC). Each sample was first extracted with 60 mL of an 80:20 methanol: 0.5 M citric acid buffer (adjusted to pH 5 with sodium hydroxide) followed by a second extraction in 40 mL of citric acid buffer. For both extractions, the solvent/sample mixture was heated to 75ºC (in 10 min) and this temperature was maintained for 5 min. The two extracts (80:20 methanol: citric acid buffer and citric acid buffer solution) were decanted from the digestion vessel into a single 100-mL beaker and then

transferred into two 50-mL polypropylene centrifuge tubes (Fisher Scientific, Canada). The extracts were centrifuged for 10 min. at 3100 rpm (52 Hz) and the supernatant from each centrifuge tube was then transferred into a 500-mL amber bottle for overnight storage at 4ºC.

3.3.5.2. Solid-Phase Extraction and Elution

The MAE extract was subjected to solid-phase extraction (SPE) cleanup using a modification of the procedure described by Amarakoon et al. (2014) for extracts arising from the pressurized liquid extraction of manure. The MAE extract (~100 mL; extracted the day before as described above) was diluted to 500 mL with milli-Q water to reduce the methanol concentration. An Oasis hydrophilic-lipophilic balance (HLB) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA) was stacked on top of an Oasis weak cation exchange (WCX) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA) and both cartridges were simultaneously conditioned with methanol (10 mL) followed by milli-Q water (10 mL). The diluted extract (500 mL) was then passed through the SPE assembly, at a flow rate of 1 mL min^{-1}, followed by rinsing with milli-Q water (10 mL) to remove salts. The cartridges were air-dried under vacuum for 1 min and maintained at 4ºC until elution.

The HLB cartridge was eluted with methanol (8 mL) into a graduated glass centrifuge tube. The WCX cartridge was eluted with methanol (4 mL) followed by methanol containing 2% formic acid (4 mL) into a separate graduated glass centrifuge tube. Each eluent was concentrated (500 µL) under a gentle stream of air, followed by dilution with milli-Q water to 1 mL and transferred into a 2-mL amber liquid chromatography vial through a 0.45 µm nylon membrane syringe filter (Chromatographic Specialities Inc., Brockville, ON). Extracts were stored at -30ºC until analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Each eluent was fortified with 100 ng 13C6-sulfamethazine (Cambridge Isotope Laboratories, Andover, MA) prior to analysis.

3.3.5.3. LC/MS/MS Analysis

All manure and manure-amended soil sample extracts were analyzed by liquid chromatography-tandem mass spectrometry with a Waters 2965 Alliance Separation Module interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters Canada, Mississauga, ON), using operating conditions previously described by Cessna et al. (2011) and Amarakoon et al. (2014). Briefly, a C-18 stainless steel column (MS Xterra, 100-mm × 2.1-mm i.d., 3.5-µm diam. packing; Waters Canada, Mississauga, ON) was used for analyte separation. Gradient elution was performed with two mobile phases, both containing acetonitrile/water and 0.1% formic acid. The gradient elution used 90% of mobile phase A (acetonitrile:water, 10:90 v/v) and 10% of mobile phase B (acetonitrile:water, 90:10 v/v) for 1 min followed by 100% of mobile phase B for 9 min. At 10 min, reconditioning of the column for the next injection (at 15 min) was undertaken by switching back to 90% mobile phase A and 10% mobile phase B. The mobile phase flow rate was 200 µL min-1 and the injection volume was 20 µL. Retention times were 6.83 min for tylosin, 6.92 min for iso-chlortetracycline, and 7.11 min for sulfamethazine and 13C6–sulfamethazine (internal standard). The electrospray ionization interface was set to positive ion mode. Suitable multiple reaction monitoring (MRM) transitions were used for confirmation and quantification of antimicrobials (Amarakoon et al., 2014). Data were processed using MassLynx software (v 4.1, Waters Canada, Mississauga, ON) and concentrations were reported on dry-weight basis.

3.3.5.4. Antimicrobial Recovery

Freeze-dried samples (1 g) of manure and manure-amended soil from their respective control treatments were fortified with 100 µL of a 1 mg L^{-1} mixture of chlortetracycline, sulfamethazine, and tylosin in Milli-Q water. This fortification solution was prepared by diluting 100-fold a 100 mg L^{-1} stock solution of each antimicrobial in acetonitrile. Thus, the fortification

level was equivalent to 100 µg kg^{-1} of manure or manure-amended soil. The fortified samples were then extracted and antimicrobial concentrations were quantified as described above.

Average recoveries from manure-amended soil (n = 30) fortified at 100 µg kg^{-1} were 66 ± 26% for *iso*-chlortetracycline, 67 ± 21% for sulfamethazine, and 110 ± 43% for tylosin. Average recoveries from beef cattle manure (n = 4) fortified at 100 µg kg^{-1} were 71 ± 24% for *iso*-chlortetracycline, 24 ± 3% for sulfamethazine, and 47 ± 9% for tylosin. Average background interferences, most likely resulting from co-eluting compounds from control samples of manure-amended soil were 1.7 µg kg^{-1} for iso-CTC, 0.13 µg kg^{-1} for SMZ, and 1.3 µg kg^{-1} for TYL. For the manure samples, the background interferences were ≤ 0.3 µg kg^{-1}. The limit of detection was considered to be three times that of background concentrations.

3.3.6. Statistical Analysis

Dissipation data for chlortetracycline in manured soils was evaluated using PROC NLIN in SAS 9.4 (SAS Institute Inc., 2013) and was best described by the first-order kinetic model:

$$C_t = C_0 e^{-kt}$$

where C_t is the antimicrobial concentration (µg kg^{-1}) at time t (d), C_0 is the initial antimicrobial concentration (µg kg^{-1}), and k is the first-order dissipation rate constant (d^{-1}). Dissipation kinetics was evaluated separately for each replicate (block) in each season in each year. Dissipation rate constants estimated by the NLIN procedure for chlortetracycline and the corresponding DT_{50} (= 0.693/k) data followed a lognormal distribution and were subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS with treatment (CTC and CTCSMZ) and season [non-growing (October 1 – March 30) and growing (April 1 – August 31)] as fixed effects and block and year as random effects. The Tukey multiple comparison procedure was used for

pairwise comparison of treatment means if a significant effect was indicated by the global ANOVA. Treatment differences were considered significant at $P < 0.05$.

Dissipation kinetics for sulfamethazine and tylosin were not assessed because concentrations of these antimicrobials in manure-amended soil were low. Mean, maximum, and minimum soil and air temperatures were determined with PROC MEANS in SAS.

3.4. Results and Discussion

3.4.1. Temperature and Precipitation

During the monitoring period, soil temperature at the 5-cm depth ranged from -5 to 23 ºC (mean 6.5 ºC) in 2010/11 and -3.2 to 27 ºC (mean 8.0 ºC) in 2011/12 (Fig. 3.1). The soil temperatures during this period were ≤ 0 ºC for 108 d in 2010/11 and for 81 d in 2011/12. Because soil temperatures ≤ 0 ºC occurred mainly in the non-growing season months of November to February, the temperature of the upper soil layer into which manure was incorporated would have been frozen for much of this period. Air temperature during the study ranged from -25 to 23 ºC (mean 3.5 ºC) in 2010/11 and -28 to 25 ºC (mean 6.6 ºC) in 2011/12. Total precipitation was 486 mm in 2010/11 and 311 mm in 2011/12, with daily maxima of 30 mm on 7 June 2011 and 34 mm on 12 Apr. 2012 (Fig. 3.2).

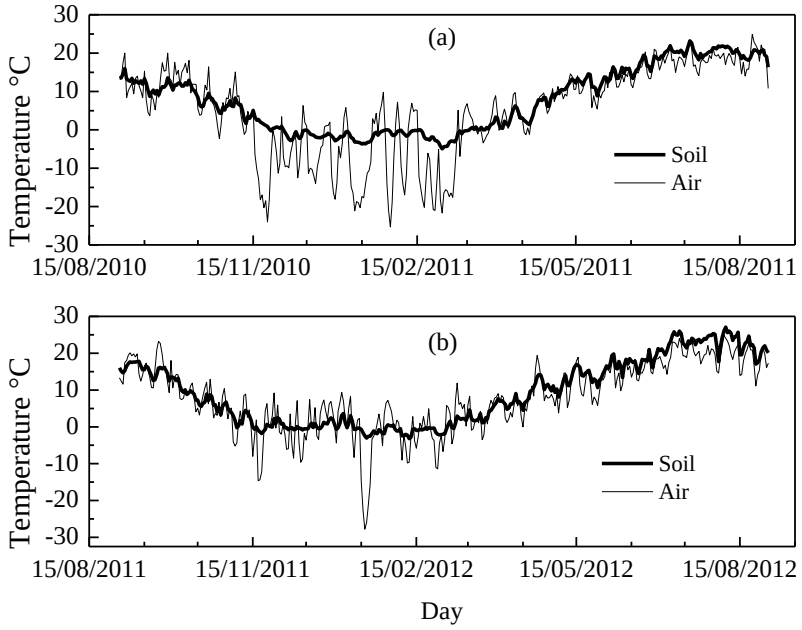


Figure 3.1: Temporal changes in daily mean soil (5-cm depth) and air temperature during the period spanning field monitoring of antimicrobial concentrations in (a) 2010/11 and (b) 2011/12. The weather station was located~ 500 m from the experimental plots.

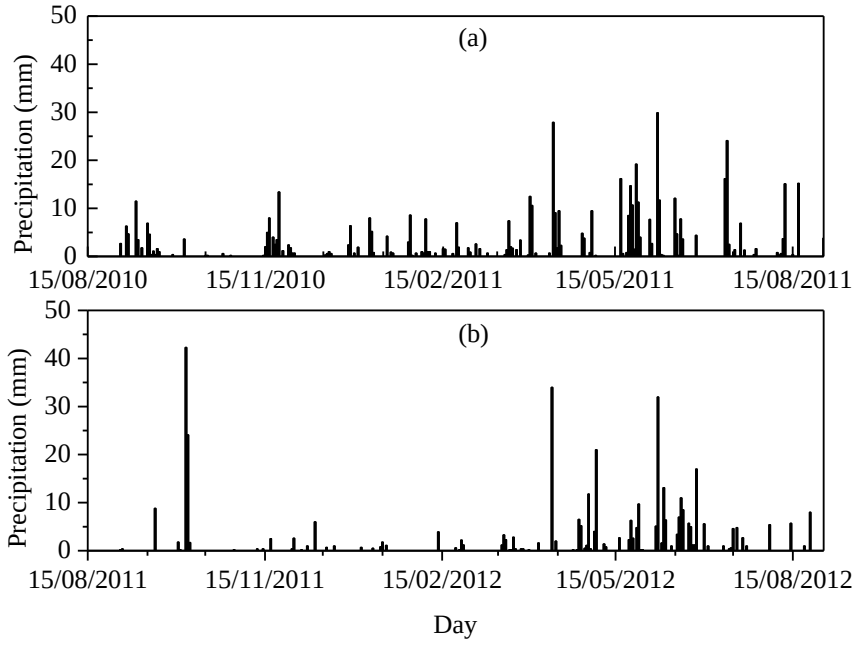


Figure 3.2: Temporal changes in daily mean precipitation during the period spanning field monitoring of antimicrobial concentrations in (a) 2010/11 and (b) 2011/12. The weather station was located ~ 500 m from the experimental plots.

3.4.2. Antimicrobial Concentrations in Manure

Manure accumulated in the feedlot pens for 111 d in 2010 and 105 d in 2011. The period of manure accumulation was drier in 2011 (105 mm of rain) than in 2010 (138 mm). Concentrations of all three antimicrobials tended to be higher in 2011 (Table 3.1).

Mean antimicrobial concentrations in manure decreased in the order chlortetracycline > sulfamethazine > tylosin (Table 1). The concentration of chlortetracycline in manure did not differ significantly ($P > 0.05$) between CTC and CTCSMZ in each year of manure application. Sulfamethazine concentration was more than an order of magnitude (13- to 20-fold) lower than that of chlortetracycline in manure from CTCSMZ even though it was administered at the same concentration in feed. These relative antimicrobial concentrations are consistent with those observed by Cessna et al. (2011) in beef cattle feedlot manure but are in contrast to the higher excretion rates reported by Kim et al. (2011) for sulfamethazine (~ 90%) than for chlortetracycline (~ 65-75%). The lower concentration of sulfamethazine in the manure in the present study may reflect its greater mobility than chlortetracycline (Chee-Sanford et al., 2009); thus, some sulfamethazine may have been transported off-site in rainfall runoff during the period of manure accumulation in feedlot pens. Rainfall amounts capable of producing surface runoff from the feedlot pens were recorded in both 2010 (13.6 and 24.5 mm) and 2011 (15.4, 15.6, and 37.0 mm). A fraction of sulfamethazine may have converted to its metabolite, N4-acetyl-sulfamethazine (Haller et al., 2002), which was not analyzed in the present study; this likely reduced the sulfamethazine concentration detected in manure. Sulfamethazine forms strong hydrogen (Teixido et al., 2011) and covalent (Bialk et al., 2005; Gulkowska et al., 2013) bonds with manure, and the bonding gets stronger with extended contact time (Carstens et al., 2013; Stoob et al., 2006; Stoob et al., 2007; Yang et al., 2009), such as occurred in this study during

manure accumulation in the feedlot; this may also have reduced the sulfamethazine concentration measured in manure. Tylosin was administered at a lower concentration in the feed than chlortetracycline and sulfamethazine, resulting in its lower initial concentration in manure. Further, rapid dissipation of tylosin in manure has previously been reported (De Liguoro et al., 2003; Hamscher et al., 2002; Kay et al., 2004).

3.4.3. Initial Antimicrobial Concentrations in Manure-Amended Soil

Antimicrobial concentrations in manure-amended soil generally reflected the antimicrobial concentrations in the applied manure (Table 1) and decreased in the order chlortetracycline > sulfamethazine > tylosin. However, the high standard deviations (± 20 to ± 96%) among initial samples is reflective of the fact that manure was not homogeneously distributed throughout the 0- to 10-cm soil layer after incorporation. In addition, the initial concentration of chlortetracycline in the manure-amended soil relative to that in the applied manure from both CTC and CTCSMZ showed that incorporation into soil diluted the concentration of chlortetracycline by approximately two orders of magnitude. A similar dilution was observed for sulfamethazine and tylosin. The maximum concentrations of chlortetracycline in the manure-amended soil from CTC (140 µg kg-1) and CTCSMZ (210 µg kg-1) occurred in 2011. Maximum initial concentrations of 16 µg kg-1 for sulfamethazine and 11 µg kg-1 for tylosin were also measured in manure-amended soil in 2011 when corresponding concentrations in the manure tended to be higher.

Table 3.1: Antimicrobial concentrations (dry wt. basis) in manure and soil.

Antimicrobial	Treatment	Year of application	Antimicrobial concentration‡	
			Manure	Soil
			——— µg kg^{-1} ———	
Chlortetracycline	CTC	2010	9180 (1610)‡	94(19)
		2011	15700 (960)	135(100)
	CTCSMZ†	2010	6970 (1330)	46(11)
		2011	13400 (1400)	213(140)
Sulfamethazine	CTCSMZ†	2010	520 (110)	7.0(6.7)
		2011	600 (64)	16(10)
Tylosin	TYL	2010	210 (51)	ND
		2011	230 (49)	11(6.6)

† CTC, chlortertracycline administered alone in the animal feed; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine

‡ Antimicrobial concentrations in manure (day of manure application) and in soil (1 d after manure application). Concentrations are arithmetic means of four replicates (four sub samples per replicate); values in parenthesis are standard deviations.

3.4.4. Dissipation Kinetics for Chlortetracycline

Dissipation of chlortetracycline during the study period following manure application was best described by the first-order kinetic model. Similarly, previous studies have demonstrated first-order kinetics for chlortetracycline in soil (Carlson and Mabury, 2006; Halling-Sørensen et al., 2005) and during composting of beef cattle manure (Cessna et al., 2011). There was a significant ($P = 0.04$) treatment by season (growing vs. non-growing season) interaction for the DT_{50} for chlortetracycline. Chlortetracycline from the CTCSMZ treatment dissipated significantly faster ($P = 0.03$) during the growing season ($DT_{50} = 77$ d) than during the non-

growing season (DT_{50} = 648 d) (Fig.3.3). By comparison, dissipation of chlortetracycline from the CTC treatment did not differ significantly between the two seasons (mean DT_{50} = 121 d). During the non-growing season, chlortetracycline from the CTC treatment dissipated faster (P = 0.004) than that from the CTCSMZ treatment, whereas the DT_{50} values did not differ significantly between CTC and CTCSMZ during the growing season. This result indicates that chlortetracycline in manure-amended soil dissipates faster when applied alone (CTC) than when applied as a 1:1 mixture of chlortetracycline and sulfamethazine (CTCSMZ) in the non-growing season and suggests that the presence of sulfamethazine may have altered the dissipation of chlortetracycline. This is the first time that a study has shown that the presence of sulfamethazine in the medium decreased the dissipation of chlortetracycline. It is not clear, however, why this effect was not evident in the growing season. As noted earlier, all previous field studies that we are aware of were conducted during the growing season.

To our knowledge, this study is the first to examine the dissipation of the three antimicrobials over a lengthy period (10 mo) that included winter months, during which soils were frozen for up to 108 d. The DT_{50} values estimated for chlortetracycline during the growing season are in general agreement with results from similar field studies conducted elsewhere during warm months (Carlson and Mabury, 2006; Halling-Sorensen et al., 2005). The greater persistence we observed for chlortetracycline from the CTCSMZ treatment during the non-growing season relative to the growing season reflects low mean annual temperatures in this seasonally-frozen soil. However, it is not clear why the seasonal effect was not significant for the chlortetracycline from the CTC treatment.

Temperature dependency of chlortetracycline dissipation has previously been reported in a 30-d laboratory incubation study (Gavalchin and Katz, 1994) in which 56% of the added

chlortetracycline was dissipated at 30 ºC compared with just 12% at 20 ºC and no significant dissipation at 4 ºC. Also, Loftin et al. (2008) showed that the DT_{50} of chlortetracycline in soil increased as temperature decreased from 35 ºC through 22 ºC to 7ºC. Chlortetracycline has soil sorption coefficients (K_d) of 1208 to 2386 L kg^{-1} (Sarmah et al., 2006) and as it has three acid dissociation constants (pKa1 = 3.3, pKa2 = 7.4, and pKa3 = 9.3), it can exist as a cation (+ 0 0), a zwitterion (+ – 0), and/or an anion (+ – –). Consequently, cation exchange would be the main mechanism of chlortetracycline sorption in soils with high cation exchange capacities (Pils and Laird, 2007; Sarmah et al., 2006; Sassman and Lee, 2005), such as those on the Canadian prairies (Soil Classification Working Group, 1998). Although the sorption of chlortetracycline to soil tends to decrease with increasing pH (Essington et al., 2010), a significant fraction of chlortetracycline remains sorbed at environmentally-relevant pH values (Figueroa-Diva et al., 2010). Thus, the persistence of chlortetracycline observed in the present study was likely due in part to the low soil temperatures during the non-growing season. Additionally, chlortetracycline may have become strongly sorbed to soil components post-application in the soil, thus making it less available for microbial degradation.

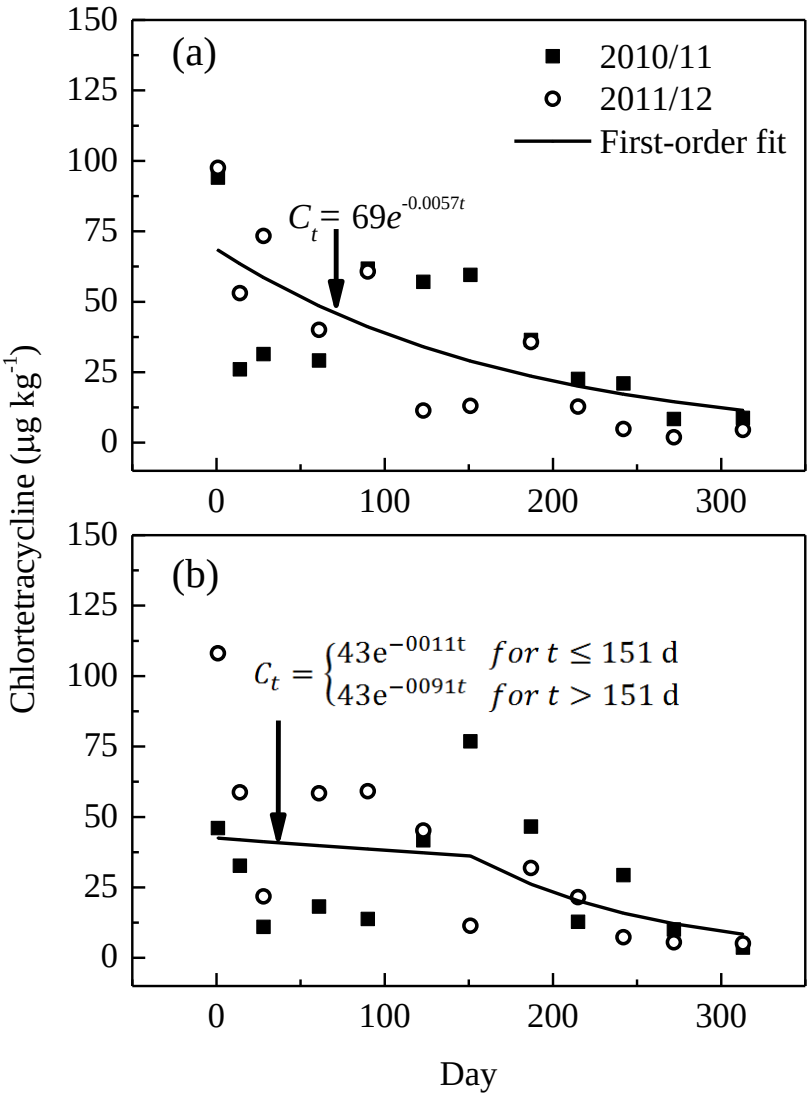


Figure 3.3: First-order dissipation of chlortetracycline in manure-amended soil from (a) CTC (chlortetracycline administered alone in the animal feed) and (b) CTCSMZ (1:1 mixture of chlortetracycline and sulfamethazine in cattle feed) treatments. C_t = antimicrobial concentration at time t; t = time elapsed (d) since the field application of manure. Root mean square error (RMSE) is 38 µg kg^{-1} for CTC and 32 µg kg^{-1} for CTCSMZ. Each data point in the figure is a mean of four replicates.

3.4.5. Sulfamethazine and Tylosin

Because the concentrations of both sulfamethazine and tylosin in soil were low throughout the study periods, with no apparent trends over time (Fig. 3.4), it was not possible to determine dissipation kinetics. However, both sulfamethazine and tylosin were detected in the manure-amended soil in the month of manure incorporation in each year, and sulfamethazine was continually detected in soil at low concentrations during both 10-mo monitoring periods. Tylosin concentration in soil was also low, but unlike sulfamethazine, tylosin was gradually dissipated.

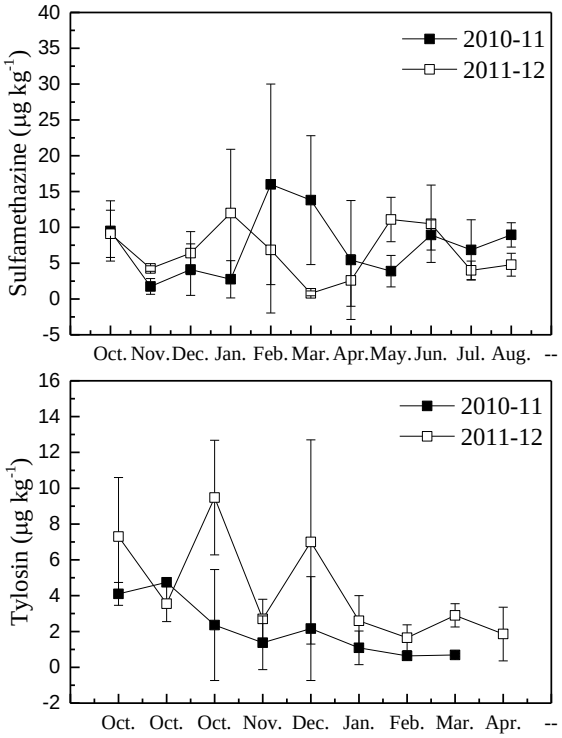


Figure 3.4: Sulfamethazine and tylosin concentrations in manure-amended soil during the study period. Each data point is an average of four replicates. Error bars represent the standard deviation of mean.

3.4.6. Antimicrobial Transport to Surface Waters

Although much of the Prairie region of Canada is semi-arid, rainfall can generate surface runoff. In these situations, raw manure from feedlots applied to crop and pasture land could act as a source of antimicrobials that could flow into surface waters. This possibility was confirmed in a recent study in which manure from beef cattle administered the same antimicrobials as in the present study were incorporated into soils just prior to simulated rainfall (Amarakoon et al., 2014). The manure was surface-applied or soil-incorporated at the same rate (60 Mg ha-1) as in the present study. All three antimicrobials were detected in the simulated rainfall runoff from the manure-amended plots. Relative to surface-applied manure in which 5 to 6% of chlortetracycline and sulfamethazine in manure was lost in the runoff, incorporation of the manure reduced runoff losses of these antimicrobials by approximately 50%. Consequently, incorporation of manure into soil and the application of manure onto cropland at the start of the active growing season could be viable strategies for reducing the risk of antimicrobial loss in surface runoff.

3.5. Conclusions

Antimicrobial concentrations in manure-amended soil generally reflected antimicrobial concentrations in the applied manure and decreased in the order chlortetracycline > sulfamethazine > tylosin. The first-order dissipation half-life of chlortetracycline when added as a 1:1 mixture with sulfamethazine was 77 d during the growing season and 648 d during the non-growing season when topsoil was frozen for an extended period. By comparison, dissipation of chlortetracycline from the CTC treatment did not differ significantly between the two seasons (DT_{50} = 121 d). Both sulfamethazine and tylosin were detected at low concentrations in manure-amended soil; sulfamethazine was detected throughout the monitoring period while tylosin was

gradually dissipated. Chlortetracycline had greater persistence than reported in previous studies, reflecting the slow dissipation in the seasonally-frozen soil. Chlortetracycline was measured in the soil 10 mo after manure application, indicating a potential risk for residue build-up in the soil and for off-site contamination if manure is applied repeatedly to the same field.

3.6. References

Accinelli, C., W.C. Koskinen, J.M. Becker, and M.J. Sadowsky. 2007. Environmental fate of two sulfonamide antimicrobial agents in soil. J. Agric. Food Chem. 55:2677-2682.

Alberta Agriculture and Forestry. 2016. Agriculture: Manure application [Online]. Available: http://www.agric.gov.ab.ca (verified 8 June 2016).

Amarakoon, I.D., F. Zvomuya, A.J. Cessna, D. Degenhardt, F.J. Larney, and T.A. McAllister. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. J. Environ. Qual. 43:549-557.

Amarakoon, I.D., F. Zvomuya, S. Sura, F.J. Larney, A.J. Cessna, S. Xu, and T.A. McAllister. 2015. Dissipation of antimicrobials in feedlot manure compost following oral administration vs. fortification post-excretion. J. Environ. Qual. doi:10.2134/jeq2015.07.0408.

Bialk, H.M., A.J. Simpson, and J.A. Pedersen. 2005. Cross-coupling of sulfonamide antimicrobial agents with model humic constituents. Environ. Sci. Technol. 39:4463-4473.

Boxall, A., D. Kolpin, B. Halling-Sorensen, and J. Tolls. 2003. Are veterinary medicines causing environmental risks? Environ. Sci. Technol. 37:286A-294A.

Carlson, J.C. and S.A. Mabury. 2006. Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environ. Toxicol. Chem. 25:1-10.

Carstens, K.L., A.D. Gross, T.B. Moorman, and J.R. Coats. 2013. Sorption and photo-degradation processes govern distribution and fate of sulfamethazine in freshwater-sediment microcosms. Environ. Sci. Technol. 47:10877-10883.

Cessna, A.J., F.J. Larney, S.L. Kuchta, X. Hao, T. Entz, E. Topp, and T.A. McAllister. 2011. Veterinary antimicrobials in feedlot manure: Dissipation during composting and effects on composting processes. J. Environ. Qual. 40:188-198.

Chee-Sanford, J.C., R.I. Mackie, S. Koike, I.G. Krapac, Y.F. Lin, A.C. Yannarell, S. Maxwell, and R.I. Aminov. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38:1086-1108.

Davis, J.G., C.C. Truman, S.C. Kim, J.C. Ascough II, and K. Carlson. 2006. Antibiotic transport via runoff and soil loss. J. Environ. Qual. 35:2250-2260.

De Liguoro, M., V. Cibin, F. Capolongo, B. Halling-Sorensen, and C. Montesissa. 2003. Use of oxytetracycline and tylosin in intensive calf farming: Evaluation of transfer to manure and soil. Chemosphere 52:203-212.

Dolliver, H. and S. Gupta. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. 37:1227-1237.

Dolliver, H., K. Kumar, and S. Gupta. 2007. Sulfamethazine uptake by plants from manure-amended soil. J. Environ. Qual. 36:1224-1230.

Essington, M.E., J. Lee, and Y. Seo. 2010. Adsorption of antibiotics by montmorillonite and kaolinite. Soil Sci. Soc. Am. J. 74:1577-1588.

Fang, H., Y. Han, Y. Yin, X. Pan, and Y. Yu. 2014. Variations in dissipation rate, microbial function and antibiotic resistance due to repeated introductions of manure containing sulfadiazine and chlortetracycline to soil. Chemosphere 96:51-56.

Figueroa-Diva, R.A., D. Vasudevan, and A.A. MacKay. 2010. Trends in soil sorption coefficients within common antimicrobial families. Chemosphere 79:786-793.

Gavalchin, J. and S.E. Katz. 1994. The persistence of fecal-borne antibiotics in soil. J. AOAC Int. 77:481-485.

Ghosh, S. and T.M. LaPara. 2007. The effects of sub-therapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. Isme J. 1:191-203.

Gulkowska, A., M. Sander, J. Hollender, and M. Krauss. 2013. Covalent binding of sulfamethazine to natural and synthetic humic acids: Assessing laccase catalysis and covalent bond stability. Environ. Sci. Technol. 47:6916-6924.

Haller, M.Y., S.R. Muller, C.S. McArdell, A.C. Alder, and M.J.F. Suter. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. 952:111-120.

Halling-Sorensen, B., G. Sengelov, and J. Tjornelund. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch. Environ. Contam. Toxicol. 42:263-271.

Halling-Sorensen, B., A.M. Jacobsen, J. Jensen, G. Sengelov, E. Vaclavik, and F. Ingerslev. 2005. Dissipation and effects of chlortetracycline and tylosin in two agricultural soils: A field-scale study in southern Denmark. Environ. Toxicol. Chem. 24:802-810.

Hamscher, G., H.T. Pawelzick, H. Hoper, and H. Nau. 2005. Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. Environ. Toxicol. Chem. 24:861-868.

Hamscher, G., S. Sczesny, H. Hoper, and H. Nau. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. 74:1509-1518.

Hoese, A., S.A. Clay, D.E. Clay, J. Oswald, T. Trooien, R. Thaler, and C.G. Carlson. 2009. Chlortetracycline and tylosin runoff from soils treated with antimicrobial containing manure. J. Environ. Sci. Heal. B. 44:371-378.

Kay, P., P.A. Blackwell and A.B.A. Boxall. 2004. Fate of veterinary antibiotics in a macro porous tile drained clay soil. Environ. Toxicol. Chem. 23:1136-1144.

Kemper, N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Ind. 8:1-13.

Kim, S.C., J.G. Davis, C.C. Truman, J.C. Ascough II, and K. Carlson. 2010. Simulated rainfall study for transport of veterinary antibiotics-mass balance analysis. J. Hazard. Mater. 175:836-843.

Kim, K., G. Owens, S. Kwon, K. So, D. Lee, and Y.S. Ok. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut. 214:163-174.

Kumar, K., S.C. Gupta, S.K. Baidoo, Y. Chander, and C.J. Rosen. 2005. Antibiotic uptake by plants from soil fertilized with animal manure. J. Environ. Qual. 34:2082-2085.

Kummerer, K. 2003. Significance of antibiotics in the environment. J. Antimicrob. Chemother. 52:317-317.

Martinez-Carballo, E., C. Gonzalez-Barreiro, S. Scharf, and O. Gans. 2007. Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ. Pollut. 148:570-579.

Pils, J.R.V. and D.A. Laird. 2007. Sorption of tetracycline and chlortetracycline on K- and ca-saturated soil clays, humic substances, and clay-humic complexes. Environ. Sci. Technol. 41:1928-1933.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725-759.

Sassman, S.A. and L.S. Lee. 2005. Sorption of three tetracyclines by several soils: Assessing the role of pH and cation exchange. Environ. Sci. Technol. 39:7452-7459.

Sassman, S.A., A.K. Sarmah, and L.S. Lee. 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environ. Toxicol. Chem. 26:1629-1635.

Schluesener, M.P. and K. Bester. 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. Environ. Pollut. 143:565-571.

Soi1 Classification Working Group. 1998. The Canadian system of soil classification. Agriculture and Agri-Food Canada Publ. 1646, Ottawa, ON.

Stoob, K., H.P. Singer, S.R. Mueller, R.P. Schwarzenbach, and C.H. Stamm. 2007. Dissipation and transport of veterinary sulfonamide antibiotics after manure application to grassland in a small catchment. Environ. Sci. Technol. 41:7349-7355.

Stoob, K., H.P. Singer, S. Stettler, N. Hartmann, S.R. Mueller, and C.H. Stamm. 2006. Exhaustive extraction of sulfonamide antibiotics from aged agricultural soils using pressurized liquid extraction. J. Chromatogr. A 1128:1-9.

Teixido, M., J.J. Pignatello, J.L. Beltran, M. Granados, and J. Peccia. 2011. Speciation of the ionizable antibiotic sulfamethazine on black carbon (biochar). Environ. Sci. Technol. 45:10020-10027.

Topp, E., R. Chapman, M. Devers-Lamrani, A. Hartmann, R. Marti, F. Martin-Laurent, L. Sabourin, A. Scott, and M. Sumarah. 2013. Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine-degrading micro bacterium sp. J. Environ. Qual. 42:173-178.

Toth, J.D., Y. Feng, and Z. Dou. 2011. Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. Soil Biol. Biochem. 43:2470-2472.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: Environment. Int. J. Antimicrob. Agents 14:321-325.

Yang, J.F., G.G. Ying, L.H. Yang, J.L. Zhao, F. Liu, R. Tao, Z.Q. Yu, and P. Peng. 2009. Degradation behavior of sulfadiazine in soils under different conditions. J. Environ. Sci. Heal. B 44:241-248.

4. DISSIPATION OF ANTIMICROBIALS IN FEEDLOT MANURE COMPOST FOLLOWING ORAL ADMINISTRATION VS. FORTIFICATION POST-EXCRETION

4.1. Abstract

Fortification of manure with antimicrobials is one approach to studying their dissipation. However, fortified antimicrobials may not accurately model dissipation that occurs after antimicrobials have been administered to livestock in feed and excreted in manure. This study examined the dissipation of antimicrobials excreted in manure versus those added directly to manure (fortified). Steers were fed a diet containing (kg^{-1} feed) (i) 44 mg chlortetracycline (CTC); (ii) 44 mg each of chlortetracycline and sulfamethazine (CTCSMZ); (iii) 11 mg tylosin (TYL); and (iv) no antimicrobials (control). Fortified antimicrobial treatments were prepared by adding antimicrobials to control manure. Manure was composted for 30 d, sampled every 2-3 d and analyzed for antimicrobials and compost properties. Antimicrobial dissipation followed first-order kinetics. The dissipation rate constant (k) was significantly greater (based on 95% confidence limit) for excreted (0.29 d^{-1} - 0.54 d^{-1}) than for fortified chlortetracycline (0.11 d^{-1} - 0.13 d^{-1}). In contrast, dissipation rate constants were significantly greater for fortified sulfamethazine (0.47 d^{-1}) and tylosin (0.31 d^{-1}) than when the same antimicrobials were excreted (0.08 d^{-1} and 0.07 d^{-1}, respectively). On average, 85–99% of the initial antimicrobial concentrations in manure were dissipated after 30 d of composting. The degree of dissipation was greater ($P < 0.0001$) for fortified (99%) than for excreted tylosin (85%). Composting can be used to reduce environmental loading of antimicrobials prior to field application of beef cattle

manure. Dissipation rates of fortified antimicrobials during manure composting may not accurately reflect those of antimicrobials that are consumed and excreted by cattle.

4.2. Introduction

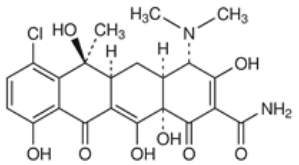
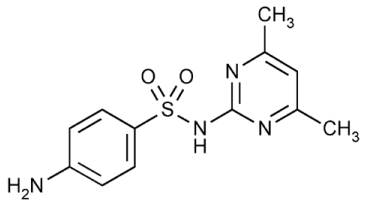
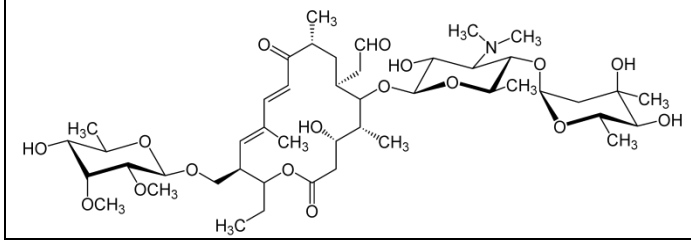
Livestock operations in North America routinely use antimicrobials to treat infections, control disease, and promote growth. Antimicrobial use in livestock in the United States is estimated to be 11–16 million kg yr^{-1} (Kim et al., 2011; Sarmah et al., 2006). The amount of antimicrobials excreted after ingestion varies with feeding conditions and the type of antimicrobial, and has been reported to be 65–75% of chlortetracycline, 90% of sulfamethazine, and 50–100% of tylosin (Kim et al., 2011). Manure containing excreted antimicrobials is often applied to agricultural land, resulting in the transfer of antimicrobial residues to soil, surface water and ground water (Boxall et al., 2004; Kemper et al., 2008; Sarmah et al., 2006). Antimicrobial dispersal in the environment increases the risk of selection for antimicrobial resistant bacteria (Chee-Sanford et al., 2009; Kemper et al., 2008; Zhang et al., 2009).

Composting entails the aerobic degradation of organic matter by a wide array of microorganisms. It reduces the mass and volume of manure and concentrates plant nutrients (Larney et al., 2006). Composting also reduces pathogen populations and the viability of weed seeds (Larney et al., 2003; Larney and Hao, 2007), making compost a preferred agricultural amendment over fresh manure. Composting has also shown the potential to enhance the dissipation of antimicrobials administered to livestock (Arikan et al., 2009; Bao et al., 2009; Cessna et al., 2011).

Numerous studies on the dissipation of antimicrobials have traditionally employed the addition of antimicrobials to antimicrobial-free manure (Burkhardt et al., 2005; Dolliver and

Gupta, 2008; Ho et al., 2013), a technique known as fortification. Manure fortification with antimicrobials is more economical and efficient than administering antimicrobials to livestock and collecting manure containing excreted antimicrobials. However, the dissipation of antimicrobials in fortified manure may not accurately reflect those of antimicrobials excreted in manure following oral administration via cattle feed. To date, a comparison of the fate of fortified vs fed antimicrobials in beef cattle manure has not been undertaken. Thus, this study was conducted to characterize the dissipation of chlortetracycline, sulfamethazine, and tylosin in feedlot manure compost following oral administration in feed or fortification post-excretion. The antimicrobials we tested are those that have relevance to human health and are most commonly fed at sub-therapeutic levels to feedlot cattle (Table 4.1). Further, the antimicrobials were administered at concentrations used in commercial feedlots. The study also allowed assessment of the impact of these antimicrobials (both excreted and fortified) on the composting process (temperature, C, N, and dry matter (DM) losses).

Table 4.1: Physical and chemical properties and applications of the selected antimicrobials.

Antimicrobial	MW (g mol^{-1})	pK_a	Log K_{ow}	Solubility (mg L^{-1})	Applications
Chlortetracycline (a tetracycline) 	479	3.3 7.5 9.3	0.41	600	Active against gram positive and gram negative bacteria, Mycoplasma, Chlamydia, etc. Used in human and animal therapy and at sub-therapeutic levels for growth promotion
Sulfamethazine (a sulfonamide) 	278	2.1 7.5	0.8	1500	Active against gram positive and negative bacteria. Used in human and animal therapy and at sub-therapeutic levels for growth promotion.
Tylosin (a macrolide)‡ 	917	7.5	3.4	5000	Active against mainly gram positive bacteria, Vibrio, Spirochete, Coccidian etc. Used in animal therapy and at sub-therapeutic levels for growth promotion.

Modified after Essington et al. (2010), Sarmah et al. (2006), and Thiele-Bruhn (2003).

†MW = molecular weight; pKa = acidity constant; LogKow = octanol-water partition coefficient.
‡Structure is for tylosin A, which accounts for 80-90% of the parent compound mixture (Sarmah et al., 2006).

4.3. Materials and Methods

4.3.1. Antimicrobial Administration to Cattle

Four pens housing nine steers each at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada research feedlot were randomly assigned to four antimicrobial treatments (kg^{-1} feed) from June to August 2010 (Amarakoon et al., 2014): (i) 44 mg of chlortetracycline, CTC (Aureomycin-100 G, Alpharma Inc., Bridgewater, NJ); (ii) a mixture of 44 mg each of chlortetracycline and sulfamethazine, CTCSMZ (Aureo S-700 G, Alpharma Inc., Bridgewater, NJ) ; (iii) 11 mg of tylosin, TYL (Tylan, Elanco Animal Health, Calgary, AB, Canada); and (iv) no antimicrobials (control). Cattle were fed a barley (*Hordeum vulgare* L.) grain–barley silage diet, in which the antimicrobials were administered as previously described (Amarakoon et al., 2014). A manure: bedding ratio of ~4:1 was maintained in each pen.

4.3.2. Composting Vessel Construction

Composting vessels were constructed as described by Xu et al., (2010). Briefly, cylindrical polyethylene barrels (110 L; 0.7 m in height and 0.45 m in width) and their lids were covered with a ~5 cm layer of polyurethane foam for insulation. At the bottom of each barrel, a 0.1-m height air plenum was created to enable passive aeration by placing a perforated (~1-cm diam. holes) polyethylene disk 0.1-m from the bottom supported by three legs made from polyethylene pipes (~5 cm in diameter). Holes (2.5 cm diam.) were drilled on the side near the bottom of the barrel and on the lid to facilitate aeration through the air plenum created.

4.3.3. Experimental Design and Treatments

Manure was composted in the composting vessels described above. Factorial combinations of antimicrobial treatment (CTC, CTCSMZ, and TYL) and mode of addition to manure (excreted vs. fortified) were tested along with manure containing no antimicrobials (control), resulting in 7 treatments. The experiment was conducted over two 30-d composting cycles, with each cycle containing all 7 treatments and consisting of 2 replicates per treatment. The experimental design was a randomized complete block, with composting cycle (two 30-d periods) as the blocking factor, each of which had two replicate composting vessels per treatment.

In August 2010, manure containing excreted chlortetracycline, sulfamethazine, and tylosin were collected from the feedlot pens where steers were orally administered the corresponding antimicrobials, while manure for the control and fortification treatments was collected from pens in which steers were not administered antimicrobials. Each composting vessel was filled with 45 kg of wet manure (moisture content 0.48 – 0.68 kg kg^{-1}) at the beginning of composting. Manure destined for each composting vessel was mixed separately for 5 min in a 0.34 m^3 capacity mortar mixer (Model 12SGH9, Crown Construction Equipment, Winnipeg, MB, Canada) and immediately transferred to a composting vessel to begin composting.

For the fortified treatments, antimicrobials were sprinkled onto manure during mixing of manure from the control pens. The amount of antimicrobial added to each composter for fortified treatments was calculated based on the amount of antimicrobial administered to the animal in the feed, assuming the feed was 63% digestible and no antimicrobial degradation occurred in the digestive tract. For the fortified CTC treatment, 1.36 g of the chlortetracycline premix Aureomycin 220G (Zoetis Canada, Kirkland, QC; 220 g chlortetracycline kg^{-1} of premix) was

added to 45 kg of wet manure. For the fortified CTCSMZ treatment, 3.89 g of Aureo700G premix (Zoetis Canada, Kirkland, QC; 77 g of chlortetracycline and sulfamethazine kg^{-1} of premix) was added to 45 kg of wet manure. The fortified TYL treatment was prepared by adding 4.69 g of Tylosin 40 premix (Bio Agri Mix, Mitchell, ON, Canada; 88 g of tylosin kg^{-1} of premix) to 45 kg of wet manure. Moisture content of the control manure used for the fortified treatments averaged 0.63 kg kg^{-1}. To facilitate uniform mixing with manure, each antimicrobial premix aliquot was mixed with approximately 100 g of purified fine sand and then sprinkled onto manure in a mortar mixer at a rate of 20 g min^{-1} of mixing.

Mixed manure (300 g) from each treatment was placed in each of six retrievable ~ 15 × 15-cm nylon mesh bags with 3-mm diameter holes. Polyethylene twine was then attached to each mesh bag to facilitate retrieval at sampling. After filling composting vessels to 50% capacity, the six mesh bags were placed in the center of each vessel, with retrieval twines protruding from the top of the vessels. Two thermocouples (T-thermocouples, Thermo Electric, West Chester, PA) were also placed in proximity to the nylon mesh bags in each composting vessel. Each vessel was then filled with a total of 45 kg of manure (including manure in the mesh bags), leaving 5-cm of headspace. The vessels were closed with insulated lids and placed in an unheated room. The temperature in the room was monitored using two thermocouples and ranged from 9 to 25°C, during the 30-d experiment.

4.3.4. Composting Process and Sampling

In each of the two composting cycles described above, manure was composted for 30 d. On Day 0 (i.e., at the start of composting), two subsamples were collected immediately after manure mixing for each composting vessel. Manure was subsequently sampled on after 3, 7, 10, 14 and

16 days of composting. On each sampling day, one nylon mesh bag was retrieved from each vessel and split into two subsamples: one was processed immediately for compost properties, while the second was freeze-dried and stored at -40ºC until antimicrobial extraction. After sampling on Day 16, moisture content in each vessel was adjusted to 0.5 kg kg^{-1} dry manure by adding deionized water. To simulate windrow turning, the contents of each vessel were then transferred to a mortar mixer and mixed for 5 min. Another six nylon mesh bags were filled with composted manure from the same composter following mixing and placed in the middle of each composting vessel during re-filling as described above. Further compost samples were retrieved in mesh bags on Days 18, 22, 24, 28, and 30. Temperature in each vessel was continuously measured at 1-min intervals with 15-min averages recorded for the 30-d of composting and the mixer was thoroughly washed between treatments.

4.3.5. Compost Properties

Fresh manure (150 g) was placed into 500 mL Whirl-Pak® bags, weighed and stored at -25ºC. Prior to analysis, the sample was freeze-dried for 7 d and weighed to determine moisture content. The sample was then ground to pass through a 2 mm screen using a Model 4 Wiley Mill (GMI Inc., Ramsey, MN). A subsample was further ground to < 150 µm using a ball-and-capsule grinder (Model MM2000, Retsch, Haan, Germany). The fine-ground subsample was used to determine total C and N by dry combustion gas chromatography (Model NC 2100, Carlo Erba, Milan, Italy).

4.3.6. Antimicrobial Extraction

4.3.6.1. Pressurized Liquid Extraction

Antimicrobials were extracted from freeze-dried manure samples by pressurized liquid extraction (PLE) followed by solid-phase extraction (SPE) and antimicrobial elution and analysis, as previously described (Sura et al., 2014). Briefly, 2 g of freeze-dried manure were mixed with 20 g of Ottawa sand (Fischer Scientific, Ottawa, ON, Canada) and placed into a stainless steel PLE cell (33 mL). The packed cell was subjected to PLE with an ASE 200 (Dionex, Sunnyvale, CA) using a citric acid buffer solution (pH 5.0) followed by an 80/20 mixture of methanol: citric acid buffer. Temperature was increased from room temperature (22±2ºC) to 75ºC in 5 min where it was held for 2 min during extraction. Each cell was flushed immediately after extraction with the series of solvents described above and purged with N_2 for 90 s. Pressure during extraction was held at 105.5 kg cm^{-2}. Two extraction cycles per sample generated ~44 mL of extract, which was diluted to 250 mL with Milli-Q water prior to SPE.

4.3.6.2. Solid-Phase Extraction

Solid-phase extraction was performed to concentrate antimicrobials extracted from manure by passing the diluted PLE extract through an assembly of an Oasis weak cation exchange (WCX) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA) stacked on top of an Oasis hydrophilic-lipophilic balance (HLB) cartridge (225 mg of sorbent, 60 µm particle size; Waters, Milford, MA) at a flow rate of 100 mL h^{-1}. The cartridge assembly was conditioned with 10 mL of methanol followed by 10 mL of milli-Q water for SPE. After passing the 250 mL of extract through the assembly, the cartridges were rinsed with 10 mL of milli-Q water to remove salt. The cartridges were then dried for 30 s under vacuum and maintained at -10 ºC until elution.

The Oasis HLB cartridge was eluted with 10 mL of methanol while the Oasis WCX cartridge was eluted with 10 mL of methanol followed by 8 mL of methanol containing 2% formic acid. Each eluate was concentrated to a volume of ~200 µL and milli-Q water was used to bring the volume to 1 mL, after which the contents were transferred to a 2-mL amber liquid chromatography vial through a 0.45-µm nylon membrane syringe filter (Chromatographic Specialities Inc., Brockville, ON). The extract was stored at -15ºC until analysis. Each eluent was fortified with a 100 ng $^{13}C_6$-sulfamethazine as an internal standard (Cambridge Isotope Laboratories, Andover, MA) prior to analysis.

4.3.6.3. Antimicrobial Analysis

Antimicrobial concentrations were determined with a liquid chromatography-tandem mass spectrometry (LC-MS-MS; Waters 2965 Alliance Separation Module interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer, Waters Canada, Mississauga, ON). The conditions for LC-MS-MS were adapted from Cessna et al. (2011) as previously described (Amarakoon et al., 2014). Briefly, a C-18 stainless steel column (MS Xterra, 100-mm × 2.1-mm i. d., 3.5 µm diam. packing; Waters Canada, Mississauga, ON) was used for analyte separation. Gradient elution was performed utilizing two mobile phases, both containing 0.1% formic acid. Mobile phase A contained 10:90 acetonitrile/water (v/v) and mobile phase B contained 90:10 acetonitrile/water (v/v). Mobile phase flow rate was 200 µL min^{-1} and the sample extract injection volume was 20 µL. Retention times were 6.83 min for tylosin, 6.92 min for *iso*-chlortetracycline, 7.11 min for sulfamethazine, and 7.11 min for ^{13}C6–sulfamethazine (internal standard). Electrospray ionization mass spectrometry was operated in positive ion mode. Suitable multiple reaction monitoring transitions were used for conformation and quantification of antimicrobials. Data were processed using MassLynx software (v 4.1, Waters

Canada, Mississauga, ON). Antimicrobial concentrations in the samples were corrected for background concentrations in the control samples.

4.3.7. Statistical Analysis

Mean daily temperature data were analyzed with PROC MIXED for repeated measures in SAS 9.4 (SAS Institute Inc., 2013), with sampling time as a repeated fixed factor, antimicrobial treatment as a fixed factor, and composting cycle as a random factor. Based on the Akaike Information Criterion (Littell et al., 2006), the first-order autoregressive [AR(1)] covariance structure was the most suitable of six covariance structures tested in the model.

Data for antimicrobial loss and for percentage losses of dry matter, C, and N, determined as described by Larney and Buckley (2007) were subjected to ANOVA using PROC MIXED, with antimicrobial treatment as a fixed factor and composting cycle as a random factor. The Tukey multiple comparison procedure was used for pairwise comparisons of treatment means. Treatment differences were considered significant at $P < 0.05$.

Dissipation kinetics of antimicrobials during composting were evaluated using PROC NLIN in SAS. Parameter estimates were considered significant if their 95% confidence intervals did not overlap. Dissipation half-lives were computed for each replicate, and the results were then subjected to analysis of variance (ANOVA) using PROC MIXED as described above.

4.4. Results and Discussion

4.4.1. Composting Parameters

4.4.1.1. Temperature

Temperature increased and peaked twice during the 30 d composting – initially during the first 4 d of composting and again during the 4-d period immediately following watering and mixing of compost on Day 16 (Fig. 4.1). The highest temperature ranged from 67ºC to 69ºC and was recorded during the first 4 d of composting (data not presented). The observed increase in temperature reflects an increase in biological activity and in turn successful composting (Larney et al., 2006). The high temperature itself may also play a role in the degradation of organic compounds (Arikan et al., 2009).

Composting is mediated by microorganisms and therefore the presence of antimicrobials could conceivably reduce the rate of composting due to their inhibitory properties. There was an effect of day ($P < 0.0001$) on composting temperature (Fig. 1), but the treatment and treatment × day effects were not significant. Further, the temperature of fortified antimicrobial treatments did not differ from excreted antimicrobial treatments and also the temperature of the control compost did not differ from any of the antimicrobial treatments, indicating that the composting process was not adversely affected by the presence, or the method of addition (excreted vs. fortified), of antimicrobials.

Our results corroborate previous studies, which showed that the presence of chlortetracycline at 113 mg kg^{-1} (dry wt) in beef cattle manure (Arikan et al., 2009) and both chlortetracycline and sulfamethazine at 100 mg kg^{-1} in swine manure (Xun et al., 2013) did not suppress the composting process. However, in contrast, Cessna et al. (2011), using comparable antimicrobial concentrations in manure during windrow composting, observed lower mean

composting temperatures in manure containing tylosin (20.5°C) compared with the control (35°C) and treatments containing either chlortetracycline (35°C) or a mixture of chlortetracycline and sulfamethazine (36°C).

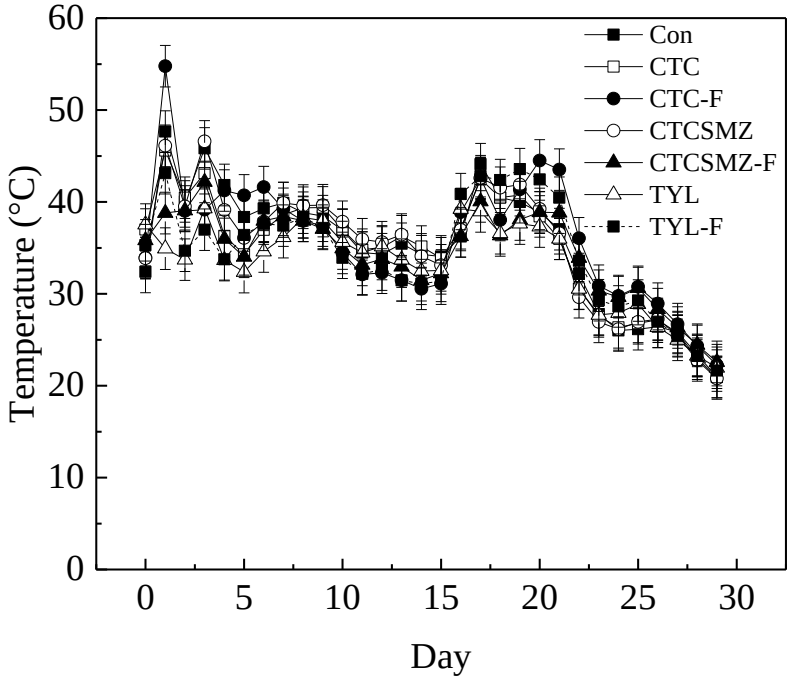


Figure 4.1: Changes in temperature during composting of beef cattle feedlot manure. Con, control treatment (manure containing no antimicrobial); CTC, chlortetracycline treatment; CTC-F, fortified CTC treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine; CTCSMZ-F, fortified CTCSMZ treatment; TYL, tylosin treatment; and TYL-F, fortified TYL treatment. Antimicrobials were either included in the diet or fortified in manure just prior to composting.

4.4.1.2. Carbon, Nitrogen, and Dry Matter Losses

The effects of antimicrobial treatment, including method of introduction into manure (excreted vs. fortified), did not impact C (P = 0.45, mean = 400 g kg^{-1}) or N concentrations (P = 0.73, mean = 31.3 g kg^{-1}) in the final compost. Likewise, treatment effects were non-significant for mass losses of C (P = 0.22, mean = 17%), N (P = 0.28, mean = 7.8%) and DM (P = 0.20, mean = 13%) during composting. Thus, the main physical and chemical properties of compost in this study did not appear to be altered by the presence of antimicrobials or the route to which they were introduced into manure.

4.4.2. Antimicrobial Dissipation

4.4.2.1. Antimicrobial Recovery

Antimicrobial recoveries, determined from freeze-dried control manure (1 g) subsamples fortified with 100 ng of each antimicrobial, were 57 ± 19% for *iso*-chlortetracycline, 32 ± 12% for sulfamethazine, and 57 ± 18% for tylosin. Background concentrations of the antimicrobials in the control manure were 16 µg kg^{-1} for *iso*- chlortetracycline, 2.2 µg kg^{-1} for sulfamethazine, and 9.9 µg kg^{-1} for tylosin. These background levels may reflect migration of residual antimicrobials among pens within the feedlot or residual levels that remained in the pens from previous feeding experiments in the feedlot.

4.4.2.2. Initial Concentrations

The amount of antimicrobial used in fortified treatments did not yield the same antimicrobial concentrations in fortified manure as was achieved when the antimicrobials were fed to and excreted by animals (Table 4.2). In this study, we measured *iso*-chlortetracycline, main metabolite detected in beef cattle feedlot manure formed by irreversible isomerization (Cessna et al., 2011), and the lower concentration of *iso*-chlortetracycline in the fortified

treatments compared to the excreted treatments may be due to insufficient time for conversion to take place in the fortified treatments. However, these differences in antimicrobial concentrations had no significant effect on the composting process, as indicated by the compost properties described above. However, it is not clear whether the differences in the initial concentrations altered the properties of the compost microbiome. Coefficients of variation for initial antimicrobial concentrations in manure in this study were 3 – 58% for subsamples and 7 – 45% for replicates in excreted antimicrobial treatments and were 0.04 – 95% for subsamples and 24 – 64% for replicates in fortified antimicrobial treatments. The higher variability of fortified antimicrobials in subsamples reflects the challenge of achieving uniform mixing during the fortification of cattle manure, a problem identified by others (Carlson and Mabury, 2006; Hamscher et al., 2002).

Table 4.2: Initial antimicrobial concentrations in beef cattle manure used for composting.

Antimicrobial	Antimicrobial formulation†	Method of antimicrobial introduction to manure‡	Antimicrobial concentration in manure prior to composting
			$\mu g\ kg^{-1}$
Chlortetracycline	CTC	Excreted	3138 (1003)§
		Fortified	239 (57)
	CTCSMZ†	Excreted	3211 (212)
		Fortified	183 (94)
Sulfamethazine	CTCSMZ	Excreted	360 (137)
		Fortified	783 (501)
Tylosin	TYL†	Excreted	107 (49)
		Fortified	4453 (1448)

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.

‡ Excreted antimicrobials are antimicrobials excreted in manure after being fed to beef cattle; Fortified antimicrobials are antimicrobials added directly to manure just prior to composting.

§ Concentrations are means of four replicates. Each replicate consisted of two subsamples. Values in parentheses are standard deviations.

4.4.2.3. Dissipation Kinetics

Dissipation data for chlortetracycline, sulfamethazine, and tylosin during 30 d of composting were best described by a first-order kinetic model (Fig 4.2; Table 4.3):

$$C_t = C_0 e^{-kt}$$

where C_t is the antimicrobial concentration (µg kg^{-1}) at time t (d), C_0 is the initial antimicrobial concentration (µg kg^{-1}), and k is the first-order rate constant (d^{-1}). The first-order kinetics observed in the present study were consistent with findings from a previous study using the same antimicrobials in beef cattle manure (Cessna et al., 2011). Dissipation rate constants were significantly greater for excreted (0.29 for CTC and 0.54 d^{-1} for CTCSMZ) than for fortified (0.11 and 0.13 d^{-1}) chlortetracycline. Similar to our results for chlortetracycline, excreted chlortetracycline was found to dissipate faster than fortified chlortetracycline during anaerobic digestion of swine wastewater (Huang et al., 2014). In contrast, dissipation rate constants of fortified sulfamethazine (0.47 d^{-1}) and tylosin (0.31 d^{-1}) in the present study were significantly greater than those of the excreted compounds (0.08 for sulfamethazine and 0.07 d^{-1} for tylosin).

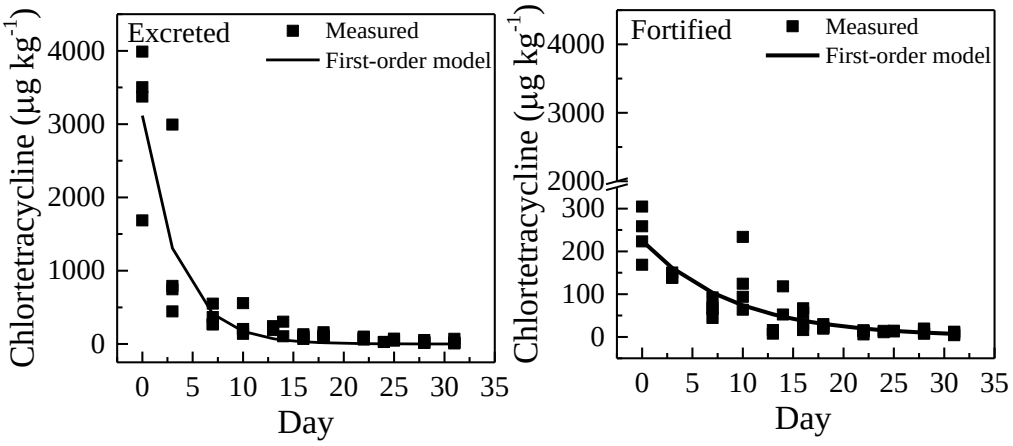


Figure 4.2: First-order dissipation of excreted chlortetracycline [$C_t = 3116e^{-0.29t}$] and fortified chlortetracycline [$C_t = 225e^{-0.11t}$] from the treatment containing chlortetracycline alone (i.e., treatment CTC). C_t, antimicrobial concentration; t, days since the start of composting.

Table 4.3: First-order kinetic model parameters for antimicrobial dissipation over 30 d in vessel composters.

Antimicrobial	Formulation†	Method of AV introduction	Model‡	*k*§	RMSE#	DT_{50}††
				d^{-1}	$\mu g\ kg^{-1}$	d
Chlortetracycline	CTC	Excreted	$C_t = 3116e^{-0.29t}$	0.2898 (0.20-0.37)a¶	995	1.9b
		Fortified	$C_t = 225e^{-0.11t}$	0.1115 (0.08-0.14)b	77	6.3ab
	CTCSMZ†	Excreted	$C_t = 3197e^{-0.54t}$	0.5382 (0.42-0.65)a	920	2.0b
		Fortified	$C_t = 172e^{-0.13t}$	0.1305 (0.09-0.17)b	63	5.6ab
Sulfamethazine	CTCSMZ	Excreted	$C_t = 322e^{-0.08t}$	0.0825 (0.06-0.10)b	105	9.0ab
		Fortified	$C_t = 781e^{-0.46t}$	0.4652 (0.21-0.72)a	269	7.2ab
Tylosin	TYL†	Excreted	$C_t = 119e^{-0.07t}$	0.0722 (0.04-0.10)b	51	12a‡‡
		Fortified	$C_t = 4460e^{-0.31t}$	0.3069 (0.21-0.41)a	1439	1.5b

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.

‡ C_t, antimicrobial concentration; t, day of composting.

§ Values in parentheses are 95% confidence limits for rate constant (*k*) means.

¶ *k* means followed by the different letters within each antimicrobial treatment differ based on the 95% confidence interval.

RMSE, root mean square error.

†† DT_{50}, time for 50% dissipation. DT_{50} means followed by the same letter are not significantly different according to the Tukey-Kramer pairwise comparison procedure ($P < 0.05$).

Chlortetracycline shows strong sorption to clay minerals, with soil partition coefficients (K_d) of 1,208 – 2,386 L kg^{-1}, which are greater than those reported for tylosin (66 – 92 L kg^{-1}) and sulfamethazine (0.6 – 3.2 L kg^{-1}) (Sarmah et al., 2006). However, chlortetracycline sorption has been shown to decrease with increasing organic matter content. Dissolved organic matter reduced the sorption of chlortetracycline to kaolinite and montmorillonite (Essington et al., 2010). Chlortetracycline rapidly forms complexes with clay minerals, but this affinity decreases in the presence of humic substances (Pils and Laird, 2007).

Chlortetracycline in excreted manure is exposed to microbial activity both in the animal's digestive tract and also during the accumulation of manure on the feedlot pen floor. Introduction of an antimicrobials selects for resistance in the microbial community (Schmitt et al., 2006; Thiele-Bruhn and Beck, 2005; Westergaard et al., 2001). Mechanisms of antimicrobial resistance in bacteria include expression of genes that are capable of enzymatically modifying and/or degrading antimicrobials (Speer et al., 1992). Thus, the comparatively greater bioavailability of chlortetracycline in manure, along with the presence of bacteria capable of breaking it down, may have resulted in the faster dissipation of the excreted than the fortified compound.

In contrast to our observations, others have reported lower dissipation percentages for excreted chlortetracycline in broiler manure (92%) and hog manure (27%) than for fortified chlortetracycline in layer-hen manure (94–100%)durng composting (Bao et al., 2009). In the same study, most of the fortified chlortetracycline dissipated during the first 3 d of composting whereas most of the excreted chlortetracycline dissipated over 4–10 d of composting. Chlortetracycline can form strong complexes with divalent cations, such as Ca^{2+} and Mg^{2+}, and subsequently can sorb strongly to organic matter via cation bridging (Parolo et al., 2012; Loke et

al., 2002; Pils and Laird, 2007), a mechanism which may result in strong sorption and contribute to the recalcitrance of excreted chlortetracycline in manure.

Sulfamethazine has been shown to form strong hydrogen (Teixido et al., 2011) and covalent (Bialk et al., 2005; Gulkowska et al., 2013) bonds with organic matter. Sulfamethazine can also form recalcitrant residual fractions with extended contact time (Forster et al., 2009), and its recovery has been shown to decrease with increasing contact time with soil and organic matter (Carstens et al., 2013; Stoob et al., 2007). Similarly, tylosin sorbs strongly to manure, with partition coefficients of 175–840 L kg^{-1} (Clay et al., 2005; Loke et al., 2002; Sarmah et al., 2006). Tylosin is a comparatively large organic molecule (molecular weight = 917 g mol^{-1}) with a log K_{ow} (octanol water partition coefficient) of 3.41 (Sarmah et al., 2006), indicating its strong affinity for organic matter. The sorption of tylosin to manure can exceed its sorption to soil, even with tylosin being predominantly cationic at environmentally relevant pH values and readily participating in cation exchange processes (Essington et al., 2010; Sassman et al., 2007). Thus, the decrease in the rate of microbial and chemical degradability in excreted sulfamethazine and tylosin compared with the fortified compounds could have resulted from the strong sorption to manure during the extended contact time inside the animal and also on the feedlot floor where manure accumulated for 3 mo (June – August).

A wide range of values have previously been reported for the time to 50% dissipation (DT_{50}) during the composting of manure containing antimicrobials. In a laboratory composting study, Bao et al. (2009) reported a DT50 value of 12 d for excreted chlortetracycline in broiler manure, while DT50 values for fortified chlortetracycline were 4–12 d in layer manure and 87 d in swine manure. In another laboratory study, DT_{50} values of 4–5 d were reported for excreted chlortetracycline in beef cattle manure (Arikan et al., 2009). Indoor laboratory composting of

broiler manure containing excreted antimicrobials, further fortified with the same antimicrobial, yielded DT_{50} values of 1 d for sulfadiazine and 2 d for tylosin (Ho et al., 2013). In piled and in-vessel composting studies of turkey litter fortified with antimicrobials, DT_{50} values were 1 d for chlortetracycline and 19 d for tylosin, but there was no measurable degradation for sulfamethazine (Dolliver et al., 2008). In a windrow composting study using beef cattle manure, DT_{50} for excreted antimicrobials ranged from 14 to 27 d for chlortetracycline, 20 to 44 d for tylosin, and 27 to 237 d for sulfamethazine (Cessna et al., 2011).

In a parallel study conducted to characterize antimicrobial resistance determinants in manure treatments tested in this study, the resistance genes *tet*(B), *tet*(L), *tet*(W), *erm*(A), *erm*(B), *erm*(F), *erm*(X), *sul*(1) and *sul*(2) were detected (Xu et al.. unpublished data, 2015). Many of these genes had higher occurrence in excreted than in fortified compost treatments. Field incorporation of manure containing antimicrobials can lead to development of antimicrobial resistance in bacteria in environmental media (Esiobu et al., 2002; Ghosh et al., 2007; Sengelov et al. 2003). The development of resistant phenotypes is a complex process, and the duration for resistance to emerge is a function of many factors such as the concentration of antimicrobial in the environment, the bacterial species, the type of antimicrobial, and the prevailing conditions in the soil. A study by Halling-Sorensen et al. (2005) showed a ~1 – 5% increase in the tylosin- and tetracycline-resistant population in the first set of samples collected 3 d following the field application of manure containing 30-50 $\mu g\ kg^{-1}$ of these antimicrobials in a sandy and a sandy loam soil.

The DT_{50} of excreted antimicrobials in this study decreased in the order tylosin > sulfamethazine > chlortetracycline from CTC > chlortetracycline from CTCSMZ, while that of fortified antimicrobials decreased in the order sulfamethazine > chlortetracycline from CTC >

chlortetracycline from CTCSMZ > tylosin. Our results for excreted antimicrobials are consistent with those from previous studies, which showed DT_{50} values decreasing in the order sulfamethazine > tylosin > chlortetracycline in windrow compost (Cessna et al., 2011), stockpiled beef cattle manure (Sura et al., 2014) and stockpiled and composted turkey litter (Dolliver et al., 2008). The greater persistence of excreted sulfamethazine and tylosin compared with excreted chlortetracycline could have resulted from the strong bonding of sulfamethazine (Bialk et al., 2005; Gulkowska et al., 2013; Teixido et al., 2011) and tylosin (Clay et al., 2005; Loke et al., 2002; Sarmah et al., 2006) with organic matter as discussed above. The opposite was observed for fortified antimicrobials, with chlortetracycline being more persistent than tylosin. The lack of persistence of fortified tylosin as compared to that of fortified chlortetracycline may be due to insufficient time for the formation of strong bonding with manure.

4.4.2.4. Percentage Antimicrobial Dissipation

Concentrations of all antimicrobials in manure decreased by more than 85% during the 30-d indoor composting period (Table 4.4). We found no difference in percent dissipation (i.e., percent reduction in antimicrobial concentration relative to initial concentration) between fortified and excreted chlortetracycline and sulfamethazine at the end of composting. However, for tylosin, the percent dissipation was significantly greater when this antimicrobial was fortified (99%) than when it was excreted (85%). Strong sorption of tylosin to manure during the extended contact time in feedlot pens, as discussed earlier, may have resulted in the lower percent dissipation of excreted tylosin.

Table 4.4: Antimicrobial dissipation (percent of initial concentration) during 30 d of composting.

Antimicrobial	Formulation†	Method of antimicrobial introduction to manure	Percentage dissipation
			%
Chlortetracycline	CTC	Excreted	99a‡
		Fortified	97a
	CTCSMZ†	Excreted	94a
		Fortified	99a
Sulfamethazine	CTCSMZ	Excreted	93a
		Fortified	99a
Tylosin	TYL	Excreted	85b
		Fortified	99a
			P value
Antimicrobial			<0.0001

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.

‡ Means followed by the same letter are not significantly different according to the Tukey-Kramer multiple comparison procedure ($P < 0.05$).

4.5. Conclusions

Composting reduced concentrations of chlortetracycline, sulfamethazine, and tylosin in manure by 85-99% over a 30-d period, indicating its potential to reduce environmental loading of these antimicrobials and hence the risk of antimicrobial resistance development in bacteria within the environment. Temporal changes in concentrations of the three antimicrobials during composting were adequately described by first-order kinetics. Our results showed that first-order dissipation rate constants for excreted chlortetracycline were higher than those for fortified chlortetracycline, whereas fortification produced greater dissipation rate constants for fortified than for excreted sulfamethazine and tylosin. These results indicate that fortified chlortetracycline, sulfamethazine, and tylosin may not accurately reflect the dissipation of these antimicrobials in manure when they are administered in the feed and excreted in feces.

Therefore, caution should be exercised when decision-making is based on the dissipation rates of fortified antimicrobials. To our knowledge, this is the first time that a study of this scale has examined dissipation kinetics of the three antimicrobials in excreted vs. fortified beef cattle manure.

4.6. References

Amarakoon, I.D., F. Zvomuya, A.J. Cessna, D. Degenhardt, F.J. Larney, and T.A. McAllister. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. J. Environ. Qual. 43:549-557.

Arikan, O.A., W. Mulbry, and C. Rice. 2009. Management of antibiotic residues from agricultural sources: Use of composting to reduce chlortetracycline residues in beef manure from treated animals. J. Hazard. Mater. 164:483-489.

Arikan, O.A., L.J. Sikora, W. Mulbry, S.U. Khan, and G.D. Foster. 2007. Composting rapidly reduces levels of extractable oxytetracycline in manure from therapeutically treated beef calves. Bioresour. Technol. 98:169-176.

Bao, Y.Y., Q.X. Zhou, L.Z. Guan, and Y.Y. Wang. 2009. Depletion of chlortetracycline during composting of aged and spiked manures. Waste Manage. 29:1416-1423.

Bialk, H.M., A.J. Simpson, and J.A. Pedersen. 2005. Cross-coupling of sulfonamide antimicrobial agents with model humic constituents. Environ. Sci. Technol. 39:4463-4473.

Boxall, A.B.A., L.A. Fogg, P.A. Blackwell, P. Kay, E.J. Pemberton, and A. Croxford. 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. 180:1-91.

Burkhardt, M., C. Stamm, C. Waul, H. Singer, and S. Muller. 2005. Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. J. Environ. Qual. 34:1363-1371.

Carlson, J.C. and S.A. Mabury. 2006. Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environ. Toxicol. Chem. 25:1-10.

Carstens, K.L., A.D. Gross, T.B. Moorman, and J.R. Coats. 2013. Sorption and photo degradation processes govern distribution and fate of sulfamethazine in freshwater-sediment microcosms. Environ. Sci. Technol. 47:10877-10883.

Cessna, A.J., F.J. Larney, S.L. Kuchta, X. Hao, T. Entz, E. Topp, and T.A. McAllister. 2011. Veterinary antimicrobials in feedlot manure: Dissipation during composting and effects on composting processes. J. Environ. Qual. 40:188-198.

Chee-Sanford, J.C., R.I. Mackie, S. Koike, I.G. Krapac, Y.F. Lin, A.C. Yannarell, S. Maxwell, and R.I. Aminov. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38:1086-1108.

Clay, S., Z. Liu, R. Thaler, and H. Kennouche. 2005. Tylosin sorption to silty clay loam soils, swine manure, and sand. J. Environ. Sci. and Health B 40:841-850.

Dolliver, H. and S. Gupta. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. 37:1227-1237.

Dolliver, H., S. Gupta, and S. Noll. 2008. Antibiotic degradation during manure composting. J. Environ. Qual. 37:1245-1253.

Esiobu, N., L. Armenta, and J. Ike. 2002. Antibiotic resistance in soil and water environments. Int. J. Environ. Health Res. 12:133-144.

Essington, M.E., J. Lee, and Y. Seo. 2010. Adsorption of antibiotics by montmorillonite and kaolinite. Soil Sci. Soc. Am. J. 74:1577-1588.

Forster, M., V. Laabs, M. Lamshoft, J. Groeneweg, S. Zuhlke, M. Spiteller, M. Krauss, M. Kaupenjohann, and W. Amelung. 2009. Sequestration of manure-applied sulfadiazine residues in soils. Environ. Sci. Technol. 43:1824-1830.

Ghosh, S. and T.M. LaPara. 2007. The effects of sub-therapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME J. 1:191-203.

Gulkowska, A., M. Sander, J. Hollender, and M. Krauss. 2013. Covalent binding of sulfamethazine to natural and synthetic humic acids: Assessing laccase catalysis and covalent bond stability. Environ. Sci. Technol. 47:6916-6924.

Halling-Sørensen, B., A.M. Jacobsen, J. Jensen, G. Sengelov, E. Vaclavik, and F. Ingerslev. 2005. Dissipation and effects of chlortetracycline and tylosin in two agricultural soils: A field-scale study in southern Denmark. Environ. Toxicol. Chem. 24:802-810.

Hamscher, G., S. Sczesny, H. Hoper, and H. Nau. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. 74:1509-1518.

Ho, Y.B., M.P. Zakaria, P.A. Latif, and N. Saari. 2013. Degradation of veterinary antibiotics and hormone during broiler manure composting. Bioresour. Technol. 131:476-484.

Huang, L., X. Wen, Y. Wang, Y. Zou, B. Ma, X. Liao, J. Liang, and Y. Wu. 2014. Effect of the chlortetracycline addition method on methane production from the anaerobic digestion of swine wastewater. J. Environ. Sci. (China) 26:2001-2006.

Kemper, N., H. Farber, D. Skutlarek, and J. Krieter. 2008. Analysis of antibiotic residues in liquid manure and leachate of dairy farms in northern Germany. Agric. Water Manage. 95:1288-1292.

Kim, K., G. Owens, S. Kwon, K. So, D. Lee, and Y.S. Ok. 2011. Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut. 214:163-174.

Larney, F.J. and K.E. Buckley. 2007. Dry matter mass balance estimates for composted feedlot manure. Compost Sci. Util. 15:222-227.

Larney, F.J., K.E. Buckley, X. Hao, and W.P. McCaughey. 2006. Fresh, stockpiled, and composted beef cattle feedlot manure: Nutrient levels and mass balance estimates in Alberta and Manitoba. J. Environ. Qual. 35:1844-1854.

Larney, F.J. and X. Hao. 2007. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta, Canada. Bioresour. Technol. 98:3221-3227.

Larney, F.J., L.J. Yanke, J.J. Miller, and T.A. McAllister. 2003. Fate of coliform bacteria in composted beef cattle feedlot manure. J. Environ. Qual. 32:1508-1515.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D.Wolfinger (eds.) 2006. SAS system for mixed models. 2nd ed. SAS Inst. Inc., Cary, NC.

Loke, M.L., J. Tjornelund, and B. Halling-Sorensen. 2002. Determination of the distribution coefficient (log K-d) of oxytetracycline, tylosin A, olaquindox and metronidazole in manure. Chemosphere 48:351-361.

Parolo, E.M., M.J. Avena, G.R. Pettinari, and M.T. Baschini. 2012. Influence of Ca^{2+} on tetracycline adsorption on montmorillonite. J. Colloid Interface Sci. 368:420-426.

Pils, J.R.V. and D.A. Laird. 2007. Sorption of tetracycline and chlortetracycline on K- and Ca-saturated soil clays, humic substances, and clay-humic complexes. Environ. Sci. Technol. 41:1928-1933.

Ramaswamy, J., S.O. Prasher, R.M. Patel, S.A. Hussain, and S.F. Barrington. 2010. The effect of composting on the degradation of a veterinary pharmaceutical. Bioresour. Technol. 101:2294-2299.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725-759.

Sassman, S.A., A.K. Sarmah, and L.S. Lee. 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environ. Toxicol. Chem. 26:1629-1635.

Schmitt, H., K. Stoob, G. Hamscher, E. Smit, and W. Seinen. 2006. Tetracyclines and tetracycline resistance in agricultural soils: Microcosm and field studies. Microb. Ecol. 51:267-276.

Sengeløva, G., Y. Agersøa, B. Halling-Sørensenb, S.B. Balodaa, J.S. Andersena, and L.B. Jensen. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environ. Int. 28:587–595.

Speer, B.S., N.B. Shoemaker, and A.A. Salyers. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin. Microbiol. Rev. 5:387-399.

Stoob, K., H.P. Singer, S.R. Mueller, R.P. Schwarzenbach, and C.H. Stamm. 2007. Dissipation and transport of veterinary sulfonamide antibiotics after manure application to grassland in a small catchment. Environ. Sci. Technol. 41:7349-7355.

Sura, S., D. Degenhardt, A.J. Cessna, F.J. Larney, A.F. Olson, and T.A. McAllister. 2014. Dissipation of three veterinary antimicrobials in beef cattle feedlot manure stockpiled over winter. J. Environ. Qual. 43:1061-1070.

Teixido, M., J.J. Pignatello, J.L. Beltran, M. Granados, and J. Peccia. 2011. Speciation of the ionizable antibiotic sulfamethazine on black carbon (biochar). Environ. Sci. Technol. 45:10020-10027.

Thiele-Bruhn, S. 2003. Pharmaceutical antibiotic compounds in soils - A review. J. Plant Nutr. Soil Sc. 166:546-546.

Thiele-Bruhn, S. and I.C. Beck. 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. Chemosphere 59:457-465.

Westergaard, K., A.K. Muller, S. Christensen, J. Bloem, and S.J. Sorensen. 2001. Effects of tylosin as a disturbance on the soil microbial community. Soil Biol. Biochem. 33:2061-2071.

Xu, S., T.A. McAllister, J.J. Leonard, O.G. Clark, and M. Belosevic. 2010. Assessment of microbial communities in decomposition of specified risk material using a passively aerated laboratory-scale composter. Compost Sci. Util. 18:255-265.

Xun, P., Q. Zhi-min, and B. Wei-wei. 2013. Effects of high-temperature composting on degradation of antibiotics in swine manure. J. Ecol. Rural Environ. 29:64-69.

Zhang, X.X., T. Zhang, and H. Fang. 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82:397-414.

5. OVERALL SYNTHESIS

5.1 Summary Findings and Contributions to the Knowledge

Antimicrobials are widely used in North America's livestock industry (Sarmah et al. 2006), thus a large volume of manure containing antimicrobials is generated annually (Roe and Pillai 2003; Chee-Sanford et al. 2009) which is often land-applied to recycle nutrients and also as a means of livestock waste disposal (Boxall et al. 2004; Sarmah et al. 2006). Management of the antimicrobials, once released to the environment is important to prevent their potential adverse effects on bacteria within the environment (Halling-Sorensen 2001; Halling-Sorensen et al. 2002; Toth et al. 2011; Fang et al. 2014) and their ability to promote the development of antimicrobial resistance in bacteria that could be pathogenic to livestock or humans or both (Chee-Sanford et al. 2009). Information on the transport behavior of antimicrobials and the environmental persistence of antimicrobials are key management tools that were the focus of the studies reported in Chapters 2 and 3 of this thesis. The antimicrobials studied (chlortetracycline, sulfamethazine and tylosin), are commonly used in the livestock industry, both therapeutically and sub-therapeutically in beef cattle and have relevance to human health (Sarmah et al. 2006).

The simulated rainfall runoff study reported in Chapter 2 showed that antimicrobial concentrations in runoff from plots receiving manure generally reflected the concentrations of the antimicrobials in the manure applied. This study is one of a handful of studies that have examined surface runoff of antimicrobials in a field following the application of beef cattle manure.

Both chlortetracycline and sulfamethazine were persistent in the seasonally-frozen soil tested (Chapter 3), increasing the risk of runoff and plant uptake during the growing season and

the build-up of residues as a result of repeated manure applications. Chlortetracycline from the CTCSMZ treatment dissipated faster during the growing season (DT_{50} = 77 d) than during the non-growing season when the soil was frozen for an extended period (DT_{50} = 648 d). By comparison, dissipation of chlortetracycline added alone did not differ significantly between the two seasons (mean DT_{50} = 121 d). Sulfamethazine was detected at concentrations of up to 16 ± 10 μg kg^{-1} throughout the study, contrary to evidence from short term experiments, which suggests that sulfonamides have low persistence. Tylosin concentration was ≤ 11 ± 6.6 μg kg^{-1} and gradually dissipated, indicating a comparatively low risk for residue build-up and subsequent offsite contamination.

Composting is a relatively new management tool in feedlots (Larney and Hao 2007) that is gaining acceptance owing to the benefits that it brings to manure handling. The composting study reported in Chapter 4 of this thesis showed that composting dissipated 85–99% of initial antimicrobial concentrations in manure within 30 d.

Fortification with antimicrobials is one approach commonly used in studies on the dissipation behavior of antimicrobials in manure, with the assumption that the behavior of fortified antimicrobials is the same as that of the excreted antimicrobials. We tested the dissipation behaviour of fortified vs. that of excreted antimicrobials (Chapter 4) and found the first order dissipation rate constant (k) to be significantly greater for excreted chlortetracycline (0.29 d^{-1} - 0.54 d^{-1}) than for the fortified (0.11 d^{-1} - 0.13 d^{-1}) compound. In contrast, dissipation was significantly greater for fortified sulfamethazine (0.47 d^{-1}) and tylosin (0.31 d^{-1}) than when these antimicrobials were excreted (0.08 d^{-1} for sulfamethazine and 0.07 $d^{-1}$for tylosin).

5.2 Implications of the Research

Information on the transport behavior (Chapter 2) and persistence (Chapter 3) of chlortetracycline, sulfamethazine and tylosin can be used in future to develop models and make recommendations on suitable application rates of manure containing antimicrobials so as to reduce the risk of residue build-up in receiving soils and increasing the risk of antimicrobial transport to surface and ground water. As information on the existing levels of resistance in the environment and threshold concentrations for resistance development in environmental bacteria becomes available, it can be used in conjunction with results on the transport behavior and persistence of the antimicrobials to make informed decisions on minimizing or even discontinuing the use of a particular antimicrobial in livestock. We observed a greater persistence of chlortetracycline and sulfamethazine than reported in previous short-term studies that were conducted under warm soil conditions, reflecting the effect that frozen soils may have on the dissipation of antimicrobials in the Canadian prairies (Chapter 3). This finding highlights the importance of region-specific antimicrobial persistence information in model development, guideline formulation, and decision making.

The observed reduction in chlortetracycline and sulfamethazine concentrations in runoff and in mass loss of chlortetracycline with manure incorporation compared to surface application (Chapter 2) strengthens the rationale behind the recommendation to incorporate manure into soil immediately after application. Prevention of antimicrobial dispersal to agricultural soils is more desirable than attempting to contain the antimicrobials in the receiving soil or accelerate their in-situ degradation. Evidence reported in Chapter 4 of this thesis points to the effectiveness of composting as a management tool for controlling antimicrobial dispersal to cropland. Composting also has other documented benefits, including reduction of pathogens and weed seed

viability, and concentration of plant nutrients via reduction of the mass and volume of manure, hence increasing the economical distance for transportation (Larney et al. 2003; Larney et al. 2006). Composting should therefore be promoted as a routine manure handling practice. Nonetheless, as pointed out by other researchers (Dolliver et al. 2008; Sura et al. 2014), the economics and feasibility of composting needs further evaluation versus options such as stockpiling and minimally managed composting, which reduce labour and associated costs.

5.3 Recommendations

5.3.1 Research Focused Recommendations

Our antimicrobial runoff field study was conducted under a simulated high intensity rainfall event (Chapter 2). To our knowledge, runoff losses of the three antimicrobials during natural rainfall and snow melt events have not been fully examined and should therefore be a focus of future studies. Our understanding of dissolved and particulate bound antimicrobial losses in runoff is also currently limited and warrants investigation to provide an insight into the loss processes as well as to inform the design of effective mitigation strategies. Compared to surface movement of antimicrobials, even fewer studies have investigated the potential leaching of antimicrobials field applied with manure.

The greater persistence observed for chlortetracycline and sulfamethazine in the seasonally-frozen soil (Chapter 3) points to the importance of developing a region-specific persistence database for antimicrobials commonly used in livestock. Such information could be used to minimize crop exposure to chlortetracycline and sulfamethazine residues in manured soils. In comparison with animal/poultry-based meat products, contamination of plant based produce with veterinary antimicrobials has received limited attention (Kumar et al. 2005;

Dolliver et al. 2007) and therefore calls for greater focus in future studies. Such studies should focus on environmentally relevant concentrations such as presented in this thesis (Chapter 3), and should culminate in the setting of maximum residue limits for antimicrobial compounds.

As demonstrated by our composting study (Chapter 4), dissipation rates of fortified chlortetracycline, sulfamethazine and tylosin during composting does not accurately reflect those of the excreted antimicrobials. Results from studies employing fortification should therefore be interpreted with caution. Importantly, our results point to the need for future studies to utilize excreted antimicrobials in dissipation studies so that more reliable results can be realized.

Beef cattle feedlot manure typically contains a mixture of antimicrobials rather than a single antimicrobial at a given time, often in conjunction with other organic compounds, such as steroids. Understanding the effect of a mixture of these compounds should be a paramount consideration in future studies. Studies reported in this thesis (Chapters 2-4) are a step in that direction and should inspire future studies.

Metabolites of sulfamethazine were not included in the studies presented in this thesis. Future studies should include these metabolites in the analyses to avoid any potential underestimation of dissipation rates. Following administration the animal, sulfamethazine can undergo inactivation inside the host liver by conjugation with acetyl, forming N^4-acetyl-sulfamethazine. Upon excretion, microbes can degrade the acetyl, converting it back to the parent form (Sarmah et al. 2006). Although limited, the presence of this metabolite was reported in cattle and swine manure (Haller et al. 2002; Heuer et al. 2008), waste water (Garcia-Galan et al. 2012), soil (Garcia-Galan et al. 2013), groundwater (Garcia-Galan et al. 2010), and surface water (Stoob et al. 2005). The environmental significance of this de-conjugation is that it

transforms a biologically inactive metabolite back to the bioactive form, thereby increasing the risk of antimicrobial resistance that may arise as a result of the presence of sulfonamides.

Uniform mixing of beef cattle manure is always a challenge due to its heterogeneous nature, largely a result of the bedding material used in feedlot pens. Fortification of such manure with antimicrobials is therefore inevitably associated with high coefficients of variation (Chapter 4). To minimize the variability, we mixed the antimicrobials with purified sand prior to sprinkling onto manure in a motor mixer. Variability is particularly large and a greater challenge in field studies when solid beef cattle manure is applied to plots (Chapter 3), as has been acknowledged in previous studies (Hamscher et al. 2002; Kay et al. 2004; Carlson Mabury 2006). We used four replicates per treatment and four subsamples per replicate, but still the statistical power was not high enough to detect differences that were evidently large. Future studies should therefore consider the use of more replicates, if feasible, to optimize the statistical power.

5.3.2 General Recommendations

The use of antimicrobials is integral to today's livestock industry. Zero emissions of antimicrobials from livestock use to the environment would be ideal to reduce environmental contamination, but very difficult to achieve in confined livestock operations without incurring significant economic losses. Sweden took the initiative to ban the use of antimicrobial as growth promoters in livestock in 1986. Denmark discontinued the use of antimicrobial growth promoters in March 1998. The use of antimicrobials for growth promotion was banned in the European Union in January 2006, with a few exceptions. North America is also considering restrictions on the routine sub-therapeutic administration of antimicrobials to healthy animals for growth

promotion at the potential expense of human health. Assessments should be conducted to determine whether significant performance benefits can be gained by the use of sub-therapeutic antimicrobials in low risk livestock; such information would help in deciding on whether to limit the sub-therapeutic use in livestock (Stanford et al. 2015).

It has been argued that a decrease in sub-therapeutic use may increase therapeutic use of antimicrobials in livestock due to less protection from diseases. Denmark exhibited approximately a 5% increase in therapeutic use following the elimination of sub-therapeutic use (Dibner and Richards 2005). However, it is difficult to attribute the total increase in antimicrobial use to the banning of sub-therapeutic antimicrobials as therapeutic use itself is continuously increasing with time regardless of changes to the sub-therapeutic use (Sarmah et al. 2006). Either way, antimicrobials should not be a replacement for improving management practices that reduce infectious diseases in livestock. Alternatives to antimicrobials should be further explored that can bring comparable performance benefits to livestock (Narvaez et al. 2013; Kolotilin et al. 2014).

Overall, studies presented in this thesis represent a significant contribution to the knowledge base of the environmental fate of chlortetracycline, sulfamethazine, and tylosin. This contribution will assist policy makers in making informed decisions on the use of antimicrobials in livestock. The thesis also identifies existing knowledge gaps that should be the subject of future studies.

5.1 References

Boxall, A. B. A., Fogg, L. A., Blackwell, P. A., Kay, P., Pemberton, E. J. and Croxford, A. 2004. Veterinary medicines in the environment. Rev. Environ. Contam. Toxicol. **180:** 1-91.

Carlson, J. C. and Mabury, S. A. 2006. Dissipation kinetics and mobility of chlortetracycline, tylosin, and monensin in an agricultural soil in Northumberland County, Ontario, Canada. Environ. Toxicol. Chem. **25:** 1-10.

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S. and Aminov, R. I. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. **38:** 1086-1108.

Dibner, J. J. and Richards, J. D. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. **84:** 634-643.

Dolliver, H. and Gupta, S. 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. J. Environ. Qual. **37:** 1227-1237.

Dolliver, H., Gupta, S. and Noll, S. 2008. Antibiotic degradation during manure composting. J. Environ. Qual. **37:** 1245-1253.

Dolliver, H., Kumar, K. and Gupta, S. 2007. Sulfamethazine uptake by plants from manure-amended soil. J. Environ. Qual. **36:** 1224-1230.

Fang, H., Han, Y., Yin, Y., Pan, X. and Yu, Y. 2014. Variations in dissipation rate, microbial function and antibiotic resistance due to repeated introductions of manure containing sulfadiazine and chlortetracycline to soil. Chemosphere **96:** 51-56.

Garcia-Galan, M., Diaz-Cruz, S. and Barcelo, D. 2013. Multiresidue trace analysis of sulfonamide antibiotics and their metabolites in soils and sewage sludge by pressurized liquid extraction followed by liquid chromatography-electrospray-quadrupole linear ion trap mass spectrometry. J. Chromatogr. A **1275:** 32-40.

Garcia-Galan, M., Froemel, T., Mueller, J., Peschka, M., Knepper, T., Diaz-Cruz, S. and Barcelo, D. 2012. Biodegradation studies of N (4)-acetylsulfapyridine and N (4)-acetylsulfamethazine in environmental water by applying mass spectrometry techniques. Anal. Bioanal. Chem. **402:** 2885-2896.

Garcia-Galan, M., Garrido, T., Fraile, J., Ginebreda, A., Silvia Diaz-Cruz, M. and Barcelo, D. 2010. Simultaneous occurrence of nitrates and sulfonamide antibiotics in two ground water bodies of Catalonia (Spain). J. Hydrol. **383:** 93-101.

Haller, M. Y., Muller, S. R., McArdell, C. S., Alder, A. C. and Suter, M. J. F. 2002. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. J. Chromatogr. A **952:** 111-120.

Halling-Sorensen, B. 2001. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Arch. Environ. Contam. Toxicol. **40:** 451-460.

Halling-Sorensen, B., Sengelov, G. and Tjornelund, J. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch. Environ. Contam. Toxicol. **42:** 263-271.

Hamscher, G., Sczesny, S., Hoper, H. and Nau, H. 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. **74:** 1509-1518.

Heuer, H., Focks, A., Lamshoeft, M., Smalla, K., Matthies, M. and Spiteller, M. 2008. Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol. Biochem. **40:** 1892-1900.

Kay, P., Blackwell, P. A. and Boxall, A. B. A. 2004. Fate of veterinary antibiotics in a macroporous tile drained clay soil. Environ. Toxicol. Chem. **23:** 1136-1144.

Kolotilin, I., Topp, E., Cox, E., Devriendt, B., Conrad, U., Joensuu, J., Stger, E., Warzecha, H., McAllister, T., Potter, A., McLean, M. D., Hall, J. C. and Menassa, R. 2014. Plant-based solutions for veterinary immunotherapeutics and prophylactics. Vet. Res. **45:** 117.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y. and Rosen, C. J. 2005. Antibiotic uptake by plants from soil fertilized with animal manure. J. Environ. Qual. **34:** 2082-2085.

Larney, F. J., Buckley, K. E., Hao, X. and McCaughey, W. P. 2006. Fresh, stockpiled, and composted beef cattle feedlot manure: Nutrient levels and mass balance estimates in Alberta and Manitoba. J. Environ. Qual. **35:** 1844-1854.

Larney, F. J. and Hao, X. 2007. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta, Canada. Bioresour. Technol. **98:** 3221-3227.

Larney, F. J., Yanke, L. J., Miller, J. J. and McAllister, T. A. 2003. Fate of coliform bacteria in composted beef cattle feedlot manure. J. Environ. Qual. **32:** 1508-1515.

Narvaez, N., Wang, Y. and McAllister, T. 2013. Effects of extracts of Humulus lupulus (hops) and Yucca schidigera applied alone or in combination with monensin on rumen fermentation and microbial populations in vitro. J. Sci. Food Agric. **93:** 2517-2522.

Roe, M. T. and Pillai, S. D. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. Poult. Sci. **82:** 622-626.

Sarmah, A. K., Meyer, M. T. and Boxall, A. B. A. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere **65:** 725-759.

Stanford, K., Gibb, D. J., Schwartzkopf-Genswein, K. S., Van Herk, F. and McAllister, T. A. 2015. Feeding sub-therapeutic antimicrobials to low-risk cattle does not confer consistent performance benefits. Can. J. Anim. Sci. **95:** 589-597.

Stoob, K., Singer, H. P., Goetz, C. W., Ruff, M. and Mueller, S. R. 2005. Fully automated online solid phase extraction coupled directly to liquid chromatography-tandem mass spectrometry - Quantification of sulfonamide antibiotics, neutral and acidic pesticides at low concentrations in surface waters. J. Chromatogr. A **1097:** 138-147.

Sura, S., Degenhardt, D., Cessna, A. J., Larney, F. J., Olson, A. F. and McAllister, T. A. 2014. Dissipation of three veterinary antimicrobials in beef cattle feedlot manure stockpiled over winter. J. Environ. Qual. **43:** 1061-1070.

Toth, J. D., Feng, Y. and Dou, Z. 2011. Veterinary antibiotics at environmentally relevant concentrations inhibit soil iron reduction and nitrification. Soil Biol. Biochem. **43:** 2470-2472.

APPENDIX I

MICROWAVE-ASSISTED EXTRACTION OF CHLORTETRACYCLINE, SULFAMETHAZINE AND TYLOSIN FROM MANURE AND MANURE-AMENDED SOILS

Abstract

Microwave-assisted extraction (MAE) is an emerging technique used in the extraction of organic compounds from various matrices. The objective of this study was to develop an MAE method to simultaneously extract the antimicrobials, chlortetracycline, sulfamethazine, and tylosin, from beef cattle manure and manure-amended soil. Manure samples were collected from beef cattle feedlot pens at Agriculture and Agri-Food Canada, Lethbridge Research Center (LRC), Canada, receiving chlortetracycline, both chlortetracycline and sulfamethazine, tylosin or no antimicrobial (control) in feed. Manure-amended soil samples were collected from LRC research plots in which manure from the feedlot pens described above had been incorporated into the 0- to 10 cm soil layer at a rate of 60 Mg ha^{-1}. Antimicrobial recovery from fortified beef cattle manure was 71 ± 24% for chlortetracycline, 24 ± 3% for sulfamethazine and 47 ± 9% for tylosin. Antimicrobial recovery from fortified manure-amended soil was 35 ± 9% for chlortetracycline, 48 ± 5% for sulfamethazine and 82 ± 18% for tylosin. Microwave-assisted extraction and pressurized liquid extraction (PLE) were then compared using manure and manure-amended soil containing excreted antimicrobials, and MAE produced greater recoveries for chlortetracycline than PLE, but both methods were equally efficient for the extraction of sulfamethazine and tylosin from manure. Both PLE and MAE were equally efficient in extracting all three antimicrobials listed from soil.

Introduction

Antimicrobials are emerging contaminants in soil and water. Once distributed in the environment, antimicrobials can be directly toxic to environmental bacteria, but they may also increase the level of antimicrobial resistance in human and livestock pathogens (Boxall et al., 2003; Chee-Sanford et al., 2009; Sarmah et al., 2006). Livestock manure is a significant source of antimicrobials in the environment via direct runoff from stored manure containing antimicrobials and from cropland receiving such manure (Amarakoon et al., 2014; Dolliver and Gupta, 2008). A fast and reliable, multi residue technique for antimicrobial determination in environmental solids is needed to generate data that can be used to develop management recommendations for these compounds in the environment. Antimicrobial extraction and quantification techniques have been largely developed in the last decade primarily for water samples. Techniques to extract residues from environmental solids are important, yet the extraction and quantification of the target analytes from manure and soils is challenging due to the complex nature of these environmental matrices. This partly explains why only a handful of studies on such techniques have been reported to date. The majority of published studies on the extraction of antimicrobials from environmental solids (soil, manure and sediment) have employed mechanical shaking (Hoese et al., 2009; Hoese et al., 2009; Raich-Montiu et al., 2010; Sassman et al., 2007), pressurized liquid extraction (PLE) (Amarakoon et al., 2014; Raich-Montiu et al., 2010; Stoob et al., 2006; Sura et al., 2014), and microwave-assisted extraction (MAE) (Balakrishnan et al., 2014; Chen et al., 2009; Hu et al., 2010; Raich-Montiu et al. 2007; Raich-Montiu et al. 2010; Speltini et al., 2011).

Microwave systems heat up rapidly using microwave energy, partitioning the target analyte from the sample matrix into the solvent. The microwave system, with its multi vessel carousel

system, can accommodate a larger number of samples per run compared with PLE. Its closed vessel system enables the heating of solvents to temperatures higher than their boiling points and also often uses low solvent volumes, reducing the volume of chemical waste produced (Eskilsson and Bjorklund, 2000). Automation of the analytical process with MAE is feasible and has been demonstrated from antimicrobial extraction up to cleaning and pre-concentration of antimicrobials via solid-phase extraction (SPE) (Chen et al., 2009), with potential for automation up to the detection stage in the future. Microwave-assisted extraction therefore has the potential to be a reliable and rapid extraction procedure for antimicrobials from environmental solids such as soil, manure, sediments, biosolids and sludge (Balakrishnan et al., 2014; Hu et al., 2010; Raich-Montiu et al., 2010; Speltini et al., 2011). The main objective of this study was to develop an MAE method to simultaneously extract chlortetracycline, sulfamethazine, and tylosin, three antimicrobials commonly used in beef cattle, from beef cattle manure and manure-amended soils. Study was then extended to a comparison MAE vs. PLE of cattle excreted antimicrobial residues in manure and manure-amended soil.

Materials and Methods

Collection of Manure and Manure-Amended Soil

Manure was sourced from beef cattle feedlots receiving chlortetracycline (44 mg kg^{-1} feed), a 1:1 mixture of chlortetracycline and sulfamethazine (each at 44 mg kg^{-1} feed), tylosin (11 mg kg^{-1} feed) or no antimicrobial (control pens) at Agriculture and Agri-Food Canada Lethbridge Research Centre, AB, Canada. Manure-amended soil was sampled (0-10 cm layer) from a field at the Lethbridge Research Center where manure from the feedlot pens described above was incorporated into the 0–10 cm soil layer at a rate of 60 Mg wet weight ha^{-1}. Both

manure and manure-amended soil samples were freeze-dried following collection and then ground to pass through a 2-mm screen.

Microwave-Assisted Extraction

Conditions for extraction were adapted from Amarakoon et al. (2014). Manure or manure-amended soil (1 g) was weighed into a 10-mL beaker and transferred into a 75-mL Teflon microwave digestion vessel. Two solvents used in the extraction process were i) 0.2 M citric acid buffer (pH 4.7) and ii) 80/20 methanol: citric acid buffer. Microwave-assisted extraction was performed with a Mars Xpress microwave system (CEM Corporation, NC), which had a 40 vessel carousel and operated in closed vessel mode. Two extraction cycles per sample were used, first with 60 mL of 80/20 methanol: citric acid buffer solution followed by 40 mL of citric acid buffer. The sample/solvent mixture was held at 75 °C for 5 min during each extraction cycle. Ramping time (time taken to reach the temperature) was 10 min. The two extracts (80:20 methanol: citric acid buffer and citric acid buffer solution) were decanted from the digestion vessel into a single 100-mL beaker and then transferred into two 50-mL polypropylene centrifuge tubes (Fisher Scientific, Canada). The extracts were centrifuged for 10 min. at 3100 rpm (52 Hz) and the supernatant from each centrifuge tube was then transferred into a 500-mL amber bottle for overnight storage at 4ºC. On the following day, the extract (~ 100 mL) was diluted to 500 mL with milli-Q water prior to SPE to dilute the methanol in the sample extract.

Solid-Phase Extraction, Elution and Sample Analysis

The diluted MAE extract (500 mL) was subjected to SPE and subsequently to elution as previously described (Amarakoon et al., 2014). Briefly, SPE was carried out using an Oasis hydrophilic-lipophilic balance (HLB) cartridge (225 mg of sorbent, 60 µm particle size; Waters) stacked on top of an Oasis weak cation exchange (WCX) cartridge (225 mg of sorbent, 60 µm particle size; Waters). Both cartridges were pre-conditioned in tandem with methanol (10 mL), followed by deionized water (10 mL). The diluted MAE extract was passed through the cartridge assembly under vacuum at a flow rate of 1 mL min^{-1}. Cartridges were then washed with deionized water (10 mL) to remove excess salts and then dried under vacuum for 1 min, after which it was maintained at 4°C for up to 7 d until elution.

The cartridges were separated after SPE and eluted into two separate graduated test tubes. The HLB cartridge was eluted with 8 mL of methanol while the WCX cartridge was eluted with 4 mL of methanol followed by 4 mL of methanol containing 2% formic acid. Both eluents were evaporated to 200 µL under a steady stream of air. The concentrated eluents were brought to 1 mL with deionized water, vortexed, and transferred to a 2-mL amber liquid chromatography vial through a 0.45-µm nylon membrane syringe filter (Chromatographic Specialities Inc.) equipped with a 3-mL disposable syringe (BD Diagnostics). All residue extracts were maintained at –30°C until analysis. Residue extracts were analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS, Waters 2965 Alliance Separation module interfaced with the Micromass Quattro Ultima triple quadrupole mass spectrometer, Waters). The conditions for LC-MS/MS analysis have previously been reported (Amarakoon et al., 2014). Data analysis was performed using MassLynx software (v4.1, Waters).

Fortification with Antimicrobials for Testing Recovery

Control (no antimicrobials) manure or control manure-amended soil (1 g) was weighed into a beaker and spiked with *iso*-chlortetracycline, sulfamethazine and tylosin (Fisher Scientific, Canada) at a rate of 100 µg kg^{-1} manure or soil. An aliquot (100 µL) of solution containing the three antimicrobials, each at 1 mg L^{-1} in milli-Q water was added to 1 g of manure or manure-amended soil. A solution of 1 mg L^{-1} containing the three antimicrobials in milli-Q water was prepared via 10-fold dilution of stock solutions (100 mg L^{-1} in acetonitrile) of each antimicrobial (Fisher Scientific, Canada). The spiked sample was then transferred to a 75-mL Teflon microwave digestion vessel for extraction, elution and sample analysis (as described above).

Comparison of MAE vs PLE Extraction

Manure containing excreted antimicrobials following administration and manure-amended soil samples collected from research plots were extracted with both PLE and MAE for comparison, followed by SPE, elution and sample analysis. For PLE extraction, the method was adapted from Amarakoon et al. (2014). Briefly, 1 g of freeze-dried and ground (< 2 mm) manure or manure-amended soil was mixed with ~20 g of Ottawa sand and placed in a PLE cell. The contents were extracted with a citric acid buffer solution followed by 80/ 20 methanol: citric acid buffer solution at pH 4.7, using an ASE 200 (Dionex Canada Ltd.). The solvent and soil were heated to 75 ºC for 5 min and maintained at that temperature for 2 min. Each extraction cycle produced ~60 mL of solvent, giving a total of ~120 mL.

Statistical Analysis

The differences in antimicrobial concentration between extraction methods (MAE vs. PLE) and also among different elution methods and extraction solvents were assessed using PROC MIXED in SAS (SAS Institute Inc., 2013). The Tukey multiple comparison procedure was used for pair-wise comparisons. Effects were considered significant if $P < 0.05$.

Results and Discussion

Recovery of Fortified Antimicrobials in Manure and Soil

Manure-amended soil had initial antimicrobial recoveries, following fortification, of ~33% for sulfamethazine and ~92% for tylosin. However, recovery of chlortetracycline was < 10%. Successful extraction of chlortetracycline from chicken manure was achieved by Hu et al. (2010) by adding ethylenediaminetetraacetic acid (EDTA) to the solvent. Authors suggested that EDTA likely reduced the chelation of chlortetracycline, making chlortetracycline more soluble in solution and available for extraction. A study by Parolo et al. (2012) showed tetracycline forming Ca-bridging, resulting in adsorption to clay particles in the soil. Thus, disodium-EDTA (Fisher Scientific, Canada) was tested to improve chlortetracycline recovery via addition of 100 µg of 0.02 M EDTA per 100 mL of solvent. The incorporation of disodium-EDTA to the solvent increased chlortetracycline recovery to 27 ± 1% with no change in the recovery of sulfamethazine and tylosin.

It was noted that there was an inverse relationship between the final concentration of eluate, following evaporation, and the recovery of antimicrobial during elution. The reduction in antimicrobial recovery with increase in concentration (or the reduction in the volume) during elution may have been due to the irreversible attachment of antimicrobials onto the inner wall of

the graduated test tube. Eluates were concentrated to near dryness during elution at the beginning of the experiment but this was subsequently modified to 50 µL and then to 200 µL over the duration of our work (data not shown). In this study, with MAE, we compared 200 µL vs. 500 µL during elution. Concentration to 500 µL by evaporation instead of up to 200 µL further improved (P = 0.08*)* the recovery of antimicrobials. Final recoveries for antimicrobials fortified into beef cattle manure were 71 ± 24% for chlortetracycline, 24 ± 3% for sulfamethazine and 47 ± 9% for tylosin, and the final recoveries for antimicrobials fortified into manure-amended soil were 35 ± 9% for chlortetracycline, 48 ± 5% for sulfamethazine and 82 ± 18% for tylosin using MAE extraction.

Comparison of MAE vs. PLE

Microwave-assisted extraction was significantly more efficient (P = <0.001) than PLE in extracting excreted chlortetracycline residues from manure, but the extraction efficiency did not differ between sulfamethazine and tylosin in manure (Table 1). Antimicrobial recoveries did not differ (P = 0.74) between PLE and MAE, indicating both methods are equally efficient in extracting all three antimicrobials from manure-amended soil. Similar to our results, Raich-Montiu et al. (2010) reported similar recoveries for PLE and MAE during the extraction of soil spiked with sulfonamides, including sulfamethazine. Sample aging reduces the recovery of sulfonamides (Stoob et al., 2006), and MAE was shown to give better recovery than PLE for spiked and aged soil samples (Raich-Montiu et al., 2010). This makes MAE a preferred method for the extraction of sulfonamides. Methanol was adopted as the solvent in this study as it was successful with the antimicrobials used in our previous experiments, an observation consistent with those reported by others (Balakrishnan et al., 2014; Raich-Montiu et al., 2010).

Table 1. Recovery of excreted antimicrobials when extracted using microwave-assisted extraction and pressurized liquid extraction from manure and manure-amended soil.

Antimicrobial	Manure		Manure-Amended Soil	
	Extraction method		Extraction method	
	MAE‡	PLE‡	MAE‡	PLE‡
	Concentration (μg kg^{-1})		Concentration (μg kg^{-1})	
Chlortetracycline	9182(388)§a¶	3682(388)b	50(13)§	31(13)
Chlortetracycline (CTCSMZ)†	6974(388)a	3229(388)b	52(12)	71(12)
Sulfamethazine	524(388)a	246(388)a	6.1(12)	8.1(12)
Tylosin	208(388)a	699(388)a	5.1(10)	14.1(10)

	P value	*P* value
Extraction method	<0.0001	0.74
Antimicrobial	<0.0001	<0.0001
Extraction method × Antimicrobial	<0.0001	0.41

†Chlortetracycline (CTCSMZ) represent chlortetracycline from the treatment consists of equal parts of chlortetracycline and sulfamethazine.
‡MAE, Microwave-assisted extraction; PLE, Pressurized liquid extraction
§Value in parenthesis is the standard error of each treatment.
¶Different letters within each row (between MAE and PLE) for medium manure represent significant differences

Conclusion

Antimicrobial recovery, following fortification, with MAE followed by SPE and detection with LC-MS/MS was 71 ± 24% for chlortetracycline, 24 ± 3% for sulfamethazine and 47 ± 9% for tylosin in beef cattle manure and 35 ± 9% for chlortetracycline, 48 ± 5% for sulfamethazine and 82 ± 18% for tylosin in manure-amended soil. Microwave-assisted extraction produced greater recoveries than PLE for cattle excreted chlortetracycline in manure, but both methods were equally efficient at extracting sulfamethazine and tylosin from manure and for all three antimicrobials from manure-amended soil.

References

Amarakoon, I.D., F. Zvomuya, A.J. Cessna, D. Degenhardt, F.J. Larney, and T.A. McAllister. 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. J. Environ. Qual. 43:549-557.

Balakrishnan, V.K., K.N. Exall, and J.M. Toito. 2014. The development of a microwave-assisted extraction method for the determination of sulfonamide antibiotics in sediments and soils. Can. J. Chem. 92:369-377.

Boxall, A., D. Kolpin, B. Halling-Sorensen, and J. Tolls. 2003. Are veterinary medicines causing environmental risks? Environ. Sci. Technol. 37:286A-294A.

Chee-Sanford, J.C., R.I. Mackie, S. Koike, I.G. Krapac, Y.F. Lin, A.C. Yannarell, S. Maxwell, and R.I. Aminov. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38:1086-1108.

Chen, L., Q. Zeng, H. Wang, R. Su, Y. Xu, X. Zhang, A. Yu, H. Zhang, and L. Ding. 2009. On-line coupling of dynamic microwave-assisted extraction to solid-phase extraction for the determination of sulfonamide antibiotics in soil. Anal. Chim. Acta 648:200-206.

Dolliver, H.A.S. and S.C. Gupta. 2008. Antibiotic losses from unprotected manure stockpiles. J. Environ. Qual. 37:1238-1244.

Eskilsson, C.S. and E. Bjorklund. 2000. Analytical-scale microwave-assisted extraction. J. Chromatogr. A 902:227-250.

Hoese, A., S.A. Clay, D.E. Clay, J. Oswald, T. Trooien, R. Thaler, and C.G. Carlson. 2009. Chlortetracycline and tylosin runoff from soils treated with antimicrobial containing manure. J. Environ. Sci. Heal. B 44:371-378.

Hu, X., Y. Luo, and Q. Zhou. 2010. Simultaneous analysis of selected typical antibiotics in manure by microwave-assisted extraction and LC-MS (n). Chromatographia 71:217-223.

Parolo, E.M., M.J. Avena, G.R. Pettinari, and M.T. Baschini. 2012. Influence of Ca^{2+} on tetracycline adsorption on montmorillonite. J. Colloid Interface Sci. 368:420-426.

Raich-Montiu, J., J.L. Beltran, M.D. Prat, and M. Granados. 2010. Studies on the extraction of sulfonamides from agricultural soils. Anal. Bioanal. Chem. 397:807-814.

Raich-Montiu, J., J. Folch, R. Compano, A. Granados, and M.D. Prat. 2007. Analysis of trace levels of sulfonamides in surface water and soil samples by liquid chromatography-fluorescence. J. Chromatogr. A 1172:186-193.

Sarmah, A.K., M.T. Meyer, and A.B.A. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725-759.

Sassman, S.A., A.K. Sarmah, and L.S. Lee. 2007. Sorption of tylosin A, D, and A-aldol and degradation of tylosin A in soils. Environ. Toxicol. Chem. 26:1629-1635.

Speltini, A., M. Sturini, F. Maraschi, A. Profumo, and A. Albini. 2011. Analytical methods for the determination of fluoroquinolones in solid environmental matrices. TrAc Trends Anal. Chem. 30:1337-1350.

Stoob, K., H.P. Singer, S. Stettler, N. Hartmann, S.R. Mueller, and C.H. Stamm. 2006. Exhaustive extraction of sulfonamide antibiotics from aged agricultural soils using pressurized liquid extraction. J. Chromatogr. A 1128:1-9.

Sura, S., D. Degenhardt, A.J. Cessna, F.J. Larney, A.F. Olson, and T.A. McAllister. 2014. Dissipation of three veterinary antimicrobials in beef cattle feedlot manure stockpiled over winter. J. Environ. Qual. 43:1061-1070.