ANTIBIOTIC TRANSPORT VIA RUNOFF AND SOIL LOSS FROM MANURED FIELDS

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ABSTRACT

Previous research has verified the occurrence of veterinary antibiotics in manure, agricultural fields, and surface water bodies, yet little research has evaluated transport of antibiotics from agricultural fields. The objective of this project is to quantify the transportability of veterinary antibiotics from agricultural fields where manure or effluent is applied. Our hypothesis was that there would be significant differences among antibiotics in the partitioning of losses between runoff and sediment. An antibiotic solution including tetracycline (TC), chlortetracycline (CTC), sulfathiazole (STZ), sulfamethazine (SMZ), erythromycin (ERY), tylosin (TYL), and monensin (MNS) was sprayed on the soil surface 1 hr prior to rainfall simulation (intensity = 60 mm hr^{-1} for 1 h). Runoff samples were collected continuously and analyzed for aqueous and sediment antibiotic concentrations. All of the antibiotics lost <0.1% of the total applied in this 1 hr extreme rainfall event only 1 hr after antibiotic application. Macrolides (ERY and TYL) had the highest sediment concentrations and percent loss associated with sediment, tetracyclines (TC and CTC) had the lowest aqueous and sediment concentrations, ionophores (MNS) had the highest aqueous concentrations and amounts of loss, and sulfonamides (STZ and SMZ) had low sediment concentrations but moderate aqueous concentrations. If agricultural runoff is proven to result in development of resistance genes or toxicity to aquatic organisms, then erosion control practices are expected to reduce macrolide losses leaving agricultural fields, but other methods will be needed to reduce sulfonamide and ionophore transport.

INTRODUCTION

Veterinary antibiotics have long been used for treating and protecting the health of animals and to promote growth and feed efficiency (Thiele-Bruhn, 2003). According to the Animal Health Institute's 2002 Market Sales Report, livestock producers use 11.2 million kg of antimicrobials every year in the absence of disease for non-therapeutic purposes (Mellon *et al.*, 2001). The relatively high usage of veterinary antibiotics and their potential persistence in the environment has led to an interest in the fate and transport of these compounds. Major pathways for antibiotic transport into the environment include seepage from manure storage facilities or runoff from fields fertilized with manure (Boxall *et al.*, 2003). Although detailed transport mechanisms have not been studied, previous research has verified the occurrence of veterinary antibiotics in manure, agricultural fields, and water resources (Campagnolo et al., 2002; Haller et al., 2002; Hamscher et al., 2002; Schlussener et al., 2003; and Yang and Carlson, 2003).

Antibiotics in the environment may lead to the development of antibiotic resistance, a critical concern as it relates to the efficacy of antibiotics in the treatment of human disease. In addition to human health concerns, there are possible toxic effects of antibiotics on aquatic and edaphic organisms. Although most measured concentrations have been well below (an order of magnitude lower) levels that have been shown to be toxic to standard testing organisms (Boxall *et al.,* 2003), degradation products and interactions among compounds have not been adequately evaluated and could result in synergistic toxic effects.

The objective of this study was to quantify the transportability of veterinary antibiotics from agricultural fields (where manure or effluent is applied). Our hypothesis was that there would be significant differences among antibiotics in the partitioning of antibiotic losses between runoff and sediment.

MATERIALS AND METHODS

The research site was located in north central Colorado at the Colorado State University Agricultural Research, Development, and Educational Center (ARDEC) north of Fort Collins, CO. The soil was a Fort Collins sandy clay loam (fine-loamy, mixed, superactive, mesic Aridic Haplustalf) with a 2% slope. Surface soil properties were 55% sand, 16% silt, 29% clay, pH 7.9, EC of 1.19 dS m^{-1}, 18 g kg^{-1} OM, CEC of 24.2 cmoles kg^{-1}, and 44 g kg^{-1} $CaCO_3$. The site was prepared to simulate typical soil preparation for corn planting.

Three rainfall simulation plots (6-m^2, 2 m wide by 3 m long) were established within a 0.5 ha area at the ARDEC complex (19 July 2004). The plots were established parallel to the slope (~2%) and row direction, and consisted of two half beds containing one row each (76 cm row spacing) and a wheel track middle. A 3 m wide by 4 m long area surrounding each 6-m^2 simulator plot was treated like the test area to allow soil material to be splashed in all directions.

Antibiotics were applied 1 h prior to rainfall simulation using a backpack sprayer to achieve an application rate of 209 mg per plot. An antibiotic solution was prepared by dissolving seven antibiotics (TC, CTC, STZ, SMT, ERY, TYL, and MNS) in methanol and then bringing it to volume with DI H2O to achieve a 1.0 mg L^{-1} concentration for each of the antibiotics. The application rate was based on measured levels of antibiotics in 18 hog and dairy lagoons (Carlson et al., 2004) with the goal of applying the approximate amount of antibiotics that would normally be applied when applying liquid manure at 220 kg N ha^{-1}, a typical N application rate for corn [assuming 0.6 g N L^{-1} effluent based on Davis et al. (2002)].

Simulated rainfall was applied to each 6-m^2 plot with an oscillating nozzle rainfall simulator (Frauenfeld and Truman, 2004) that used 80150 Veejet nozzles (median drop size=2.3 mm) placed 3 m above each plot. Well water was used in all simulations (pH=8.0, EC=0.10 dS m^{-1}). Simulated rainfall was applied at a variable rainfall intensity pattern (Fig. 1) obtained from natural rainfall data collected at Fort Collins (1975-2002) and Byers (1971-2002), CO. Rainfall during the months of March-August were analyzed to determine the most extreme event occurring during the row-crop growing season, and the simulated rainfall pattern was based on this most extreme rainfall event. Rainfall duration for each simulation was 60 min.

Runoff and sediment were collected continuously in 5-min intervals into stainless steel buckets. Bottles were weighed (bottle+water+sediment), dried at 105 C for 24 h, then weighed again (bottle+sediment) to determine runoff and sediment gravimetrically. In addition, a 1 L sample was collected in every 5 min interval for antibiotic analysis.

RESULTS AND DISCUSSION

Simulated rainfall intensity increased rapidly to a peak of 115 mm h^{-1} at 17-18 min into the simulation and then declined gradually through the rest of the 1 h rainfall event (Fig. 1). Runoff and soil loss rates followed a similar pattern with peaks a few minutes later during the 20-25 min time period, followed by a decline.

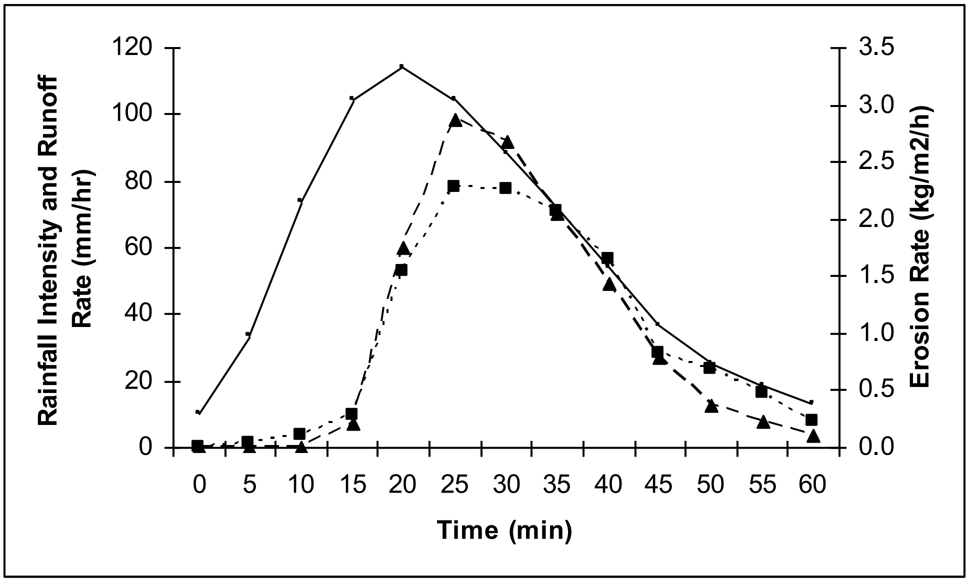

Figure 1. Rainfall intensity (·) and average runoff (▲) and erosion rates (■) for a variable intensity simulated rainstorm.

Runoff and sediment concentrations were significantly different among antibiotics and antibiotic classes (Table 1). Aqueous concentrations were highest for MNS and lowest for the tetracyclines and TYL. On the other hand, sediment concentrations were highest for ERY, followed by MNS and then TYL.

Table 1. Average antibiotic concentrations in aqueous and sediment portions of runoff collected over a 1-h simulated rainfall event.

	Concentration	
Antibiotic	Aqueous	Sediment
	--ug L^{-1}--	--ug kg^{-1}--
Tetracycline	0.03 e[†]	1.30 d
Chlortetracycline	0.04 e	1.47 d
Sulfathiazole	0.33 c	2.04 d
Sulfamethazine	0.58 b	0.57 d

Erythromycin	0.21 d	17.03 a
Tylosin	0.09 e	8.02 c
Monensin	1.20 a	10.51 b

† Antibiotics with a common letter are not significantly different by Least Significant Differences ($p<0.05$) within each column.

In addition, there were significant differences in antibiotic concentrations with time during the rainfall simulation event (Fig. 2a). Aqueous concentrations revealed significant differences only at 5 and 20 min sampling times. At 5 min, SMT had the highest aqueous concentration followed by MNS and STZ; the tetracyclines and macrolides had the lowest aqueous concentrations. At 20 min, MNS had the highest aqueous concentration followed by SMT, and the tetracyclines and TYL were the lowest. The TC concentrations were below the minimum detection limit (MDL) of 0.01 $\mu g\ L^{-1}$ from 30-60 min, and CTC concentrations also fell below the MDL from 50-60 min.

Sediment concentrations were significantly different from 10-20 min and again at 60 min (Fig. 2b). During the 10-20 min time interval, ERY consistently had the highest sediment concentrations followed by MNS and TYL. At 60 min, the sediment concentration of TYL was greater than all others. The MDL for sediment concentrations was 1 $\mu g\ kg^{-1}$, and concentrations were below this level for CTC and SMT from 20-60 min, TC from 30-60 min, and STZ and MNS from 45-60 min.

There were also significant differences among antibiotics and antibiotic classes in the total losses measured in runoff and erosion (Table 2). MNS was lost in the greatest amounts, followed by ERY, and TC and CTC were lost in the least amounts. The same differences were found when loss was calculated as a percentage of the measured amounts applied. Note that all of the antibiotics lost <0.1% of the total applied in this 1 hr extreme rainfall event only 1 hr after antibiotic application.

Table 2. Antibiotic losses in runoff and erosion as actual values and as percentage of applied.

Antibiotic	Total Lost	Lost as % of Applied	Loss Associated with Sediment
	--mg/plot--	-----------%-----------	-----------%-----------
Tetracycline	0.005 d†	0.002 d	65.4 a
Chlortetracycline	0.009 d	0.004 d	33.9 b
Sulfathiazole	0.056 c	0.021 c	23.0 bc
Sulfamethazine	0.059 c	0.024 c	5.0 d
Erythromycin	0.096 b	0.049 b	73.5 a
Tylosin	0.057 c	0.022 c	77.0 a

Monensin	0.219 a	0.081 a	8.7 cd

† Antibiotics with a common letter are not significantly different by Least Significant Differences ($p<0.05$) within each column.

The percent loss associated with sediment is a helpful indicator for evaluating management practices to reduce off-site transport. For example, when the percentage is high, erosion control practices would be expected to be effective in minimizing off-site transport. Antibiotics were significantly different in their percentages of loss associated with sediment (Table 2). TC, ERY, and TYL had the highest losses associated with sediment (>50%), and MNS and SMT had the lowest losses (<10%).

Antibiotic type significantly impacted the partitioning of antibiotic losses between runoff and sediment. MNS had the highest concentration in runoff and the second highest concentration on the sediment, resulting in the highest total loss of all the antibiotics tested. However, in spite of relatively high sediment concentration, the amount of loss associated with sediment transport was <10% for MNS since aqueous concentrations were also high. ERY had the highest concentrations in sediment, relatively high total loss (2^{nd} to MNS), and was among the antibiotics with the highest percent loss associated with sediment (>50%). TYL, the other macrolide studied, also had >50% loss associated with sediment and relatively high sediment concentration; on the other hand, TYL was among those antibiotics with the lowest aqueous concentrations in runoff. The tetracyclines (TC and CTC) also had very low aqueous concentrations, and had the lowest total losses as compared to the other antibiotics evaluated.

In the future, antibiotic transport should be monitored from fields receiving manure applications (not antibiotic solutions) since the impact of the manure matrix could alter transport characteristics. If agricultural antibiotic runoff is proven to result in the development of antibiotic resistance genes or toxicity to aquatic organisms, then erosion control practices are predicted to result in reduced loss of ERY, TYL, and TC to surface water bodies, but other tested antibiotics probably will not respond to soil conservation practices alone. Other methods will need to be developed in order to reduce the off-site transport of sulfonamides and ionophores to the environment.

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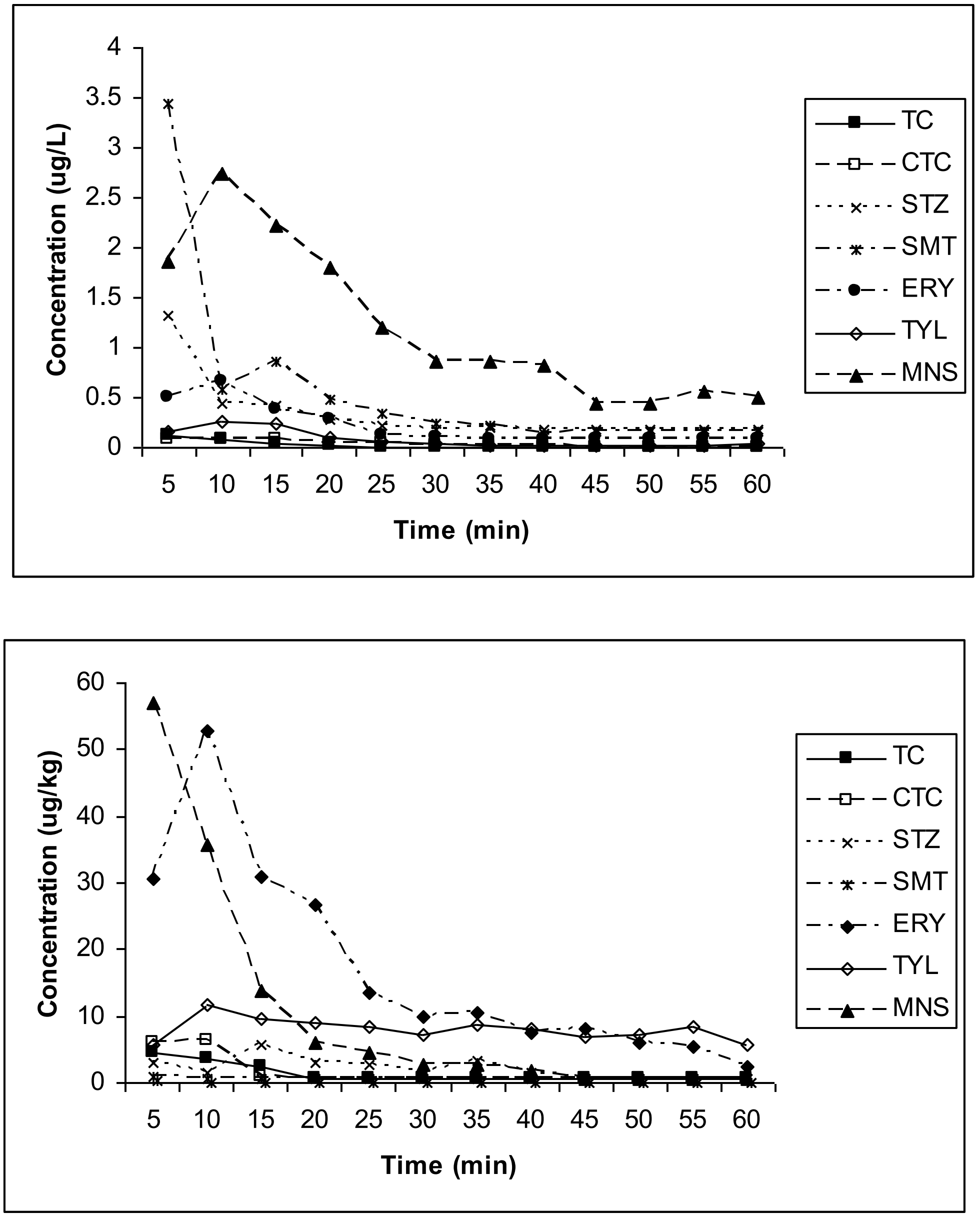

Figure 2. Average antibiotic concentrations in runoff aqueous phase (a) and sediment solid phase (b). TC=tetracycline, CTC=chlortetracycline, STZ=sulfathiazole, SMT=sulfamethazine, ERY=erythromycin, TYL=tylosin, and MNS=monensin.