RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Effect of manure application rate and timing on the leaching and runoff potential of antibiotic resistant bacteria and their associated genes -**NPB#13-113**

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Industry Summary: Antibiotics are used in swine production for both therapeutic and growth promotion purposes. Compounds are placed in feed or water and used in oral and injection treatments and encompass a broad range of antibiotic classes. It is well known that as much as 50 to 90% of administered antibiotics are passed in the feces or urine in an unchanged form and these may lead to development of antibiotic-resistant strains of bacteria in contaminated environments. Strains of antibiotic-resistant bacteria have been isolated from swine lagoon effluent from farms which used antibiotics. Because land application is the most common method of disposing of swine lagoon effluent, there exists the potential threat of contaminating the underlying groundwater with antimicrobial-resistant bacteria (ARB) and their associated genes.

1. The objective of our study was to determine the effect of liquid swine manure application rate and timing on soil leaching of ARB and their associated genes.
2. The general approach of this proposed study was to pass swine lagoon effluent known to contain ARB through soil at two manure application rates and three time intervals between manure application and first rainfall-induced leaching event using laboratory soil columns. We also conducted similar experiments using swine lagoon effluent spiked with two antibiotic resistant bacteria: *E. coli* and *Salmonella*. Time intervals between swine manure application and rainfall application were 1, 7, or 21 days. Transport and attachment behavior of the ARB and associated genes were evaluated in custom-made polycarbonate cylindrical columns packed with soil. Each treatment was conducted in triplicate using a full factorial design. Effluent from columns were plated onto tetracycline-, erythromycin-, and ampicillin-amended Reasoner's 2 Agar (R2A) to determine the extent of transport of resistant bacteria that exhibit resistance to these antimicrobials. For the experiments using spiked swine lagoon effluent we used organism-specific media with the appropriate antibiotics added.
3.

Keywords: Antibiotic resistance, E. coli, Salmonella, leaching, microbial transport, tetracycline, erythromycin, ampicillin

Scientific Abstract

Antibiotics are used in swine production for therapeutic and for growth promotion purposes. It is well established that as much as 50 to 90% of antibiotics administered to animals are passed in the feces or urine in an unchanged form and this may lead to development of antibiotic-resistant strains of bacteria in the manure. Because land application is the most common method of disposing of swine lagoon effluent, there exists the potential threat of contaminating the underlying groundwater with antimicrobial-resistant bacteria (ARB) and their associated genes. Our study centered on improving our scientific understanding on the effect of liquid swine manure application rate and timing on soil leaching of ARB and associated genes. We hypothesized that two commonly used manure management practices – manure application rate and timing – will have significant effects on the leaching potential of ARB and associated genes through soil. Swine manure solutions were added to laboratory soil columns at rates of 5,000 or 30,000 gallons/acre. For both manure application rate, three columns were randomly selected to have rainfall applied (1.3 in/hr for 2 hours) at 1, 7, or 21 days after manure application. Column effluent and the top section of soil in the columns were sampled for cultivable bacteria and quantitative PCR (qPCR) was used to analyze and quantify tetracycline, methicillin, β-lactam and erythromycin resistance genetic determinants. Additionally, 16S rRNA and mobile genetic elements (class 1 integron) were measured. We also conducted similar experiments using swine lagoon effluent spiked with antibiotic resistant *E. coli* and *Salmonella*. We found that the amount of cultivable total bacteria recovered in the column effluent following application of the swine lagoon was similar in magnitude as control columns for both application rates and each rainfall timing interval indicating that the manure did not significantly elevate the risk of ARB transport in these columns. Moreover, we did not observe any significant increases in sediment-attached bacteria at the column inlet. In general, results were similar for the antibiotic resistant genetic determinants. In the experiments using swine manure spiked with *E. coli* and *Salmonella*, recovery of both microorganisms eluted from fine sand columns was similar for both manure application rates and time interval between manure application and rainfall event but differences were observed between application rates. In columns packed with loamy sand, no recovery was detected in the column effluent for either organism. Concentrations of sediment-attached bacteria showed a clear effect of manure application rate and rainfall timing. Results from the GU were similar. These findings show a clear effect of time interval between manure application and leaching event. This suggests the need to avoid manure application to fields when significant rainfall event is forecasted in the near future, even if the manure is not left on the soil surface but rather infiltrates into the soil.

Introduction:

As of 2010, there were 60,000 swine animal feed operations (AFO) in the U.S (USDA, 2011). Antibiotics are used in the feed, water, and for therapeutic oral and injection treatments of pigs in AFOs. It is well established that as much as 50 to 90% of antibiotics administered to animals are passed in the feces or urine in an unchanged form (Kumar et al. 2005) and land application of antibiotic-laden animal manure is likely a primary pathway for the release of antibiotics into the environment (Baguer et al., 2000). At each stage between farrowing and finishing, antibiotics may be mixed and matched to meet growth or therapeutic requirements and thereby influence the manure bacterial population (Sengelov et al., 2003; Rajic et al., 2006).

Public health can be compromised by overuse of antibiotics, as has been recently demonstrated with outbreaks of community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium dificile* in the U.S. and Europe (Lieberman, 2003). The over-prescription and non-judicious use of antibiotics has

brought forth and helped establish multi-agency antibiotic resistance monitoring programs in North America (National Antibiotic Resistance Monitoring System) and Europe. In Europe, these concerns and pressures have caused the abandonment of subtherapeutic (i.e., growth promoting) antibiotic use in AFOs, while domestically, industries such as the poultry industry have made strong commitments to reducing antibiotic use (Isaacson and Torrence, 2002).

In addition to being a source of antibiotics, animal manures may also contain significant concentrations of antibiotic-resistant bacteria (ARB). Sengelov et al. (2003) determined that up to 10^7, 10^6, and 10^7colony forming units (CFU) mL^{-1}, respectively, of tetracycline-, erythromycin-, and streptomycin-resistant bacteria, resided in swine lagoon effluent samples collected from farms which used these respective antibiotics. The degree to which ARB can be transported from manures vertically through the soil column and into groundwater is unknown (Chee-Sanford et al., 2009). McKeon et al. (1995) and Sapkota et al. (2007) observed ARB in groundwater and suggested the resistant strains may have been linked to a large-scale swine AFO.

Studies have suggested that antimicrobial resistance may affect the mobility of bacteria through porous media. For instance, Walczak et al. (2011) found that the mobility of manure-derived tetracycline-resistant *E. coli* through clean quartz was greater than the mobility of *E. coli* isolates which were not tetracycline resistant. The authors attributed differences in transport characteristics between resistant and non-resistant strains to differences in cell surface properties. Liu et al. (2011) reported that the attachment of 203 different *E. coli* isolates to quartz sand was correlated to their antibiotic resistance profile. A limitation to these studies, however, is that they used clean quartz sand under water-saturated conditions, conditions not representative of actual fields where swine manure is applied.

Two common manure management decisions that may affect ARB and associated gene movement in the environment are manure application rate and timing. These decisions may be guided or even determined by manure management plans. Decision factors affecting manure application rates include crop nutrient needs, nutrients available in the manure, and in the context of manure management planning, the risk of phosphorus or nitrogen loss from the receiving fields. For instance, one of the purposes of the USDA-NRCS Code 590 Nutrient Management Standard is to help farmers properly utilize manure in a way that minimizes pollution of surface and groundwater resources (USDA-NRCS, 2011). For qualifying fields, this includes conducting a risk assessment for phosphorus (P) loss using a tool such as the P Index (Bolster et al, 2012; Sharpley et al., 2012). For fields with a low risk of P loss, manure can be applied up to a rate that satisfies crop N requirements. For fields with a medium risk of P loss, manure can be applied at rates to meet crop P requirements, and for high risk fields manure can be applied only at crop P removal rates. Not only will these different application rates affect the amount of nutrients applied to the field they will also affect the amount of ARB that are released into the environment and thus available to be transported to water supplies.

Another important factor affecting the risk of ARB leaching to groundwater supplies is manure application timing; in particular, the time interval between manure application and next rainfall event. A common best management practice is to avoid manure application immediately prior to a forecasted large rainfall event to reduce risk of runoff of nutrients and pathogens. For instance, Sistani et al. (2009) observed *E. coli* concentrations in surface runoff from fields where poultry litter was applied to be ~ 50% lower in fields where rainfall occurred 1 week after manure application compared with fields where rainfall occurred 1 day after application. One possible explanation for their observation is that the 1-week interval allowed for greater die-off of the *E. coli* by UV radiation, dessication, or by other means. For liquid manure, which is expected to infiltrate into the soil following application, an additional factor to consider is time for the bacteria and manure particles to sorb to the soil surfaces prior to the first leaching event.

Objectives:

The overall objectives in this study were to evaluate whether common manure management strategies such as manure application rate and timing affect the leaching potential of swine manure ARB and associated genes through soil. Specifically, this study aimed to evaluate whether these common management practices can be utilized to maximize the retention of ARB and genes in soils in an effort to reduce the risk of contamination of groundwater supplies by ARB and their associated genes. Tetracyline and macrolide resistance genes were chosen based on historical data that suggests that these two antibiotic classes represent two highly used antibiotic types in swine AFOs. The particular objectives and research questions to address in this study are:

Objective 1: To evaluate whether manure application rate affects retention of ARB and associated genes.
Objective 2: To evaluate how rainfall timing affects risk of leaching of ARB and associated genes through soil.

Materials and Methods:

Soil Preparation and Characterization: Two soils were selected for this experiment, a fine sand (Crevasse series: 99% sand and 1% clay as determined by the hydrometer method) and a loamy sand (Lakin series: 88% sand, 5% silt, and 8% clay). Prior to use, soil was air-dried, passed through a 2-mm sieve and mixed thoroughly. Soil chemical properties were determined from filtered water and Mehlich-3 extracts of each soil (2 g porous material in 20 mL distilled water). Soil extracts were analyzed for pH, specific conductivity, dissolved organic carbon (DOC) by loss on ignition, and major cations and anions by ion chromatography.

Lagoon effluent and antimicrobial resistance bacteria and genes: Prior to each set of experiments (3 in total), swine lagoon liquid was collected from a commercial swine farm located in North Central Mississippi. Lagoon effluent was stored at 4°C and transported on ice to the Bowling Green, KY laboratory for experimental studies. The manure was analyzed for antibiotic resistant bacterial (ARB) populations using a plating method with clinically relevant antibiotic levels added to agar. Preliminary experiments showed no differences in growth between R2A plates with and without 200 μg ml^{-1} of cycloheximide (to control for fungal growth). Therefore, we did not cycloheximide to R2A plates in subsequent experiments.

Bacterial Transport and Retention Experiments: Transport and attachment behavior of the ARB and associated genes were evaluated in unsaturated soil columns in triplicate at two manure application rates and three rainfall intervals using a full factorial design. The columns were completely randomized. Manure solutions were added to the columns at a rate of 5,000 or 30,000 gallons/acre. For each manure application rate, 9 column experiments were conducted. Three columns were randomly selected to have rainfall (1 mM KCl solution) applied at: 1 day after manure application, 7 days after manure application, or 21 days after manure application. For each set of columns, rainfall was applied at a rate of 1.3 in/hr for 2 hours. During each application of the rainfall solution, column effluent was collected in sterilized flasks. Concentrations of ARB and associated genes in the swine lagoon effluent applied to the columns and in the column effluent following application of the precipitation solutions were determined as described below.

Following completion of each of the leaching experiments, the top 1 cm and 1-3 cm sections of soil were removed to determine the concentration of reversibly-attached antimicrobial resistant bacteria and genes attached at the soil surface following the method of Bolster and Abit (2012). Briefly, the soil was placed in pre-weighed 50-mL centrifuge tubes followed by addition of 20 mL of 1mM KCl solution and weighed. The resulting suspension was then mixed with a vortex shaker for 20 seconds and allowed to stand for 15 minutes. One mL of suspension drawn from each tube was used to prepare predetermined dilutions (10^0 to 10^{-4}) and

plated to determine culturable concentrations of bacteria and total cell concentrations by quantitative PCR as described below.

Quantification of Genes Associated with Antimicrobial Resistance: Quantitative PCR (qPCR) was used to analyze and quantify resistant determinants in column soil and effluent water samples. Column effluent and soil samples were placed into sterile tubes or sample packs. All samples remained frozen (-80 °C) until analysis. Total genomic DNA was extracted from samples (0.3 g) using the Q-Biogene FastDNA® Spin Kit for soil according to manufacturer's specifications with optimized extraction methods as described by Cook and Britt (2007). Primers for tetracycline, methicillin, β-lactam and erythromycin resistance genetic determinants were applied using previously designed and tested assays. Additionally, 16S rRNA and mobile genetic elements (class 1 integron) were also measured. Tetracycline resistance was assessed using *tetA* (Fan et al., 2007). Methicillin resistance was measured using mecA primers (Sabet et al., 2007), while erythromycin resistance was assessed using ermF primers (Chen et al., 2007). Beta lactam resistance was measured using primers targeting blaTEM (Lachmayr et al., 2009). 16S rRNA and class 1 integron genes were targeted using primers targeting 16S (Nadkarni et al., 2002) and int1 (Hardwick et al., 2008), respectively. Syber-green qPCR assays were operated on an ABI step-one plus RT system following referenced qPCR conditions. All previously defined PCR conditions, controls, and assay condition quality assurance were followed as previously described (Brooks et al., 2016).

Experiment 1: In the first set of experiments (Exp 1), swine manure collected from the swine lagoon was added at two application rates to columns packed with fine sand. Concentrations of total bacteria, *E. coli*, and *Enterococci* leached from the columns and retained within the top 0-1 and 1-3 cm of the columns were determined by plating on R2A, mTEC, and Enterococcosel (ENT) agar, respectively. Concentrations of antibiotic resistant bacteria were determined by adding 100 mg/L ampicillin, 100 mg/L erythromycin, and 16 mg/L tetracycline to the different agar plates. Survival of the bacteria over the course of the experiment were determined by measuring bacterial concentrations in lagoon samples held at room temperature for 1, 7, and 21 days.

Experiments 2 and 3: In these experiments we spiked swine manure with antibiotic resistant strains of *E. coli* and *Salmonella Typhimurium* which were isolated from swine effluent. Prior to spiking, each isolate was grown in Luria Broth containing low doses of the appropriate antibiotics until reaching stationary phase, after which the bacteria were harvested by centrifugation and resuspended in the swine solution at a concentration of ~ 1 $x10^6$ cells mL^{-1}. The inoculated manure was applied to fine sand (Exp 2) and loamy sand (Exp 3) columns at the same rates and were rained on at the same time intervals as described earlier. Concentrations of antibiotic resistant *E. coli* and *Salmonella* in the column leachate and attached to the soil at the column inlet were determined by adding 100 □g/L ampicillin and erythromycin to mFC and Xylose lysine deoxycholate (XLD) agar, respectively. Plates were incubated for ~16 hr at 35°C followed by another 8 as needed at 35°C. Survival of the bacteria over the course of the experiment were determined by measuring bacterial concentrations in lagoon samples held at room temperature for 1, 7, and 21 days.

Results and Discussion

Several of the measured soil chemical properties varied significantly between the two soils (Table 1). Mehlich-3 extractable concentrations for most elements were significantly greater in the sandy loam. Total concentrations (as determined by acid digestion) were all significantly greater for the sandy loam compared with the fine sand.

Of note are the significantly greater concentrations of Fe and Al for the loamy sand as bacterial retention has been shown to increase with increasing concentrations of Fe and Al oxyhydroxides (Bolster et al. 2000, 2006).

In general, manure properties were similar for the three experiments (Table 2). The most notable difference in the manure solutions is the relatively low SpC of 0.39 mS/cm for the Exp 2 solution compared with values of 4.6 and 4.9 mS/cm for Exps 1 and 3, respectively.

Experiment 1 (Exp 1)

Initial concentrations of cultivated bacteria in the swine lagoon effluent ranged from 1.9×10^6 CFU/mL for total bacteria (R2A), 2.2×10^3 CFU/mL for *Escherichia coli* (mTEC), and 1.5×10^3 CFU/mL for *enterococci* (Fig 1). Concentrations of ampicillin- and erythromycin-resistant bacteria were generally lower by 1 to 2 orders of magnitude for all 3 agars. For total bacteria, concentrations of tetracycline-resistant bacteria were ~ 1 order of magnitude lower whereas for *E. coli* and *Enterococci*, nearly all of the cultivated bacteria were resistant to tetracycline at the concentrations (16 mg/L) used in this study.

Concentrations of cultivated bacteria in the manure solutions held at room temperature generally declined with time, with decreases in total bacteria being the lowest. Most of the decreased concentrations were not statistically significant.

Concentrations of genomic units (GU) in the liquid manure for 16S, tetA, blaTEM, mecA, ermF, and intl genes are shown in Table 3. Concentrations ranged from 7.0×10^5 GU/mL for blaTEM to 3.4×10^{10} GU/mL for 16S. Methicillin-resistant genes (mecA) were not detected in the manure sample. Concentrations of 16S, blaTEM, and intl GU remained relatively constant throughout the 21-day survival period whereas tetA GU increased by nearly an order of magnitude at 7 days then increased again slightly at 21 days. Concentrations of ermF GU also increased by nearly an order of magnitude after 7 days but returned to the initial concentration at 21 days.

The amount of total bacteria recovered in the effluent draining the 17-cm fine sand columns following application of the swine lagoon effluent is shown in Figs 2 and 3. While the total number of bacteria eluted from the columns was similar to the total amount applied to the columns, it was also similar to the amount recovered in the control columns indicating that the application of the swine manure did not appreciably increase the amount of total cultivated bacteria eluted from the 17-cm columns. The amount of both indicator organisms (*E. coli* and enterococci) applied to the columns was much lower than the number of total bacteria applied and the amounts eluted from the columns were below detection limit for all treatments. These results indicate that the concentrations of total bacteria and indicator organisms in the manure were not elevated enough to result in significant changes in bacteria eluted from these columns. As such, it was not possible to assess the effects of manure application rate and rainfall timing on leaching risks for these microorganisms. Given these results, we did not repeat these experiments using the loamy sand soil as preliminary results indicated that removal of bacteria from these columns was substantially greater than the bacteria removed in the fine sand columns.

Only 16S and ermF GU were consistently measured in the column effluent (Table 3). For both the low and high manure application rates, the total 16S GU recovered in the column effluent for the 1 and 7 day rainfall intervals were similar in magnitude to the amount of 16S GU eluted from the control column, results consistent with the cultivated bacteria. For the 21 day interval, however, concentrations of 16S were greater than the control column by approximately 1 order of magnitude. At the low application rate, the concentrations of ermF GU were similar to the concentrations in the control column. At the high application rate, however, recovery of ermF GU in the column effluent exceeded the concentration of GU eluted from the control column by a factor

of 10 or more. These concentrations, however, were 2 to 3 orders of magnitude below the concentrations found in the manure indicating that a significant amount of the ermF GU were removed from solution following transport through the fine sand soil. tetA, blaTEM, and mecA GU were not detected in any of the column effluent samples and intI GU were only detected in the high manure treatments following 7 and 21 day rainfall interval.

Concentrations of total cultivated bacteria attached to the top 1 cm and 1-3 cm of sediment following manure application are shown in Table 4. Concentrations of cultivated total bacteria ranged from 2.6×10^5 to 1.8×10^6 CFU/g for the low manure application rate and from 3.9×10^5 to 4.7×10^6 CFU/g for the high application rate. Similar with the effluent data, concentrations of sediment-attached *E. coli* and *enterococci* were at or below detection limit for both application rates and each time interval. For the 1 day rainfall interval, concentrations of ampicillin-resistant total bacteria were similar to the total number of bacteria for both application rates whereas concentrations of tetracycline- and erythromycin-resistant bacteria were generally 1 to 2 orders of magnitude lower than total bacteria, results consistent with the column effluent data. At 7 and 21 days, however, concentrations of ampicillin-resistant bacteria were ~ 2 to 3 orders of magnitude lower than the antibiotic-free agar plates. While sediment concentrations of total bacteria remained relatively constant over time for both manure application rates and sampling depths, concentrations of antibiotic-resistant bacteria generally declined with increasing time interval between manure application and leaching event. No significant differences in sediment attached bacterial concentrations were observed between the low and high manure application rates. Day 1 concentrations were generally 1 to 3 orders of magnitude greater than concentrations measured in the control column; at Day 21, however, concentrations in the treatment columns were similar to those measured in the control columns.

Concentrations of GU attached to the top 1 cm of soil following manure application are shown in Table 5. Concentrations of 16S GU increased by 1 to 2 orders of magnitude over the control column while concentrations of tetA and intl generally increased by 2 orders of magnitude. Concentrations of blaTEM increased from below detection limit (1×10^4 GU/g) in the control column to 3 to 9×10^4 GU/g whereas ermF increased from below detection limit to 6×10^7 to 1×10^8 GU/g, an increase of at least 3 to 4 orders of magnitude. No significant differences in GU were observed between manure application rates and time interval between manure application and leaching event. No mecA genes were detected within the control or treatment columns. This increased concentration of antibiotic-resistant genes has potential implications in that these genes near the soil surface are vulnerable to wash off in surface runoff during rainfall events and exposure by grazing animals.

Experiment 2 (Exp 2)

For the spiked manure solution applied to the fine sand columns, concentrations (CFU) of cultivated *E. coli* and *Salmonella* were between 3 and 4×10^7 CFU/mL, depending on added antibiotic (Figure 4). Concentrations of *E. coli* and *Salmonella* both decreased by ~ 1 order of magnitude after 7 days at room temperature. After 21 days at room temperature, *E. coli* concentration dropped by ~5 orders of magnitude whereas for *Salmonella* the concentration dropped by ~3 orders of magnitude. At each time interval, we observed very little difference between the plates with and without antibiotics.

Concentrations of GUs in the liquid manure applied to the fine sand columns ranged from 1.6×10^7 GU/mL for tetA to 5.3×10^{10} GU/mL for 16S (Table 6). Methicillin-resistant genes (mecA) were not detected in the manure

sample. Concentrations of 16S, tetA, ermF, and intl remained relatively constant throughout the 21-day survival period whereas blaTEM GU decreased by two orders of magnitude after 7 days and remained at the same concentration after 21 days in the spiked manure solution at room temperature.

Recovery of *E. coli* and *Salmonella* in the column effluent is shown in Figure 5. Recovery of both microorganisms was similar for both manure application rates and time interval between manure application and rainfall event. Minimal differences were observed between the unamended and ampicillin- and tetracycline-amended agars. The amount of bacteria recovered decreased noticeably with time interval. For instance, the percent recovery of *E. coli* at the low application rate ranged from 2.2 to 2.4 % at Day 1 then decreased to 0.04 to 0.06 % at day 7 and below detection limit at 21 days, representing a maximum recovery of 0.01%. At the high application rate, recovery decreased from 13 to 18 % at day 1 to 0.73 to 0.94 % at day 7 with recovery at the detection limit for day 21. Similar reductions were observed for *Salmonella*. For instance, *Salmonella* recovery ranged from 3.1 to 3.6 % for day 1 for the low manure application rate to 0.04 to 0.05 % at day 7 and below detection limit after 21 days. At the high application rate, recoveries ranged from 8-10% at day 1 to 0.06 to 0.08% at day 7 followed by no detectable recovery after 21 days. These findings show a clear effect of time interval between manure application and leaching event. This suggests the need to avoid manure application to fields when significant rainfall event is forecasted in the near future, even if the manure is not left on the soil surface but rather infiltrates into the soil.

The decrease in the amount of *E. coli* and *Salmonella* recovered in the column effluent with increasing rainfall interval time, likely has several mechanisms. One possible explanation is increased sorption of bacteria to the soil. Depending on the sorption rate, increasing contact time will increase the sorption of bacteria to the soil surface. However, it is likely that the majority of sorption occurred in the first 24 hr as most bacterial sorption studies assume equilibrium is reached within a few hours. Another potential reason why the time between application and leaching can affect microbial transport is that the longer the bacteria remain in the column the greater time for diffusion and settling to occur. Based on colloid filtration theory, the physical removal of bacteria traveling through a porous medium will increase as flow rates decrease due to increased settling and diffusion of the bacteria to the collector surface. Similar to sorption, it is likely that most of this effect will occur within the first few hours the bacteria are in the column with a pore-water velocity of zero. Another possibility is that as the time of the bacteria in the column increases, the bacteria become more actively attached to the soil surfaces by making biofilms. Increasing contact time will increase biofilm formation thus increasing bacterial removal. Based on survival data, some of this reduction in the effluent concentrations is likely due to die off. While survival in the liquid manure is not the same as within the column, the large reductions in both *E. coli* and *Salmonella* following incubation in the manure indicates that die-off of both microorganisms likely occurred within the columns.

As expected, for each treatment, the amount of cultivated *E. coli* and *Salmonella* recovered from the column effluent was greater for the high manure application treatments. The percent recoveries, however, varied depending on application rate. For instance, for the 1 day rainfall interval, recovery of *E. coli* at the low application rate was 2 % whereas at the high application rate, recovery ranged from 13 to 18 %. For *Salmonella*, recovery increased from 3 % to 8-10% for the high application rate. For the 7 day rainfall interval, *E. coli* recovery was ~ 0.05 % at the low application rate and ~ 1 % at the high application rate. Differences in percent recovery between application rates for the 7 day rainfall interval were not observed for *Salmonella*. One possible explanation for the increased relative recovery at the high application rate is due to the saturation of

bacterial sorption sites at the higher concentrations. Reduced sorption rates have been reported with increasing bacterial concentrations.

For the low manure application rates, the concentration of 16S GU collected in the column effluent was reduced by approximately 6 orders of magnitude compared with the spiked manure solution and the concentration of blaTEM GU was reduced by 4 to 5 orders of magnitude (Table 6). For tetA, mecA, ermF, and intl GU, recovery was generally below detection limit. At the high manure application rates, concentrations of 16S GU were only reduced 1 to 3 orders of magnitude compared with the spiked manure solution, resulting in much greater concentrations in the effluent than the low manure application treatments. Concentrations of tetA and ermF were notably greater in the high manure application treatments compared with the low application treatments, though reductions of 2 to 3 orders of magnitude from the applied manure solution were observed. Similar to the low application rates, mecA and intI were not detected in the column effluent of the high application columns. No consistent trends in GU concentrations and rainfall timing were observed, though concentrations of tetA and ermF were significantly greater after 21 day rainfall interval at the high manure application rate.

Sediment-attached concentrations of cultivated *E. coli* and *Salmonella* in the top 1 cm and 1-3 cm sections of the fine sand columns following manure application are presented in Table 7. Concentrations of *E. coli* after 1 day rainfall interval ranged from 9.8×10^5 to 4.4×10^5 CFU/g, respectively, for the low manure application rate and approximately 1×10^6 CFU/g for the high application rate. For *Salmonella*, concentrations in the top 1 and 1-3 cm of soil were approximately 2×10^5 CFU/g at the low application rate and 1×10^5 CFU/g at the high application rate on day 1. At day 7, concentrations of sediment-attached E. coli decreased by 2 orders of magnitude in the top 1 cm and by 3 orders of magnitude in the 1-3 cm depth. Following the 7 day rainfall interval, *Salmonella* concentrations were generally below detection limit representing a minimum of 3 orders of magnitude reduction. After 21 day rainfall interval, concentrations of both *E. col*i and *Salmonella* were near or below detection limits. In general, minimal differences were observed between the unamended and antibiotic-amended agar (Table 7). No significant differences in sediment-attached bacterial concentrations were observed between the low and high manure application rates.

With the exception of mecA, concentrations of GU attached to the top 1 cm of soil increased 1 to 3 orders of magnitude following manure application to the soil column. Concentrations of 16S GU generally increased by a factor of 10 whereas blaTEM increased from below detection limit in the control column to values ranging between 4.0×10^5 to 1.8×10^6 GU/g for the low manure application columns and between 2.2×10^6 to 1.1×10^7 GU/g for the high manure application columns. Differences in GU concentrations between low and high manure application rates were minimal for 16S and tetA. Concentrations of blaTEM at the 1 and 7 day rainfall intervals were approximately 10-fold greater for the high application rate. For ermF and intI, GU concentrations for the 7 day interval columns were a factor of 10 greater for the high manure application rate columns.

Experiment 3 (Exp 3)

For the inoculated manure solution applied to the loamy sand columns, day 1 concentrations (CFU) of *E. coli* ranged from 3.8×10^7 to 4.3×10^7 CFU/mL. For *Salmonella*, concentrations on day 1 ranged from 1.9×10^7 to 2.0×10^7 CFU/mL. Following 7 days at room temperature, *E. coli* concentrations dropped by approximately 2 orders of magnitude whereas *Salmonella* concentrations dropped by 5 orders of magnitude. Concentrations of *E. coli* and *Salmonella* were below detection limit following 21 days of incubation in the swine effluent. At each time interval, we observed very little difference between the plates with and without antibiotics. It is not clear

why survival was greatly reduced in the manure solution applied to the loamy fine sand columns compared with the manure solution applied to the fine sand columns.

Concentrations of GUs in the liquid manure applied to the loamy sand columns ranged from 1.2×10^7 GU/mL for tetA to 1.7×10^{10} GU/mL for intl (Table X). Methicillin-resistant genes (mecA) were not detected in the manure sample. Concentrations of 16S, tetA, and ermF remained relatively constant throughout the 21-day survival period whereas blaTEM GU decreased by two orders of magnitude after 7 days and another two orders of magnitude after 21 days. Conversely, ermF GU increased by nearly an order of magnitude after 7 days and remained at that level after 21 days.

Concentrations of sediment-attached *E. coli* and *Salmonella* in the loamy sand following manure application are presented in Table 10. Concentrations of both microorganisms were consistently greater in the high application treatments. For instance, the concentrations of sediment-attached cultivated *E. coli* in the top 1 cm and 1-3 cm sections of the loamy sand for the 1 day rainfall interval were 2.3×10^5 and 3.5×10^4 CFU/g, respectively, for the low manure application treatments and 1.2×10^6 and 2.7×10^5 CFU/g, respectively, for the high manure application treatments. Concentrations of ampicillin- and tetracycline-resistant *E. coli* were not significantly different. Concentrations of sediment-attached cultivated *Salmonella* in the top 1 cm and 1-3 cm sections for the 1 day rainfall interval were 1.6×10^5 and 5.2×10^4 CFU/g, respectively, for the low manure application treatments and 3.7×10^6 and 2.5×10^5 CFU/g, respectively, for the high manure application treatments. Concentrations of ampicillin- and tetracycline-resistant *Salmonella* were also not significantly different. In addition to manure application rate, there was a clear effect on rainfall timing. At the low manure application rate, sediment-attached concentrations of both microorganisms were all below detection limit at both the 7 and 21 day rainfall intervals. At the high manure application rates, concentrations following the 7 day rainfall interval were reduced by 2 to 3 orders of magnitude and were below detection limit for the 21 day rainfall interval.

With the exception of 16S and mecA, concentrations of GU attached to the top 1 cm of the loamy sand soil increased following manure application from below detection limit (1×10^4 GU/g) to concentrations ranging from 7.7×10^4 to 1.1×10^{10} GU/g (Table 1). Concentrations of tetA, blaTEM, ermF, and intI GU were generally higher by approximately an order of magnitude for the high application rate. Concentrations of GU generally decreased between the 1 day rainfall interval and the 7 and 21 day intervals. A notable exception was the ermF GU concentration which remained relatively unchanged for the three rainfall timing intervals.

References

Baguer, A.J., J. Jensen, and P.H. Krogh. 2000. Effects of antibiotics oxytetracycline and tylosin on soil fauna. *Chemosphere*. 40: p. 751-757.

Bolster, C.H. and S.M. Abit. In Press. Effect of Biochar Pyrolysis Temperature on E. coli Transport Through a Biochar-amended Soil. *Journal of Environmental Quality*.

Bolster, C.H., P.A. Vadas, A.N. Sharpley, and J.A. Lory. 2012. Using a P loss model to evaluate and improve P indices. *Journal of Environmental Quality*. 41: p. 1758-1766.

Brooks, J.P., McLaughlin, M.R., Adeli, A., and Miles, D.M. 2016. Cultivation and qPCR detection of pathogenic and antibiotic resistant bacterial establishment in naïve broiler houses. J. Environ. Qual. 45:958-966.

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

Chen, J., Z. Yu, F.C. Michel Jr., T. Wittum, and M. Morrison. 2007. Development and application of real-time PCR assays for quantification of erm genes conferring resistance to macrolides-lincosamides-streptogramin B in livestock manure and manure management systems. *Applied and Environmental Microbiology*. 73: p. 4407-4416.

Cook, K.L. and J.S. Britt. 2007. Optimization of methods for detecting *Mycobacterium avium* subsp. *paratuberculosis* in environmental samples using quantitative, real-time PCR. *Journal of Microbiological Methods*. 69(1): p. 154-160.

Fan, W., T. Hamilton, S. Webster-Sesay, M.P. Nikolich, and L.E. Lindler. 2007. Multiplex real-time SYBR green I PCR assay for detection of tetracycline efflux genes of Gram-negative bacteria. *Molecular and Cellular Probes*. 21: p. 245-256.

Hardwick, S.A., Stokes, H.W., Findlay, S., Tayloer, M., and Gillings, M.R. 2008. Quantification of class 1 integron abundance in natural environments using real-time quantitative PCR. FEMS Microbiol. Lett. 278:207-212.

Isaacson, R.E. and M.E. Torrence, *The Role of Antibiotics in Agriculture*. 2002: Washington, DC.

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

Lachmayr, K.L., Kerkhof, L.J., DiRienzo, A.G., Cavanaugh, C.M., and Ford, T.E. 2009. Quantifying nonspecific TEM β-lactamase (blaTEM) genes in a wastewater stream. Appl. Environ. Microbiol. 75:203-211.

Lieberman, J.M. 2003. Appropriate antibiotic use and why it is important: The challenges of bacterial resistance. *Pediatric Infectious Disease Journal*. 22: p. 1143-1151.

Liu, P., M.L. Soupir, M. Zwonitzer, B. Huss, and L.R. Jarboe. 2011. Association of antibiotic resistance in agricultural *Escherichia coli* isolates with attachment to quartz. *Applied and Environmental Microbiology*. 77(19): p. 6945-6953.

McKeon, D.M., J.P. Calabrese, and G.K. Bissonnette. 1995. Antibitoic resistant gram-negative bacteria in rural groundwater supplies. *Water Research*. 29: p. 1902-1908.

Nadkarni, M.A., Martin, F.E., Jacques, N.A., and Hunter, N. 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology, 148:257-266.

Rajic, A., R. Reid-Smith, A.E. Deckert, C.E. Dewey, and S.A. McEwen. 2006. Reported antibiotic use in 90 swine farms in Alberta. *Canadian Veterinary Journal*. 47: p. 446-452.

Sabet, N.S., G. Subramaniam, P. Navaratnam, and S.D. Sekaran. 2007. Detection of methicillin- and aminoglycoside-resistant genes and simultaneous identification of *S. aureus* using triplex real-time PCR Taqman assay. *Journal of Microbiological Methods*. 68: p. 157-162.

Sapkota, A.R., F.C. Curriero, K.E. Gibson, and K.J. Schwab. 2007. Antibiotic resistant enterococci and fecal indicators in surface and groundwater impacted by a concentrated swine feeding operation. *Environmental Health Perspectives*. 115: p. 1040-1045.

Sengelov, G., Y. Agerso, B. Halling-Sorensen, S.B. Baloda, J.S. Anderson, and L.B. Jensen. 2003. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. *Environment International*. 28: p. 587-595.

Sharpley, A., et al. 2012. Phosphorus Indices: Why we need to take stock of how we are doing. *Journal of Environmental Quality*. 41: p. 1711-1719.

Sistani, K.R., H.A. Torbert, T.R. Way, C.H. Bolster, D.H. Pote, and J.G. Warren. 2009. Broiler litter application method and runoff timing effects on nutrient and *Escherichia coli* losses from tall fescue pasture. *Journal of Environmental Quality*. 38: p. 1216-1223.

USDA. 2011. Farms, Land in Farms, and Livestock Operations 2010 Summary National Agricultural Statistics Service.

USDA-NRCS, *Title 190*. 2011.

Walczak, J.J., S.L. Bardy, L. Feriancikova, and S. Xu. 2011. Comparison of the transport of tetracycline-resistant and tetracycline-susceptible *Escherichia coli* isolated from Lake Michigan. *Water, Air, & Soil Pollution*. 222: p. 305-314.

Table 1. Soil characteristics

Table 1. Soil properties for fine sand and loamy fine sand soils. Probability (p) that differences between the two soils are statistically significant are also shown.

	Fine Sand		**Sandy Loam**		
	avg (mg/kg)	std (mg/kg)	avg (mg/kg)	std (mg/kg)	p value
			Mehlich-3 extractable		
Al	42	0.8	2.6E+02	19	0.040
Ca	2.4E+02	2.6	8.2E+02	18	0.014
Fe	23	0.1	79	5.9	0.047
K	91	1.5	1.0E+02	1.8	0.026
Mg	10	0.4	97	3.4	0.018
Mn	22	0.8	28	1.9	0.15
Na	2.7E+02	1.9	2.7E+02	3.7	0.34
P	2.5	0.1	28	1.7	0.030
			Total		
Al	8.4E+02	1.7E+02	1.3E+04	2.2E+03	0.011
Ca	7.1E+02	1.5E+02	1.4E+03	50	0.017
Fe	1.5E+03	2.0E+02	1.3E+04	2.3E+02	< 0.001
K	52	10	2.5E+03	6.8E+02	0.025
Mg	40	5.4	1.6E+03	93	0.001
Mn	56	5.5	3.8E+02	15	< 0.001
Na	BDL	BDL	77	35	*n/a*
P	55	23	4.2E+02	17	< 0.001

Table 2. Manure characteristics

NO3-N	**PO4-P**	**SO4-S**	**Cl**	**Na**	**NH4**	**K**	**Mg**	**Ca**	**pH**	**SpC**	**TC**	**TN**	**Total bacteria (R2A)**
mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L		mS/cm	mg/L	mg/L	CFU/mL
						Experiment 1							
1.7	16.3	13.0	279	168	63.0	370	30.9	76.3	8.11	4.6	1095	650	5.1E+05
						Experiment 2							
1.2	29.8	44.2	469	151	BDL	530	43.7	28.4	7.88	0.39	1276	229	9.3E+05
						Experiment 3							
0.4	20.9	BDL	351	89	134	337	BDL	3.1	7.96	4.9	1282	338	7.0E+04

Table 3. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/mL) in the uninoculated liquid swine manure and column effluent for Experiment 1. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Liquid Manure			
1	3.4E+10	3.1E+07	7.0E+05	BDL	3.8E+08	2.0E+07
7	4.1E+10	1.7E+08	2.4E+05	BDL	1.6E+09	2.4E+07
21	2.7E+10	3.8E+08	5.5E+05	BDL	3.3E+08	2.9E+07
			Control Column			
1	6.0E+09	BDL	BDL	BDL	BDL	BDL
7	6.8E+09	BDL	BDL	BDL	1.7E+04	BDL
21	4.5E+08	BDL	BDL	BDL	3.2E+03	BDL
			Low Manure Application Rate			
1	5.1E+09	BDL	BDL	BDL	3.1E+04	BDL
7	1.0E+10	BDL	BDL	BDL	4.0E+04	BDL
21	6.4E+09	BDL	BDL	BDL	2.4E+03	BDL
			High Manure Application Rate			
1	6.5E+09	BDL	BDL	BDL	1.4E+06	BDL
7	6.6E+09	BDL	BDL	BDL	1.4E+05	1.7E+06
21	1.0E+10	BDL	BDL	BDL	2.9E+05	2.7E+06

[a] Rainfall timing interval

[b] 16S – 16S rRNA gene; tetA – tetracycline A resistance gene; blaTEM – Beta lactamase gene; mecA – methicillin resistance gene; ermF – erythromycin resistance gene; intI – class I integron gene.

Table 4. Concentrations (CFU/g of soil) of cultivated total sediment-attached bacteria from dissections of fine sand columns for control, low application rate, and high application rate columns for Experiment 1. Concentrations of *E. coli* and *Enterococci* were below detection limit in all columns and thus are not presented. BDL is below detection limit (110 CFU/g).

	CFU/g recovered in Section 1 (0-1 cm)			**CFU/g recovered in Section 2 (1-3 cm)**		
	Rainfall timing interval (days)			Rainfall timing interval (days)		
	1	7	21	1	7	21
			Control Column			
R2A	8.56E+04	1.15E+05	4.23E+05	1.04E+05	1.04E+05	2.74E+05
R2A + Amp	1.58E+03	1.13E+03	4.61E+03	1.21E+03	1.14E+03	8.99E+03
R2A + Ery	9.86E+01	9.45E+01	BDL	BDL	BDL	BDL
R2A + Tet	7.89E+02	1.89E+02	BDL	2.53E+03	BDL	BDL
			Low Manure Application Rate			
R2A	1.7E+06 (1.3E+05-2.1E+07)	1.8E+06 (9.8E+05-3.5E+06)	3.6E+05 (1.5E+04-8.9E+06)	6.1E+05 (1.3E+04-2.9E+07)	1.7E+06 (4.2E+05-6.5E+06)	2.6E+05 (7.4E+03-9.4E+06)
R2A + Amp	1.4E+06 (1.4E+05-1.5E+07)	3.8E+04 (2.6E+03-5.3E+05)	4.8E+03 (4.9E+02-4.7E+04)	4.8E+05 (1.7E+04-1.4E+07)	4.8E+04 (2.5E+04-8.9E+04)	1.7E+03 (4.4E+02-6.3E+03)
R2A + Eryth	3.8E+02 (2.4E+01-6.0E+03)	2.2E+03 (4.0E+02-1.2E+04)	BDL	4.5E+01 (8.4E-03-2.4E+05)	2.0E+03 (1.2E+03-3.3E+03)	BDL
R2A + Tet	1.7E+04 (6.2E+02-4.6E+05)	6.8E+03 (3.4E+03-1.4E+04)	BDL	2.9E+04 (1.4E+03-5.7E+05)	6.6E+03 (3.0E+03-1.4E+04)	BDL
			High Manure Application Rate			
R2A	2.5E+06 (2.5E+05-2.6E+07)	4.0E+06 (1.0E+06-1.6E+07)	1.5E+06 (2.2E+05-9.6E+06)	1.6E+06 (6.3E+04-3.9E+07)	4.7E+06 (2.6E+06-8.6E+06)	3.9E+05 (6.3E+04-2.4E+06)
R2A + Amp	1.2E+06 (4.4E+05-3.4E+06)	7.2E+04 (2.8E+04-1.9E+05)	5.6E+01 (8.2E-03-3.8E+05)	1.1E+06 (5.6E+04-2.2E+07)	6.2E+04 (9.7E+02-4.0E+06)	4.2E+02 (2.6E+01-6.8E+03)
R2A + Eryth	1.1E+03 (1.9E+01-6.1E+04)	1.2E+03 (3.1E+01-4.3E+04)	BDL	1.1E+02 (2.8E-03-4.3E+06)	1.8E+03 (1.0E+02-3.0E+04)	1.1E+02 (8.6E+01-1.4E+02)
R2A + Tet	1.3E+05 (1.2E+04-1.4E+06)	6.6E+03 (2.4E+03-1.8E+04)	1.1E+02 (4.4E-03-2.9E+06)	3.8E+04 (1.8E+03-8.2E+05)	7.4E+03 (1.8E+03-3.0E+04)	1.1E+01 (4.0E-04-2.9E+05)

Table 5. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/g) in the top 1 cm of soil Experiment 1. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Control Column			
1	3.8E+10	4.5E+03	BDL	BDL	BDL	1.9E+04
7	6.7E+10	4.7E+04	BDL	BDL	6.4E+05	2.6E+06
21	1.0E+10	1.2E+04	BDL	BDL	BDL	8.7E+05
			Low Manure Application Rate			
1	1.2E+12	2.8E+06	5.9E+04	BDL	9.4E+07	3.5E+07
7	1.2E+12	2.6E+06	5.4E+04	BDL	8.4E+07	4.6E+07
21	7.1E+11	2.2E+06	3.1E+04	BDL	5.8E+07	1.3E+07
			High Manure Application Rate			
1	8.4E+11	3.8E+06	8.5E+04	BDL	6.9E+07	4.3E+07
7	1.1E+12	4.1E+06	2.8E+04	BDL	1.4E+08	6.9E+07
21	8.6E+11	5.9E+06	5.7E+04	BDL	1.0E+08	5.2E+07

Table 6. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/mL) in the spiked liquid swine manure and column effluent for Experiment 2. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Liquid Manure			
1	5.3E+10	1.6E+07	3.3E+07	BDL	5.0E+08	7.9E+07
7	1.0E+11	2.5E+07	2.1E+05	BDL	1.2E+09	2.3E+07
21	1.0E+11	2.9E+07	2.1E+05	BDL	1.4E+09	4.1E+07
			Control Column			
1	2.4E+06	BDL	BDL	BDL	BDL	BDL
7	9.3E+07	BDL	BDL	BDL	BDL	BDL
21	6.0E+04	BDL	BDL	BDL	BDL	BDL
			Low Manure Application Rate			
1	2.1E+04	BDL	6.9E+02	BDL	BDL	BDL
7	7.7E+03	1.3E+05	1.1E+03	BDL	4.5E+05	BDL
21	1.5E+04	BDL	7.1E+03	BDL	BDL	BDL
			High Manure Application Rate			
1	1.2E+09	1.7E+05	6.1E+04	BDL	1.2E+06	BDL
7	2.6E+08	3.8E+05	6.4E+03	BDL	6.7E+06	BDL
21	8.3E+09	2.3E+06	2.0E+04	BDL	3.2E+08	BDL

[a] Rainfall timing interval

[b] 16S – 16S rRNA gene; tetA – tetracycline A resistance gene; blaTEM – Beta lactamase gene; mecA – methicillin resistance gene; ermF – erythromycin resistance gene; intI – class I integron gene.

Table 7. Concentrations (CFU/g of soil) of cultivated sediment-attached *E. coli* and *Salmonella* from dissections of fine sand columns for low and high application rates for Experiment 2. BDL is below detection limit (110 CFU/g).

	CFU/g recovered in Section 1 (0-1 cm)			**CFU/g recovered in Section 2 (1-3 cm)**		
	Rainfall timing interval (days)			Rainfall timing interval (days)		
	1	7	21	1	7	21
			Low Manure Application Rate			
mFC	9.8E+05 (9.8E+04-9.8E+06)	2.1E+03 (1.2E+01-3.6E+05)	6.0E+00 (2.7E-03-1.3E+04)	4.4E+05 (1.1E+05-1.7E+06)	1.2E+02 (3.3E-03-4.7E+06)	BDL
mFC + Amp	5.9E+05 (7.5E+04-4.6E+06)	1.4E+03 (5.2E+01-3.6E+04)	6.8E+00 (1.7E-03-2.7E+04)	4.2E+05 (6.8E+04-2.6E+06)	1.5E+02 (3.0E-03-7.3E+06)	BDL
mFC + Tet	5.6E+05 (3.7E+05-8.4E+05)	1.3E+02 (2.3E-03-7.1E+06)	6.9E+00 (1.7E-03-2.7E+04)	6.1E+05 (3.7E+05-1.0E+06)	5.5E+02 (1.2E+01-2.5E+04)	BDL
XLD	2.3E+05 (5.5E+04-9.7E+05)	4.8E+00 (5.7E-03-4.0E+03)	BDL	2.2E+05 (7.2E+04-6.8E+05)	BDL	BDL
XLD + Amp	3.0E+05 (4.6E+04-1.9E+06)	BDL	BDL	1.3E+05 (1.4E+04-1.3E+06)	BDL	BDL
XLD + Tet	1.8E+05 (8.3E+04-4.0E+05)	4.8E+00 (5.7E-03-4.0E+03)	BDL	1.8E+05 (3.2E+04-1.0E+06)	BDL	BDL
			High Manure Application Rate			
mFC	1.4E+06 (5.8E+05-3.2E+06)	6.0E+04 (1.8E+04-2.1E+05)	4.2E+02 (1.7E+01-1.0E+04)	1.3E+06 (1.0E+06-1.7E+06)	8.3E+02 (4.1E-04-1.7E+09)	7.6E+00 (1.2E-03-4.6E+04)
mFC + Amp	1.1E+06 (4.8E+05-2.7E+06)	6.1E+04 (1.8E+04-2.1E+05)	7.4E+00 (1.3E-03-4.1E+04)	1.0E+06 (5.2E+05-1.9E+06)	8.6E+02 (3.6E-04-2.1E+09)	BDL
mFC + Tet	1.1E+06 (5.1E+05-2.3E+06)	8.1E+04 (2.8E+04-2.3E+05)	2.6E+02 (1.5E+02-4.5E+02)	9.2E+05 (5.5E+05-1.5E+06)	1.6E+04 (2.0E+03-1.3E+05)	BDL
XLD	1.1E+05 (2.8E+04-4.1E+05)	BDL	BDL	1.4E+05 (4.6E+03-3.9E+06)	5.9E+00 (2.8E-03-1.2E+04)	BDL
XLD + Amp	1.2E+05 (2.9E+04-4.8E+05)	6.8E+00 (1.8E-03-2.6E+04)	BDL	1.9E+05 (1.3E+04-2.7E+06)	BDL	BDL
XLD + Tet	1.5E+05 (5.2E+04-4.6E+05)	9.8E+00 (5.3E-04-1.8E+05)	BDL	4.8E+05 (1.0E+05-2.2E+06)	BDL	BDL

Table 8. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/g) in the top 1 cm of soil Experiment 2. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Control Column			
1	3.8E+10	4.5E+03	BDL	BDL	BDL	1.9E+04
7	6.7E+10	4.7E+04	BDL	BDL	6.4E+05	2.6E+06
21	1.0E+10	1.2E+04	BDL	BDL	BDL	8.7E+05
			Low Manure Application Rate			
1	1.7E+11	1.2E+07	1.8E+06	BDL	4.3E+07	BDL
7	2.2E+11	8.0E+06	4.0E+05	BDL	1.8E+07	2.4E+07
21	4.1E+11	1.8E+07	1.8E+06	BDL	1.6E+08	5.7E+07
			High Manure Application Rate			
1	2.9E+11	1.1E+07	1.1E+07	BDL	1.1E+08	8.3E+07
7	4.8E+11	4.0E+07	2.2E+06	BDL	2.2E+08	1.4E+08
21	5.1E+11	3.5E+07	7.6E+06	BDL	2.5E+08	1.2E+08

Table 9. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/mL) in the spiked liquid swine manure and column effluent for Experiment 3. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Liquid Manure			
1	2.6E+08	1.2E+07	5.7E+08	BDL	6.9E+07	1.9E+10
7	1.3E+08	6.8E+07	7.0E+06	BDL	4.8E+07	3.3E+09
21	4.5E+08	4.0E+07	7.3E+04	BDL	2.2E+08	3.5E+09
			Control Column			
1	3.7E+07	BDL	BDL	BDL	BDL	BDL
7	5.7E+07	BDL	BDL	BDL	BDL	BDL
21	1.9E+06	BDL	BDL	BDL	BDL	BDL
			Low Manure Application Rate			
1	1.3E+06	BDL	BDL	BDL	BDL	BDL
7	9.4E+05	BDL	BDL	BDL	BDL	BDL
21	7.6E+05	BDL	BDL	BDL	BDL	BDL
			High Manure Application Rate			
1	3.9E+05	BDL	4.9E+03	BDL	BDL	BDL
7	3.0E+05	BDL	2.8E+03	BDL	BDL	BDL
21	2.9E+05	BDL	BDL	BDL	BDL	BDL

[a] Rainfall timing interval

[b] 16S – 16S rRNA gene; tetA – tetracycline A resistance gene; blaTEM – Beta lactamase gene; mecA – methicillin resistance gene; ermF – erythromycin resistance gene; intI – class I integron gene.

Table 10. Concentrations (CFU/g of soil) of cultivated sediment-attached *E. coli* and *Salmonella* from dissections of fine sand columns for low and high application rates (Exp 3). BDL is below detection limit (140 CFU/g).

	CFU/g recovered in Section 1 (0-1 cm)			**CFU/g recovered in Section 2 (1-3 cm)**		
	Rainfall timing interval (days)			Rainfall timing interval (days)		
	1	7	21	1	7	21
			Low Manure Application Rate			
mFC	2.3E+05 (1.0E+05-5.2E+05)	BDL	BDL	3.5E+04 (4.2E+02-2.9E+06)	BDL	BDL
mFC + Amp	6.4E+04 (4.7E+03-8.5E+05)	BDL	BDL	2.7E+04 (9.4E+02-7.8E+05)	BDL	BDL
mFC + Tet	5.7E+04 (7.9E+03-4.1E+05)	BDL	BDL	6.6E+03 (3.7E+03-1.2E+04)	BDL	BDL
XLD	1.6E+05 (8.2E+04-3.2E+05)	BDL	BDL	5.2E+04 (1.8E+04-1.5E+05)	BDL	BDL
XLD + Amp	1.8E+05 (1.5E+05-2.2E+05)	BDL	BDL	4.8E+04 (1.5E+04-1.6E+05)	BDL	BDL
XLD + Tet	1.7E+05 (1.2E+05-2.2E+05)	BDL	BDL	4.3E+04 (8.2E+03-2.3E+05)	BDL	BDL
			High Manure Application Rate			
mFC	1.2E+06 (2.3E+05-6.8E+06)	2.7E+03 (1.6E+03-4.5E+03)	BDL	2.7E+05 (7.8E+04-9.6E+05)	1.6E+03 (7.1E+02-3.6E+03)	BDL
mFC + Amp	7.5E+05 (1.7E+05-3.2E+06)	1.7E+03 (6.6E+02-4.3E+03)	BDL	3.0E+05 (1.9E+05-4.6E+05)	6.9E+02 (5.5E+02-8.6E+02)	BDL
mFC + Tet	9.9E+05 (3.9E+05-2.5E+06)	1.2E+03 (6.6E+02-2.2E+03)	BDL	4.5E+05 (2.3E+05-8.9E+05)	5.7E+02 (2.2E+02-1.5E+03)	BDL
XLD	3.7E+05 (7.1E+04-1.9E+06)	3.6E+03 (1.6E+03-7.7E+03)	BDL	2.5E+05 (4.2E+04-1.5E+06)	1.6E+03 (2.3E+02-1.1E+04)	BDL
XLD + Amp	3.4E+05 (7.0E+04-1.7E+06)	7.7E+02 (2.6E+02-2.3E+03)	BDL	2.8E+05 (1.5E+04-5.4E+06)	9.8E+02 (4.6E+02-2.1E+03)	BDL
XLD + Tet	5.1E+05	BDL	BDL	2.1E+05	BDL	BDL

	(1.4E+05-1.9E+06)	(4.9E+04-8.8E+05)

Table 11. Concentrations of 16S, tetA, blaTEM, mecA, ermF, and intl genes (genomic units, GU/g) in the top 1 cm of soil Experiment 3. BDL represents below detection limit.

Day[a]	16S[b]	tetA	blaTEM	mecA	ermF	intI
			Control Column			
1	3.9E+10	BDL	BDL	BDL	BDL	BDL
7	2.3E+10	BDL	BDL	BDL	BDL	BDL
21	8.6E+09	BDL	BDL	BDL	BDL	BDL
			Low Manure Application Rate			
1	3.4E+09	6.1E+07	7.6E+06	BDL	8.7E+07	1.9E+09
7	3.2E+09	1.9E+07	5.3E+05	BDL	2.5E+07	3.1E+08
21	1.7E+09	5.2E+06	7.7E+04	BDL	9.8E+06	2.0E+07
			High Manure Application Rate			
1	7.0E+09	4.3E+08	7.6E+07	BDL	2.3E+08	1.1E+10
7	2.6E+10	6.6E+07	1.2E+07	BDL	4.1E+08	2.6E+09
21	1.8E+10	8.2E+07	2.6E+06	BDL	1.1E+08	8.9E+08

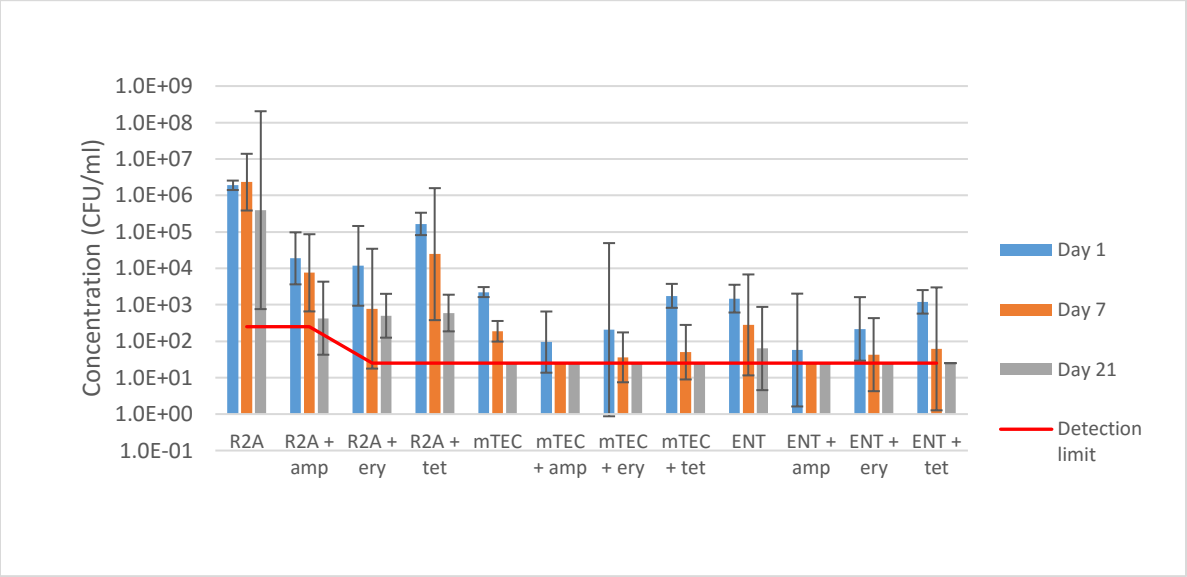

Figure 1. Geometric mean concentrations (CFU/mL) of total bacteria (R2A), E. coli (mTEC), and enterococci (ENT) in swine lagoon manure held at room temperature for 1, 7, and 21 days. Error bars represent 95 % confidence intervals. Concentrations of antibiotic-resistant bacteria were determined by plating on agar amended with ampicillin (amp; 100 mg/L), erythromycin (ery; 100 mg/L), and tetracycline (tet; 16 mg/L). Bars aligned with the detection limit of 250 or 25 CFU/mL denote concentrations that were below the detection limit.

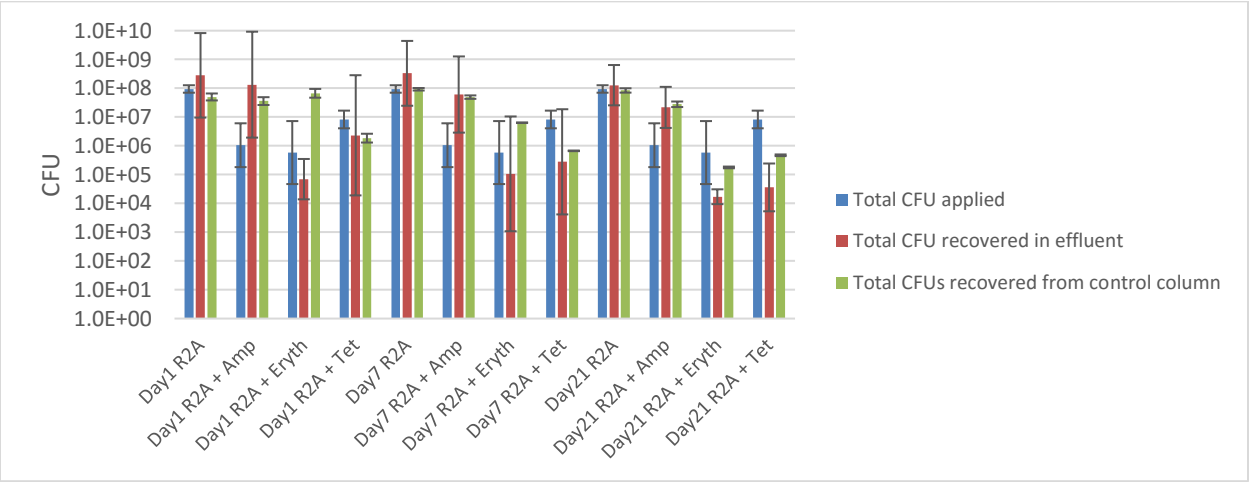

Figure 2. Geometric means of total CFU of bacteria applied to each column and recovered in column effluent following 1, 7, or 21 day interval between manure application (low rate) and rainfall event. Error bars represent 95 % confidence intervals. Also included are total CFU recovered from control columns following application of sterilized manure. Concentrations of antibiotic-resistant bacteria were determined by plating on agar amended with ampicillin (amp; 100 mg/L), erythromycin (ery; 100 mg/L), and tetracycline (tet; 16 mg/L).

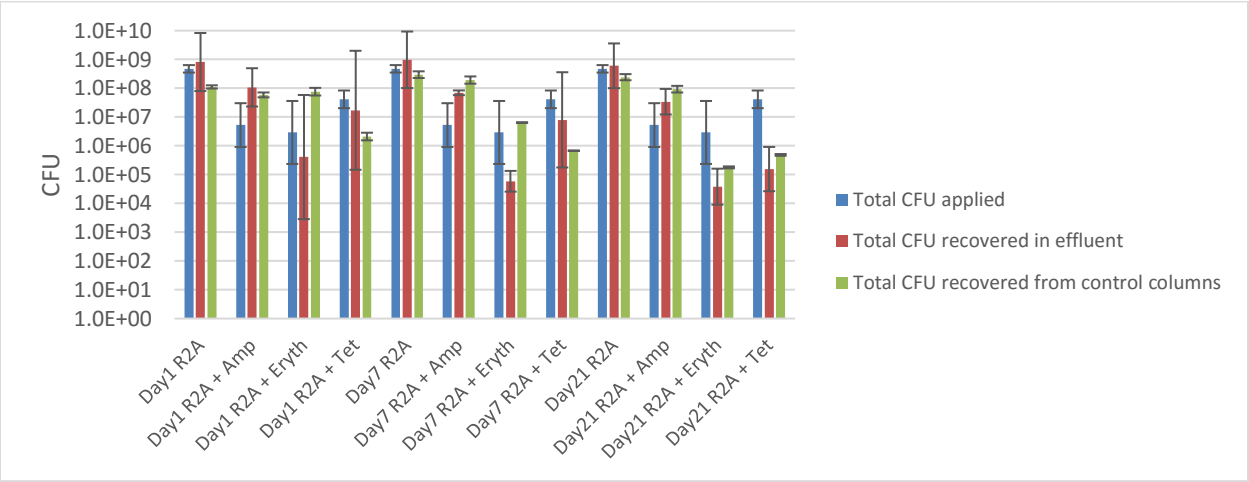

Figure 3. Geometric means of total CFU of bacteria applied to each column and recovered in column effluent following 1, 7, or 21 day interval between manure application (high rate) and rainfall event. Error bars represent 95 % confidence intervals. Also included are total CFU recovered from control column following application of sterilized manure. Concentrations of antibiotic-resistant bacteria were determined by plating on agar amended with ampicillin (amp; 100 mg/L), erythromycin (ery; 100 mg/L), and tetracycline (tet; 16 mg/L).

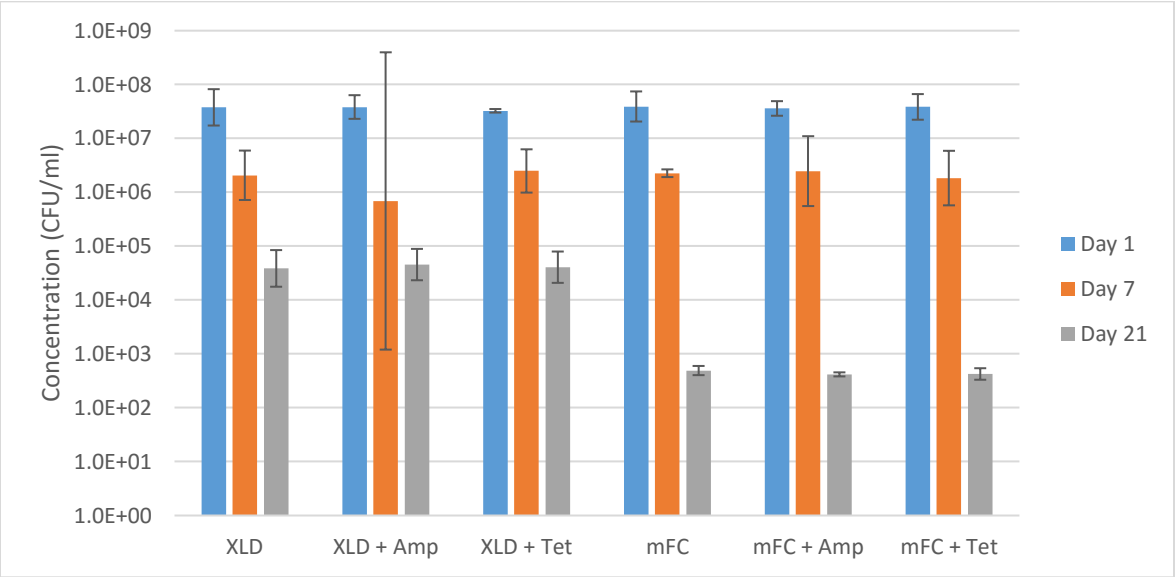

Figure 4. Geometric means of cultivated *E. coli* (mFC) and *Salmonella* (XLD) in swine effluent (CFU/mL) applied to fine sand columns (Exp 2). Error bars represent 95 % confidence intervals.

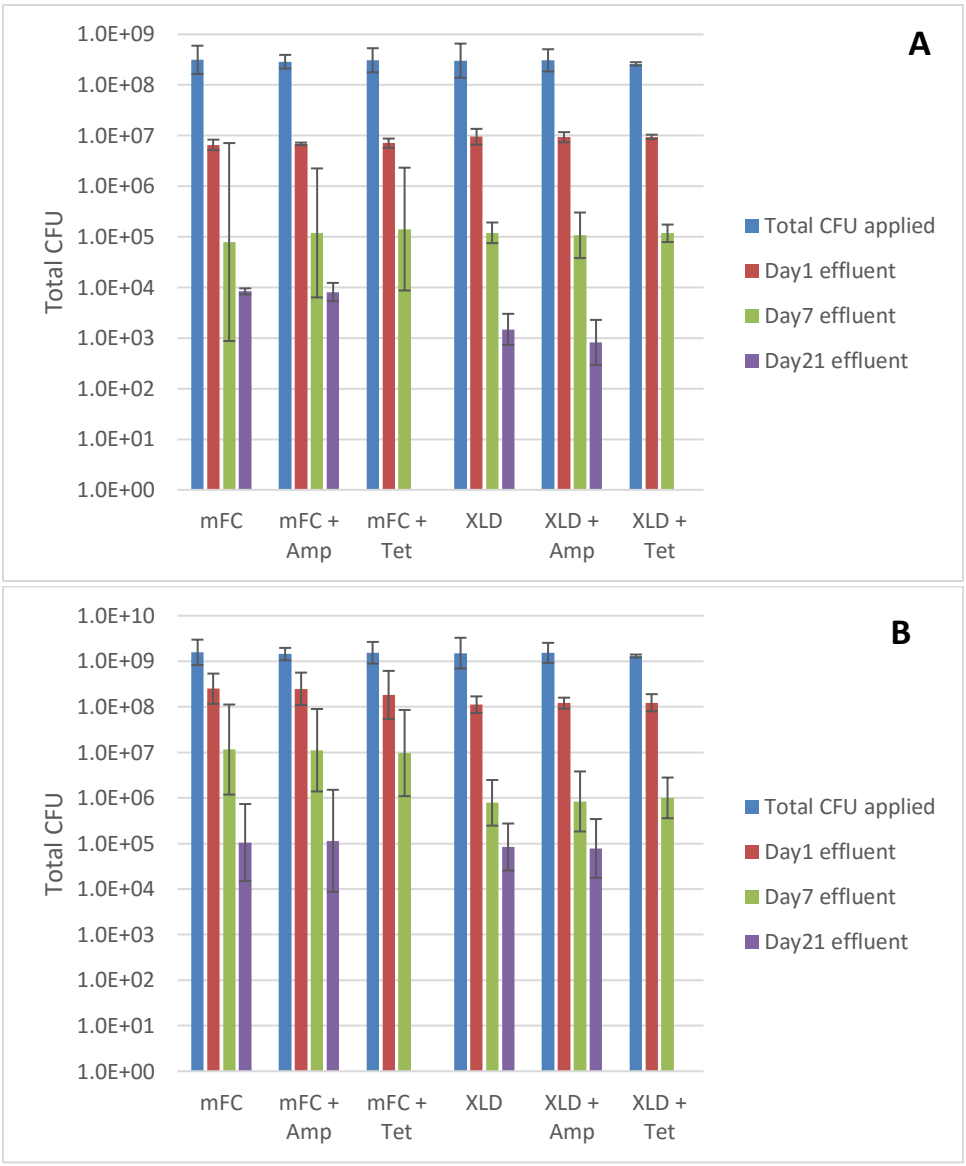

Figure 5. Geometric means of total CFU of *E. coli* (mFC) and *Salmonella* (XLD) applied to each column and recovered in column effluent following 1, 7, or 21 day interval between manure application and rainfall event for A) low and B) high application rates (Exp 2). Error bars represent 95 % confidence intervals. Concentrations of antibiotic-resistant bacteria were determined by plating on agar amended with ampicillin (amp; 100 mg/L) and tetracycline (tet; 16 mg/L). Tetracycline-resistant bacteria were not measured at day 21.

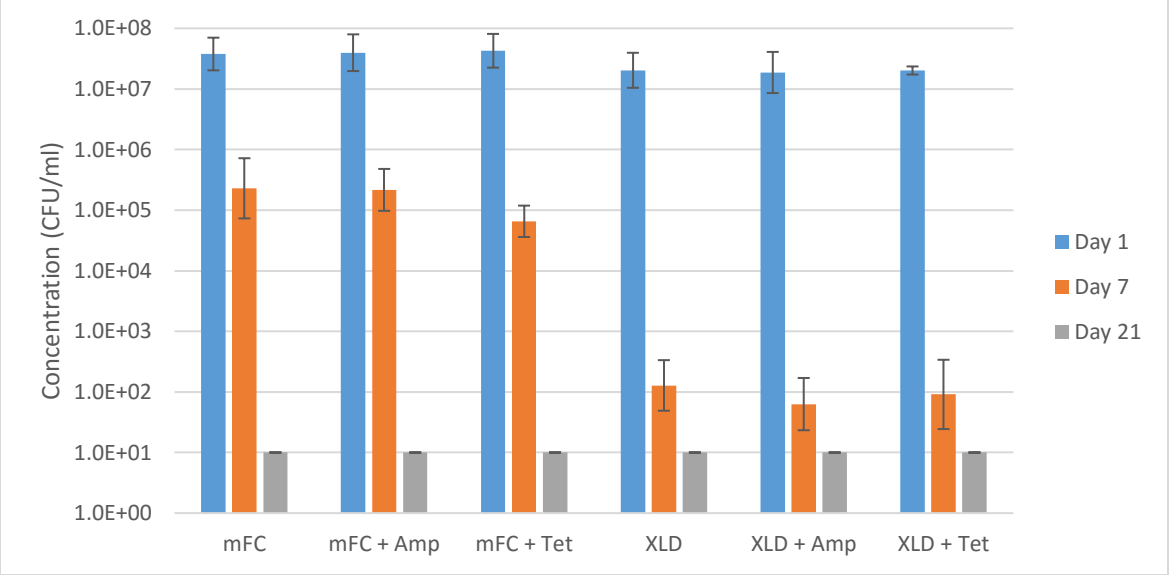

Figure 6. Geometric means of cultivated *E. coli* (mFC) and *Salmonella* (XLD) in swine effluent (CFU/mL) applied to loamy sand columns (Exp 3). Error bars represent 95 % confidence intervals. Concentrations at day 21 were below detection limit for all agar and antibiotic combinations.