University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Civil and Environmental Engineering Faculty
Publications

Civil and Environmental Engineering

4-2020

Fate and transport of antibiotics and antibiotic resistance genes in runoff and soil as affected by the timing of swine manure slurry application

Renys Enrique Barrios

University of Nebraska-Lincoln, renbar7@yahoo.com

Himanshu Khuntia

University of Nebraska - Lincoln

Shannon L. Bartelt-Hunt

University of Nebraska - Lincoln, sbartelt2@unl.edu

John E. Gilley

USDA ARS, john.gilley@ars.usda.gov

Amy M. Schmidt Schmidt

University of Nebraska-Lincoln, aschmidt@unl.edu

Follow this and additional works at: <https://digitalcommons.unl.edu/civilengfacpub>

See next page for additional authors

Part of the [Bioresource and Agricultural Engineering Commons](#), and the [Environmental Engineering Commons](#)

Barrios, Renys Enrique; Khuntia, Himanshu; Bartelt-Hunt, Shannon L.; Gilley, John E.; Schmidt, Amy M. Schmidt; Snow, Daniel D.; and Li, Xu, "Fate and transport of antibiotics and antibiotic resistance genes in runoff and soil as affected by the timing of swine manure slurry application" (2020). *Civil and Environmental Engineering Faculty Publications*. 198.
<https://digitalcommons.unl.edu/civilengfacpub/198>

This Article is brought to you for free and open access by the Civil and Environmental Engineering at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Civil and Environmental Engineering Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Renys Enrique Barrios, Himanshu Khuntia, Shannon L. Bartelt-Hunt, John E. Gilley, Amy M. Schmidt
Schmidt, Daniel D. Snow, and Xu Li

Fate and transport of antibiotics and antibiotic resistance genes in runoff and soil as affected by the timing of swine manure slurry application

Renys E. Barrios^a, Himanshu K. Khuntia^a, Shannon L. Bartelt-Hunt^a, John E. Gilley^b, Amy M. Schmidt^c, Daniel D. Snow^d, Xu Li^{a,*}

^a Department of Civil Engineering, University of Nebraska-Lincoln, Lincoln, NE 68588, United States

^b USDA-ARS, Agroecosystem Management Research Unit, Lincoln, NE 68583, United States

^c Departments of Biological Systems Engineering and Animal Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, United States

^d School of Natural Resources, Water Sciences Laboratory, University of Nebraska-Lincoln, Lincoln, NE 68583, United States

HIGHLIGHTS

- Following manure application two weeks are needed to yield low ARG levels in runoff.
- Two weeks are also needed to yield low antibiotic levels in runoff.
- ARG levels in soil hardly changed in the four weeks following manure application.
- Antibiotic levels in soil dropped in the first two weeks after manure application.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:

Received 1 November 2019

Received in revised form 29 December 2019

Accepted 2 January 2020

Available online 7 January 2020

Editor: Jay Gan

Keywords:

Timing

Manure application

Antibiotics

Antibiotic resistance genes

Runoff

Soil

ABSTRACT

Land application of swine manure slurry is a common practice to supplement nutrients to soil for crop production. This practice can introduce antibiotic residues and antibiotic resistance genes (ARGs) into the environment. Field testing is critical in identifying manure management practices effective in minimizing the environmental impacts of manure-borne antibiotic and ARGs. The objective of this study was to determine how the timing of swine manure application relative to rainfall events impacts the fate and transport of antibiotics and ARGs in surface runoff and manure-amended soil. Swine manure slurry was either broadcast or injected on test plots in the field. A set of three 30-min simulated rainfall events, 24 h apart, were initiated on manured plots 1 day, 1 week, 2 weeks, or 3 weeks after the manure application. Results showed that an interval longer than 2 weeks between application and rainfall often significantly reduced the levels of antibiotics and ARGs tested in runoff with the exception of *tet(X)*. For soil samples from broadcast plots, concentrations of two of the three antibiotics tested (lincomycin and tiamulin) decreased substantially in the first two weeks after manure application. In contrast, concentrations of most of the ARGs tested (*tet(Q)*, *tet(X)*, and *erm(A)*) in soil did not change significantly during the test period. Information obtained from the study can be beneficial in designing manure management practices and estimating the environmental loading of antibiotics and ARGs resulting from manure application.

© 2020 Published by Elsevier B.V.

* Corresponding author at: 844 N. 16th St., N117 SLNK, Department of Civil Engineering, UNL, Lincoln, NE 68588-6105, United States.

E-mail address: xuli@unl.edu (X. Li).

1. Introduction

Livestock manure has long been used as a soil conditioner in crop production for its nutrient content and positive effects on soil structure. In the US, the number of pigs raised in confined housing over the past four decades has increased as climate control, feed and water intake, health and safety of the animals can be more closely monitored. These systems are defined by regulatory standards as animal feeding operations (AFOs) and can be further defined as concentrated animal feeding operations (CAFOs) based on the quantity of animals maintained. In most confined swine production systems, antibiotics and other pharmaceuticals are used for disease treatment, prophylaxis, and in some production settings, for growth promotion (Gaskins et al., 2002). The antibiotics added in animal feed are often not completely absorbed in the animal gut; therefore, antibiotic residues may be excreted in the animals' wastes. The antibiotic residues in the animal gut and in animal manure may contribute to the emergence of antibiotic resistance among commensal and pathogenic bacteria (Salyers et al., 2004). Antibiotic residues and antibiotic resistance genes (ARGs), the genetic materials that confer antibiotic resistance to bacteria, have been documented in water bodies adjacent to CAFO sites (Chee-Sanford et al., 2009; Dolliver and Gupta, 2008; Koike et al., 2007).

Many factors can influence the fate and transport of manure-borne antibiotics and ARGs in the environment, including manure management practices such as species from which manure originates, application rate, land application method, and timing of manure application relative to precipitation. For example, the application of swine and poultry manure can result in higher diversity and concentrations of ARGs in the soil compared to cattle manure (Sandberg and LaPara, 2016; Zhang et al., 2017). Higher application rates of manure can yield greater concentrations of antibiotics and greater abundance of resistant bacteria in soil receiving manure (Lin et al., 2016; Xu et al., 2015) as well as in drainage water from manured soils (Luby et al., 2016). Different land application methods of swine manure slurry (e.g., broadcast, incorporation, and injection) can result in varying ARG concentrations in runoff (Joy et al., 2013). However, the impacts of application timing relative to rainfall on the fate and transport of antibiotics and ARGs have not been systematically studied.

The objective of this study was to determine how the timing of swine manure land application in relation to rainfall events affects the concentrations of manure-borne antibiotics and ARGs in runoff and in soil. In this study, swine manure slurry was either broadcast (i.e., surface applied) or injected on test plots that were established in the field using a factorial design. A set of three 30-min simulated rainfall events, 24 h apart, were initiated on the manure amended plots 1 day, 1 week, 2 weeks, or 3 weeks after the manure application. Analysis of variance (ANOVA) was used to evaluate the effect of two treatment factors (i.e., timing of manure application relative to rainfall and the order of rainfall event) on the concentrations of antibiotics and ARGs in runoff and soil. Results from the study will be helpful in guiding manure application practices and estimating the environmental loads of manure-borne antibiotics and ARGs to soil and surface water.

2. Materials and methods

2.1. Study site characteristics

Field tests were conducted from June through August 2014 at the University of Nebraska-Lincoln (UNL) Rogers Memorial Farm, located 18 km east of Lincoln, NE. The Aksarben silty clay loam soil at the site (fine, smectitic, mesic Typic Argiudoll) contained 16% sand, 48% silt, 36% clay, 4.0% organic matter, 1.8% total carbon, and had a mean slope of 9.8% (Kettler et al., 2001). This soil developed in loess deposits under prairie vegetation and is considered a bench mark soil within the Corn Belt. The study site has been cropped using a no-till management system under a corn (*Zea mays* L.), grain sorghum (*Sorghum*

bicolor (L.) Moench), soybean (*Glycine max* (L.) Merr.), and winter wheat (*Triticum aestivum* L. cv. Pastiche) rotation. The site where field tests were conducted had not had manure applied since 1966. Total cumulative precipitation during the study period was 4.8 mm (0.19 in.).

2.2. Slurry collection and plot preparation

Swine slurry was collected from the deep pit of a commercial 8000-head wean-to-finish swine operation in north central Nebraska just prior to field application. Antibiotic administration information was obtained from the facility operator. A commercial manure applicator was hired to broadcast and inject slurry at the experimental site. The slurry was applied at approximately 46,800 L/ha (5000 gal/ac), an application rate that is usually used in commercial application. For broadcasting, the applicator was lifted above the soil while maintaining a steady speed and flow rate to ensure uniform slurry distribution on the soil surface. For injection, a v-shaped chisel (horizontal sweep) implement was used on an 8-row applicator for manure placement.

Thirty-two 0.75-m wide × 2-m long plots were established along the slope, sixteen receiving manure through broadcast and sixteen through injection. Within the plots receiving manure through the same application method, there were four replicate plots for each of the four application timings relative to rainfall (initial simulated rainfall started 1 day, 1 week, 2 weeks, or 3 weeks after manure land application) with plots assigned to the same timing adjacent to each other.

2.3. Rainfall simulation procedures

The rainfall simulation procedures used in the study were adopted from the National Phosphorous Research Project (Sharpley and Kleinman, 2003). Well water was applied to paired plots at an intensity of approximately 70 mm/h (2.75 in/h) for 30 min using a portable rainfall simulator, based on a published design (Humphry et al., 2002). After manure application, three runs of simulated rainfall were conducted on the plots approximately 24-h apart. Precipitation application rates were measured with two rain gauges placed along the outer edge of the plots and one placed in the center between the plots. The measured and target rainfall simulation rates were similar.

Field rainfall simulations were initiated 1 day (i.e., hereafter referred to as 0 week), 1 week, 2 weeks, and 3 weeks following slurry application. Eight plots were tested each week, and each plot was examined only once throughout the course of the study. Additional details of the rainfall simulation tests can be found in the Supplementary information (SI) file.

A composite runoff sample from each plot was collected into a large container. The runoff sample was transferred to three 1-L plastic bottles for ARG analyses and to one 250-mL amber glass jar for antibiotic analysis. All sample containers were held on ice until delivery to the laboratories at UNL. The plots were not protected from natural rainfall, which happened twice during the study period: July 3rd 2014 (2.5 mm) and July 18th 2014 (2.3 mm).

2.4. Soil core collection

Soil cores were obtained from four plots just prior to the application of swine manure slurry to identify the background concentrations of antibiotics and ARGs in soil. Each week soil cores were collected from eight plots before rainfall simulation tests (four broadcast plots and four injection plots, Table S1, lightly shaded cells) and soil cores were collected again from the same eight plots after the three simulated rainfall events (Table S1, heavily shaded cells). A 5 cm diameter × 30 cm long core sampler (AMS Inc.) with a plastic liner was used to collect soil cores. The soil samples were placed on ice and quickly transferred to UNL where they were stored in a −20 °C freezer.

During processing, frozen soil cores were pushed out of the plastic liners after thawing and then divided into three subsamples: top

(0–7.5 cm from the soil surface), middle (7.5–15 cm), and bottom (15–30 cm). Each subsample was then homogenized in a plastic bag by hand. Homogenized soil samples were placed in sterile 50-mL centrifuge tubes for ARG analyses and in 250-mL amber glass jars for antibiotic analyses.

2.5. Antibiotic analyses

Antibiotics concentrations were quantified for soil, runoff, and manure slurry samples. Target compounds, chosen based on the usage data from the facility operator and expected persistence or chemical properties of the parent compounds, were chlortetracycline, lincomycin, tiamulin, penicillin G and its degradation product penillic acid. Analytes were extracted and purified from liquid and solid samples and concentrated using polymeric (Oasis HLB 200 mg, Waters Corporation, Milford, MA) solid phase extraction. Oleandomycin was used as surrogate, while roxithromycin, doxycycline, and penicillin V were used as internal standards. Results of method validation indicated detection limits ranged between 0.005 and 0.014 µg/L in water, and between 0.14 and 0.68 ng/g in soil. Concentrated extracts were analyzed on a Waters Quattro Micro triple quadrupole mass spectrometer coupled with a Waters 2695 high pressure liquid chromatography (HPLC). Additional detail about the processing and analytical methods is reported in the Supplementary information file. Surrogate recovery averaged $107 \pm 62\%$ in runoff samples and $80 \pm 35\%$ in soil samples. Method detection limits for the analytes in various sample matrices are reported in Table S2.

2.6. ARG analyses

Well mixed runoff and manure slurry samples were centrifuged at $15,000 \times g$ in 50 mL centrifuge tubes for 10 min. The pellet collected from 300 mL runoff or 50 mL manure slurry was stored at -80°C until DNA extraction. DNA from manure, runoff, and soil was extracted using the MoBio PowerSoil® DNA Isolation Kit (Carlsbad, CA) and purified using the ZYMO OneStep™ PCR Inhibitor Removal Kit (Irvine, CA) according to the manuals. DNA extracts were quantified using a NanoDrop spectrometer (Thermo Scientific, Wilmington, DE). Polymerase chain reaction (PCR) was used to determine which tetracycline and tylosin resistance genes existed in the manure samples (Aminov and Mackie, 2001; Koike et al., 2007; Ng et al., 2001). Quantitative PCR (qPCR) was used to quantify the ARGs that consistently appeared in manure and runoff samples (Aminov and Mackie, 2001; Ghosh et al., 2009a; Koike et al., 2007). The 16S rRNA gene was also quantified using qPCR (Suzuki et al., 2000).

2.7. Statistical analysis

The statistical analysis was based on analysis of variance (ANOVA) using SAS (Cary, NC). Residual plots showed that the raw data for the concentrations of antibiotics and ARGs in both runoff and soil samples were skewed (non-symmetric) and had instable variance. Therefore, antibiotic and ARG data were transformed using log transformation. The treatment means obtained from the transformed data were transformed back to the original scale. For runoff samples, ANOVA tests were used to determine the effects of two treatment factors, application timing (week 0, 1, 2, and 3) and rainfall event (#1, #2, and #3), on the concentration of antibiotics and ARGs in runoff. For soil samples, ANOVA tests were conducted only on the top soil from broadcast plots, and the two treatment factors tested were application timing and rainfall event. If a treatment method was determined as significant ($p < 0.05$), least significant difference (LSD) tests were conducted to determine the significance of the differences among the treatment levels.

3. Results

3.1. Antibiotics and ARGs in swine manure slurry

According to the facility operator, the antibiotics used at the swine facility included chlortetracycline (CTC), lincomycin (LCM), tiamulin (TML), and penicillin G (PNG). Mean antibiotic concentrations in the swine manure slurry were 754.0 ng/g (wet weight, ww) for CTC, 80.8 ng/g for LCM, 189.8 ng/g for TML, and 1.7 ng/g for PNG. Penillic acid (PA), a metabolite of PNG, was also detected in manure slurry samples at 4.6 ng/g.

Nine tetracycline resistance genes (i.e., *tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(E)*, *tet(O)*, *tet(Q)*, *tet(W)*, and *tet(X)*) and five lincomycin resistance genes (i.e., *erm(A)*, *erm(B)*, *erm(C)*, *erm(F)*, and *erm(G)*) were tested for manure samples using PCR. Gel electrophoresis results showed that four ARGs (i.e., *tet(Q)*, *tet(X)*, *erm(A)*, and *erm(C)*) occurred consistently in manure samples, so they were further quantified using qPCR for runoff and soil samples. The absolute abundances of *tet(Q)*, *tet(X)*, *erm(A)*, *erm(C)* and the 16S rRNA gene in manure slurry were $(4.18 \pm 1.41) \times 10^6$, $(2.23 \pm 1.61) \times 10^4$, $(5.57 \pm 0.39) \times 10^5$, $(6.63 \pm 2.73) \times 10^6$ and $(1.25 \pm 0.05) \times 10^7$ copies per g ww, respectively.

3.2. Antibiotics in runoff

Application timing had a significant effect on the concentrations of three antibiotics (i.e., CTC, LCM, and TML) in runoff from both broadcast (Table 1) and injection plots (Table 2), as reflected by the p -values corresponding to the treatment factor Application Timing in these two tables. A longer time between manure application and initial rainfall events is associated with lower concentrations of antibiotics in runoff (Fig. 1). For broadcast plots, virtually no CTC was detected in runoff when the rainfall events occurred two weeks after the manure application (Fig. 1A). LCM and TML were still detected in runoff even when rainfall events occurred three weeks after manure application (Fig. 1C and E) at average concentrations of 0.597 ± 0.256 µg/L and 0.115 ± 0.044 µg/L, respectively. For injection plots, the antibiotic concentrations in runoff were generally lower than those in runoff from broadcast plots (Fig. 1). CTC was rarely ever detected in runoff, and TML was absent in runoff when rainfall events occurred two weeks after manure application (Fig. 1B and F). LCM was still detected in runoff when rainfall events occurred three weeks after manure application at an average concentration of 0.081 ± 0.051 µg/L (Fig. 1D). Unlike the other parent compounds of the antibiotics tested, PNG and its degradation product (PA) were not detected in runoff or soil samples.

The percentage of the total antibiotics in the applied manure that was lost in runoff from both broadcast and injection plots decreased with subsequent runoff events (i.e., Rainfall #1 vs. #2 vs. #3, Table S3). For CTC, the percentage loss to runoff was negligible, regardless of land application method and timing of rainfall events. For both LCM and TML, the percentage loss was smaller in the runoff from injection plots than in the runoff from the broadcast plots (Table S3). The percentage losses were the highest in runoff from rainfall events that occurred 0 and 1 week after manure application and decreased substantially for rainfall events that occurred at least 2 weeks after manure application (Table S3).

The order of the rainfall events only had significant impacts on antibiotic concentrations in runoff from broadcast plots (Table 1) but not on those from injection plots (Table 2), as reflected by the p -values corresponding to the treatment factor Rainfall Event in the two tables. For broadcast plots, the average concentrations of CTC and LCM decreased after each rainfall event (Table 1 and Fig. 1A and C). The trend was less obvious for TML concentrations in runoff (LSD test in Table 1 and Fig. 1E). In contrast, the order of the rainfall had no significant effects on antibiotic concentrations in runoff from the injection plots (Table 2 and Fig. 1B, D, and F).

Table 1

ANOVA results on the effects of application timing and rainfall event on the concentrations of antibiotics and ARGs in runoff from broadcast plots.

	CTC (µg/L)	LCM (µg/L)	TML (µg/L)	<i>tet</i> (Q) (copy/mL)	<i>tet</i> (X) (copy/mL)	<i>erm</i> (A) (copy/mL)	<i>erm</i> (C) (copy/mL)
<i>Application timing</i> ^{a,b}							
0 week	0.067 a	1.565 a	0.553 a	1.59×10^5 a	3.79×10^4 a	7.69×10^4 a	2.72×10^6 a
1 week	0.014 b	2.802 b	0.270 b	2.01×10^2 b	3.99×10^6 b	3.71×10^3 b	2.30×10^5 b
2 weeks	0.001 c	0.959 c	0.093 c	3.78×10^1 c	1.32×10^6 c	9.41×10^2 c	9.46×10^4 c
3 weeks	0.000 c	0.572 d	0.113 c	1.10×10^2 d	1.77×10^6 c	6.97×10^2 c	2.16×10^5 b
<i>Rainfall event</i>							
1	0.036 a	2.037 a	0.290 a	8.54×10^2 a	5.45×10^5	5.32×10^3 a	6.21×10^5 a
2	0.018 b	1.396 b	0.246 ab	7.07×10^2 a	7.74×10^5	3.85×10^3 ab	3.20×10^5 b
3	0.006 c	0.728 c	0.179 b	3.65×10^2 b	1.09×10^6	2.47×10^3 b	1.91×10^5 c
<i>ANOVA value for</i> ^c							
Application timing	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rainfall event	<0.0001	<0.0001	0.0138	0.0020	0.0786	0.0053	<0.0001
Timing × rainfall	<0.0001	0.0001	0.0017	0.0029	<0.0001	0.0524	0.0002

^a Value reported under "application timing" and "rainfall event" are treatment averages, which were calculated based on all the data for one particular treatment level. For example, 0.067 µg/L was calculated using CTC concentrations of all runoff samples from broadcast plots, regardless of whether they were from either rainfall event 1, 2, or 3.

^b Values followed by different letters are significantly different at the 0.05 probability level based on LSD tests.

^c ANOVA values are displayed as *p* values.

3.3. ARGs in runoff

Application timing had significant impacts on the concentrations of all four ARGs in the runoff from both broadcast and injection plots, as shown in the *p*-values associated with this treatment factor (Tables 1 and 2). For broadcast plots, the concentration of *tet*(Q) decreased by three orders of magnitude as the interval between manure application and rainfall events increased (Fig. 2A). Similarly, the concentrations of *erm*(A) and *erm*(C) decreased by one to two orders of magnitude as the interval increased (Fig. 3A and C). In contrast, the concentration of *tet*(X) in runoff increased by two orders of magnitude when the interval increased from 0 to 1 week and then maintained steady (Fig. 2C). In general, the trends of the relative abundance of the ARGs, which was calculated by normalizing the absolute abundance of the ARGs by the 16S rRNA gene, were similar to those of the absolute abundance of the ARGs (Figs. S2 and S3). For injection plots, the concentration trends of the four genes are very similar to their counterparts from the broadcast plots (Figs. 2 and 3).

The order of the rainfall events had significant ($p < 0.05$) or marginally significant ($p < 0.10$) impacts on the ARG concentrations in runoff from broadcast plots. Except for *tet*(X), subsequent rainfall events led to lower ARG concentrations in runoff than did the initial rainfall (Table 1 and Figs. 2 and 3). For *tet*(X), the growth of its

bacterial host(s) led to an increase in runoff concentrations immediately following manure application (Fig. 2C). For injection plots, *tet*(Q) and *tet*(X) genes behaved similarly to their counterparts from the broadcast plots (Table 2). In contrast, the order of rainfall events had no significant impact on the concentrations of *erm*(A) or *erm*(C) in runoff (Table 2), suggesting that injection of swine manure slurry may lead to different fates of the bacteria carrying *erm* genes from those carrying *tet* genes (Figs. S3 and S4).

3.4. Antibiotics in soil of broadcast plots

Soil samples collected from injection plots were not included in this study, as it was impossible to get representative soil samples from injection plots due to the nature of this land application method: soil cores taken either within the injection slots or within the space between injection slots would not adequately represent the plots.

For broadcast plots, application timing had significant impacts on the concentrations of LCM and TML, but not CTC, in top soil (Table 3). A longer interval between manure application and rainfall events led to lower concentrations of LCM and TML in top soil. The concentration of LCM and TML in top soil, prior to simulated rainfalls, dropped from 7.2 ± 5.0 and 15.9 ± 11.0 ng/g dw at week 0 to 0.8 ± 0.7 and 3.1 ± 2.9 ng/g dw at week 3, respectively (Fig. 4). In contrast, the

Table 2

ANOVA tests on the effects of application timing and rainfall event on the concentrations of antibiotics and ARGs in runoff from injection plots.

	CTC (µg/L)	LCM (µg/L)	TML (µg/L)	<i>tet</i> (Q) (copy/mL)	<i>tet</i> (X) (copy/mL)	<i>erm</i> (A) (copy/mL)	<i>erm</i> (C) (copy/mL)
<i>Application timing</i> ^{a,b}							
0 week	0.004 a	0.519 a	0.060 a	2.07×10^4 a	4.61×10^3 a	1.41×10^4 a	1.12×10^5 a
1 week	0.000 b	1.112 b	0.012 b	5.38×10^2 b	2.58×10^5 b	3.76×10^2 b	1.32×10^4 b
2 weeks	0.000 b	0.433 a	0.007 b	1.14×10^2 c	9.26×10^5 c	2.37×10^2 b	1.68×10^4 b
3 weeks	0.000 b	0.080 c	0.007 b	1.44×10^2 c	1.63×10^5 b	5.84×10^1 c	4.22×10^3 c
<i>Rainfall event</i>							
1	0.001	0.593	0.021	1.08×10^3 a	3.72×10^4 a	5.42×10^2	1.66×10^4
2	0.001	0.389	0.019	6.01×10^2 b	1.76×10^5 b	6.63×10^2	2.32×10^4
3	0.002	0.441	0.023	4.33×10^2 b	2.70×10^5 b	3.93×10^2	1.52×10^4
<i>ANOVA value for</i> ^c							
Application timing	0.0217	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rainfall event	0.7073	0.1361	0.9269	<0.0001	<0.0001	0.1002	0.1912
Timing × rainfall	0.9250	0.1497	0.9920	0.0269	0.8740	0.0091	0.0949

^a Value reported under "application timing" and "rainfall event" are treatment averages, which were calculated based on all the data for one particular treatment level. For example, 0.004 µg/L was calculated using CTC concentrations of all runoff samples from injection plots, regardless of whether they were from either rainfall event 1, 2, or 3.

^b Values followed by different letters are significantly different at the 0.05 probability level based on LSD tests.

^c ANOVA values are displayed as *p* values.

Fig. 1. Concentrations of chlortetracycline, lincomycin and tiamulin in runoff from broadcast (A, C, E) and injection (B, D, F) plots when three consecutive simulated rainfall events occurred 1 day (0 week), 1 week, 2 weeks, and 3 weeks after manure application. Error bars represent the standard deviations from four replicate plots.

concentration of CTC in top soil did not decrease significantly during the study period (Fig. 4).

Rainfall events significantly impacted the concentrations of LCM and TML in soil (Table 3), with lower antibiotic concentrations in soil following rainfall (Fig. 4). The greatest reduction in antibiotic concentrations in top soil occurred following the rainfall events in week 0. For example, the average concentration of LCM was 7.2 ± 5.0 ng/g dw before rainfall and decreased to 1.9 ± 3.3 ng/g dw after the rainfall events were over in week 0 (Fig. 4).

3.5. ARGs in soil of broadcast plots

Application timing exhibited significant ($p < 0.05$) or marginally significant ($p < 0.10$) impacts on the concentration of *tet*(Q), *erm*(A) and *erm*(C) in soil (Table 3). While the concentrations of these three genes decreased with an increased interval between manure application and subsequent rainfall events, the decreases were within one order of magnitude (Table 3 and Fig. 5). In comparison, the concentration of *tet*(X) in

soil exhibited a slight increase (Fig. 5), although the trend is not statistically significant (Table 3).

The rainfall events, in general, did not significantly impacts ARG concentrations in soil (Table 3). The gene *erm*(C) was the only ARG that was marginally impacted by rainfall event (Table 3 and Fig. 5D). The concentration of *tet*(X) increased by four orders of magnitude in the top soil after the rainfall event occurred during week 0 and then maintained steady (Fig. 5B). This increase in *tet*(X) concentration in the soil during week 0 agreed with the increase observed in its concentration in runoff when the interval increased from 0 to 1 week (Fig. 2C), suggesting the initial growth of *tet*(X) carrying bacteria in manure amended soil.

4. Discussion

4.1. Effect of application timing on the fate of antibiotics in runoff and soil

The concentration of antibiotics in runoff was generally lower from injection plots than from broadcast plots. Injection of manure slurry

Fig. 2. The absolute abundances of tetracycline resistance genes, *tet(Q)* and *tet(X)*, in runoff from broadcast (A and C) and injection (B and D) plots when three consecutive simulated rainfall events occurred 1 day (0 week), 1 week, 2 weeks, and 3 weeks after manure application. Error bars represent the standard deviations from four replicate plots.

Fig. 3. The absolute abundances of lincosamide resistance genes, *erm(A)* and *erm(C)*, in runoff from broadcast (A and C) and injected (B and D) plots when three consecutive simulated rainfall events occurred 1 day (0 week), 1 week, 2 weeks, and 3 weeks after manure application. Error bars represent the standard deviations from replicate plots.

Table 3

ANOVA tests on the effects of application timing and rainfall event on the concentrations of antibiotics and ARGs in top soil from broadcast plots.

	CTC (ng/g dw)	LCM (ng/g dw)	TML (ng/g dw)	<i>tet</i> (Q) (copy/g dw)	<i>tet</i> (X) (copy/g dw)	<i>erm</i> (A) (copy/g dw)	<i>erm</i> (C) (copy/g dw)
Application timing^{a,b}							
0 week	31.48	1.75a	3.89a	1.46×10^6	3.77×10^3	6.96×10^4	9.61×10^5 a
1 week	15.52	1.87a	5.50a	2.24×10^5	5.98×10^4	5.22×10^4	4.46×10^6 ab
2 weeks	6.60	0.23b	1.18b	1.53×10^5	2.60×10^4	1.37×10^4	1.89×10^5 a
3 weeks	9.27	0.30b	0.83b	7.72×10^5	2.66×10^4	2.40×10^4	8.22×10^5 a
Rainfall event							
Before	25.27	1.61a	5.33a	6.05×10^5	2.71×10^4	4.32×10^4	1.72×10^6
After	6.84	0.30b	0.86b	3.25×10^5	1.45×10^4	2.53×10^4	4.74×10^5
ANOVA value for^c							
Application timing	0.3860	0.0011	0.0025	0.0856	0.6745	0.0924	0.0223
Rainfall event	0.0571	0.0005	<0.0001	0.3630	0.7061	0.2743	0.0624
Timing $\times$ rainfall	0.7930	0.0915	0.0190	0.0085	0.0637	0.0841	0.8890

^a Value reported under “application timing” and “rainfall event” are treatment averages, which were calculated based on all the data for one particular treatment level. For example, 31.48 ng/g soil dw was calculated using CTC concentrations of all soil samples from broadcast plots sampled at week 0, regardless of whether they were collected before or after rainfalls.

^b Values followed by different letters are significantly different at the 0.05 probability level based on LSD tests.

^c ANOVA values are displayed as *p* values.

below the soil surface prevents direct loss through surface runoff, as we showed previously (Joy et al., 2013). For CTC, one previous study reported 4.59% and 2.28% loss to runoff from cattle manure broadcast on and incorporated in soil, respectively (Amarakoon et al., 2014). For pirlimycin, a lincosamide antibiotic, another study showed that 4.94% and 0.17% of the antibiotics in dairy manure broadcast on and injected in soil, respectively, were lost to runoff (Kulesza et al., 2016). Therefore, injection may help prevent antibiotic losses in surface runoff.

As expected, the longer the interval between land application of manure and rainfall events, the lower the antibiotic concentrations in runoff. In this study, the mean concentration of CTC, LCM, and TML in runoff was approximately 67, 3, and 5 times higher when rainfall occurred in

week 0 following manure application than when rainfall occurred in week 3, respectively (Table 1). Previous studies have also reported similar findings for pirlimycin (Kulesza et al., 2016), tylosin (Le et al., 2018), and sulfamethazine (Le et al., 2018). The reason for this decrease in antibiotic concentration in runoff with time is due to processes such as sorption and degradation occurring in the manure/soil layer (see below).

The order of the concentration of antibiotics in manure and soil was CTC > TML > LCM, while the order in runoff was LCM > TML > CTC. The difference is likely due to the sorption and water solubility of each chemical (Sura et al., 2015). For instance, CTC has shown great affinity to manure and soils and limited transport in runoff (Amarakoon et al.,

Fig. 4. Concentrations of chlortetracycline, lincomycin and tiamulin in top of the soil from broadcast plots (A, B, C) before and after simulated rainfall events that occurred 1 day (0 week), 1 week, 2 weeks, and 3 weeks after manure application. Error bars represent the standard deviations from four replicate plots.

Fig. 5. The absolute abundances of *tet(Q)*, *tet(X)*, *erm(A)* and *erm(C)* in top of the soil from broadcast plots (A, B, C, D) before and after simulated rainfall events occurred 1 day (0 week), 1 week, 2 weeks, and 3 weeks after manure application. Error bars represent the standard deviations from four replicate plots.

2016; Bair et al., 2017; Li et al., 2016; Popova et al., 2013). LCM is highly soluble and has been detected in leachate (Domínguez et al., 2014), surface water (Kuchta et al., 2009), and snowmelt runoff (Kuchta and Cessna, 2009) from manure-amended soil.

The concentration of CTC in the soil did not decrease significantly during the four weeks of our experiments, while the concentrations of TML and LCM decreased significantly with time (Table 3). Although specific loss mechanisms were not determined in this study, other studies have reported microbially-mediated degradation or possibly irreversible sorption as mechanisms for observed decreases of antibiotics in soil as a function of time. Half-lives of CTC, TML and LCM in soil are reported as 25, 16, and 9 days, respectively (Albero et al., 2018; Chen et al., 2018; Schlüsener and Bester, 2006). These half-lives can help explain what we observed in our experiments (Fig. 4).

The predicted no effect concentrations (PNEC), concentrations below which no resistance selection would occur, for lincomycin and tiamulin are 2 µg/L and 1 µg/L, respectively (Bengtsson-Palme and Larsson, 2016). While the PNEC for chlortetracycline was not reported in that study, it reports 1 µg/L as the PNEC for tetracycline. In this study, chlortetracycline concentrations in runoff from both broadcast and injection plots were always below 1 µg/L (Fig. 1). For runoff from broadcast plots, lincomycin exceeded its PNEC when rainfall occurred at or less than two weeks after manure application, and tiamulin concentrations were well below its PNEC when there was at least a 1-week interval between rainfall and manure application. In the runoff from injection plots, neither lincomycin nor tiamulin had concentrations exceeding their PNECs, regardless of the timing of rainfall events to manure application (Fig. 1).

4.2. Effect of application timing on the fate of ARGs in runoff and soil

Following manure land application, the growth and die-off of manure-borne bacteria in soil likely had direct impacts on the

concentrations of ARGs in runoff. Following land application, manure-borne bacteria are often exposed to higher oxygen concentrations than those they were exposed to during storage. The increase in oxygen concentration often results in initial growth of facultative bacteria, including many indicator bacteria (Brooks et al., 2012; Çekiç et al., 2017; Martinez et al., 2013; Meals and Braun, 2006; Soupier et al., 2008; Stocker et al., 2015). Previous studies have shown that the concentration of *E. coli* in poultry litter or cattle manure increased by as much as 1–2 orders of magnitude during the first days or weeks following land application to soil (Brooks et al., 2012; Soupier et al., 2008; Stocker et al., 2015).

After the initial increase, manure-borne bacteria start to die off in soil (Meals and Braun, 2006). The die off is likely caused by the antagonistic interactions with indigenous soil microbes, exposure to UV light, and desiccation. Following land application, manure-borne bacteria compete with indigenous soil microorganisms for nutrients (Erickson et al., 2014; Jiang et al., 2002). UV light exposure also increases the decay rate of manure-borne bacteria significantly. The die-off of *E. coli* and fecal coliforms was significantly higher in unshaded cowpats than in shaded ones in one study (Van Kessel et al., 2007). In another study, the decay rate of *E. coli* in fresh cow pats was -0.176 day^{-1} and -0.297 day^{-1} for shaded and unshaded condition, respectively (Oladeinde et al., 2014).

The concentration of ARGs in runoff was usually lower from injection plots than from broadcast plots. Compared to broadcast, injection kept most of the manure-borne bacteria below soil surface and prevented direct loss to surface runoff, as was reported in our previous study (Joy et al., 2013). Conversely, injection may allow manure-borne bacteria to survive longer in soil than broadcast (Hodgson et al., 2016; Mostofa Amin et al., 2013; Truhlar et al., 2018), and be more prone to transport through leaching and runoff (Mostofa Amin et al., 2013; Saini et al., 2010).

For broadcast plots, the impact of timing was more evident for the ARGs in runoff than ARGs in soil. In a previous study, longer interval between manure application and rainfall event resulted in a more significant decrease in the *E. coli* in leachate than that in soil (Saini et al., 2010), suggesting that soil became a reservoir that retained an increasing portion of *E. coli* cells. Hence, we think that most of the manure-borne bacteria likely remained in the soil-manure zone and became less available to runoff as the time interval increased. In contrast, some studies proposed that the attachment of *E. coli* and fecal coliform to soil decreased with increased manure content and manure-borne bacteria are likely to be transported as free-cells in runoff (Guber et al., 2005, 2007). Likewise, another study found that most of the *E. coli* and enterococci transported from cattle manure into runoff were in the unattached state (Soupir and Mostaghimi, 2011). They observed that after the first rainfall event, the portion of *E. coli* and enterococci associated with particles in the runoff was 4.8% and 13%, respectively. In a subsequent rainfall event, the portion in the runoff decreased to 0.06% and 0.98%, respectively.

As reported in other papers, different genes behaved differently in this study. For instance, *tet(X)* behaved differently from the other ARGs tested. *tet(X)* encodes an oxidoreductase that can inactivate tetracycline in the presence of oxygen (Bartha et al., 2011). The gene has been suggested in other studies to be hosted in *Bacteroides* (Bartha et al., 2011), *Sphingobacterium* (Ghosh et al., 2009b), and *Firmicutes* (Wang et al., 2017). *tet(Q)* encodes a ribosomal protection protein (Bartha et al., 2011). The gene is included in the transposon Tn5030 and has been mainly found in anaerobic bacteria (Chung et al., 1999). *erm(A)* and *erm(C)* have been found in facultative anaerobes such as *Staphylococcus aureus* (Lodder et al., 1997; Murphy, 1985), *Helicobacterium* (P.C.Y. et al., 2005), and *Bacillus subtilis* (Bechhofer and Zen, 1989; Bussiere et al., 1998). Among *tet* genes, those encoding ribosomal protection proteins (e.g., *tet(Q)*, *tet(M)* and *tet(O)*) are more likely to be eliminated through aerobic processes, such as aerobic composting or aerobic lagoons (Sui et al., 2017; Wang et al., 2018), while those encoding efflux pumps (e.g., *tet(C)* and *tet(G)*) and enzymatic inactivation genes (e.g., *tet(X)*) tend to be effectively reduced through anaerobic digestion (Sui et al., 2016; Wang et al., 2017). In fact, other studies also found that *tet(X)* increased in aerobic environments (Qian et al., 2016).

5. Conclusions

An interval longer than 2 weeks between application and rainfall often significantly reduced the levels of antibiotics and ARGs tested in runoff with the exception of *tet(X)*. For soil samples from broadcast plots, concentrations of two of the three antibiotics tested (lincomycin and tiamulin) decreased substantially in the first two weeks after manure application. In contrast, concentrations of most of the ARGs tested (*tet(Q)*, *tet(X)*, and *erm(A)*) in soil did not change significantly during the test period. Information obtained from the study can be beneficial in improving manure land application strategies by taking into account the effects of natural attenuation on the loading of manure-borne antibiotics and ARGs in runoff from manured soil.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding for the project was provided in part by the National Pork Board (#15-045). Himanshu K. Khuntia was supported by the WARI program through the Daugherty Water for Food Global Institute and Indo-US Science and Technology Forum. The authors also thank Marzieh Khedmati and Amin Mohebbi for technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.136505>.

References

- Albero, B., Tadeo, J.L., Escario, M., Miguel, E., Pérez, R.A., 2018. Persistence and availability of veterinary antibiotics in soil and soil-manure systems. *Sci. Total Environ.* 643, 1562–1570. <https://doi.org/10.1016/j.scitotenv.2018.06.314>.
- Amarakoon, I.D., Zvomuya, F., Cessna, A.J., Degenhardt, D., Larney, F.J., McAllister, T.A., 2014. Runoff losses of excreted chlortetracycline, sulfamethazine, and tylosin from surface-applied and soil-incorporated beef cattle feedlot manure. *J. Environ. Qual.* 43, 549. <https://doi.org/10.2134/jeq2013.02.0039>.
- Amarakoon, I.D., Surac, S., Zvomuya, F., Cessna, A.J., Larney, F.J., McAllister, T.A., 2016. Dissipation of antimicrobials in a seasonally frozen soil after beef cattle manure application. *J. Environ. Qual.* 45, 1644. <https://doi.org/10.2134/jeq2016.01.0012>.
- Aminov, R.I., Mackie, R.I., 2001. Molecular Ecology of Tetracycline Resistance: Development and Validation of Primers for Detection of Tetracycline Resistance Genes Encoding Ribosomal Protection Proteins. <https://doi.org/10.1128/AEM.67.1.22>.
- Bair, D.A., Popova, I.E., Tate, K.W., Parikh, S.J., 2017. Transport of oxytetracycline, chlortetracycline, and ivermectin in surface runoff from irrigated pasture. *J. Environ. Sci. Health B* 52, 631–640. <https://doi.org/10.1080/03601234.2017.1330069>.
- Bartha, N.A., Söki, J., Urbán, E., Nagy, E., 2011. Investigation of the prevalence of *tetQ*, *tetX* and *tetX1* genes in *Bacteroides* strains with elevated tetracycline minimum inhibitory concentrations. *Int. J. Antimicrob. Agents* 38, 522–525. <https://doi.org/10.1016/j.ijantimicag.2011.07.010>.
- Bechhofer, D.H., Zen, K.H., 1989. Mechanism of erythromycin-induced *ermC* mRNA stability in *Bacillus subtilis*. *J. Bacteriol.* 171, 5803–5811. <https://doi.org/10.1128/jb.171.11.5803-5811.1989>.
- Bengtsson-Palme, J., Larsson, D.G.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environ. Int.* 86, 140–149. <https://doi.org/10.1016/j.envint.2015.10.015>.
- Brooks, J.P., Adeli, A., McLaughlin, M.R., Miles, D.M., 2012. The effect of poultry manure application rate and AlCl₃ treatment on bacterial fecal indicators in runoff. *J. Water Health* 10, 619–628. <https://doi.org/10.2166/wh.2012.033>.
- Bussiere, D.E., Muchmore, S.W., Dealwis, C.G., Schluckebier, G., Nienaber, V.L., Edalji, R.P., Walter, K.A., Lador, U.S., Holzman, T.F., Abad-Zapatero, C., 1998. Crystal structure of *ErmC*, an rRNA methyltransferase which mediates antibiotic resistance in bacteria. *Biochemistry* 37, 7103–7112. <https://doi.org/10.1021/bi973113c>.
- Çekiç, S.K., De, J., Jubair, M., Schneider, K.R., 2017. Persistence of indigenous *Escherichia coli* in raw bovine manure-amended soil. *J. Food Prot.* 80, 1562–1573. <https://doi.org/10.4315/0362-028x.jfp-17-033>.
- Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.-F., Yannarell, A.C., Maxwell, S., 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. <https://doi.org/10.2134/jeq2008.0128>.
- Chen, C., Ray, P., Knowlton, K.F., Pruden, A., Xia, K., 2018. Effect of composting and soil type on dissipation of veterinary antibiotics in land-applied manures. *Chemosphere* 196, 270–279. <https://doi.org/10.1016/j.chemosphere.2017.12.161>.
- Chung, W.O., Young, K., Leng, Z., Roberts, M.C., 1999. Mobile elements carrying *ermF* and *tetQ* genes in Gram-positive and Gram-negative bacteria. *J. Antimicrob. Chemother.* 44, 329–335. <https://doi.org/10.1093/jac/44.3.329>.
- Dolliver, H., Gupta, S., 2008. Antibiotic losses in leaching and surface runoff from manure-amended agricultural land. *J. Environ. Qual.* 37, 1227. <https://doi.org/10.2134/jeq2007.0392>.
- Domínguez, C., Flores, C., Caixach, J., Mita, L., Piña, B., Comas, J., Bayona, J.M., 2014. Evaluation of antibiotic mobility in soil associated with swine-slurry soil amendment under cropping conditions. *Environ. Sci. Pollut. Res.* 21, 12336–12344. <https://doi.org/10.1007/s11356-014-3174-3>.
- Erickson, M.C., Habteselassie, M.Y., Liao, J., Webb, C.C., Mantripragada, V., Davey, L.E., Doyle, M.P., 2014. Examination of factors for use as potential predictors of human enteric pathogen survival in soil. *J. Appl. Microbiol.* 116, 335–349. <https://doi.org/10.1111/jam.12373>.
- Gaskins, H.R., Collier, C.T., Anderson, D.B., 2002. Antibiotics as growth promotants: mode of action. *Anim. Biotechnol.* 13, 29–42. <https://doi.org/10.1081/ABIO-120005768>.
- Ghosh, S., Ramsden, S.J., Lapara, T.M., 2009a. The Role of Anaerobic Digestion in Controlling the Release of Tetracycline Resistance Genes and Class 1 Integrons From Municipal Wastewater Treatment Plants. , pp. 791–796. <https://doi.org/10.1007/s00253-009-2125-2>.
- Ghosh, S., Sadowsky, M.J., Roberts, M.C., Gralnick, J.A., LaPara, T.M., 2009b. *Sphingobacterium* sp. strain PM2-P1-29 harbours a functional *tet(X)* gene encoding for the degradation of tetracycline. *J. Appl. Microbiol.* 106, 1336–1342. <https://doi.org/10.1111/j.1365-2672.2008.04101.x>.
- Guber, A.K., Shelton, D.R., Pachepsky, Y.A., 2005. Transport and retention of manure-borne coliforms in soil. *Vadose Zone J.* 4, 828. <https://doi.org/10.2136/vzj2004.0097>.
- Guber, A.K., Pachepsky, Y.A., Shelton, D.R., Yu, O., 2007. Effect of bovine manure on fecal coliform attachment to soil and soil particles of different sizes. *Appl. Environ. Microbiol.* 73, 3363–3370. <https://doi.org/10.1128/AEM.02434-06>.
- Hodgson, C.J., Oliver, D.M., Fish, R.D., Bulmer, N.M., Heathwaite, A.L., Winter, M., Chadwick, D.R., 2016. Seasonal persistence of faecal indicator organisms in soil following dairy slurry application to land by surface broadcasting and shallow injection. *J. Environ. Manag.* 183, 325–332. <https://doi.org/10.1016/j.jenvman.2016.08.047>.

- Humphry, J.B., Daniel, T.C., Edwards, D.R., Sharpley, A.N., 2002. A portable rainfall simulator for plot-scale runoff studies. *J. Agric. Saf. Health* 18, 199–204. <https://doi.org/10.13031/2013.7789>.
- Jiang, X., Morgan, J., Doyle, M.P., 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl. Environ. Microbiol.* 68, 2605–2609. <https://doi.org/10.1128/AEM.68.5.2605>.
- Joy, S.R., Bartelt-Hunt, S.L., Snow, D.D., Gilley, J.E., Woodbury, B.L., Parker, D.B., Marx, D.B., Li, X., 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. *Environ. Sci. Technol.* 47, 12081–12088. <https://doi.org/10.1021/es4026358>.
- Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified method for soil particle size determination to accompany soil-quality analyses. *Soil Sci. Soc. Am. J.* 65, 849–852.
- Koike, S., Krapac, I.G., Oliver, H.D., Yannarell, A.C., Chee-Sanford, J.C., Aminov, R.I., Mackie, R.I., 2007. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Appl. Environ. Microbiol.* 73, 4813–4823. <https://doi.org/10.1128/AEM.00665-07>.
- Kuchta, S.L., Cessna, A.J., 2009. Fate of lincomycin in snowmelt runoff from manure-amended pasture. *Chemosphere* 76, 439–446. <https://doi.org/10.1016/j.chemosphere.2009.03.069>.
- Kuchta, S.L., Cessna, A.J., Elliott, J.A., Headley, J.V., Canada, E., 2009. Transport of lincomycin to surface and groundwater from manure-amended cropland. *J. Environ. Qual.* 38, 1719–1727. <https://doi.org/10.2134/jeq2008.0365>.
- Kulesza, S.B., Maguire, R.O., Xia, K., Cushman, J., Knowlton, K., Ray, P., 2016. Manure injection affects the fate of pirlimycin in surface runoff and soil. *J. Environ. Qual.* 45, 1466. <https://doi.org/10.2134/jeq2015.06.0266er>.
- Le, H.T.V., Maguire, R.O., Xia, K., 2018. Method of dairy manure application and time before rainfall affect antibiotics in surface runoff. *J. Environ. Qual.* 47, 1310. <https://doi.org/10.2134/jeq2018.02.0086>.
- Li, Y., Wang, H., Liu, X., Zhao, G., Sun, Y., 2016. Dissipation kinetics of oxytetracycline, tetracycline, and chlortetracycline residues in soil. *Environ. Sci. Pollut. Res.* 23, 13822–13831. <https://doi.org/10.1007/s11356-016-6513-8>.
- Lin, H., Sun, W., Zhang, Z., Chapman, S.J., Freitag, T.E., Fu, J., Zhang, X., Ma, J., 2016. Effects of manure and mineral fertilization strategies on soil antibiotic resistance gene levels and microbial community in a paddy-upland rotation system. *Environ. Pollut.* 211, 332–337. <https://doi.org/10.1016/j.envpol.2016.01.007>.
- Lodder, G., Werckenthin, C., Schwarz, S., Dyke, K., 1997. Molecular analysis of naturally occurring ermC-encoding plasmids in staphylococci isolated from animals with and without previous contact with macrolide/lincosamide antibiotics. *FEMS Immunol. Med. Microbiol.* 18, 7–15. [https://doi.org/10.1016/S0928-8244\(97\)00016-3](https://doi.org/10.1016/S0928-8244(97)00016-3).
- Luby, E.M., Moorman, T.B., Soupir, M.L., 2016. Fate and transport of tylosin-resistant bacteria and macrolide resistance genes in artificially drained agricultural fields receiving swine manure. *Sci. Total Environ.* 550, 1126–1133. <https://doi.org/10.1016/j.scitotenv.2016.01.132>.
- Martinez, G., Pachepsky, Y.A., Shelton, D.R., Whelan, G., Zepp, R., Molina, M., Panhorst, K., 2013. Using the Q10 model to simulate *E. coli* survival in cowpats on grazing lands. *Environ. Int.* 54, 1–10. <https://doi.org/10.1016/j.envint.2012.12.013>.
- Meals, D.W., Braun, D.C., 2006. Demonstration of methods to reduce runoff from dairy manure application sites. *J. Environ. Qual.* 35, 1088. <https://doi.org/10.2134/jeq2005.0380>.
- Mostafa Amin, M.G., Forslund, A., Bui, X.T., Juhler, R.K., Søren Petersen, O., 2013. Persistence and leaching potential of microorganisms and mineral N in animal manure applied to intact soil columns. *Appl. Environ. Microbiol.* 79, 535–542. <https://doi.org/10.1128/AEM.02506-12>.
- Murphy, E., 1985. Nucleotide sequence of *ermA*, a macrolide-lincosamide-streptogramin B determinant in *Staphylococcus aureus*. *J. Bacteriol.* 162, 633–640.
- Ng, L., Martin, I., Alfa, M., Mulvey, M., 2001. Multiplex PCR for the Detection of Tetracycline Resistant Genes. , pp. 209–215. <https://doi.org/10.1006/mcpr.2001.0363>.
- Oladeinde, A., Bohrmann, T., Wong, K., Purucker, S.T., Bradshaw, K., Brown, R., Snyder, B., Molina, M., 2014. Decay of fecal indicator bacterial populations and bovine-associated source-tracking markers in freshly deposited cow pats. *Appl. Environ. Microbiol.* 80, 110–118. <https://doi.org/10.1128/aem.02203-13>.
- P.C.Y., W., H., T., S.S.Y., W., C.W.S., T., A.M.Y., F., D.M.W., T., S.K.P., L., K.-Y., Y., 2005. Life-threatening invasive *Helicobacter* infections in intravenous-drug users and *ermA*-mediated erythromycin resistance. *J. Clin. Microbiol.* 43, 6205–6208. <https://doi.org/10.1128/JCM.43.12.6205-6208.2005>.
- Popova, I.E., Bair, D.A., Tate, K.W., Parikh, S.J., 2013. Sorption, leaching, and surface runoff of beef cattle veterinary pharmaceuticals under simulated irrigated pasture conditions. *J. Environ. Qual.* 42, 1167. <https://doi.org/10.2134/jeq2013.01.0012>.
- Qian, X., Sun, W., Gu, J., Wang, X.J., Sun, J.J., Yin, Y.N., Duan, M.L., 2016. Variable effects of oxytetracycline on antibiotic resistance gene abundance and the bacterial community during aerobic composting of cow manure. *J. Hazard. Mater.* 315, 61–69. <https://doi.org/10.1016/j.jhazmat.2016.05.002>.
- Saini, R., Halverson, L.J., Lorimer, J.C., 2010. Rainfall timing and frequency influence on leaching of RS2G through soil following manure application. *J. Environ. Qual.* 32, 1865. <https://doi.org/10.2134/jeq2003.1865>.
- Salyers, A.A., Gupta, A., Wang, Y., 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol.* 12, 412–416. <https://doi.org/10.1016/j.tim.2004.07.004>.
- Sandberg, K.D., LaPara, T.M., 2016. The fate of antibiotic resistance genes and class 1 integrons following the application of swine and dairy manure to soils. *FEMS Microbiol. Ecol.* 92, 1–7. <https://doi.org/10.1093/femsec/fiw001>.
- Schlüsener, M.P., Bester, K., 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. *Environ. Pollut.* 143, 565–571. <https://doi.org/10.1016/j.envpol.2005.10.049>.
- Sharpley, A., Kleinman, P., 2003. Effect of rainfall simulator and plot scale on overland flow and phosphorus transport. *J. Environ. Qual.* 32, 2172–2179. <https://doi.org/10.2134/jeq2003.2172>.
- Soupir, M.L., Mostaghimi, S., 2011. *Escherichia coli* and enterococci attachment to particles in runoff from highly and sparsely vegetated grassland. *Water Air Soil Pollut.* 216, 167–178. <https://doi.org/10.1007/s11270-010-0524-8>.
- Soupir, M.L., Mostaghimi, S., Lou, J., 2008. Die-off of *E. coli* and enterococci in dairy cowpats. *Trans. ASABE* 51, 1987–1996.
- Stocker, M.D., Pachepsky, Y.A., Hill, R.L., Shelton, D.R., 2015. Depth-dependent survival of *Escherichia coli* and enterococci in soil after manure application and simulated rainfall. *Appl. Environ. Microbiol.* 81, 4801–4808. <https://doi.org/10.1128/AEM.00705-15>.
- Sui, Q., Zhang, J., Chen, M., Tong, J., Wang, R., Wei, Y., 2016. Distribution of antibiotic resistance genes (ARGs) in anaerobic digestion and land application of swine wastewater. *Environ. Pollut.* 213, 751–759. <https://doi.org/10.1016/j.envpol.2016.03.038>.
- Sui, Q., Zhang, J., Tong, J., Chen, M., Wei, Y., 2017. Seasonal variation and removal efficiency of antibiotic resistance genes during wastewater treatment of swine farms. *Environ. Sci. Pollut. Res.* 24, 9048–9057. <https://doi.org/10.1007/s11356-015-5891-7>.
- Sura, S., Degenhardt, D., Cessna, A.J., Lamey, F.J., Olson, A.F., McAllister, T.A., 2015. Transport of three veterinary antimicrobials from feedlot pens via simulated rainfall runoff. *Sci. Total Environ.* 521–522, 191–199. <https://doi.org/10.1016/j.scitotenv.2015.03.080>.
- Suzuki, M.T., Taylor, L.T., Long, E.F.D.E., 2000. Quantitative Analysis of Small-subunit rRNA Genes in Mixed Microbial Populations via 5'-Nuclease Assays. 66 pp. 4605–4614.
- Truhlar, A.M., Denes, T.G., Cantilina, K.K., Leung, S.K., Todd Walter, M., Hay, A.G., 2018. Absence of genetic selection in a pathogenic *Escherichia coli* strain exposed to the manure-amended soil environment. *PLoS One* 13, 1–14. <https://doi.org/10.1371/journal.pone.0208346>.
- Van Kessel, J.S., Pachepsky, Y.A., Shelton, D.R., Karns, J.S., 2007. Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. *J. Appl. Microbiol.* 103, 1122–1127. <https://doi.org/10.1111/j.1365-2672.2007.03347.x>.
- Wang, R., Chen, M., Feng, F., Zhang, J., Sui, Q., Tong, J., Wei, Y., Wei, D., 2017. Effects of chlortetracycline and copper on tetracyclines and copper resistance genes and microbial community during swine manure anaerobic digestion. *Bioresour. Technol.* 238, 57–69. <https://doi.org/10.1016/j.biortech.2017.03.134>.
- Wang, J., Sui, B., Shen, Y., Meng, H., Zhao, L., Zhou, H., Li, R., Ding, J., Cheng, H., Zhang, X., 2018. Effects of different biochars on antibiotic resistance genes during swine manure thermophilic composting. *Int. J. Agric. Biol. Eng.* 11, 166–171. <https://doi.org/10.25165/j.jjabe.20181106.4667>.
- Xu, Y., Yu, W., Ma, Q., Zhou, H., 2015. Occurrence of (fluoro)quinolones and (fluoro)quinolone resistance in soil receiving swine manure for 11 years. *Sci. Total Environ.* 530–531, 191–197. <https://doi.org/10.1016/j.scitotenv.2015.04.046>.
- Zhang, Y.J., Hu, H.W., Gou, M., Wang, J.T., Chen, D., He, J.Z., 2017. Temporal succession of soil antibiotic resistance genes following application of swine, cattle and poultry manures spiked with or without antibiotics. *Environ. Pollut.* 231, 1621–1632. <https://doi.org/10.1016/j.envpol.2017.09.074>.