TRANSPORT OF ANTIBIOTICS, ANTIBIOTIC RESISTANT BACTERIA, AND ANTIBIOTIC RESISTANCE GENES THROUGH PRAIRIE STRIPS DURING SIMULATED RUNOFF

Laura M. Alt[1,*], Andrew J. Craig[1], Jena L. Congilosi[2], Diana S. Aga[2],
Adina Howe[1], Michelle L. Soupir[1]

[1] Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa, USA.
[2] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York, USA.
[*] Correspondence: lmalt@iastate.edu

HIGHLIGHTS

- A physical model system was used to track resistance contaminants through prairie strips.
- Resistance contaminants included antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes.
- Subsurface filtration caused the highest concentration reduction of resistance contaminants.
- Infiltration is highlighted as a key feature in concentration and mass load reductions.

ABSTRACT. *Antibiotic use in production animals can drive the development and spread of antibiotic resistance within environmental systems. Transport pathways of resistance contaminants from animals to the environment are known, but there is no regulatory consensus on which specific contaminants should be monitored. Furthermore, there is a need for management practices to combat the transport of these resistance contaminants. The objective of this study was to evaluate Conservation Practice 43 prairie strips for their reduction of three resistance indicators: antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes, from manure-impacted overland flow under varying run-on flow rates. High, medium, and low run-on flow rates were tested with the use of a laboratory-scale flume containing a field-extracted section of prairie strip. Grab samples were collected immediately upstream, downstream, and beneath the prairie strip, representing run-on, runoff, and infiltrated flows, respectively. Percent reductions in the median concentration of each resistance contaminant were calculated for each flow experiment at both the runoff and infiltration sampling locations to demonstrate the impact of the prairie strip. However, in instances where an increase in the median concentration of a contaminant occurred between the run-on sampling location and either the runoff or infiltration sampling locations, a negative percent reduction is reported. Across all flows, the percent reductions in median concentrations at the runoff sampling location ranged from -11.4%–0.8% for antibiotics, 1.2%–28.2% for bacteria, and -4.2%–30.5% for genes. In comparison, the percent reductions in median concentrations at the infiltration sampling location ranged from -23.9%–43.6% for antibiotics, 12.9%–65.5% for bacteria, and -12.6%–43.5% for genes. The dataset from this study supports the use of appropriately designed prairie strips to decrease the concentrations and loads of resistance contaminants that are transported downstream from manured fields and can be used to inform in-field design parameters.*

Keywords. *Antibiotic resistance, Flume, Swine manure, Water quality.*

The emergence, evolution, and spread of antibiotic-resistant pathogens represents one of the world's most urgent public health crises (Murray et al., 2022). Increasingly, bacteria are developing resistance to currently available antibiotics, making it more difficult to treat resulting infections. Government agencies at both national and global levels have identified antibiotic resistance as a danger to public health and have characterized the need to surveil, study, and respond to this threat (FTFCARB, 2020; O'Neill, 2016; WHO, 2014).

Although antibiotic resistance will occur naturally within microbial communities, selective pressures caused by antibiotic use, and misuse, can increase the prevalence of both antibiotic resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) (Allen et al., 2010; Boerlin and White, 2013).

Submitted for review on 29 March 2023 as manuscript number NRES 15611; approved for publication as a Research Article by Associate Editor Dr. Mahmoud Sharara and Community Editor Dr. Kati Migliaccio of the Natural Resources & Environmental Systems Community of ASABE on 17 July 2024.

Citation: Alt, L. M., Craig, A. J., Congilosi, J. L., Aga, D. S., Howe, A., & Soupir, M. L. (2024). Transport of antibiotics, antibiotic resistant bacteria, and antibiotic resistance genes through prairie strips during simulated runoff. *J. Nat. Resour. Agric. Ecosyst., 2*(4), 167-178. https://doi.org/10.13031/jnrae.15611

ISSN 2835-2351 https://doi.org/10.13031/jnrae.15611

In agriculture, the administration of antibiotics to livestock can select for resistance within an animal's gastrointestinal tract, leading to a higher presence of ARBs and ARGs in the animal's manure (Looft et al., 2012; Varga et al., 2009; Zhu et al., 2013). Moreover, many of the antibiotics commonly distributed to production animals are poorly metabolized and can be excreted in biologically active forms as parent compounds or dangerous metabolites in manure, leading to a continued possibility for resistance selection (Xie et al., 2018).

Within the Midwestern U.S., the concentration of swine production generates a high availability of swine manure, which is commonly used as an organic fertilizer (Andersen and Pepple, 2017). Often stored as a liquid or slurry, the application methods of swine manure typically include broadcast or banded surface application followed by incorporation or direct injection (Laguë et al., 2005). The nature of these application methods keeps swine manure on or near the surface of the soil, allowing opportunities for the transport of manure-derived antibiotics, ARBs, and ARGs during rainfall events. The land application of swine manure is a well-documented pathway that creates environmental reservoirs of resistance contaminants, where transportation downstream, and subsequent exposure to humans and other animals, can occur (Garder et al., 2014; Joy et al., 2013; Neher et al., 2020a).

Prairie strips, a recent addition to the USDA's Conservation Reserve Program as Conservation Practice 43 (CP 43), are a best management practice in which 9–37 m (30–120 ft) bands of prairie species native to the U.S. Midwest are planted perpendicular to water flows within and at the edge of cropland (USDA FSA, 2019). Comparable to other vegetative filter strips, prairie strips can promote the deposition of particulate-attached compounds and the adsorption of unattached compounds by slowing runoff and increasing infiltration (Dillaha et al., 1989; Liu et al., 2008). Prairie strips are already acknowledged as an effective management practice to reduce sediments and nutrients from cropland runoff (Helmers et al., 2012; Zhou et al., 2010; Zhou et al., 2014); however, their ability to reduce resistance contaminants is less established (Flater et al., 2022).

The goal of this study was to quantify the removal of swine manure-derived antibiotics (tetracycline), ARBs (tetracycline-resistant *Enterococci*), and ARGs (*ermB*, *ermF*, and *tetM*) from the surface runoff and through the shallow subsurface infiltration of prairie strips. This study further extends the potential benefits of adopting prairie strips by characterizing their ability to mitigate resistance contaminant movement downstream of swine manure treated cropland.

MATERIALS AND METHODS

FLUME FACILITY

Experiments were conducted using a state-of-the-art tempered glass flume (fig. 1) housed in the Buss Hydrology Laboratory at Iowa State University (ISU). Design and operation of the flume are described by Craig et al. (2016). Briefly, the flume is a 0.6 m high by 1.2 m wide by 11.5 m long open channel of uniform cross-section. The slope of the flume can be altered between ±5%, and the tailgate can be adjusted to vary the flow depth for a particular discharge.

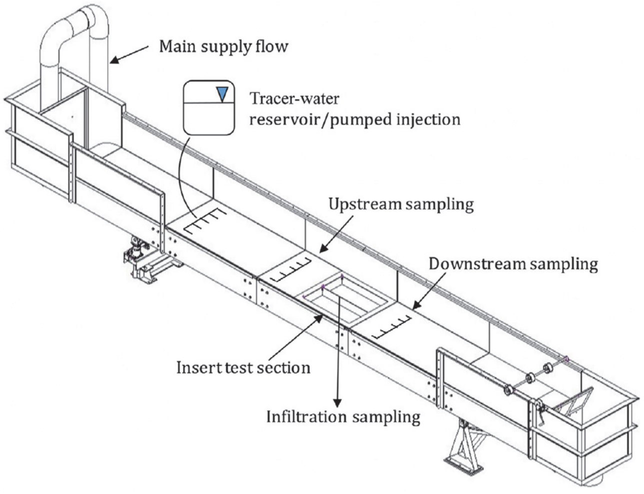

Figure 1. Schematic of the state-of-the-art tempered glass flume used for overland flow experiments (used with permission from Craig et al., 2021).

Water is drawn from a 37.5 m^3 sump at a peak flow rate of 0.25 m^3 s^{-1} using a Hydroflow two-stage mixed flow vertical turbine pump and 60 horsepower motor and supplied to the flume's headbox through a 0.3 m inflow line. Additional manual flow rate control within the flume is provided by a variable-frequency drive and electronically actuated butterfly valves. Flow rates are measured within the flume using an electromagnetic flow meter (magflow, Badger M2000). As seen in figure 1, an essential component of this flume is the 1.16 m long by 0.99 m wide by 0.15 m deep recessed test section, where extracted sections of prairie strips were inserted to create a laboratory-scale physical model system.

PRAIRIE STRIP SITE, EXTRACTION, AND INSTALLATION

Sections of prairie strip were extracted from the ISU WOR research site located in Ames, Iowa. This site incorporates prairie strips into a corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] cropping system and is characterized by gently to strongly sloping Nicollet and Clarion loam soils. Original seeding of the in-field prairie strips occurred on 1 April 2015, using the Stateside Mesic 10-30 Iowa Pollinator Mix.

On three separate occasions, intact sections of an in-field prairie strip were extracted, transported to the ISU Buss Hydrology Laboratory undisturbed, and installed into the recessed test section of the experimental flume as described by Craig et al. (2021). Extractions occurred on 15 April 2019, 25 October 2019, and 4 August 2020, with all sections coming from a large, uniform, flat area of the same in-field prairie strip. To minimize plant senescence within the extracted section of prairie strip, all flume runs took place within three weeks after removal from the field.

EXPERIMENTAL DESIGN

Experimental conditions and sample collection methods follow those provided in Craig et al. (2023), where they are described in much greater detail. Briefly, three experiments were conducted in which overland runoff events were simulated using a range of run-on flow rates. The average run-on flow rates of these experiments included 1.51 (±0.002), 0.36 (±0.01), and 0.24 (±0.006) L s^{-1}, representing a high (1-year, 10-min), medium (1-year, 2-h), and low (1-year, 6-h) flow run-on event, respectively. The approximate equivalent storm return period for each experiment was calculated using the rational method and the conditions of the sample extraction site, making the high, medium, and low flow experiments typical of events that could routinely occur in the field setting. At the start of each experiment, saturated conditions were achieved by running water over the prairie strip until constant infiltration and runoff rates were established.

Following saturation of the prairie strip section, a premade, diluted swine manure mixture was injected upstream of the prairie strip section into the flume's already existing run-on flow (fig. 1), in the case of the high flow experiment, or was added as the sole source of run-on flow, in the case of the medium and low flow experiments. It is recognized that the continual addition of a diluted swine manure mixture may represent extreme environmental conditions, such as the occurrence of a rainfall event promptly following manure application to a field. However, this approach ensured consistent detection of each targeted resistance contaminant at every sampling timepoint throughout the duration of each experiment. Prior to each experiment, swine manure slurry was obtained from a local, tunnel-ventilated, deep pit, wean-to-finish facility known to supplement both tetracycline and tiamulin antibiotics (facility owner, personal communication, 5 December 2018). The diluted swine manure mixture for all experiments was created using a ratio of 11.4 L swine manure slurry added to 481 L of water. In the case of the low flow experiment, this mixture also contained an antibiotic spike that brought the concentration of tetracycline to a minimum of 20 ng mL^{-1}. This designated concentration of tetracycline ensured consistent detection at the downstream sampling locations while still being environmentally relevant (Chee-Sanford et al., 2009; Kim et al., 2011; Xie et al., 2018). Subsamples of the manure were collected before dilution or the addition of antibiotics. During all experiments, the diluted swine manure mixture was continually added, either as a supplement to the already existing run-on or as the sole source of run-on, until the termination of a run.

Throughout all experiments, water run-on, runoff, and infiltration samples were collected immediately upstream, downstream, and below the prairie strip section, respectively. As well, immediately preceding the first flume experiment, as saturated conditions were being achieved in the prairie strip, a run-on, a runoff, and an infiltration sample of water without swine manure were collected to determine a baseline representation of the prairie strip as a potential source for the analyzed antibiotic and antibiotic resistance contaminants.

In order to ensure the diluted swine mixture had reached the appropriate sampling locations, delays after the onset of manure addition were instituted prior to the first runoff and infiltration sample collection of each experiment. The delay at the runoff sampling location accounted for the average run-on flow rate and advection, while the delay at the infiltration sampling location accounted for the average run-on flow rate and the infiltration rate. The total recorded run-on flow rate consisted of either the sum of the base flow rate and the injected flow rate, for the high flow experiment, or the injected flow rate only, for the medium and low flow experiments. For all experiments, the flow rate of infiltration was measured volumetrically throughout the duration of a run, and the runoff flow rate was recorded as the difference between the flow rates of run-on and infiltration. A summary of the conditions for each experiment is shown in table 1.

Samples collected at each location throughout the entirety of an experiment were considered biological replicates. However, the sampling effort of each experiment varied and resulted in different sample sizes. A breakdown of each experiment's sampling effort can be found in supplemental table S1.

ANTIBIOTIC ANALYSIS OF MANURE AND WATER SAMPLES

Prior to the performance of any experiment, preliminary manure samples were freeze dried at ISU and then shipped on ice to the University at Buffalo (UB), where they were analyzed for the presence of antibiotic residues from the Macrolide, Pleuromutilin, Sulfonamide, and Tetracycline drug classes. This initial characterization of the manure antibiotic profile allowed for a selection of ARB and ARG targets with corresponding resistance to be considered during the subsequent flume runs. A full list of specific antibiotic targets is provided in supplemental table S2. All water samples were concentrated using solid phase extraction (SPE) at ISU following the method described by Wallace and Aga (2016). SPE cartridges were then individually wrapped in aluminum foil and shipped on ice to UB for liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. A full description of the chemicals and reagents used, the glassware and plastic preparation, the sample collection and storage, the manure and water extraction methods, the LC-MS/MS methods and optimization, and the criteria used for quality assurance can be found in supplemental material S3–S10. Any sample not considered positive detection based on defined quality assurance criteria was excluded from further analysis.

TOTAL AND TETRACYCLINE-RESISTANT *ENTEROCOCCI* ENUMERATION

Enumeration of *Enterococci* (ENT) and tetracycline-resistant *Enterococci* (TET-ENT) was performed on all manure and water samples using membrane filtration and selective plating as described by APHA (1998). Selective agar included m*Enterococcus* (Difco) without antibiotics (ENT) or m*Enterococcus* agar infused with tetracycline (Sigma-

Table 1. Summary of experimental conditions for each experimental flume run (modified from Craig et al., 2023). Approximate equivalent storm return periods were calculated for the strip extraction site in Ames, Iowa, using the run-on rate of each experiment and the rational method.

Experiment	Average Flow Rates (L s-1) Run-on	Infiltration	Runoff	Approximate Equivalent Storm
High Flow	1.51	0.48	1.04	1-yr, 10-min
Medium Flow	0.36	0.10	0.26	1-yr, 2-h
Low Flow	0.24	0.09	0.15	1-yr, 6-h

Aldrich) at 16 μg L^{-1} (TET-ENT). This tetracycline concentration represents the tetracycline resistance breakpoint for ENT established by the Clinical and Laboratory Standards Institute (CLSI, 2021). Samples were analyzed within 24 h of collection.

DNA EXTRACTION AND QPCR ANALYSIS

Manure subsamples (250 μL) were stored at -20°C until DNA extraction. Water subsamples (75 mL–500 mL) were filtered through 0.22 μm sterile filters within 24 h of sample collection. Filters were then frozen at -20°C in bead tubes until the full extraction process could be completed. DNA extractions were performed using the DNeasy PowerSoil Pro Kit (Qiagen) and DNeasy PowerWater Kit (Qiagen) for the manure subsamples and water filters, respectively. Concentrations of extracted DNA were quantified using the Quant-it dsDNA Assay Kit, high sensitivity (Thermo Fisher Scientific). After quantification, the concentrations of several DNA samples were recorded as 0 ng μL^{-1}; these samples were excluded from further analysis based on evidence that the DNA extraction had failed. Remaining DNA samples were stored at -80 °C.

Quantitative PCR assays were performed on the remaining DNA to quantify the concentrations of the 16S rRNA bacterial gene, two Macrolide-Lincosamide-Streptogramin B (MLSB) resistance genes (*ermB* and *ermF*), and one Tetracycline resistance gene (*tetM*). These specific gene targets were selected based on their previously reported abundance in swine manure (Alt et al., 2021). Standard curves were generated for each gene target using linear regression analysis of tenfold serial dilutions of synthetic template DNA standards (Integrated DNA Technologies) versus their quantification cycles in order to calculate gene concentrations (gene copies 100 mL^{-1}). Each qPCR reaction was performed in triplicate using a 96-well plate format on a CFX96 Touch Real-Time PCR Detection System (BioRad) using methods previously described in Alt et al. (2021). A detailed description of the primer sequences, individual reaction summaries, thermocycle conditions, and limits of quantification (LOQ) are described in supplemental material S11–S12.

STATISTICAL ANALYSIS

All data analyses, including statistical analysis and figure generation, were completed using the RStudio software package with R version 4.2.1 (R Core Team, 2022). Percent reductions in median concentration were calculated for each targeted resistance contaminant along the surface (runoff) and shallow subsurface (infiltration) of the prairie strip insert by comparing the median concentration of a contaminant in the runoff and infiltration samples to its median concentration in the run-on samples. Mass loads of each targeted resistance contaminant were determined for every sample by multiplying a sample's contaminant concentration by its respective flow rate. Percent reductions in median surficial mass loads were calculated for each targeted resistance contaminant by comparing the median mass load of a contaminant in the runoff samples to its median mass load in the run-on samples. The non-parametric Wilcoxon rank-sum test was used to determine if statistically significant reductions occurred between the run-on and runoff concentrations, the run-on and infiltration concentrations, or the run-on and runoff mass loads of any targeted resistance contaminant. An alpha criterion of 0.05 was used to define statistical significance. Quantitative PCR data and code for statistical analyses and figure generation are available at https://github.com/LauraAlt/STRIPS_Flume_AMR_Manuscript.

RESULTS AND DISCUSSION

RESISTANCE CONTAMINANTS WITHIN THE SWINE MANURE AND DILUTED SWINE MANURE MIXTURE

LC-MS/MS analysis confirmed the presence of Tetracyclines, Pleuromutilins, and Macrolides in the swine manure (supplemental fig. S1). As was expected, based on the communicated antibiotic administration to the animals, both the specific antibiotics tetracycline and tiamulin were detected in the manure, with average concentrations of 4,433 ± 1,401 and 2,642 ± 2,105 ng mL^{-1}, respectively. In addition, low levels of the Macrolide antibiotic erythromycin were also detected, with an average concentration of 30 ± 17 ng mL^{-1}. Although antibiotics belonging to the Sulfonamide drug class were included in the target analytes, no member of this antibiotic class was detected, an unsurprising result as there was no communicated history of Sulfonamide administration at the manure source.

When averaged across all three flow experiments, ENT were present in the liquid swine manure at a concentration of 1.32 (± 1.15) x 10^6 cfu 100 mL^{-1}, of which 9.31 (± 7.35) x 10^5 cfu 100 mL^{-1} (70.4%) were resistant to tetracycline. This concentration of ENT was comparable to previously reported values present in swine manure sources, which suggest a range of 10^5–10^7 cfu 100 g^{-1} or mL^{-1} of waste (Hoang et al., 2013; Garder et al., 2014; Luby et al., 2016). The average concentration of the 16S rRNA gene across all three flow experiments was 3.74 (± 3.66) x 10^{12} copies 100 mL^{-1}, while the average concentrations of *ermB*, *ermF*, and *tetM* were 5.92 (± 8.62) x 10^{10}, 1.59 (± 0.96) x 10^{10}, and 1.72 (± 1.64) x 10^{11} copies 100 mL^{-1}, respectively. Comparison of these values with those previously reported is difficult as the concentrations of the 16S rRNA gene and ARGs within manure sources are typically the product of various factors including antibiotic presence, moisture content, pH, and manure storage and handling (Flores-Orozco et al., 2022; Smith et al., 2019). Furthermore, the concentrations of 16S rRNA within any source are likely to be drastically impacted by that source's underlying microbial composition, as gene copy numbers per cell can vary based on bacterial species or metabolic state (Větrovský and Baldrian, 2013).

ENT concentrations within the diluted swine manure mixture ranged from 2.12 x 10^3 to 1.76 x 10^4 throughout all run-on samples collected during the three flow experiments. Serving as the oncoming source of resistance contaminants, the diluted swine manure mixture was created with the intent of achieving ENT concentrations typical of runoff waters leaving manure-impacted agricultural areas. Although dependent on seasonality, previous investigations have reported peak ENT concentrations ranging from 10^3–10^4 cfu 100 mL^{-1} within the surface runoff and subsurface tile drainage of swine manured fields (Hoang et al., 2013; Jaffrezic et al., 2011; Neher et al., 2020b). The calculated ratio of swine manure slurry to water accounted for dilution as well as a previously observed tendency for bacterial die-off, hypothesized to be caused by cell lysis upon dilution of the hypertonic manure with hypotonic water.

In comparison, the concentrations of the select antibiotic and antibiotic resistance contaminants in the non-manured storm reuse water samples taken prior to the first flume experiment were either universally low or non-existent. Concentrations of tetracycline were below the limit of detection in the run-on, runoff, and infiltration samples. ENT concentrations were 3, 0, and 88 cfu 100 mL^{-1} in the run-on, runoff, and infiltration samples, respectively, while no TET-ENT were detected at any of the sample locations. Similarly, the concentrations of the 16S rRNA gene were 3.47 x 10^{8}, 3.52 x 10^{8}, and 5.41 x 10^{8} copies 100 mL^{-1} for the run-on, runoff, and infiltration samples, respectively, but concentrations of *ermB*, *ermF*, and *tetM* were below the limits of quantification at all the sample locations. The significant detection of these antibiotic and antibiotic resistance contaminants in the swine manure slurry, coupled with their absence in non-manured water samples processed through the flume system, made them appropriate targets for quantifying the specific movement of manure-derived antibiotic resistance during the flume experiments.

ANTIBIOTIC CONCENTRATION REDUCTIONS

Among the antibiotics tested, only tetracycline was consistently present above its limit of detection (0.32 ng mL^{-1}) in water samples from all three flow experiments. Consequently, only tetracycline was investigated for removal by the prairie strip insert. During both the high and medium flow experiments, the concentrations of tetracycline within water samples were often low ($\leq$ 1 ng mL^{-1}) and approached the limit of detection. To ensure that tetracycline concentrations would be reliably present above the limit of detection in all samples during the low flow experiment, an environmentally relevant antibiotic spike was added to the diluted swine manure mixture at a concentration of 20 ng mL^{-1}.

Attributed to its high sorption coefficient (K_d) value (>400–1,620 L kg^{-1}), tetracycline has a strong tendency to complex with the cations present in organic matter (OM) and soil clay minerals (Lou et al., 2018; Tolls, 2001). Reductions in tetracycline concentrations were therefore expected to occur through the deposition of particulate OM along the surface of the prairie strip, and consequently the deposition of any particulate OM-bound tetracycline, or via the adsorption of tetracycline within the prairie strip soil during subsurface filtration. The predicted deposition of OM-bound tetracycline was not supported, as no significant decrease in tetracycline concentrations were observed in runoff during any of the flow experiments (fig. 2). Conversely, in the case of the low flow experiment, median tetracycline concentrations in infiltrated flows did show a statistically significant reduction, decreasing by 43.6% (table 2).

Currently, little is known about the ability of vegetative filter strips to reduce antibiotic concentrations or loads from surface runoff. Within those studies that have been conducted (Moody et al., 2022; Soni et al., 2015), the effectiveness of vegetative filter strips at removing antibiotics is typically limited by either the length of the strip or by a tendency for the antibiotic targets to be predominantly transported within the dissolved phase of runoff. Because the sole source of run-on contaminants was an injection of diluted swine manure rather than a section of manure applied to cropland, it could be hypothesized that these mechanisms similarly limited antibiotic reductions from surface runoff through the prairie strips.

Without the addition of field soil to the diluted manure mixture, the vast majority (70%–90%) of the solids within the injected run-on were likely to be smaller than 45 µm (Zhang et al., 2018). While it has been shown that larger particles, >40 µm, can be almost completely removed from run-on within the first five meters of a vegetative filter strip, smaller particles tend to either stay completely in suspension or require much longer residence times for capture within the strips (Gharabaghi et al., 2006; Liu et al., 2008). Also, although the partitioning of tetracycline between the dissolved phase and the sediment-bound phase was not measured during this study, tetracycline within swine manure may preferentially bind with dissolved OM over particulate OM.

Within infiltration, the significant reductions of tetracycline concentrations were likely linked to tetracycline's strong adsorption to organic matter as the flow passed through the prairie strip soil. This observation was similarly documented in a previous study where the highest adsorption of tetracycline occurred in soils that contained the highest

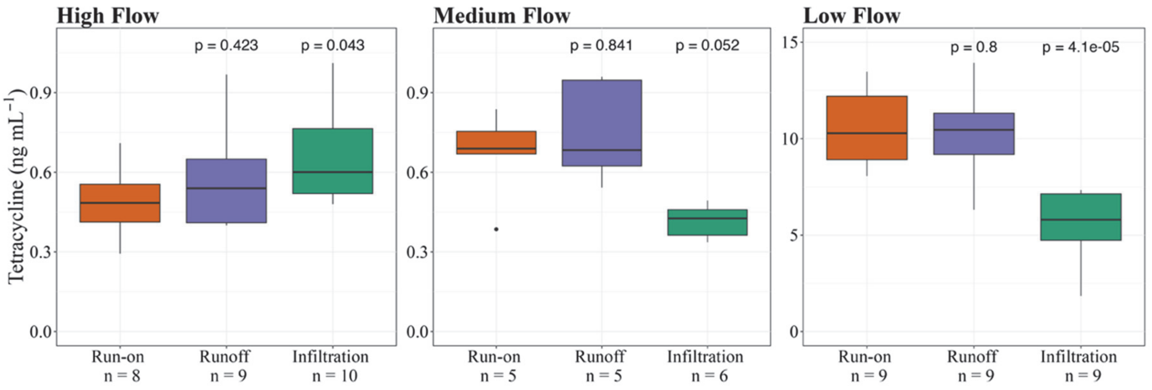

Figure 2. Box and whisker plots of tetracycline concentrations at each sampling location for the three flow experiments. P-values generated from pairwise comparisons made between the median concentrations in run-on and runoff or run-on and infiltration during each flow are displayed.

Table 2. Summary of sample size (n), median concentration (C_{median}), and percent median concentration reduction (% C_{median} Reduction) along the surface (runoff) and through the shallow subsurface (infiltration) of the prairie strip insert during each flow experiment.

				Run-on		Run-off		Infiltration		% C_{median} Reduction (p-value)	
				n	C_{median}	n	C_{median}	n	C_{median}	Runoff	Infiltration
Antibiotics	(ng mL $^{-1}$)	Tetracycline	High	8	0.48	9	0.54	10	0.60	-11.4% (0.42)	-23.9% (0.043)
			Med	5	0.69	5	0.68	6	0.43	0.8% (0.84)	38.1% (0.052)
			Low	9	10.3	9	10.5	9	5.80	-1.7% (0.80)	43.6% (0.00004)
Bacteria	(cfu 100 mL^{-1})	ENT	High	9	3480	9	3220	10	3030	7.5% (0.093)	12.9% (0.020)
			Med	5	14200	5	10200	7	4900	28.2% (0.016)	65.5% (0.0025)
			Low	9	10700	9	9500	9	7350	11.2% (0.16)	31.3% (0.0009)
		TET-ENT	High	9	3260	9	3220	10	2610	1.2% (0.83)	19.9% (0.0007)
			Med	5	6200	5	5550	7	2680	10.5% (0.40)	56.8% (0.0057)
			Low	9	8300	9	7900	9	5750	4.8% (0.085)	30.7% (0.0020)
Genes	(copies 100 mL^{-1})	16S rRNA	High	9	5.54E+09	9	5.30E+09	9	6.24E+09	4.4% (0.67)	-12.6% (0.55)
			Med	5	1.33E+10	5	1.07E+10	7	7.50E+09	19.3% (0.31)	43.5% (0.010)
			Low	6	2.97E+09	8	2.96E+09	8	2.33E+09	0.3% (0.75)	21.4% (0.75)
		ermB	High	9	1.16E+08	9	1.08E+08	9	9.77E+07	7.7% (0.60)	16.1% (0.44)
			Med	5	4.58E+07	5	3.81E+07	7	2.70E+07	16.9% (0.31)	41.1% (0.0025)
			Low	6	9.60E+07	8	7.30E+07	8	7.37E+07	23.9% (0.80)	23.2% (1.0)
		ermF	High	9	1.10E+07	9	8.90E+06	9	8.11E+06	19.5% (0.30)	26.6% (0.19)
			Med	5	1.10E+08	5	7.63E+07	7	7.59E+07	30.5% (0.15)	31.0% (0.0051)
			Low	6	2.01E+08	8	2.09E+08	8	1.56E+08	-4.2% (0.66)	22.5% (0.85)
		tetM	High	9	2.85E+08	9	2.87E+08	9	3.10E+08	-0.6% (0.80)	-9.0% (0.34)
			Med	5	2.01E+08	5	1.44E+08	7	1.35E+08	28.3% (0.15)	33.0% (0.073)
			Low	6	4.82E+08	8	4.83E+08	8	4.14E+08	-0.05% (0.85)	14.1% (0.85)

organic matter (Conde-Cid et al., 2019). Because the storage of soil organic carbon has been shown to be positively correlated with plant diversity (Chen et al., 2018; Lange et al., 2015), the polyculture of prairie strips may offer an advantage over the monoculture of other typical filter strips in the adsorption of tetracycline during infiltration.

ARB CONCENTRATION REDUCTIONS

Similar to the transport of antibiotic targets, it was expected that bacterial targets could move within runoff as either unattached cells, freely suspended within the water column, or as attached cells, bound to particulates. In runoff experiments performed by Soupir et al. (2010), an average of 28%–49% of ENT were reported to move as particulate-attached cells, adsorbed across a range of particle soil size. As well, Hoang et al. (2013) reported a strong positive correlation between the concentrations of ENT and the total suspended solids in drainage water. It was therefore again expected that both ENT and TET-ENT would be sensitive to concentration reductions produced by particulate deposition along the surface of the prairie strip.

The same overall trend of bacterial concentrations was seen for both ENT and TET-ENT, with reductions observed at both the runoff and infiltration sampling locations when compared to the run-on sampling location (table 2). In runoff, the median concentration reductions across all three flow experiments ranged from 1.2%–28.2%; however, the only significant drop in concentration between runoff and run-on was in ENT concentrations during the medium flow experiment. In comparison, the median concentrations of both ENT and TET-ENT in infiltration were statistically lower than the corresponding concentrations in run-on during all three flow experiments, with the reductions ranging from 12.9%–65.5%. (fig. 3).

Although only one statistically significant reduction in median concentrations was observed for ENT and TET-ENT in runoff, it was again hypothesized that these reductions could have been greater had the bacteria originated from manure fertilized soil rather than directly from a diluted swine

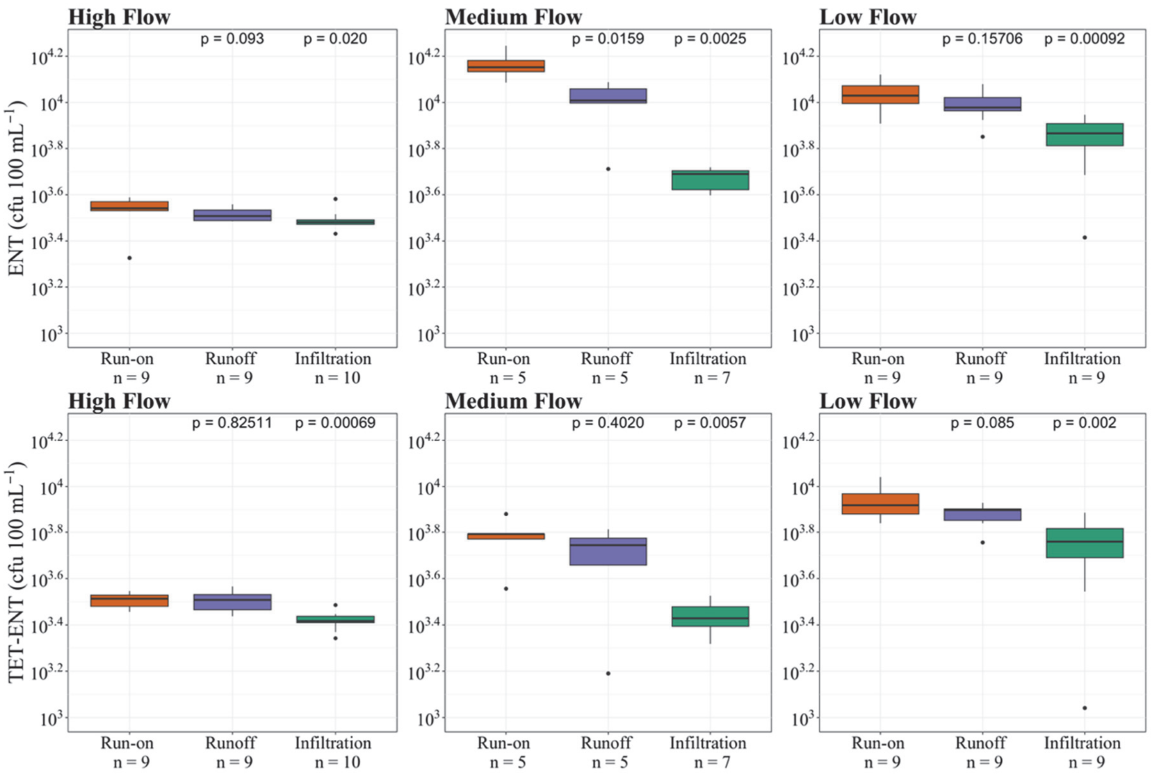

Figure 3. Box and whisker plots of *Enterococci* (ENT) and tetracycline-resistant *Enterococci* (TET-ENT) concentrations at each sampling location for the three flow experiments. P-values generated from pairwise comparisons made between the median concentrations in run-on and runoff or run-on and infiltration during each flow are displayed.

manure source. As well, during this entire experiment the identification of statistical significance in the concentration reductions of contaminants was generally limited by small sample sizes.

In a partner experiment aimed at informing prairie strip design via the measurement of a fecal indicator bacteria with known regulatory standards, the same flume system was used to examine the ability of prairie strips to reduce *E. coli* from swine manure impacted run-on. During this experiment, median concentrations of *E. coli* were significantly reduced at the runoff sampling location under the medium and low flow conditions by 23.3% and 45.3%, respectively (Craig et al., 2023). The combined results from these two experiments indicate that prairie strips may be only marginally effective at removing ENT under the described conditions but may be more effective at removing other bacterial targets or may be more effective at removing resistance contaminants under in-field conditions. This finding is in line with previous studies that have indicated biofilters may be less efficient at removing ENT than other fecal indicator bacteria (Rippy, 2015) and highlights the need for more targeted investigation into the sorption of specific bacterial species within environmental systems in order to determine whether their transport can be combined or must be described separately.

The significant reductions of ARB concentrations during infiltration are once again likely tied to the adsorption of freely suspended bacteria to the prairie strip soil, or via the straining of particulate attached cells as they move through pores within the soil profile. Again, during the partner experiment using the same flume system it was demonstrated that movement through the shallow subsurface of prairie strips was highly effective at lowering the concentration of *E. coli* (Craig et al., 2023). Previous studies investigating vegetative filter strips have emphasized the importance of infiltration as it contributes to surficial mass load reductions of bacteria from overland runoff. However, the concentration reductions that can occur as a portion of run-on infiltrates, and becomes groundwater recharge, has remained unexplored.

ARG CONCENTRATION REDUCTIONS

Unlike the antibiotic and ARB contaminants, ARGs have the capability of being transported in two forms: as intracellular DNA within host bacteria and as extracellular DNA separate from host bacteria. The inclusion of both forms of ARGs in monitoring transport is valuable, as only accounting for intracellular ARGs within an initial sampling could vastly underestimate the presence of intracellular ARGs at later timepoints, once the mechanisms of horizontal gene transfer are given the time and proximity to occur (Ikuma and Rehmann, 2020).

As the methodology used for DNA extraction and qPCR did not discriminate between intracellular or extracellular DNA, the presented median concentrations represent a potential combination of both sources. While there has been little research on the specific mechanisms underlying the transport of extracellular ARGs, the mobilization of intracellular ARGs could show an affinity for particulate-attached or unattached movement that is dependent on their predominant host bacterium. For example, characterization of ARGs in runoff produced during a plot-scale rainfall simulation revealed that the MLSB resistance genes *ermB* and *ermF* were evenly distributed in both the solid and aqueous phases of runoff, whereas the Tetracycline resistance gene *tetM* was present at a higher relative abundance in the dissolved, aqueous phase (Alt et al., 2023). This observation could have been the result of *tetM* being carried by bacterial hosts with weak affinities for sediment- or particulate-association. However, without much previous research on the combined transport methods of intracellular and extracellular genes, it was again expected that the targeted ARGs could be sensitive to concentration reductions at both the surface and shallow subsurface of the prairie strip.

No significant reductions in median ARG concentrations were observed between runoff and run-on during any of the three flow experiments (table 2). Significant reductions in concentration were observed in infiltration during the medium flow run for 16S rRNA, *ermB*, and *ermF*. Median concentration reductions ranged from -4.2% to 30.5% in runoff and from -12.6% to 43.5% in infiltrated flows (fig. 4). As was observed with the antibiotic contaminant, on occasion the median concentrations of ARGs increased between the run-on sampling location and the two downstream sampling locations. The largest of these increases again occurred during the high flow experiment, indicating that under sufficiently high flows, pre-existing antibiotic resistance contaminants within the prairie strip may be mobilized rather than contained.

SURFICIAL MASS LOAD REDUCTIONS OF RESISTANCE CONTAMINANTS

While the concentration reductions of the resistance contaminants in runoff were sometimes limited, the reductions in the runoff volume caused by flow partitioning, as an addition to the surficial concentration reductions, more frequently resulted in significant reductions of the mass load of resistance contaminants from runoff. Percent reductions in median surficial mass loads ranged from 23.6%–35% for tetracycline, 32.3%–51.8% for the ARBs, and 31%–54.4% for the ARGs (table 3). Previous literature has highlighted the importance of the infiltration capacity of vegetative filter strips as it provides surficial mass load reductions of contaminants (Cardoso et al., 2012; Coyne et al., 1998; Fox et al., 2011). Plantings of perennial vegetation, such as prairie strips, have been shown to have a higher macroporosity when compared to the soil profile created by a crop rotation of corn and soybeans, indicating that they can increase water infiltration and facilitate deeper water movement (Hernandez-Santana et al., 2013; Udawatta et al., 2008).

CONCLUSIONS

Commonly investigated antibiotic resistance contaminants associated with runoff from manured crop fields can include bacteria, genes, and antibiotics initially derived from the source manure that impact recipient and downstream soil and water resources. Unique to this experiment was the combined analysis of all three of these resistance contaminants, where previous experimental efforts typically focus on quantifying antibiotic and ARB targets or antibiotic and ARG targets, a fact that is likely tied to the current lack of regulatory guidance within water quality standards. Throughout the three flume runs, a wide range of median concentration reductions was observed for the various resistance contaminants in both runoff (-11.4%–30.5%) and infiltration (-23.9%–65.5%). The largest percentage reductions in median concentration of resistance contaminants occurred in infiltration, while only one instance of significant reduction in concentration occurred in runoff. Percent reductions in median surficial mass loads for the various resistance contaminants ranged from 23.6% to 54.4%, again highlighting the importance of the increased infiltration that prairie strips can provide.

While this study provides an initial investigation into the transport of resistance contaminants within run-off that has passed through a section of prairie strip, the use of a laboratory-scale flume to conduct simulated runoff is essentially representative of a set of column transport experiments. In order to further explain the transport behaviors of antibiotics, ARBs, and ARGs within these environmental systems, future areas of study should include batch sorption experiments, investigating the portion of these contaminants transported in either a particulate-attached or unattached phase.

Furthermore, it is important to note that the methodology employed within this study utilized a readily transported manure source, likely indicative of a recent manure application followed by a quick rainfall occurrence. In a field setting, if a rainfall does not occur immediately following a manure application, the manure source will be exposed to a number of environmental conditions that are likely to assist in the dissipation of these resistance contaminants as well as promote their attachment to particulates. Microcosm studies investigating the dissipation rates of these compounds post-field application as they are continuously exposed to new environmental conditions, including climate (UV light, temperature, moisture), pH, and microbial activity, may also help to characterize the dynamics of these contaminants when present in the field setting and assist in identifying their risk of transport.

The described experiments were designed to be highly controlled via the use of a flume system, which was limited to the insertion of small sections of prairie strip (1.16 m long by 0.99 m wide by 0.15 m deep). However, if the length and depth of the prairie strip insert were increased, reductions in resistance contaminant concentrations would show a level of proportional increase (Craig et al., 2023; Moody et al., 2022). The crucial balance in this management practice lies in identifying the ideal length of prairie strip that can achieve necessary reductions in resistance contaminants while minimizing the loss of profitable land.

SUPPLEMENTAL MATERIAL

The supplemental materials mentioned in this article are available for download from the ASABE Figshare repository at: https://doi.org/10.13031/26838613

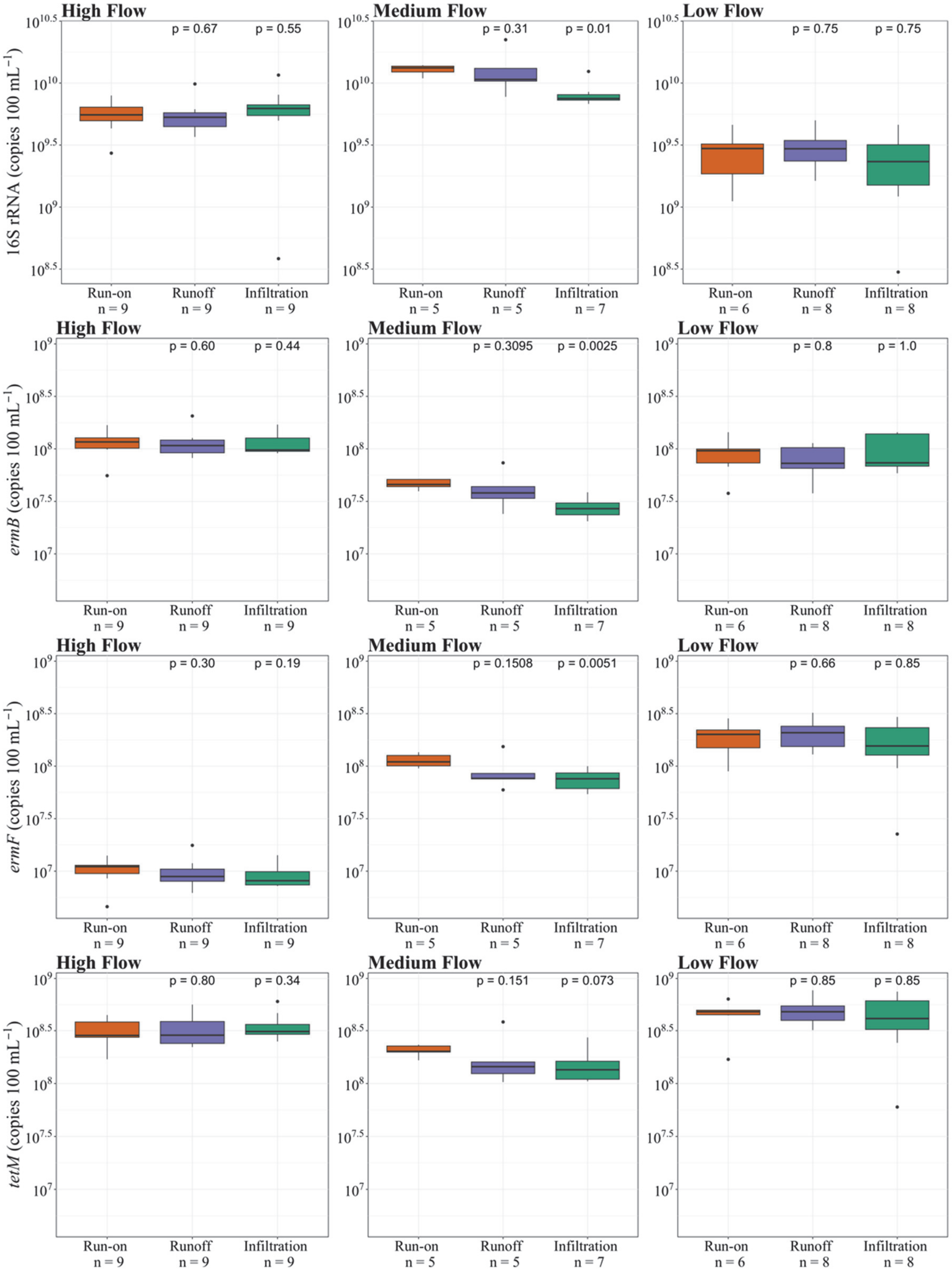

Figure 4. Box and whisker plots of 16S rRNA, *ermB*, *ermF*, and *tetM* gene concentrations at each sampling location for the three flow experiments. P-values generated from pairwise comparisons made between the median concentrations in run-on and runoff or run-on and infiltration during each flow are displayed. 16S rRNA gene concentrations are plotted on a higher scale than the concentrations of *ermB*, *ermF* and *tetM*.

Table 3. Summary of sample size (n), median mass load ($\dot{m}_{median}$), and percent median mass load reduction (% $\dot{m}_{median}$ Reduction) along the surface (runoff) of the prairie strip insert during each flow experiment.

				Run-on		Run-off		Infiltration		% $\dot{m}_{median}$ Reduction (p-value)
				n	$\dot{m}_{median}$	n	$\dot{m}_{median}$	n	$\dot{m}_{median}$	Runoff
Antibiotics	(ng mL $^{-1}$)	Tetracycline	High	8	733	9	560	10	285	23.6% (0.093)
			Med	5	257	5	181	6	27.2	29.5% (0.22)
			Low	9	2433	9	1583	9	493	35.0% (0.0003)
Bacteria	(cfu 100 mL $^{-1}$)	ENT	High	9	52618	9	33391	10	14393	36.5% (0.0027)
			Med	5	52164	5	25157	7	3109	51.8% (0.0079)
			Low	9	25316	9	14374	9	6248	43.2% (0.0004)
		TET-ENT	High	9	49291	9	33391	10	12398	32.3% (0.0004)
			Med	5	22372	5	14674	7	2083	34.4% (0.056)
			Low	9	19638	9	11953	9	4888	39.1% (0.0004)
Genes	(copies 100 mL $^{-1}$)	16S rRNA	High	9	8.38E+10	9	5.49E+10	9	2.97E+10	34.5% (0.019)
			Med	5	4.79E+10	5	2.74E+10	7	5.39E+09	42.7% (0.15)
			Low	6	7.02E+09	8	4.48E+09	8	1.98E+09	36.2% (0.23)
		ermB	High	9	1.76E+09	9	1.12E+09	9	4.64E+08	36.7% (0.024)
			Med	5	1.63E+08	5	9.39E+07	7	1.95E+07	42.2% (0.056)
			Low	6	2.27E+08	8	1.10E+08	8	6.27E+07	51.4% (0.043)
		ermF	High	9	1.67E+08	9	9.23E+07	9	3.85E+07	44.8% (0.019)
			Med	5	4.04E+08	5	2.02E+08	7	4.73E+07	50.0% (0.016)
			Low	6	4.76E+08	8	3.17E+08	8	1.32E+08	33.4% (0.14)
		tetM	High	9	4.31E+09	9	2.97E+09	9	1.47E+09	31.0% (0.050)
			Med	5	7.01E+08	5	3.19E+08	7	8.75E+07	54.4% (0.15)
			Low	6	1.14E+09	8	7.30E+08	8	3.52E+08	36.0% (0.081)

ACKNOWLEDGMENTS

This research was supported by the USDA National Institute of Food and Agriculture, with Food Safety Grant No. 2018-68003-27468, Food Safety Grant No. 2021-68015-33495, and Agriculture and Food Research Initiative project 2021-08949. The authors thank Alyssa Iverson, Tom Moorman, and Leigh Ann Long for assistance with sample collection and analysis. We would also like to thank Adam Buseman, Lindsey Hartfiel, Timothy Neher, Isaac Rempe, Abby Schaefer, and Alexis Slade for their assistance with sample collection.

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