CONVERSATIONS AND CONCENTRATIONS: A MULTIMETHOD APPROACH TO UNDERSTANDING CONTAMINANT TRANSPORT IN ANIMAL AGRICULTURE

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CONVERSATIONS AND CONCENTRATIONS:
A MULTIMETHOD APPROACH TO UNDERSTANDING CONTAMINANT
TRANSPORT IN ANIMAL AGRICULTURE

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Contaminant reduction through sustainable agricultural practices is critical for maintaining human, livestock, and environmental health. Our research sought to contribute to a holistic view of phosphorus and antibiotics as contaminants in agriculture. The hydrologic flow paths that transport these contaminants from manure connect agricultural systems with surface waters and drinking water supplies. These compounds are concerning due to their influence on soil and aquatic ecosystems and, subsequently, human and livestock heath. To understand transport of these compounds, we designed field scale, laboratory scale, and interview-based studies. We assessed the effectiveness of a cattle exclusion riparian buffer in reducing total and soluble reactive phosphorus loads (Chapter 1), and found the practice reduced stream phosphorus, but may be more effective when implemented adaptively around variable source areas. We determined adsorption characteristics of erythromycin (a macrolide antibiotic used on farms) to soil in the presence and absence of manure (Chapter 2) and found that the antibiotic is more mobile in the presence of manure and sorbs more readily to soil in the absence of manure. Lastly, we interviewed central New York dairy farmers to understand farmer perceptions of transport pathways of antibiotics on

dairy farms (Chapter 3) and found that there is a strong divide in perception of these compounds as contaminants between organic and conventional farmers, that farm size and farmer age influence perceptions and management decisions around treatment and transport, and that contamination of market products was a higher concern than environmental contamination. The scales and methods applied in this work combine to provide a broader perspective with which to guide future contaminant mitigation efforts.

BIOGRAPHICAL SKETCH

I grew up in Atlanta, GA, wandering urban streams and forests with my childhood-pal-turned-grad-school-lab-mate, Lisa Watkins, under the freedom provided by our protective white German Shepard, Chloe. My parents, Aris Georgakakos and Leslie Blythe, encouraged my brother, Philip, and my early fascination with the natural world by providing copious field guides, taking us on countless hikes, and letting us tag-along on work trips to fascinating ecosystems across the world. We split our summers exploring Georgia's humid deciduous forests, lakes, and rivers, Connecticut's coastal salt marshes and Long Island Sound, and southern Greece's dry hillsides, mountain springs, and teaming sea. Water, it's landscape scale impacts, floral and faunal inhabitants, and pathways to adventure, was the ever present connection between the places we called home.

I moved to Ithaca in 2011 to start a Bachelor of Science in Biological Engineering at Cornell, continued with a Master's of Engineering in 2014, and finally began my PhD in the Department of Biological and Environmental Engineering in 2016 after a two-part thru-hike of the Appalachian Trail. Between finishing my master's and beginning my PhD, enjoyed exploring a variety of potential trajectories beyond academia. In this 1-year break I developed my goals and motivations for returning to graduate school, through the lenses of the various positions I held: I was a substitute teacher with the Ithaca City School District, an outdoor educator with Cornell Outdoor Education, an intern at the Finger Lakes Land Trust, a brief intern at the engineering firm Wetland Studies and Solutions, a research assistant in the Soil and Water Lab, and the coach of Cornell's womxn's ultimate frisbee team, the Wild Roses. Through these experiences I confirmed my interest in teaching and mentoring, and re-inspired my interest in research.

My time in graduate school at Cornell was defined by developing my teaching and mentoring skills, understanding the logistics of running and maintaining laboratory research, and conducting a breadth of studies both included and not included in my dissertation. I'm grateful for all the opportunities provided to me during this time, both for those that led to the completion of this degree and those developed other aspects of my skillset. While at Cornell Graduate School, research I participated in was disseminated in the following peer-reviewed publications:

(1) Hofmeister, K. L., **Georgakakos, C. B.,** & Walter, M. T. (2016). A runoff risk model based on topographic wetness indices and probability distributions of rainfall and soil moisture for central New York agricultural fields. *Journal of Soil and Water Conservation*, *71*(4), 289-300.

(2) **Georgakakos, C. B.,** Morris, C. K., & Walter, M. T. (2018). Challenges and opportunities with on-farm research: total and soluble reactive stream phosphorus before and after implementation of a cattle-exclusion, riparian buffer. *Frontiers in Environmental Science*, *6*, 71. ***(Chapter 1)***

(3) **Georgakakos, C. B.,** Richards, P. L., & Walter, M. T. (2019). Tracing Septic Pollution Sources Using Synthetic DNA Tracers: Proof of Concept. *Air, Soil and Water Research*, *12*, 1178622119863794.

(4) Redding, L.E, Brooks, C., **Georgakakos, C.B.,** Habing, G., Rozenkrantz, L., Dahlstrom, M., & Plummer, P.J. (2020). Addressing individual values to impact prudent antimicrobial prescribing in animal agriculture. *Frontiers in Veterinary Medicine* 297(7). doi: 10.3389/fvets.2020.00297

(5) **Georgakakos, C.B.,** Hicks, B., Walter, M.T. (2021). Farmer perspectives of antibiotics in the dairy farm environment. *J. of Environmental Management, 281*, 111880. doi: 10.1016/j.jenvman.2020.111880 ***(Chapter 3)***

(6) **Georgakakos, C.,** Cerra, J., Allred, S., Williams, K., Walter, M.T., LoGuidice, E., Smith, G. (*in press*). Cross disciplinary learning in environmental engineering and landscape architecture. *Int. J. of Collaborative Engineering.* doi: 10.1504/IJCE.2020.10033950

(7) Pietz, O., Augenstein, M., **Georgakakos, C.B.,** Singh, K., McDonald, M., Walter, M.T. (*in review).* Macroplastic accumulation in roadside ditches of the Finger Lakes region across land uses, traffic gradients, and the COVID-19 pandemic. *Submitted to the Journal of Environmental Management.*

(8) **Georgakakos, C.B.,** Helbling, D., Martínez, C.E., Walter, M.T. (*in prep).* Increased mobility of erythromycin through agricultural soils in the presence of manure. ***(Chapter 2)***

This work is dedicated to my yiayia and pappous, Δήμητρα και Πέτρος Γεωργακάκος,

for passing on their passion for learning and exemplifying selfless work ethics.

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LIST OF ABBREVIATIONS

AMR	antimicrobial resistance
BMP	best management practice
CN	curve number
DDS	Dynamically Dimensioned Search
DEM	digital elevation model
DOM	dissolved organic matter
ELISA	enzyme-linked immunosorbent assay
ET	evapotranspiration
Gen X	Generation X
NSE	Nash-Suticliffe efficiency
NY	New York
NYS	New York State
OM	organic matter
P	phosphorus
PET	potential evapotranspiration
Q	discharge
S	soil-only
SM	soil+manure
SRP	soluble reactive phosphorus
SSE	standard square error
SWE	snow-water equivalent
TP	total phosphorus
TSS	total suspended solids
US	United States
USDA	United States Department of Agriculture

PREFACE

Human, livestock and environmental health, depend on sustainable agricultural practices that are designed to reduce environmental impacts of agricultural contaminants. Agricultural contaminant mitigation from local to global scales has drawn attention as humanity strives to support a growing population while minimizing negative environmental impacts. Mitigating nutrient and emerging contaminant pollution is a critical focus to guide sustainable agricultural operations. To do so, reducing agricultural contamination requires actions by and inclusion of farmer perceptions to effectively incorporate farmer knowledge into research and direct research to ensure on-farm applicability. Understanding farmer perceptions of contaminant transport and valuation of contaminant pathways directs effective management changes in complex, on-farm systems.

Phosphorus and antibiotics are two agricultural contaminants that are dispersed in the environment through manure application. The hydrologic flow paths that drive the movement of both phosphorus and antibiotic residues connect human, animal, and ecological systems via sediment and water transport. Some antibiotics, similar to phosphorus, are chemically 'sticky' compounds, adsorbing to sediment and organic particles with which they interact and desorbing from those particles later due to changes in their proximate environment. In part due to this complicated adsorption-desorption dynamic, these contaminants can influence ecosystems distant from their sources.

1.0 Phosphorus: A primary agricultural contaminant

Agricultural phosphorus contamination from manure to local and regional water bodies has been the focus of agricultural non-point source pollution research and mitigation for decades (e.g. Sharpley et al, 1994; Daniel et al, 1998), yet it remains one of the key challenges currently addressed by farmers across the globe. Global phosphorus utilization has been described as a anthropogenic planetary boundary, establishing a tipping point between global phosphorus flows that could dramatically alter earth's biogeochemical processes, making efficient utilization of this resource critical for continued global agricultural success (Steffen et al., 2015). Because ortho-phosphate caries a -3 charge, it is a highly reactive molecule and frequently adsorbs to sediments through ionic mechanisms and is incorporated into organic material through biological uptake (e.g. Zhou et al., 2005). Therefore, practices that target reduction of sediment loads into water bodies tend to similarly reduce total phosphorus (TP) loads. Soluble reactive phosphorus (SRP), or biologically available phosphorus, forms an equilibrium with its adsorbed forms, adsorbing and desorbing from sediment loads as SRP concentrations in the water column and mineral characteristics dictate movement toward equilibrium (Wang & Li, 2010). Due to this dynamic, sediments, with associated phosphorus, deposited in lakes can desorb later, as aqueous concentrations shift, expanding the range of influence of upstream water quality concerns.

Implementation of phosphorus- and sediment-targeted best management practices (BMPs) has been attributed with major water quality gains across both small and large watersheds (e.g. Strauss et al., 2007; Rao et al., 2009). While timing of manure application can control temporal phosphorus loads (e.g. Liu et al., 2017; Vadas et al., 2017), total phosphorus and sediment loads to local water bodies are moderated by BMPs (e.g. Sharpley et al., 2006) and manure application techniques (e.g. Jahanzad et al., 2019).

2.0 Antibiotics: Emerging agricultural contaminants

Antibiotics are a critical component of conventionally managed agriculture in the US and around the world. However, despite rigid regulations controlling transport of these compounds into food products, the environmental transport and legacy of antibiotics are not regulated. Antibiotics are of concern due to their toxic interactions with soil and water microorganisms (Välitalo et al., 2017), and their dramatic impacts to microbial community structure (Guo et al., 2016; Kumar et al., 2005), potentially leading to increased antimicrobial resistance, a concern for both human and livestock health (Singer et al., 2016).

The World Health Organization has identified management of antimicrobial compounds as a critical component of addressing the One Health objectives outlined by the Interagency Group on Antimicrobial Resistance (IGAR, 2019). The IGAR (2019) approach highlights the connectivity between human and animal antimicrobial treatments and food systems, ecological interactions and residue and gene transport. Antimicrobial residues provide the selective pressure that increase prevalence of antimicrobial resistance genes in the environment. Because human and livestock medicine rely on the efficacy of similar classes of compounds, concern over antibiotic use connects urban and rural communities across the globe. Human and animal health are both impacted by antimicrobial efficacy, which is at risk with increased loads of antimicrobials in the environment from anthropogenic and agricultural sources.

Pharmaceuticals, the broader contaminant category to which antibiotics belong along with hormones and other prescription drugs, have been found in surface waters (Carpenter et al., 2018; Carpenter et al., 2019), groundwater (Kibuye et al., 2019), wastewater treatment effluents (Vatovec et al., 2016; Lishman et al., 2006), and animal manures (Kim et al., 2011), giving unique signatures to the water in which they are transported. And though agriculture is often blamed for antibiotic contamination due to its disproportioned purchasing of these compounds over human medicine, the debate remains as to if anthropogenic or agricultural waste sources are the true sources. Antibiotics enter the waste stream largely unchanged, with estimates of 50-90% of treated antibiotics appearing in manure by mass (Kim et al, 2011; Alcock et al., 1999; Feinman & Maheson, 1978). As has been studied with nutrient best management practices (Doran et al., 2020), manure management strategies to reduce antibiotic resistant bacteria transport and antibiotic residue transport have just begun to be studied (Oliver et al, 2019). However, the specific transport dynamics of many agricultural antibiotics are largely unknown.

3.0 Farmer perceptions: The foundation of contaminant mitigation

The pathways that lead to contaminant loads from livestock farms start with human decisions. Human behaviors influence the magnitude and timing of contaminant loads, and understanding those behaviors paired with biophysical knowledge of transport allows more acute contaminant mitigation (Smith et al., 2015). This is especially true for antibiotic environmental contamination, where disease prevention strategies, treatment decisions, and manure management choices all, in turn, influence antibiotic loadings. Complicating this dynamic are the variety of personnel who make treatment decisions, from veterinarians and farm owners, to herd managers and other on-farm employees. Because human decisions are so intertwined with antibiotic contaminant loading, mitigation of this growing problem must consider the perceptions and values that underly these decisions to make impactful progress.

Redding et al., (2020) emphasize the importance of understanding each antibiotic prescriber's (i.e. veterinarians, farmers, herd managers, etc.) values before attempting to influence change in behavior in this community. Because values and motivators can be drastically different across geographic locations and farm categories, understanding the specific values and perceptions driving each farmer where a change in behavior is recommended is critical.

4.0 Research objectives

My dissertation takes a multi-pronged approach to address questions in the field of agricultural contaminants. The diversity of methodologies employed create a holistic picture of agricultural contaminants from edge of field best management practice monitoring to lab batch reactions and column experiments to qualitatively analyzed interviews with dairy farmers. Each method provides a unique set of conclusions and future research directions.

The objectives of chapter 1 were to address the effectiveness of a cattle exclusion riparian buffer BMP on improving water quality by reducing soluble and total reactive phosphorus in the water column. We assessed our objective through a pre- and post-BMP implementation monitoring experiment on a small tributary of Dutch Hollow Creek in New York State. Our study site was a small farm with both dairy and beef cattle. We found that the riparian buffer cattle exclusion successfully reduced total stream phosphorus, but potential positive water quality gains were impeded by management decisions that resulted in short circuiting the riparian buffer. This study has since been included in a cattle exclusion riparian buffer systematic review, which highlighted the highly variable but generally positive water quality results from riparian buffers on stream sediment and phosphorus (Grudzinski et al., 2020).

The objectives of chapter 2 were to characterize the adsorption and transport of erythromycin, a commonly used agricultural antibiotic, in laboratory batch reactors and column experiments to enable better prediction of off-farm transport. We assessed our objectives through controlled laboratory experiments with soil and manure solutions. The application of this study is to better predict effective mitigation strategies for the antibiotic studied.

The objectives of chapter 3 were to understand farmer perceptions of the antibiotic transport pathways, including which were prioritized to better influence on-farm usage strategies and manure management methods of reducing antibiotic environmental contamination. We assessed our objectives through a qualitative interview study with central New York dairy farmers across farm size, farmer age, farm generation, and management classifications. We found dairy farmers were most concerned with antibiotic transport into market products of milk and meat, somewhat less concerned about transport into waste milk, few were concerned with transport with manure, and none were concerned with transport associated on-farm mortality.

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CHAPTER 1 -

CHALLENGES AND OPPORTUNITIES WITH ON-FARM RESEARCH: TOTAL AND SOLUBLE REACTIVE STREAM PHOSPHORUS BEFORE AND AFTER IMPLEMENTATION OF A CATTLE EXCLUSION, RIPARIAN BUFFER [a]

Abstract

Many nutrient mitigation best management practices (BMP) are promoted by state and federal agencies to protect water quality from animal agriculture. The measured effectiveness of these is highly variable in the research literature. Here, we establish pre- and post-BMP monitoring to evaluate the effectiveness of fencing out cattle from the riparian zone on water quality, specifically, phosphorus (P) loads and concentrations. We collected water samples year-round both before and after a cattle exclusion was established at a small mixed dairy and beef cattle pasture where cattle have historically grazed with unrestricted access to first and second order streams, and analyzed for soluble reactive and total P. Immediately after fence construction, we observed a significant reduction in total P in the stream but not in soluble reactive P. We also observed the development of new runoff source areas and short-circuiting of the riparian buffer as well as repeated presence of cows in the fenced-out area, all of which may diminish the potential effectiveness of this practice. Because BMPs will perform uniquely given climate and landscape position and how managers maintain

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[a] Reproduced from *Frontiers in Environmental Science,* 2018, *6,* 71. DOI: 10.3389/fenvs.2018.00071

them, we suggest the need for more nuanced guidance for future BMP designs to ensure successful outcomes and provide installation and maintenance recommendations.

1.0 Introduction

Toxic algal blooms can impact drinking water supplies and recreation on lakes due to their effects on wildlife and humans (Correll, 1998). Harmful algal blooms are a rising issue across the US (US House of Rep, 2014), with New York State (NYS), in particular, making some important changes. New York State's Water Quality Rapid Response Team, under direction of the governor and Department of Environmental Conservation, has begun steps to implement a serious effort to reduce harmful algal blooms according to the 12th Proposal of the State of the State (NY Executive, 2018). Harmful algal blooms and associated eutrophication result from high nutrient inputs to freshwater bodies. Harmful algal blooms are defined broadly as the growth of toxin producing algae. Eutrophication results from an excessive nutrient load to a water body, often leading to anoxic conditions. Limnological systems are frequently phosphorus (P) limited (e.g. Anderson et al., 2002), leading to an acute focus on how to reduce P loadings, in particular to protect water quality. The P equilibrium in lakes complicates management of this nutrient because P sorbs to sediment particles, is incorporated into algal and bacterial biomass, and exists in biologically available ionic forms (Correll, 1998; Chislock, 2013). This equilibrium makes managing for sediment-bound and biologically available P equally important. The anoxic zones that can accompany eutrophication in lakes push the equilibrium to favor desorbing sediment-bound P into the water column, exacerbating the P problem if sediments have sequestered large amounts of P (Holdren & Armstrong, 1980; Howarth et al., 2011). Agriculture has been identified as a high contributor of P to lakes and the streams that feed them, and is therefore one important area to implement phosphorus reducing management strategies (e.g. Drolc & Zagorc Koncan, 2002; Ulén et al., 2007).

Avoiding fertilization, manure spreading, or fecal deposition in areas that are likely to generate runoff mitigates non-point source pollution (Walter, 2000; Thodsen et al., 2015; Winchell et al., 2015; Knighton et al., 2017). Within nutrient-rich parts of a watershed (e.g., areas that receive animal manure), the areas that are most prone to soil saturation, including streams, disproportionately contribute more nutrients to streams than other parts of the landscape (e.g., Archibald et al., 2014; Lerch et al., 2015; Peukert et al., 2016). In designing best management practices (BMPs) to reduce non-point source pollution, it is therefore reasonable to focus resources on these hydrologically sensitive areas.

Riparian buffers, or uncultivated borders to streams, are prescribed for several reasons to mitigate nutrient pollution from agricultural fields. One reason is because streams and their riparian zones disproportionately generate runoff due to high soil moisture. Riparian buffers therefore are prescribed to reduce transport of pollutants that could have been deposited within the buffer area, if the buffer area were not protected. A second application is allowing the buffer to slow overland flow and allow for deposition and degradation of nutrients. A cattle exclusion area as applied around a stream is referred to as a *cattle-excluded riparian buffer* and aims to address both of these nutrient transport mechanisms. This practice is generally recommended for the purpose of eliminating fecal deposition directly into streams and reducing sediment suspension from cattle traffic (Miller, 2010). Cow behavior appears to have significant impacts on site hydrology. In their search for shade and water cattle may continue to compact soils near streams and increase the risk of runoff (Trimble & Mendel, 1995).

Many studies have found riparian buffers reduce P loading to streams from cropped agricultural fields (Patty, 1997; Aguiar et al., 2015; Mander et al., 2017; Zhang et al., 2017). Suggested widths for those buffers are highly variable in the literature (e.g. Lee et al., 2003, Lee et al., 2004). Implementation of riparian buffers has traditionally followed a blanket approach of including either the flood plain of a 100-year design storm or 25 to 50 ft of undisturbed forest to buffer streams (e.g., NYS Storm Water Design Manual, 2015). However, this broad approach does not account for nuances of topography, livestock rotation, or variable source areas that may contribute more pollutant loads at different points during the year (Walter et al., 2000) and may lead to both over and under sized buffers. Walter et al., (2009) recommend creating riparian buffers of flexible widths to account for variable source areas.

Within a riparian buffer, mechanisms for P removal are sediment deposition, sorption to sediment particles, chemical precipitation or conversion, plant uptake, and biological immobilization via incorporation into organisms. However, rates and reaction potentials of these processes depend on soil type, degree of P saturation, buffer width, vegetation type, and management conditions of the buffer region (Hoffmann et al., 2009).

Total P pollution is reported to be reduced with the implementation of riparian buffers (McKergow et al., 2003; Miller et al., 2010; Shukla et al., 2011,). However, as P accumulates in buffer sediments, the ability of the buffer to reduce P pollution diminishes with time unless a management strategy, such as removing plant material that has accumulated P, is implemented (Dodd & Sharpley, 2015). Reports of soluble reactive P reduction by riparian buffers, however, are inconsistent. Some studies report soluble reactive P removal (Abu-Zrieg et al., 2003; Lee et al., 2003), while others report soluble reactive P generation (Makin et al., 2007; Hoffman et al, 2009). For a diversity of reasons, riparian buffers are sometimes ineffective in reducing nutrient loads to streams. One such reason, relevant to this study, is point sources, such as tile drains, bypassing riparian buffers and routing water directly to streams or other receiving waters.

In this study we assess the effectiveness of a cattle-excluded riparian buffer on reducing total and soluble reactive P loads and concentrations to a small headwater stream by comparing pre- and post-BMP installation. We also include qualitative observations that may be relevant to BMP effectiveness.

2.0 Methods

2.1 Site description

Our study site was a small active cattle pasture in the Finger Lakes region of NYS with a small headwater stream dividing the pasture (Figure 1.1). The farm in this study experienced several landscape alterations that are expected on a dynamic farm, such as changes in pasture boundaries, type of cow grazed, and integrity of fencing. These changes complicate nutrient runoff analysis, so we present here the results of a cattle exclusion riparian buffer in light of typical farm management and changes. The objective of this study is to characterize phosphorus and sediment dynamics under conditions seen in practice, i.e. including each of these complicating factors.

The climate is temperate with snow-influenced runoff in the spring. The annual rainfall during the study period was recorded in Auburn, NY (10.5 km from the study site) and collected from the National Oceanic and Atmospheric Administration's National Center for Environmental Information (NOAA, 2018). Pre-BMP monitoring occurred during 2014 and 2015, with 1.21 m and 1.23 m of annual precipitation respectively. Post-BMP monitoring occurred in 2017 through March 2018, with the annual 2017 precipitation at 1.29 m.

The watershed contributing flow to the inlet of the study reach was 9.5 ha, while the total watershed contributing flow at the outlet of the stream from the farm property is 65.2 ha. There are two additional springs contributing flow along the stream reach, with 12.4 and 8.3 ha in each drainage. The stream reach is 953 m long, and the cattle exclusion buffer width is 14m. The total watershed is 20% forested with the remainder being pasture and cropped land; the inlet watershed (Figure 1.1) is 100% fertilized corn and hay.

The farm is a small mixed dairy and beef cattle operation with an average of about 100 cows over the study period. This particular farm was identified as a possibly high contributor of P to the waterway, and was therefore chosen by the local Soil and Water Conservation District to receive several BMPs to reduce P pollution to the surface water system.

The initially envisioned BMP was only a cattle-excluded riparian buffer. However, as a result of identifying a highly P-enriched tile drain, the BMP also included renovation of an existing in-series settling pond system. The pond system had been degraded, presumably due to the cattle, such that water from some treatment ponds was being directed into the drain. The cattle-exclusion fence surrounds all streams that cross and border the property and the renovated settling ponds. The pond system was renovated by adding an additional in-series settling pond, and routing contaminated water from manure overflow to the first of three in-series ponds (Figure 1.1). This study focuses on the impacts of the cattle-excluded riparian buffer. However, we acknowledge that the impact of the renovations on this property on phosphorus loading reduction would be greater when accounting for all BMP renovations. To primarily analyze the impacts of the riparian buffer on the reach, all in stream nutrient calculations correspond to samples immediately upstream of where the pond point source mixes with the stream.

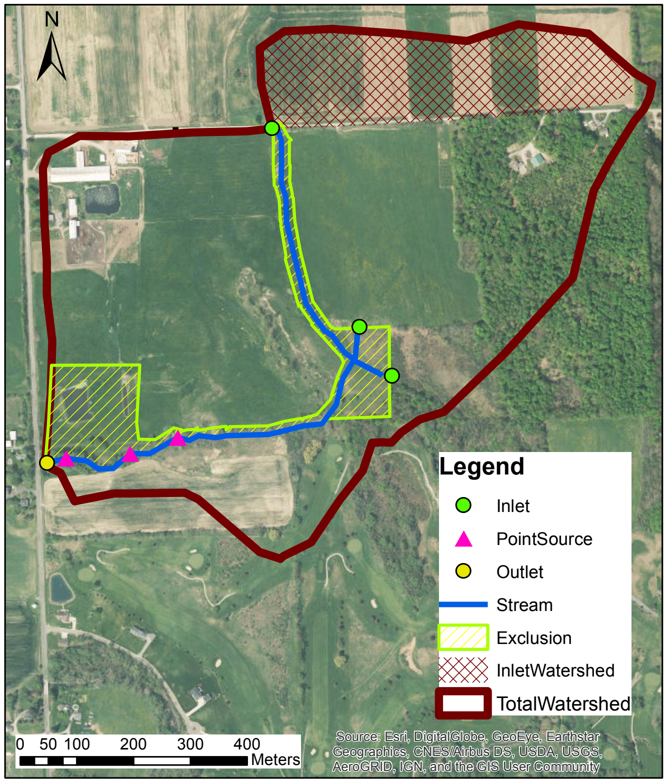

Figure 1.1: Study watershed. Inlet watershed comprises of fields draining into a road ditch that feeds the inlet of the stream. Northern watershed boundary has been significantly altered from natural flow regime by agricultural infrastructure development. In the subsequent discussion point sources of pond effluent, tile drainage, and cow generated saturated areas correspond with point sources from west to east respectively in the figure above. This image was taken pre-BMP implementation. The three renovated settling ponds are located in the boxed exclusion area near the stream outlet and collect manure overflow from the manure pit shown in the north-western corner of the watershed. Of the three inlets, only the northern-most inlet point is used as the inlet in this study.

2.2 Sampling methods

Grab samples were taken from the stream and pipes discharging into the stream year round and analyzed for total phosphorus (TP), soluble reactive phosphorus (SRP), and total suspended solids (TSS). One year of sample analysis pre-BMP was deemed sufficient in accordance with former cattle exclusion studies (e.g. Sheffield et al, 1997). A total of 78 grab samples, 51 pre-BMP and 27 post-BMP, were taken at the outlet. Pre-BMP samples were distributed seasonally: 7, 25, 11, and 4 samples for spring, summer, fall and winter months, respectively. Similarly, post-BMP samples were seasonally distributed with 4, 12, 8, and 3 samples for spring, summer, fall and winter, respectively. Winter was sampled less frequently because cows were not in the pasture. The grab sampling method was chosen to obtain non-storm event samples so to highlight the impact of the buffer during the majority of the year rather than the inability of the buffer to entrap contaminants during high flows. High flows enable suspension of more sediments, and therefore increase the total phosphorus load of the stream, which sediment bound phosphorus is the primary component of total phosphorus. Grab sampling is a method accessible to farms more so than other more continuous data collection techniques, which enables research like this study to be applied widely. Samples were collected using plastic Nalgene 200mL to 1000mL bottles, stored on ice during transport, and prepared for TP, SRP, and TSS analysis in the lab. Samples were taken at a minimum frequency of once per month and a maximum frequency of once per week during both pre and post BMP monitoring.

Instantaneous discharge measurements were taken 9 times between the two sampling periods. Because interpretation of concentration data alone is limiting, we turned to a simple stream flow model, validated for this region, and used our few flow measurements to calibrate the nine model parameters. Parameters used in this model are wind speed, forest coverage, watershed time to peak, maximum potential evapotranspiration (PET), the hydrologic baseflow recession coefficient, daily minimum curve number (CN), coefficient relating CN to soil water, initial abstraction coefficient, and ground albedo. Parameters were initialized with values used in the Owasco watershed, the basin that encompasses the farm and our catchment (Archibald et al., 2014). Calibrating a regional model with only a few measurements and prior estimates of model parameters has been used in ungauged catchments before (Rojas-Serna et al., 2006). The modeled discharge values were used to calculate P and sediment loads.

2.3 Analysis methods

2.3.1 Total phosphorus and soluble reactive phosphorus

Samples were filtered the same day of sampling for SRP and acidified using sulfuric acid to prevent algal and bacterial growth. TP samples were acidified with sulfuric acid day of sampling, then fully digested using persulfate solution paired with sulfuric acid (USEPA, 1978) and filtered after digestion. All samples were filtered using 0.45μm filter paper before analysis. All samples were stored at 4°C until sample analysis. Phosphorus analysis was done on an automated wet chemistry analyzer (FS3000; Xylem Analytics O.I. Analytical, Beverly, Massachusetts) screening for phosphate anions (PO_4^{3-}) in SRP and digested TP samples. Reagents for analysis were ammonium molybdate, ascorbic acid, sulfuric acid, and potassium antimonyl tartate (USEPA, 1978). Each run was calibrated using 0.005 ppm, 0.05 ppm, 0.5 ppm, 5 ppm, and 10 ppm potassium phosphate standards, with all R^2 values of the standard curves between 0.999800 and 0.999999.

2.3.2 Total suspended solids

At both the inlet and outlet of the stream reach, pre-BMP and post-BMP samples were analyzed for total suspended solids (TSS). Water volume filtered for TSS analysis ranged from 100ml to 500ml depending on visual clarity of the water. All filters were 0.45μm Whatman glass fiber filters (cat. no. 1826-047). Immediately following filtration, filters were oven dried at 60°C for 24 hours.

2.3.3 Statistical analysis

Data analysis was completed using R-studio (version 1.0.136) for data visualization and statistical analysis. Statistical testing for comparing samples from pre and post-BMP periods was preformed using a Wilcox Rank Sum test (two-sided Mann-Whitney test) after determination that data was not normally distributed following Shapiro-Wilks normality tests. The null hypothesis was that loads and concentrations of P and TSS did not differ, while the alternative hypotheses was that samples from pre and post-BMP monitoring experienced a shift in value. For the following discussion, significance is defined by $p<0.05$.

To normalize differences in runoff volume and changes in P flowing into the reach on any sample day, TP, SRP, and TSS load accumulations were calculated to define effectiveness of the buffer over the course of the entire reach. Load accumulation is calculated by first modeling the flow at the inlet and outlet of the system using JoFlow (Archibald et al., 2014) in the R EcoHydRology package. This model was chosen due to its representation of regionally relevant hydrological mechanisms and good agreement with distributed and watershed hydrological monitoring (Archibald et al., 2014; Knighton et al., 2017a; 2017b). The forcing data for this model are precipitation, maximum and minimum daily air temperatures, latitude, and day of the year. The parameters of mean annual wind speed, the initial abstraction for surface water pooling, minimum daily curve number, time to peak, maximum potential evapotranspiration, the recession coefficient, ground albedo, and a coefficient for the curve number's relationship to the soil water were calibrated to this watershed using the Dynamically Dimensioned Search (DDS) generated by Tolson & Shoemaker (2007). The Nash-Suticliff Efficiency Coefficient (NSE)(Nash & Sutcliffe, 1970) objective function was used to determine model fit to observed discharge. We generated the NSE from the natural log of the data to remove heteroscedasiticty of the underlying residuals. Equation 1 gives the relationship used.

$$NSE = 1 - \frac{\sum (\ln(sim_i) - \ln(Obs_i))^2}{\sum (\ln(Obs_i) - \ln(Obs_{avg}))^2} \quad (1)$$

where Sim_i refers to simulated daily flow and Obs_i refers to the observed flow on the same day.

2.3.4 Load calculations

Watershed area was delineated using ArcGIS from 1927 UTM projection 18N (NYS, 1927) initially, however modifications to the landscape by agricultural water routing and road ditch installation have drastically changed the local hydrology from what was captured in the DEM. The ArcGIS delineated watershed was manually adjusted to match the true hydrologic pattern of the region after walking the watershed boundary. Stream discharge measurements were made 9 times over the course of the study, and used to calibrate simulated continuous flow. The JoFlow (Archibald et al., 2014) model used pre-defined or calibrated input parameters shown in Table 1.1. JoFlow was used to obtain daily discharges, which were in turn used to calculate daily loads of TSS and P from grab samples on the same day. No samples were used to model P or TSS loads on days other than the days they were taken. We chose to model stream flow because discharge data can be difficult for farms to gather, and on-farm research contributes an important practical perspective missed by many laboratory run experiments.

With watershed flows from JoFlow, TP, SRP, and TSS load accumulations were then calculated from Equation 2.

$$Load\ accumulation = C_{out}Q_{out} - C_{in}Q_{in} \qquad (2)$$

where *Load Accumulation* is in g/day, C_{out} and C_{in} are concentrations flowing out of and into the system, respectively (g/L), and $Q_{out}$and Q_{in} are volumetric flows at the outlet and inlet, respectively (L/day).

The relationship between flow and loading was plotted to visually determine changes in loading between pre- and post-BMP samples across flow rates. A simple linear regression was fit to the data, and intercepts and slopes compared to address differences between conditions. All linear models were preformed on log-transformed data.

Table 1.1: Calibrated parameter values obtained by running DDS. All other parameters used the default values of model. CN refers to the curve number method of generating runoff. Numbers that appear without units are scalars. PET = potential evapotranspiration.

Parameter	**Calibrated Value**
Wind speed *(m/s)*	3.9
Forest coverage *(%)*	20
Time to peak *(hr)*	7.9
Maximum PET *(mm/day)*	5.51
Baseflow recession coefficient	0.058
Daily minimum CN *(mm)*	42
Coefficient relating CN to soil water	1.12
Initial abstraction coefficient	0.22
Ground albedo	0.10

3.0 Results

3.1 Discharge results

The calibrated JoFlow parameters are shown in Table 1.1. These parameters were the only parameters modified from default parameters outlined by Archibald et al. (2014). NSE calibration points are shown in Figure 1.2 with corresponding modeled and observed flows. After calibration, stream flow is modeled for entire study duration along with soil water and snow water equivalent (Figure 1.3).

According to Moriasi et al. (2007), an NSE value above 0.7 is accepted to fit the observed values well. Our NSE value of 0.82 allows us to be confident in the simulated flow values. Simulated flow is then used to calculate TP, SRP, and TSS load accumulations.

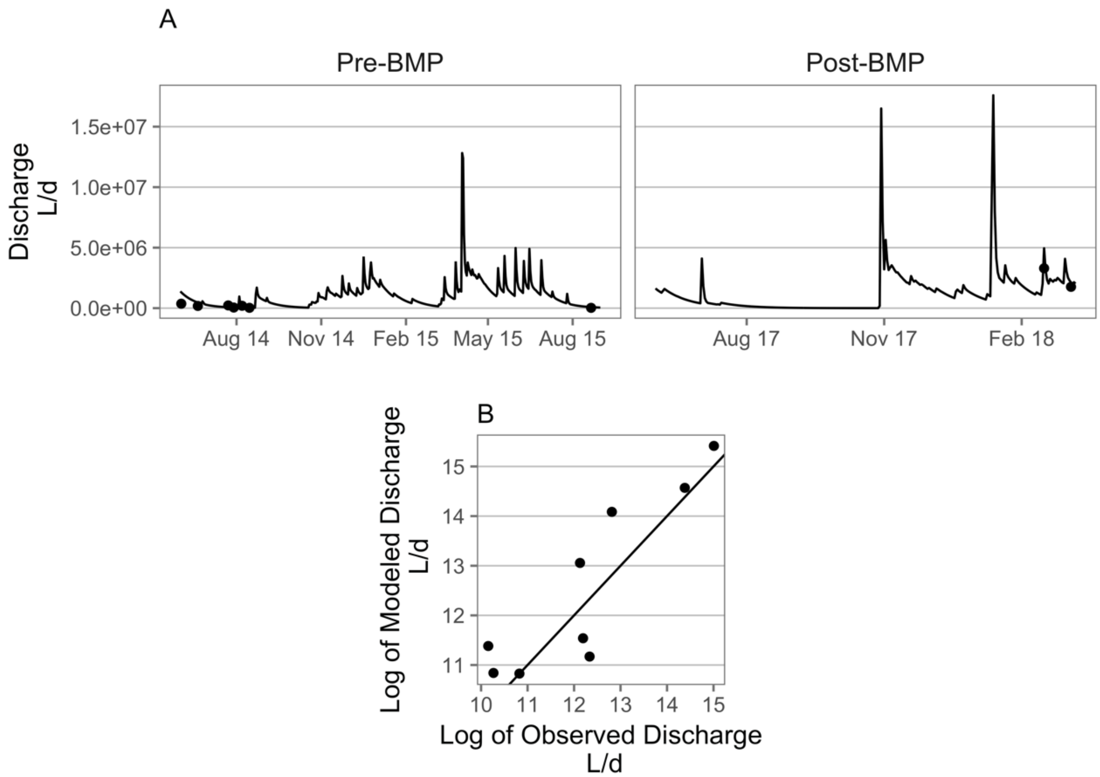

Figure 1.2: (A) Observed and simulated flow values (mm/d) (points and lines, respectively) over study duration. (B) represents the relationship between observed to simulated flow on a log-log plot to ensure the model fits well for the more frequent low flow scenarios. The line shown is the 1 to 1 line between simulated and observed flows. NSE = 0.82.

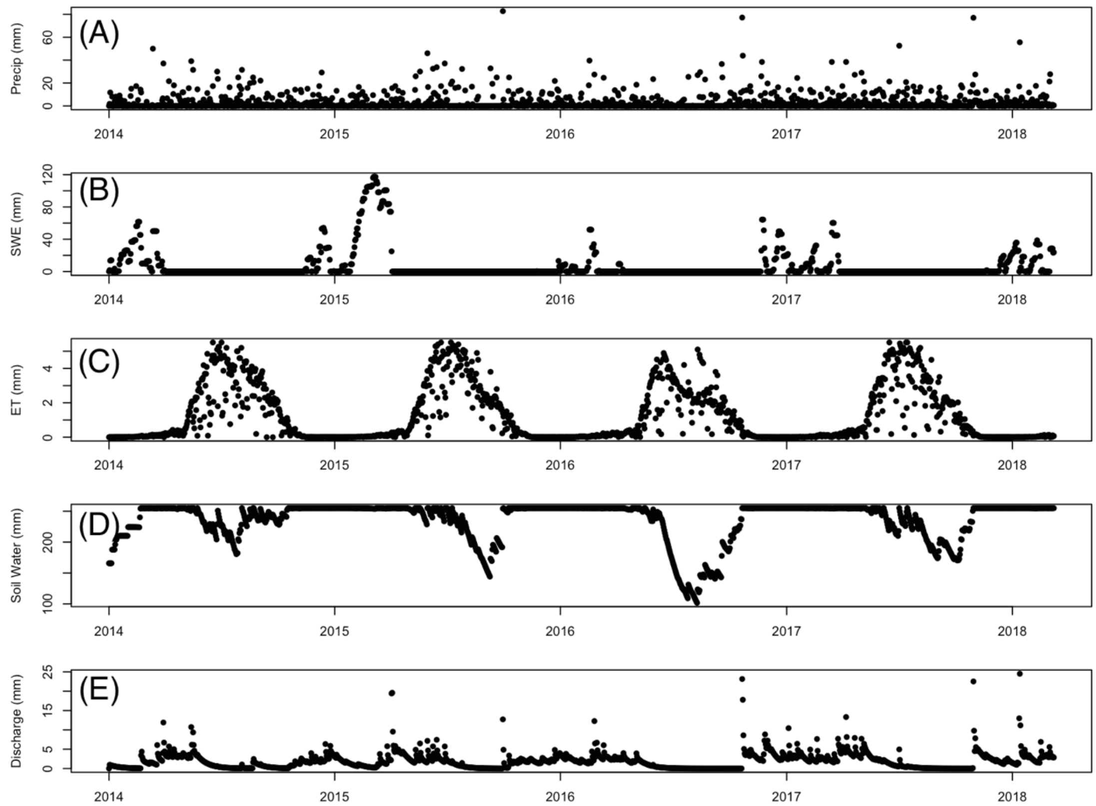

Figure 1.3: Daily precipitation data (A) used to model SWE (snow water equivalent)(B), ET (evapotranspiration)(C), soil water (D), and discharge (E). All plots include both pre- and post-BMP sampling regimes. Pre-BMP sampling occurred 2014-2015, post-BMP sampling corresponds to 2017-2018.

3.2 Water quality results

3.2.1 Concentration

SRP concentrations at the outlet after BMP implementation were not significantly different than pre-BMP conditions (Figure 1.4(A) p-value = 0.48). Pre-BMP SRP concentrations ranged from 0.025 to 0.287 mg/L. The SRP concentrations in the post-BMP period were between 0.012 and 0.786 mg/L. Median SRP concentration values at the watershed outlet are 0.07 mg/L and 0.08 mg/L for pre- and post-BMP respectively. All water quality results are summarized in Table 1.2.

TP concentrations after BMP implementation were significantly lower than pre-BMP conditions at the outlet, 0.022 - 2.06 mg/L before BMP construction and 0.024 - 1.27 mg/L after (Figure 1.4(B), p-value < 0.001). Median TP concentration values at the watershed outlet are 0.42 mg/L and 0.14 mg/L for pre- and post-BMP respectively.

Outlet TSS concentration values ranged from 2.0 to 814.0 mg/L and 1.1 to 21.0 mg/L for pre- and post-BMP respectively. The median values at the outlet were 27 and 3.4 mg/L for pre and post-BMP samples respectively, demonstrating a significant reduction in TSS (Figure 1.4(C), p-value <0.001)

Inlet concentrations of SRP ranged from 0.030 to 0.082 mg/L for pre-BMP samples and 0.002 to 0.79 for post-BMP samples. TP concentrations ranged from 0.017 to 6.09 mg/L for inlet samples from pre-BMP samples and 0.024 to 0.365 mg/L for post BMP samples. Both TP and SRP differences at the inlet were significant (p-value = 0.038 and 0.031 for SRP and TP, respectively). TSS concentrations ranged from 2.0 to 290 mg/L pre-BMP construction and 1.2 to 1096 mg/L post-BMP construction, and were not significantly different at the watershed inlet (Figure 1.4(C), p-value = 0.749). Median TSS concentrations at the inlet were 8.0 and 5.2 mg/L for pre- and post-BMP samples respectively. Due to fluctuations in inflowing P between pre- and post-BMP sampling, load accumulations (*g/day*) were calculated as a metric of buffer effectiveness in stream P reduction.

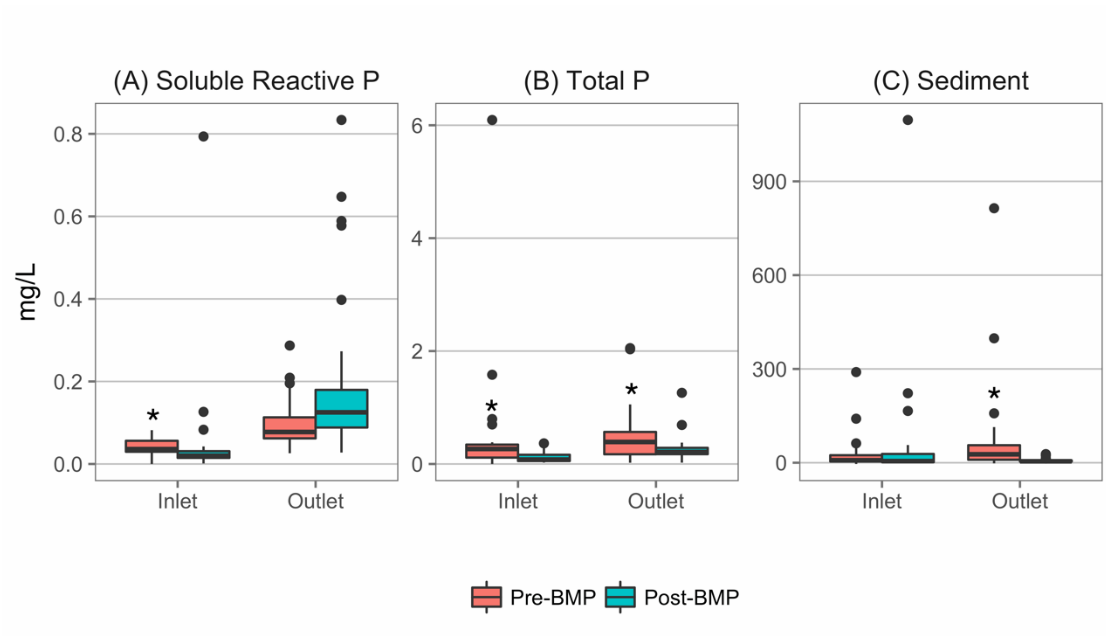

Figure 1.4: TP, SRP concentrations, and TSS observed at stream inlets and outlets. TP concentrations are significantly different (as indicated by an asterisk, *), but SRP concentrations are unchanged. N= 51 for pre-BMP samples and n=27 for post-BMP samples.

3.2.2 Load accumulations

Phosphorus loading analyses are meant to eliminate the effect of yearly variations in flow (i.e., weather) that could obscure the impact of the BMP on P loads at the outlet. The TP and TSS load accumulations over the course of the reach are significantly reduced after the installation of the cattle-excluded buffer (p-values of <0.001 and 0.033, respectively). However, the SRP load accumulation reduction is not significant (p-value = 0.396)(Figure 1.5). Because particulate P is a large component of TP, the reduction in TSS likely accounts for a large part of the TP reduction. The median load accumulations for TP are 242 g/day and 47 g/day for pre- and post-BMP respectively. Median load accumulations for SRP are 61 g/day and 28 g/day for pre- and post-BMP respectively. Median TSS load accumulations are 10 kg/day and 0.6 kg/day for pre- and post-BMP respectively. The variation in the data is also reduced in both TP and TSS from pre to post-BMP indicating a more predictable P dynamic after the BMP implementation. However, SRP again departs from the TP and TSS trend and shows an increase in variation post-BMP (Table 1.2).

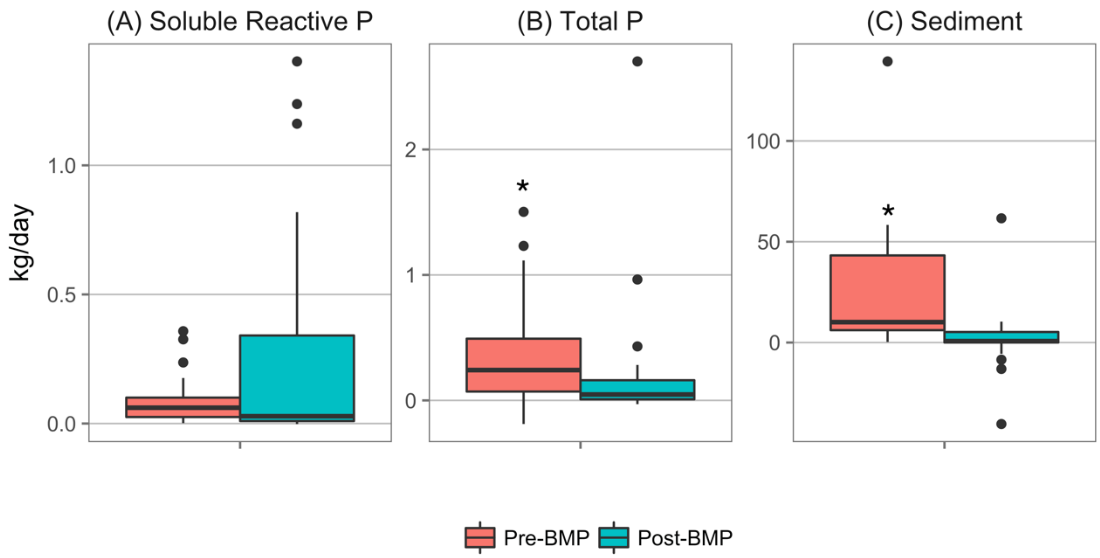

Figure 1.5: Total phosphorus, soluble reactive phosphorus, and total suspended solids loading accumulated across the site before and after the cattle excluded buffer was in place. TP and TSS accumulations are significantly different (as indicated by an asterisk, *), but SRP accumulation is unchanged. N= 25 for pre-BMP samples and n=24 for post-BMP samples. A negative load accumulation indicates reduction of P or TSS load over the course of the reach.

Table 1.2: Summary statistics for concentrations and accumulated loads for TP, SRP, and TSS; p-values refer to significance in change of observed value between pre and post-BMP samples. Absence of p-value for cow point source indicates this location was not sampled pre-BMP. Asterisks indicate statistical significance between pre and post-BMP samples at the same location.

Sample Location	Pre or Post BMP	Mean	Median	Standard Deviation	P-value
Inlet SRP *(mg/L)*	Pre	0.0393	0.0360	0.0215	0.039*
	Post	0.0689	0.0202	0.1780	
Inlet TP *(mg/L)*	Pre	0.5895	0.2660	1.3103	0.031*
	Post	0.1189	0.0786	0.1007	
Inlet TSS *(mg/L)*	Pre	38.3765	8.0	73.9192	0.749
	Post	83.3901	5.2	245.5122	
Outlet SRP *(mg/L)*	Pre	0.0950	0.0775	0.0577	0.489
	Post	0.1889	0.0868	0.2282	
Outlet TP *(mg/L)*	Pre	0.5294	0.4207	0.5048	<0.001*
	Post	0.2103	0.1402	0.2741	
Outlet TSS *(mg/L)*	Pre	70.9313	27.0	154.0879	<0.001*
	Post	2.3304	3.4	19.5008	NA
Pond point source SRP *(mg/L)*	Pre	0.4484	0.2200	0.6496	0.067
	Post	0.5731	0.3869	0.4612	
Pond point source TP *(mg/L)*	Pre	1.1904	0.9590	0.8390	0.343
	Post	1.0012	0.8384	0.7923	
Tile point source SRP *(mg/L)*	Pre	0.4804	0.3600	0.4131	0.601
	Post	0.5299	0.3958	0.7023	
Tile point source TP *(mg/L)*	Pre	1.2698	1.1912	1.3499	0.012*
	Post	0.6704	0.5660	0.4794	
Cow point source SRP *(mg/L)*	Post	0.1503	0.0476	0.2922	
Cow point source TP *(mg/L)*	Post	0.2304	0.2527	0.0520	
TSS Load Accumulation *(g/day)*	Pre	28,000	10,000	35,000	<0.001*
	Post	2000	700	18,000	
SRP Load Accumulation *(g/day)*	Pre	94	61	104	0.396
	Post	291	28	482	
TP Load Accumulation *(g/day)*	Pre	368	242	454	0.034*
	Post	272	47	652	

3.3 High phosphorus sources

Three point sources were identified along the stream reach as significant P sources: two observed in both the pre- and post-BMP periods, and one in only the post-BMP period. The pond effluent point source (Figure 1.6) discharges from the in series ponds intended to treat intercepted manure pit overflow while the tile drain collects subsurface flow and directs it to the stream. This point source discharges downstream of our outlet sample, but is included here as an example of a point source that frequently contaminates agricultural streams. The cow point source is flow from a cow-generated saturated area (currently outside of the buffer area) that short circuits the riparian buffer. Post-BMP samples from all three sources were higher in TP and SRP than the inlet, the other major direct input to the stream (Figure 1.6). Median concentrations for the point sources and inlet SRP concentrations were 0.38, 0.39, 0.04, and 0.02 mg/L for point sources from the pond, tile drain, cow-generated saturated area and inlet respectively. Median concentrations for the point sources and inlet TP concentrations were 0.83, 0.56, 0.25, and 0.07 mg/L. Data shown are concentrations rather than loads due to the difficulty in estimating discharge from these sources.

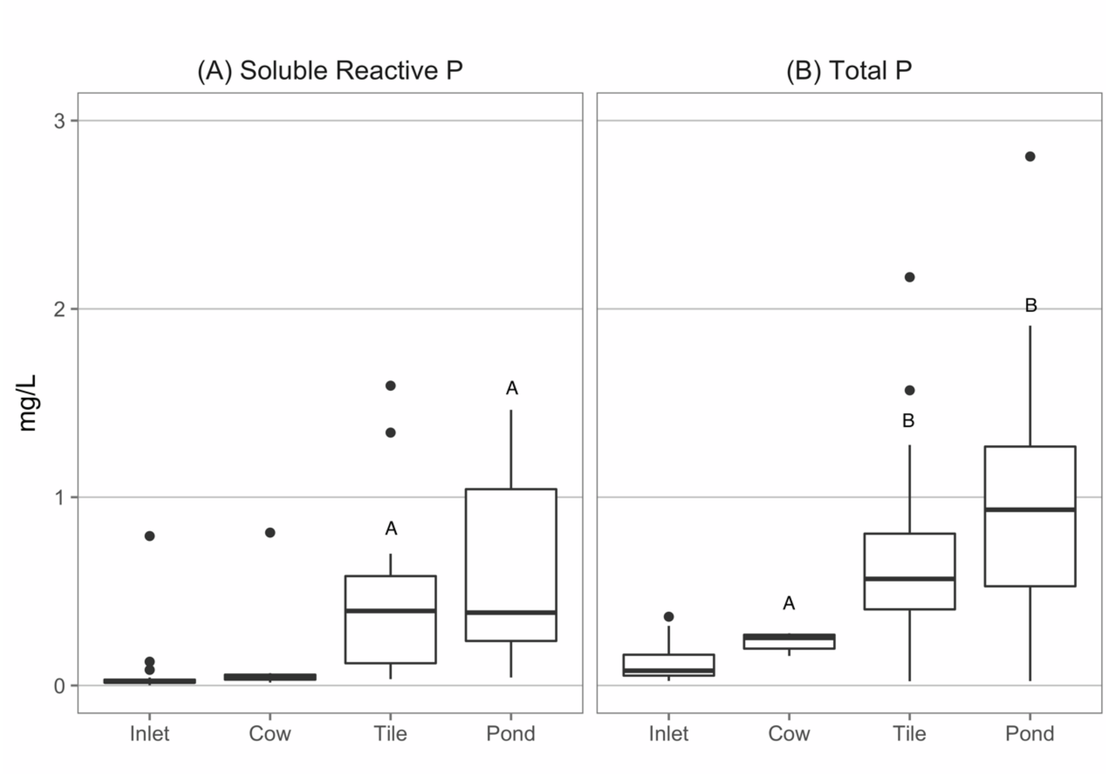

Figure 1.6: Post-BMP TP and SRP concentrations from point sources along the stream reach. Statistical difference is noted by 'A' and 'B' above (all TP data p-values <0.01). Point sources came from pond effluent (Pond, n = 29), a tile drain (Tile, n = 27), and a cow-generated saturated area (Cow, n = 8). Inlet is included for reference (n = 24).

Pre- and post-BMP point source comparisons for the pond and tile drain (Figure 1.7) indicate SRP concentrations decreased, though not significantly, in the pond (Figure 1.7, p-value= 0.067), while the tile drain did not experience a reduction in SRP (Figure 1.7, p-value=0.601). The reduction in SRP from the pond effluent indicates that pond renovations may have helped reduce P from the farm, but the tile drain continues to discharge highly polluted water directly into the stream.

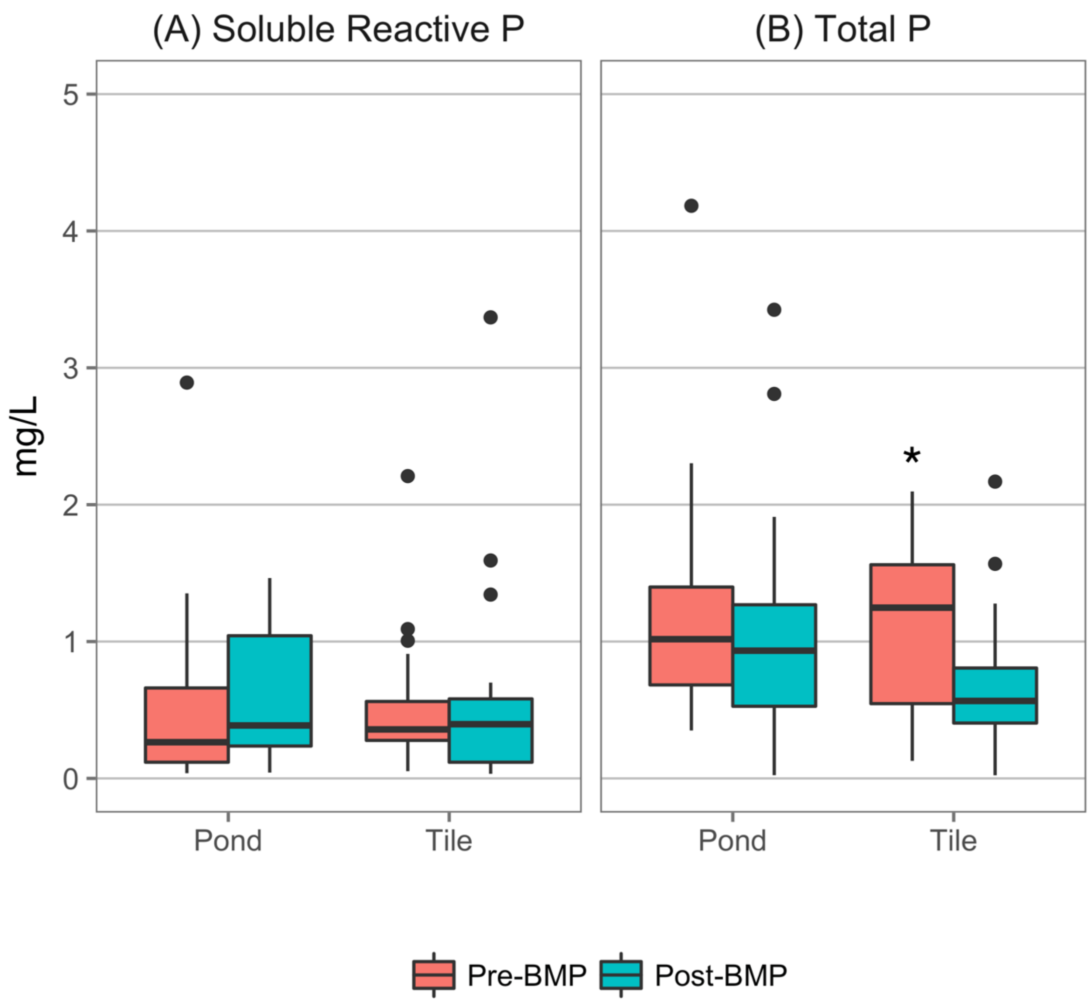

Figure 1.7: Point source SRP and TP concentrations pre- and post-BMP installation. Pond and Tile correspond with Pond and Tile sources from Figure 1.6. Statistical differences are noted by an asterisk (*). N = 24, 28 for pre- and post-BMP samples at source 1. N = 24, 28 for pre- and post-BMP samples taken from Source 2, respectively.

The cow-generated point source was generated post-BMP and does not have pre-BMP monitoring data. Median SRP concentrations for pre- and post-BMP pond point source are 0.22 mg/L and 0.38 mg/L respectively, and median SRP concentrations for pre- and post-BMP tile drain point source are 0.36 mg/L and 0.40 mg/L respectively.

Total phosphorus is not reduced when comparing pre- and post-BMP concentrations at the pond point source (p-value = 0.343). Median TP values for the pond point source are 0.96 and 0.84 mg/L respectively for pre- and post-BMP. However, total phosphorus is reduced at the tile drain point source (p-value = 0.011). Median TP values at the tile drain point source are 1.19 and 0.57 mg/L for pre- and post-BMP respectively.

Anecdotally, the discharge volume was greater in the tile drain than the pond effluent. The pond effluent was nearly zero for much of the summer during post-BMP monitoring and was dry for the summer during pre-BMP monitoring. The tile drain flowed continuously during both monitoring periods. The cow-impacted saturated area likely generated the least amount of runoff over the study period. This would indicate that the tile drain may have the largest impact on P accumulation over the reach, the pond effluent an intermediate impact, and the cow impacted area the smallest impact when considering load in addition to concentration. Of the three sources, the tile drain appears to have impacted the P accumulation over the reach the most.

There were 2 additional flow sources to the system, one from a forested wetland and one from a perennially wet spot at the toe-slope of a hill. None of these sources were not significantly different from the inlet when comparing SRP (all p-values > 0.3). The springs were sampled 24 times each in the post-BMP monitoring period. These springs were not taken to be the inlet samples because occasionally cows were pastured further upstream. All sources but one were not significantly different when considering TP (all p-values <0.1). The one source that was different (p-value = 0.03) flowed from a forested wetland with TSS comprising mostly of organic matter. This organic matter settled out before mixing with the main stem, and therefore did not influence total stream phosphorus dynamics significantly.

3.4 Load accumulation across discharges

The relationships between load and flow for TP, SRP, and TSS are not the same when comparing pre- and post-BMP data. TP and TSS load accumulations post-BMP are reduced in comparison with pre-BMP load accumulations. However, like concentration and load, we do not see a difference in the management practice in its effect on SRP as a function of discharge.

The cattle excluded riparian buffer appears to have the largest influence on TP and TSS loads at low flows, while at high flows the impact of the buffer is reduced (Figure 1.8(A),(C)). SRP appears to have no significant change as a result of the buffer system (Figure 1.8(B)). The equations for the regression lines in Figure 1.8 are in Table 1.3 with the associated errors of each estimate.

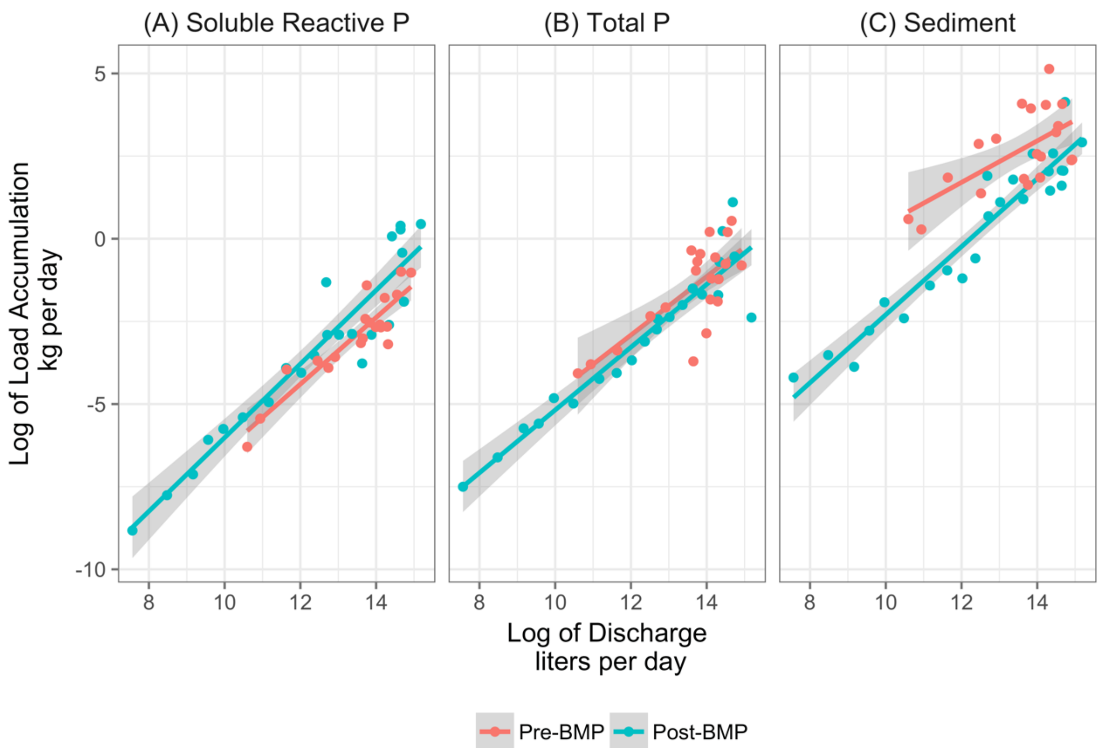

Figure 1.8: TP, SRP, TSS load accumulations over the reach are plotted against discharge on a log-log scale. Pre-BMP accumulations are plotted in red while post-BMP accumulations are plotted in blue. Points represent observed accumulations while lines represent the simple linear regression model for the data. Gray shaded zones represent error associated with the best-fit lines for each time period. Table 1.3 outlines statistics of the linear regressions.

Table 1.3: Coefficients of regression lines in Figure 1.8 (log-transformed relationships). Y-intercept is given by 'b' and slope is given by 'm'. S.E. (b) and S.E. (m) refer to standard error in the estimates of b and m respectively.

	Pre or post-BMP	b	S.E. (b)	m	S.E. (m)	R^2
Q vs. TP	Pre	-7.03	2.11	0.92	0.16	0.66
	Post	-3.70	1.94	1.13	0.15	0.79
Q vs. SRP	Pre	-9.55	1.49	1.00	0.11	0.81
	Post	-11.01	2.59	1.13	0.20	0.65
Q vs. TSS	Pre	-1.63	2.82	0.82	0.21	0.47
	Post	-7.17	2.29	1.10	0.17	0.74

4.0 Discussion

In all concentration and load comparisons, TP was significantly reduced with the addition of the cattle exclusion area. This observation follows those results found by several previous studies (e.g., McKergow et al., 2003; Miller et al., 2010; Shukla et al., 2011). The observed reduction in inflowing TP could have several explanations: (1) direct P input and sediment suspension is reduced by the limited cow access to upland streams and riparian areas, (2) mobile P and sediment is trapped by riparian buffer sediments and/or via chemically- and biologically-mediated processes, before reaching the stream. The reduction in TSS loading post-BMP implementation, suggests that either or both mechanisms are present. However, if these two factors were the only factors controlling stream P, we would expect SRP to be reduced as well. Our observation of minimal impact on SRP follows the results Hoffman et al. (2009) and Makin et al. (2007). Non-significant reduction in SRP may suggest that the source has not been completely addressed by the cattle exclusion area. For example, there could be accumulated P in the soils that continues to be slowly released over time, thus, mitigating the BMPs immediate impact on SRP.

A legacy problem is presented by soils with high P contents, as are common in this area (Ketterings et al., 2005). Legacy pollution issues arise when a certain pollutant has saturated or highly contaminated an area, such that it continues to provide pollution to the system after the incoming pollution source has stopped or been reduced. Desorption of soluble P accumulated in sediments into overland flow may continue for years beyond BMP construction (Sharpley, et al., 2013). The reach load accumulations observed in this study were collected in the year following buffer implementation, but if legacy contamination is contributing SRP at this site, we expect the load to diminish with time.

Desorption of SRP from enriched soils could explain our non-significant drop in stream SRP, but in the absence of soil P measurements, we cannot conclude its influence on stream SRP relative to the point sources. Yet, we stress that our findings demonstrate stream SRP is less influenced by the installation of riparian buffer cattle exclusions than is stream TP. Care should be exercised in concluding when and what water quality impact this BMP will have on a specific site.

We observed several point sources with high concentrations of SRP of water entering the stream. The riparian zone is short-circuited by the flow of point source 3 (Figure 1.6), bypassing the biological or physical processes expected to occur in the riparian buffer (e.g. Shultz et al., 1995). Additionally, ponded water at the edge of the pasture short-circuited the buffers in a concentrated flow paths, discharging directly to the stream. Areas where short circuiting occurred were likely wetter than average due to their relative low position in the landscape and the heavy traffic of cows, which, in combination, reduced vegetation growth and infiltration capacity in these areas and created wet spots that expanded through the season, similar to observations of Nguyen, et al. (1998).

Our monitoring after cattle exclusion buffer area installation revealed management issues that we suspect reduce the water quality gains of the riparian buffer. First, the continued, albeit unpredictable, presence of cows inside the fenced riparian area could slow observed impact of the riparian area. Of the 27 times this site was sampled after the installation of the exclusion fences, cows were found 5 times within the cow-exclusion area. No significant spikes in stream P levels accompanied these cow entrances when comparing days that the cows gained entrance to the stream and those in which they were successfully excluded. Inside the cow-exclusion area, the cows likely trample riparian vegetation, re-suspend sediment, and potentially directly defecate into the stream. Despite suspected negative impacts of cow entry into cattle-excluded buffer areas, we still calculate significant TP and TSS load reductions.

We expect that a wetter climate leads to more frequently saturated soils and greater nutrient runoff (Walter et al., 2000). Therefore, because post-BMP monitoring occurred in a wetter year compared to pre-BMP monitoring, it is possible that when assessing yearly loads the practice would demonstrate greater water quality gains across two monitoring periods with similar rainfall (in the case of this study, 2014-2015).

Another issue observed pertaining to reduced effectiveness of the cow exclusion area was the concentrated flow path generated from new saturated areas within the cow pastures, through the riparian buffers, and into the stream (e.g., point source 3). Other studies (e.g. Pankau et al, 2012) have made similar observations in cropped landscapes, indicating the dynamic management of riparian buffers is critical for their intended functionality. Changes in microtopography due to cattle traffic around fences and water sources were observed in this study. The cow-generated saturated areas (origins of concentrated flow paths) grew in area over the duration of the sampling period in 2017. Cows repeatedly trampled wet areas, expanding the saturated zones over time and likely reducing infiltration capacity through compaction (Nguyen et al., 1998). These areas tended to be near gates and walk ways, though they were also in shadier, moister, lowland areas that may have attracted cows during hot summer months. In order for the cattle-excluded riparian buffer to be maximally effective, exclusion areas need to be adjusted to include new areas likely to be saturated and those that arise. We recognize the logistical difficulty of modifying fence positions with traditional fencing methods. Solutions to this issue may include a combination of more frequent pasture rotation and movable fencing. Without inclusion of these saturated areas into cattle-excluded riparian buffer areas pollutants essentially become connected to the stream via preferential overland flow paths.

The final issue brought forth by this study, is that point sources of P that may be contributing significant loads to the system are not solved by cattle-excluded buffers. The tile-drain with high SRP discharge concentrations into the stream supports previous research on P transport in subsurface water flow (King, et al., 2015), and should be considered when assessing farm scale nutrient management. End of pipe BMPs have shown reductions on TP loads, likely as a result of sediment capture, with less consistent SRP reductions (Al-Hamdan et al., 2007). In order to significantly reduce all loads to the surface water system, these point sources need to be assessed for their potential interactions (or lack there of) with BMPs.

4.1 Limitations

As is the nature of on-farm research, there are a multitude of factors that impede the generalizability of the results. We believe that on-farm sampling encounters many of the obstacles seen, but not researched, in farm management, and tells the broader story of BMPs in the landscape.

Because the precipitation conditions of the pre-BMP sampling period were drier than post-BMP monitoring period, we will not make an estimate of percent reduction that occurred as a result of the cattle exclusion riparian buffer. Precipitation was approximately 5% higher in the post-BMP period than the pre-BMP period, suggesting reduction in phosphorus load accumulation may have been greater if our sampling periods shared the same precipitation conditions (Dougherty et al. 2004).

Point source discharges were monitored neither during pre- nor post-BMP monitoring periods due to rapid, and sometimes transient, generation of some of these points and difficulty in modeling source areas. To assess the total impact of the buffer versus these point sources, we needed to calculate P loads, which require discharges measurements for the duration of the experiment. Similarly, soil P observations, which we did not collect, could be used to parse the impact of the direct effect of cows in stream from the pollution caused by enriched soils. Regular soil P measurements over time could be useful in projecting when legacy P contamination will end.

Because grab samples did not capture storm events, these data should not be used to generate yearly averages with and without riparian buffer cattle exclusions. Our study objective was to assess buffer reduction on reach P and TSS during conditions the buffer would be anticipated to have the greatest effectiveness, i.e. moderate to low flow events (Meals & Hopkins, 2002).

5.0 Conclusion

In spite of the mixed water quality results, we continue to support the implementation of cattle-excluded riparian buffers. Although we found a lack of a significant reduction of SRP with the implementation of a cattle-excluded riparian buffer, this does not preclude the BMP from having a positive environmental benefit. We hypothesize that the reduction in TP will, over longer time scales, reduce SRP additions to the system from desorption from suspended sediments.

The construction of a riparian buffer cattle exclusion at this site showed significant reduction in TP and TSS even with the discovery of new, and unmitigated, point sources. Controlling for hydrologic patterns and landscape manipulations, this BMP could demonstrate even higher reductions of phosphorus loads. We have chosen to include the point source additions in our analysis to more accurately capture farm dynamics.

Buffers provide benefits beyond water quality including wildlife habitat and stream shading. The sediment reductions observed at this site are important to the health of this agricultural watershed. Reducing the transport of sediment-bound P is important for downstream lakes where P can later desorb and contribute to prolong eutrophic conditions via internal P cycling within lakes.

It is important to be aware of the additional, unmitigated sources of SRP on grazed pastures. To increase the water quality benefit, we recommend including the following in conjunction with the implementation of a cattle exclusion riparian buffer: (1) identify point sources along the reach, and work to reduce nutrient loads from those sources as well, (2) extend the buffered area to include likely variable source areas that create preferential flowpaths through the buffer, and (3) modify cattle exclusions as new saturated, runoff-generating areas develop within pastures.

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CHAPTER 2

INCREASED MOBILITY OF ERYTHROMCYIN THROUGH AGRICULTURAL SOIL IN THE PRESENCE OF MANURE

Abstract

Antibiotic residues in the environment pose a series of threats to soil and aquatic organisms and human and livestock health through the building of antimicrobial resistance. Manure spreading associated with animal agriculture is one source of environmental antibiotic residues. To better understand the risk of contamination from these emerging contaminants, we studied the adsorption of erythromycin, a model macrolide antibiotic used across human and animal medicine. We conducted a series of equilibrium batch experiments to determine the speed and extent of adsorption and a continuous flow column adsorption experiment to observe non-equilibrium adsorption patterns. We determined adsorption equilibration time to soil was approximately 72 hr in out batch experiments. Erythromycin adsorbed to soil strongly ($K = 8.01 \times 10^{-2}$ L/mg; $q_{max} = 1.53 \times 10^{-3}$ mg/mg), adsorbed to soil in the presence of manure with less affinity but similar maximum capacity ($K = 1.99 \times 10^{-4}$ L/mg; $q_{max} = 4.63 \times 10^{-2}$ mg/mg), and did not adsorb to manure across the solid ratios tested. We observed a multi-phased adsorption of erythromycin to soil during the non-equilibrium column experiment, that was largely absent from the treatments with both soil and manure present. These results suggest erythromycin is more mobile in the environment when introduced with manure spreading , likely the largest source of agriculturally sourced environmental antibiotics.

Keywords: Antibiotic residue, antimicrobial resistance (AMR), pharmaceuticals, adsorption isotherm, erythromycin

1.0 Introduction

Antibiotics and other emerging contaminants (e.g. pesticides, pharmaceuticals, personal care products) are concerning environmental pollutants. Antibiotic residues have been detected in surface waters (e.g. Carpenter et al., 2018, Guo et al., 2016; Vatovec et al., 2016; Kolpin, 2002) and resistance genes have been found in the soil microbiome after manure applications (e.g. Fahrenfield et al., 2014; Jacobs et al., 2019). These compounds pose a threat through their interactions with aquatic and soil organisms (Välitalo et al., 2017), inhibition of soil microbial activity, and reduction of microbial biodiversity (Cycón et al., 2019). Antibiotic residue presence selects for antibiotic resistant genes in the microbial community, allowing the buildup of resistance that subsequently reduces antibiotic efficacy in human and animal medical applications (e.g. Martínez, 2008). Agricultural antibiotic residue transport pathways are poorly understood, which encumbers mitigation efforts of these emerging contaminants (Menz et al., 2018).

In livestock operations, antibiotics are used to treat and prevent diseases and to promote livestock growth and productivity (McEwen & Fedorka-Cray, 2002). Many animals absorb antibiotics poorly, with some animals excreting 50-90% of the ingested compound depending on antibiotic type (Kim et al., 2011; Alcock, 1999; Feinman &

Maheson, 1978). The excreted compounds can then move with water and sediments and interact with surrounding microbial communities. Understanding the transport of antibiotics will inform the future engineering of farm manure management systems to minimize the distribution of these potent compounds on farm-by-farm and industry wide bases. Agricultural land has long been recognized as a major nonpoint source of nutrient pollution (e.g. Carpenter et al., 1998; Zaring, 1996), but, more recently, has been associated with antibiotic pollution (Yi et al., 2019). Broadly, 6.2 million kg of medically important (i.e. antibiotics also used in human medicine) and 5.2 million kg of non-medically important (i.e. not used in human medicine) antibiotics were sold and distributed for US agriculture use in agriculture in 2019 with 41% of all medically important and 62% all non-medically important antibiotics sold for use in cattle specifically (U.S. FDA, 2020).

However, farmer perceptions of antibiotic transport and degree of reliance upon these compounds is highly variable across farms, management styles, and farm sizes. Georgakakos et al., (2021) found that all farmers in their study considered transport of antibiotic residues into milk and meat products on dairy farms, but only certain groups considered transport into wastes (waste milk, manure, mortality). These varying perceptions of contaminant transport influence management strategies that control antibiotic environmental loads.

Partitioning of antibiotics into aqueous and adsorbed phases from manure slurries in soils likely controls transport of these compounds. Due to the wide array of chemical structures that characterize antibiotics, it is difficult to generalize the behavior of one or even several classes of these chemicals. Tolls (2001) reviewed the existing literature on adsorption of antibiotics to soils and found that the distribution coefficients (K_d) across antibiotic classes varied from 0.62 to 1030 L/kg for sandy loam. He hypothesized that the functional groups of these compounds may better indicate sorbate-sorbent interactions than the antibiotic classes themselves. Davis et al. (2006) also reinforced that solubility and adsorption vary widely among antibiotics. This within- and across-antibiotic class variability complicates antibiotic residue contamination mitigation. Policy-based initiatives as mitigation strategies will depend on the transport characteristics of the target compound.

Chandler et al. (2005) showed that soil-bound antibiotics in equilibrium with a clay loam and loamy sand retained antimicrobial properties (i.e. limited bacterial growth) after desorption. This suggests that we need to understand the sediment—organic matter—antibiotic relationship to predict not only transport but also impacts to soil and aquatic organisms. Kemper (2008) suggested post-excretion interactions of residues, metabolites, and bacterial resistance are ripe areas for additional antibiotic contamination research. Menz et al. (2018) noted that a major obstacle in determining soil-bound antibiotic impact is the lack of soil adsorption coefficients in the literature, and subsequent complex interactions with differing soils.

Erythromycin, a bacteriostatic macrolide antibiotic often used on dairy farms, inhibits the growth of a wide variety of bacteria, including both gram positive and gram negative organisms, as well as some other parasitic pathogens (Amsden, 1996). Erythromycin is used widely across both animal and human medicine, making it an important contaminant to understand due to its broad medical applications. The pK_a of erythromycin is 8.8 (McFarland et al., 1997), however, erythromycin can be expected to be predominately positively charged in the pH range expected of manure (pH = 4.6-7.4) as a result of association of a hydrogen atom with the nitrogen lone pair of electrons (**Figure 2.1**) .

Adsorption of erythromycin has been studied in relation to biochar (Ndoun et al., 2020) and activated carbon (Ghalomayan et al., 2020) with promising applications for removal of erythromycin from the water column with 35-75% removal efficiency depending of pyrolysis temperature of the biochar and low required interaction times with activated carbon. . EPI Suite™ predictions for erythromycin suggest partitioning into organic solvents over water (log K_{ow} = 3.06) and organic carbon over water (log K_{oc} = 1.41) (U.S. EPA, 2019). In comparison to other macrolide antibiotics, erythromycin has comparable log K_{OW}, log K_{OC}, and biodegradation in waste water, but somewhat higher solubility and lower sorption to wastewater sludge (**Table 2.1**, U.S. EPA, 2019). When comparing macrolides to β-lactam antibiotics, another class with many medically important antibiotics, the two classes have comparable log K_{OW} and log K_{OC}, while β-lactams have higher solubility and lower sorption to wastewater sludge (**Table 2.1,** U.S. EPA, 2019). These predictions suggest erythromycin is likely to interact with organic matter because of its adsorption to and partitioning into organic phases over aqueous phases. However, if the post excretion environment or manure application location is more alkaline, such as in sodic agricultural soils that are likely to be water-logged, the mobility of erythromycin may increase due to changes in pH dependent charge, shifting erythromycin to a negatively charged molecule and making it more soluble in water.

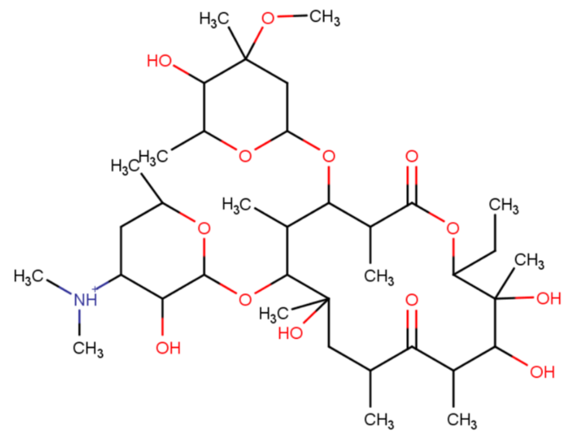

Figure 2.1: Erythromycin chemical structure, as expected in pH range 0-8.8. Visualization completed using Marvin Chem Axon (version 21.17.0, ChemAxon, https://www.chemaxon.com)

Table 2.1: EPI Suite ™ predictions for antibiotics in the macrolide and β-lactam classes.

Class	Antibiotic	Solubility [mg/L]	Log K_{OW}	Log K_{OC}	Wastewater biodegradation [%]	Wastewater sorption to sludge [%]
Macro -lide	Erythromycin	0.517	3.06	1.406	0.130	6.11
	Tildipriosin	0.0104	5.05	2.648	0.690	78.5
	Tilmicosin	0.007804	4.13	1.842	0.370	36.0
	Tulathromycin	0.0768	3.46	1.354	0.180	12.0
	Tylosin	1.59	1.05	-0.116	0.090	1.80
β-lactam	Ampicillin	439.3	1.35	0.808	0.090	1.84
	Penicillin G	210.4	1.83	1.095	0.090	2.02

This work aims to characterize the adsorption and transport processes of erythromycin to help predict risk of contamination of this compound from agricultural sites to surrounding ecosystems. We characterized the adsorption isotherms of erythromycin to a characteristic agricultural soil with and without the presence of manure through adsorption equilibrium batch reactions and column experiments to better understand mobility. We fitted Freundlich and Langmuir adsorption models to both treatments. This research fills existing gaps in the contaminant transport literature to better assess and model antibiotic contamination from animal agriculture.

2.0 Methods

2.1 Soil and manure collection

We collected soil from the top 10 cm of a fallow control plot at the Cornell Recreation Connection (Freese Road, Ithaca NY) agricultural research plots in the Caneseraga CaB soil series, an agricultural soil characteristic of New York's Finger Lakes region. We collected manure samples from four organic dairy farms in the region. On a per cow basis, farm 1 was 100% grass-fed with 0 kg grain/day, while farms 2, 3, and 4 fed 1.4 kg grain/day (3 lb/day), 4.5 kg grain/day (10 lb/day), and 17.2 kg grain/day (38 lb grain/day), respectively, at the time of manure collection. This diversity of feeding practices is representative of farms in the region. We combined manure from the four farms in a 1:1:1:1 by dry mass ratio to achieve an average manure composition for our experiments. All manures were individually analyzed for bulk parameters. Manure and soil were twice autoclaved at 135°C for 25 min to sterilize, then oven dried before experimental use.

2.2 Bulk parameter characterization

We measured bulk solid parameters to understand the environment of the adsorption experiments and allow comparison between treatments. We obtained bulk density, pH, particle size distribution, electrical conductivity, organic matter loss on ignition, surface area, pore size, and zeta potential for both manure and soil substrates (**Table 2.2**). We also obtained percent sand, silt and clay for the soil. We tested both oven-dried and autoclaved then oven dried samples for the manure and the soil to assess the effect of substrate preparation on bulk parameters.

We calculated bulk density of the soil by extracting a 7.3 cm diameter by 6.5 cm tall soil core and measured total solids content of fresh manure by measuring 250 mL of fresh manure and recorded the wet weights. Samples were oven dried at 65°C for 24 hr before recording dry weights (U.S. Soil Quality Institute, 1998). We prepared pH samples from 15 g dry soil and manure, sieved through a 2 mm sieve, and rehydrated in 30 mL deionized (DI) water after a 30 min equilibration period (Robertson et al., 1999). Using an Accument Research AR50 Dual Channel pH/Ion Conductivity Meter (S/N AR 81202286), we measured pH and electrical conductivity. We prepared electrical conductivity samples in a 1:5 ratio by mass of dry soil or manure to water and left to equilibrate for 1 hr prior to analysis (Rayment & Higginson, 1992). Particle size distribution, specific surface area, and pore size was obtained using the Brunauer-Emmett-Teller (BET) method (Micrometrics ASAP 2640) from dry, sieved (2mm) samples after subjecting the samples to a 100°C vacuum for 24 hr. We calculated percent loss on ignition by subjecting 5 g of oven dried soil or manure to 500°C for 2 hr. Solutions of 0.1 mg/mL were prepared for zeta potential analysis following Darrow et al. (2020) for soils. We obtained zeta potential from a Malvern Panalytical (zs90) Zetasizer set to a reflective index (RI) of 2 and absorbance index (A) of 1 for soil, and a RI of 1.4 and an A of 0 for manure.

2.3 Batch equilibrium adsorption experiments

Oven dried manure and soil were added to each batch and volume brought up to 150 mL with deionized water after erythromycin addition. To determine solid ratios for experimental use, differing solid masses (0.01 g, 0.05 g, 0.5 g, 5 g, 15 g) were tested to determine detectable adsorption of erythromycin (at 667 ppb) for both soil and manure. Manure tests resulted in no adsorption across the treatments, leading to the two-phased equilibrium experiment discussed below. Soil solids ratio of 5 g/150 mL was chosen as the first solid ratio tested with significant adsorption. For the combined manure and soil experiment (SM treatment), 5 g soil with 0.5 g manure was used. All batch experiments were in 250 mL amber glass vials. All amber glass vials were sonicated and autoclaved prior to usage and left on a rotating shaker in the dark for duration of experiment. Control reactors with no solids were prepared in the same conditions as treatment reactors. Because erythromycin showed no adsorption to manure-only treatments, no additional manure-only experiments were conducted.

2.3.1 Soil adsorption equilibration time

For determination of adsorption equilibration time, 10 mL of 10 ppm erythromycin solution was added to each batch reactor and total volume brought to 150 mL with a final concentration of 667 ppb. Each batch was sampled at 0 hr, 3 hr, 12 hr, 24 hr, 36 hr, 48 hr, 72 hr, 96 hr, 120 hr, and 144 hr. Samples were collected using a syringe and filtered through a 0.30 μm glass fiber filter immediately after sampling. Triplicate batch reactors were prepared for each equilibration time. We diluted samples to approximately

15 ppb erythromycin for analysis using an erythromycin enzyme-linked immunosorbent assay (ELISA) kit. All samples were analyzed within 24 hrs of filtration using a MyBioSource erythromycin ELISA kit (cat #: MBS282249) and a Molecular Devices M2 microplate reader.

We defined the adsorption equilibration time to be the first sample that was not statistically different from both the previous (t-1) and next (t+1) samples using a Wilcox Rank Sum Test (with p-value < 0.1 indicating statistical difference). We also obtained first order reaction kinetics (Eq. 1),

$$q_t = q_e(1 - e^{-k_1 t}) \qquad \textit{(Eq. 1)}$$

where q_t is the amount of erythromycin adsorbed at time t, q_e is the amount of erythromycin adsorbed at equilibrium, and k_1 is the kinetic rate constant.

2.3.2 Adsorption equilibrium isotherms

After establishment of the adsorption equilibrium time, erythromycin at 19 concentrations (10 ppb, 20 ppb, 40 ppb, 80 ppb, 100 ppb, 200 ppb, 400 ppb, 800 ppb, 1000 ppb, 2000 ppb, 5000 ppb, 10,000 ppb, 25,000 ppb, 40,000 ppb, 50,000 ppb, 65,000 ppb, 75,000 ppb, 100,000 ppb, and 150,000 ppb) was added to 5 g soil in 250 ml amber glass vials in triplicate (S treatment). Batch reactors were left on a shaker for 72 hr, filtered through a 0.3 μm glass fiber syringe filter, and analyzed within 24 hr using an ELISA kit and microplate reader. All samples were diluted (as needed) to detection range of 15 ppb from the initial concentration prior to analysis.

The SM treatment was also equilibrated for 72 hr at initial concentrations of 10 ppb, 100 ppb, 400 ppb, 1000 ppb, 5000 ppb, 10,000 ppb, 25,000 ppb, 50,000 ppb, 100,000 ppb, and 150,000 ppb. We combined 5 g soil and 0.5 g manure by dry mass in amber glass vials for a total volume of 150 mL.

We fitted 5 models (Eq. 2-6) to each set of data resulting from the adsorption isotherm experiments to determine best model fit. Standard square error and statistical significance of model parameters were used to determine best fit. Freundlich non-linear (Eq. 2) and linearized regressions (Eq. 3) and Langmuir non-linear (Eq. 4) and linearized regressions (Eq. 5,6) models were tested.

$$q = k_f \times c^n \qquad (Eq. 2)$$

$$log\, q = a + b \times log\, c \qquad (Eq. 3)$$

$$n = b;\ k_f = 10^a \qquad (Eq. 3.1)$$

$$q = q_{max} \times K \times \frac{c}{1+K*C} \qquad (Eq. 4)$$

$$\frac{1}{q} = a + b \times \frac{1}{c} \qquad (Eq. 5)$$

$$q_{max} = \frac{1}{a};\ K = \frac{1}{q_{max} \times b} \qquad (Eq. 5.1)$$

$$\frac{c}{q} = a + b \times c \qquad (Eq. 6)$$

$$q_{max} = \frac{1}{b};\ K = \frac{1}{q_{max} \times a} \qquad (Eq. 6.1)$$

In Eq. 2-6, q is the equilibrium adsorption density (mg adsorbate)/(mg adsorbent), q_{max} is the maximum adsorption density, c is aqueous concentration (mg/L), k_f is Freundlich's coefficient, n is the non-linearity coefficient, and K is the equilibrium coefficient for adsorption between the adsorbate (erythromycin) and adsorbent (solids) (L/mg). In the modified linear models (Eq. 3, 5, 6), a and b, are the intercept and slope of the linearized models, respectively. Linearized model parameters were then converted to parameters with physical meaning (Eq. 3.1, 5.1, 6.1).

To assess the impact of manure on adsorption of erythromycin to soil, we conducted a two-phased equilibration experiment. In the first equilibration, we equilibrated manure in 4 treatments (0.01 g, 0.05 g, 0.5 g, and 5 g) with erythromycin at 667 ppb for 72 hours. In the second equilibration, we added the filtrate of the first equilibration to batch reactors with 5 g soil/150 mL. The second equilibration equilibrated for an additional 72 hr. Samples were analyzed after each equilibration. SM batch experiments utilized 0.5 g manure solids following this test. We applied a semi-log transformation to the data after the second equilibration to generate a linear model:

$$c = a + b \times \log m \qquad (Eq. 7)$$

where c is the aqueous concentration of erythromycin after second equilibration, m is the initial mass of manure used in the first equilibration, and a and b are the empirically derived intercept and slope, respectively, of the resulting model.

2.4 Soil column experiments

We used 7 cm diameter columns, continuously infiltrated from below using a pump. We infiltrated about 17 liters of 500 ppb erythromycin solution through each column. Average infiltration rates ranged from 28 mL/min to 31 mL/min. Treatments were run in triplicate. All columns contained 200.00 g homogenized soil. Columns in manure treatment contained 20.00 g manure in addition to 200.00 g soil. We determined infiltration volume from a combination of initial column tests and high and low estimates of fitted adsorption isotherm models. Volumetric outflow was recorded continuously throughout the experiment for each column independently. Samples were filtered, diluted, and analyzed within 12 hours of experiment completion. Eight samples per column were analyzed for pH, 30 samples per column were analyzed for aqueous antibiotic concentration. A non-parametric smooth local regression (loess) model was used to visualize the column results.

2.5 Data analysis

The ELISA analysis method is a targeted approach, well-equipped to assess the presence of a single compound in laboratory samples. This method required a standard microplate device, making it accessible across laboratories lacking high performance liquid chromatography – mass spectrometry (HPLC-MS) set ups. Because ELISA methods are designed with a detection range of 0.2-25 ppb, this method is ideal for scenarios where low concentrations are expected. Sample dilution to reach the detection range may have introduced some of the noise in the data. We believe some of the variability between our replicates may be attributed to the high dilution ratios required to reach detection range of the ELISA.

All data were analyzed in R-studio (version 1.4.1106 for Mac). Model parameters for Eq. 1-7 were tested for statistical significance using a t-test.

3.0 Results

3.1 Bulk solid parameter data

We obtained bulk characteristic data to better understand the environment of these experiments (**Table 2.2**). We found that the soil autoclaving procedure slightly reduced pH, particle surface area, pore diameter, bulk density, loss on ignition and zeta potential, and increased nanoparticle size, soil water content, electrical conductivity, and bulk density (**Table A1.1**). Manure from farm 2 was tested to assess the effect of solid preparation, resulting in a reduction in pH, bulk density, nanoparticle size, and zeta potential and an increase in surface area, pore diameter, soil water content, loss on ignition, and electrical conductivity (**Table A1.1**).

Table 2.2: Soil and manure bulk parameters. Manure characteristics were averaged across the 4 farms from which manure was collected.

Characteristic	Soil	Manure[1]
Bulk Density/Total Solids (g/cm^3)	0.872	0.149
SWC (g water/g dry sample)	0.4752	5.9925
pH	4.37	6.68
Particle Diameter (nm)	1,216	14,296
Electrical conductivity (µScm)	370	5.33
OM loss on ignition (%)	9.81	87.55
BET Surface Area (m^2/g)	4.93	0.32
Pore Diameter (nm)	14.93	33.62
Zeta potential (mV)	-13.11	-5.87

[1] Average values. Manure characteristic data from each farm may be found in **Table S.2**.

3.2 Batch adsorption studies

3.2.1 Adsorption equilibration time

We applied a first order reaction model and determined q_e and k_1 to be 1.47×10^{-5} mg/mg (p-value = 3.64×10^{-5}) and 6.399×10^{-2} hr^{-1} (p-value = 0.020) respectively for the concentration tested (667 ppb), with both parameters significant predictors at the p-value <0.05 level. We found the adsorption equilibration time for erythromycin to adsorb to Caneserga soil was approximately 72 hours (**Figure 2.2**). The 72 hr sample as not statically different from neither the previous (48 hr, p-value = 0.4) nor the next sample (96 hr, p-value = 0.4). Some additional adsorption was observed in the 144 hr samples, and may be indicative of an additional, slower adsorption mechanism. The control reactors experienced some reduction (~50 ppb) in erythromycin concentration over the test period, but this change was not statically significant (p-value = 0.2) in a wilcox rank sum test between the first and last control sample, and therefore not considered in further analysis.

When testing adsorption of erythromycin to pure manure substrate, we observed no change in aqueous concentration over any of the range of solids ratios tested (**Figure A1.1**). Therefore, adsorption equilibration time to manure was not determined. All subsequent tests contained a mixture of manure and soil solids.

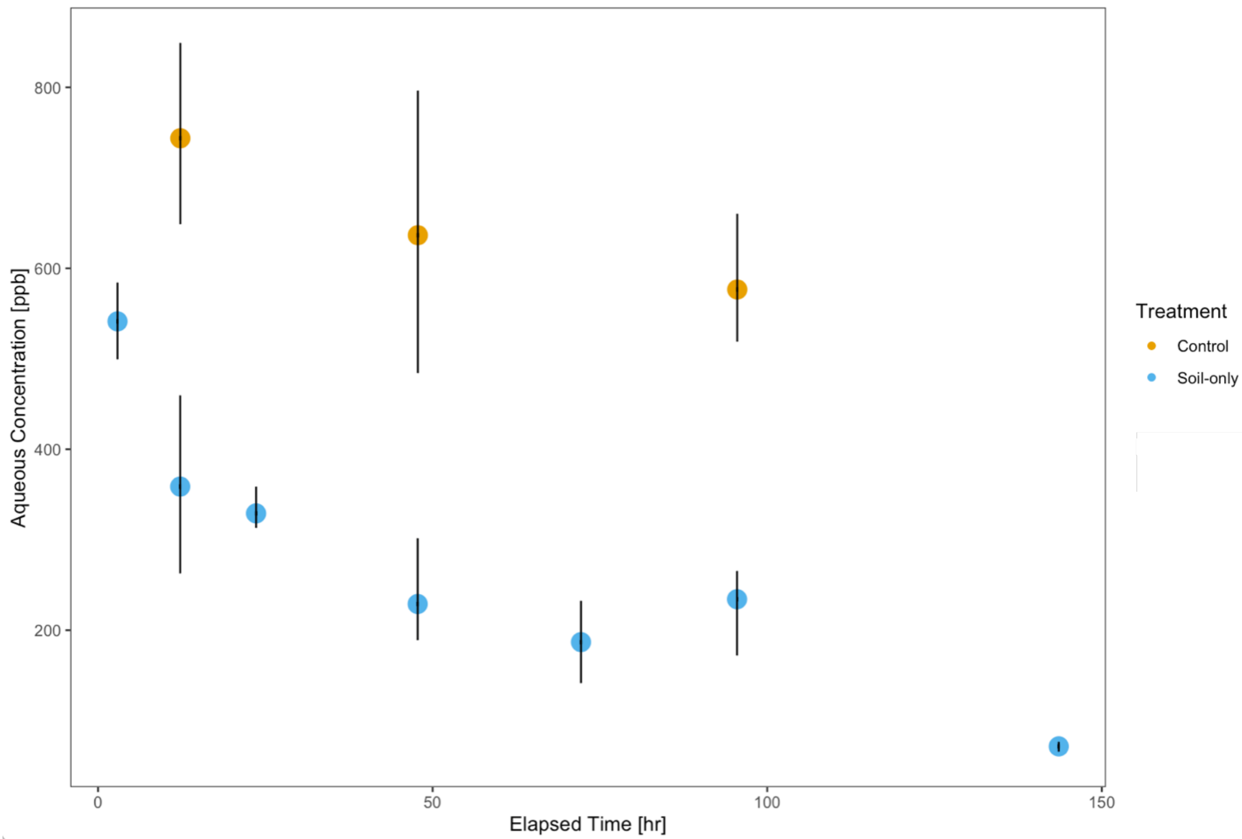

Figure 2.2: Soil-only (S) equilibration time between erythromycin and Caneseraga soil (blue) compared to control reactors (yellow) with no soil. Error bars represent the range of the 3 replicates used to obtain the points plotted.

3.2.2 Adsorption equilibrium isotherms

Both the S and SM isotherms were best fit by Langmuir models. In the S adsorption isotherm (**Figure A1.2**), the Langmuir non-linear regression model fit best (Eq. 4), with parameters of maximum adsorption capacity (q_{max}) and the equilibrium coefficient (K), were $1.5300x10^{-3}$ mg adsorbed erythromycin/mg soil and $8.0144x10^{-2}$ L/mg erythromycin respectively (**Table 2.3**). Parameters were statistically significant (p-value < 0.01) for all models when t-test statistics were computed, except for one Langmuir linearization (Eq. 5) (**Table A1.3**). The 150,000 ppb samples did not show a large enough change to be detected. The 150,000 ppb sample has therefore been removed from the analysis, and was not used to calculate best fit. The Langmuir linearization model (Eq. 6) and the Freundlich non-linear regressions (Eq. 2) were the next two closest fitting models.

Unlike the models run on the S isotherm, the SM isotherm (**Figure A1.3**) models had fewer statistically significant parameters (Eq. 2-6) (**Table A1.3**). The linearized Freundlich model (Eq. 3) was the only model with two statistically significant parameters. Of the models with at least one significant parameter, the data were best fit by the linearization of the Langmuir model (Eq.5) with SSE of $3.5x10^{-7}$, however one parameter was negative with no physical meaning. Eq 3 and Eq 6 fit the data less well than eq. 4, but had reasonable parameter values with physical interpretation (Eq. 2: k_f = 1.00, n = 1.01; Eq. 5: K =$1.99x10^{-4}$, q_{max} = $4.63x10^{-2}$) (**Table 2.3**). Both the Freundlich nonlinear regression (Eq. 2) and the Langmuir nonlinear regression (Eq.4) had neither parameter as a statically significant predictor of the data and, therefore, were omitted from further analysis.

When comparing S and SM adsorption isotherms, it is apparent that the presence of manure increases the aqueous concentration of erythromycin and reduces the adsorbed mass (**Figure 2.3**). The two isotherms are distinct until the highest concentrations tested (100,000 ppb, 150,000 ppb). At these highest concentrations, adsorption of the two treatments begin to converge around the same q_{max} as observed in the S treatments. Adsorption capacity remained constant between the two treatments, but adsorption density was reduced when manure was present.

We tested the extent of manure's influence over erythromycin adsorption and found a semi-log relationship (Eq. 7) between manure mass and aqueous concentration (**Figure 2.4**). We found that with increased manure, adsorption density decreased. At the highest manure mass tested (5.00 g), samples resembled control batch experiments' aqueous concentrations at equilibrium. While at the lowest manure mass tested (0.01 g), samples resembled S treatments' aqueous concentrations. We found the adjusted R^2 for this relationship to be 0.9629. The semi log model coefficients were 527.54 (p-value = 0.0033*) and 93.56 (p-value = 0.0124) for *a* and *b* respectively. This result suggests that at manure to soil ratios of 1:1 or greater, erythromycin is likely to remain in a more mobile, aqueous phase.

Table 2.3: Converted model parameters from linearized and non-linear regressions. Eq 2-3 are Freundlich isotherms, with k_f and n parameters, while Eq. 4-6 are Langmuir isotherms with K and q_{max} parameters.

Model	S Parameters k_f or K	n or q_{max}	SM Parameters k_f or K	n or q_{max}
Eq. 1	2.17×10^{-4}	4.52×10^{-1}	1.87×10^{-15}	5.96
Eq. 2	6.95×10^{-5}	8.87×10^{-1}	1.00×10^{-5}	1.00
Eq. 3	8.01×10^{-2}	1.53×10^{-3}	-7.59	2.14×10^{-4}
Eq. 4	1.58	5.13×10^{-5}	-1.94×10^{-3}	-5.17×10^{-3}
Eq. 5	6.38×10^{-2}	1.52×10^{-3}	1.99×10^{-4}	4.63×10^{-2}

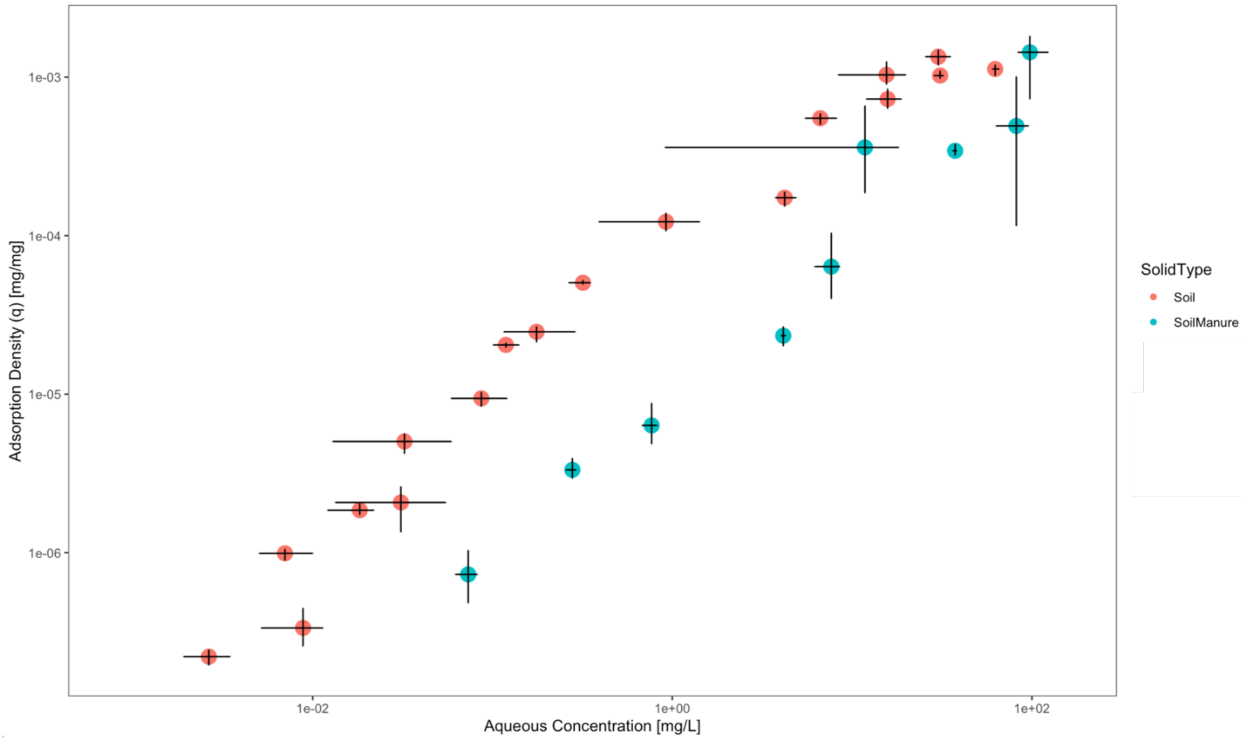

Figure 2.3: Soil-only (S) and soil + manure (SM) isotherms. Error bars represent the range in the 3 replicates averaged for each point.

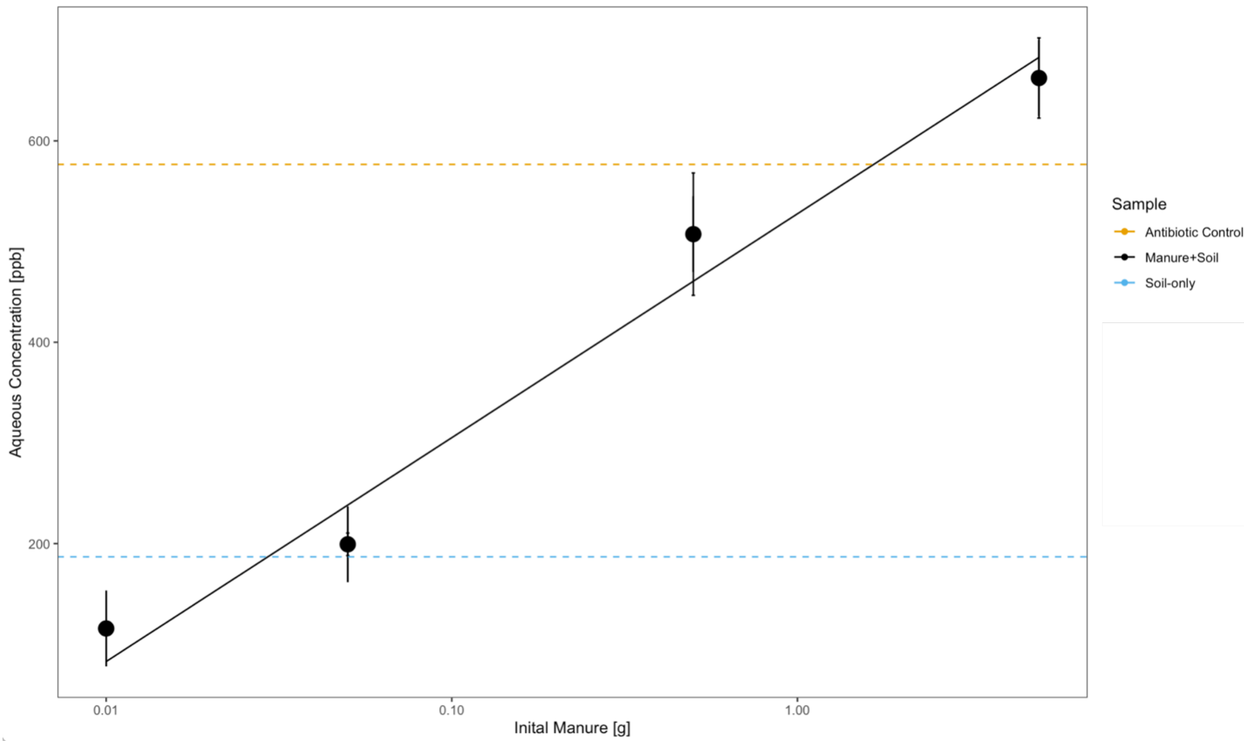

Figure 2.4: Filtered manure-DOM influence on erythromycin aqueous concentration after 72 hr reaction with 5 g soil. Error bars represent the range of the data. Fitted model follows eq.6 (black line). Antibiotic control (yellow line) and 72 hr S sample (blue line) are provided for reference.

3.3 Soil column experiments

Initial breakthrough curves for both S and SM columns occurred between 0 and 5 L erythromycin solution passed through the columns (**Figure 2.5**). The two column treatments overlap significantly between 2.5 and 6 L infiltrated after which they diverge for the remainder of the experiment (16.6-17.6 L passed through the columns). Although there is considerable overlap between the two treatments, especially in early samples, the SM smooth loess model results in a higher aqueous concentration than the S treatment model for the entire experiment. Due to the slow adsorption kinetics determined in the adsorption equilibration time experiment, these results are expected. When the columns begin to diverge, the SM columns discharged a greater amount of erythromycin, consistent with lower adsorption rates in the isotherm experiments.

The S column from 5 L through the end of the experiment displayed additional adsorption in comparison with SM columns, that may be characterized by a slower adsorption equilibration time or an adsorption mechanism with less affinity for erythromycin. This multi-phased adsorption may be a result of the complex, non-homogenous nature of soil used, and appears to be much less extensive in the SM treatment, which approaches the influent concentration for some final samples. This second phase of adsorption maybe have been captured by the 144 hr samples in the adsorption equilibration time experiment, which displayed additional adsorption in comparison to the 72 hr samples.

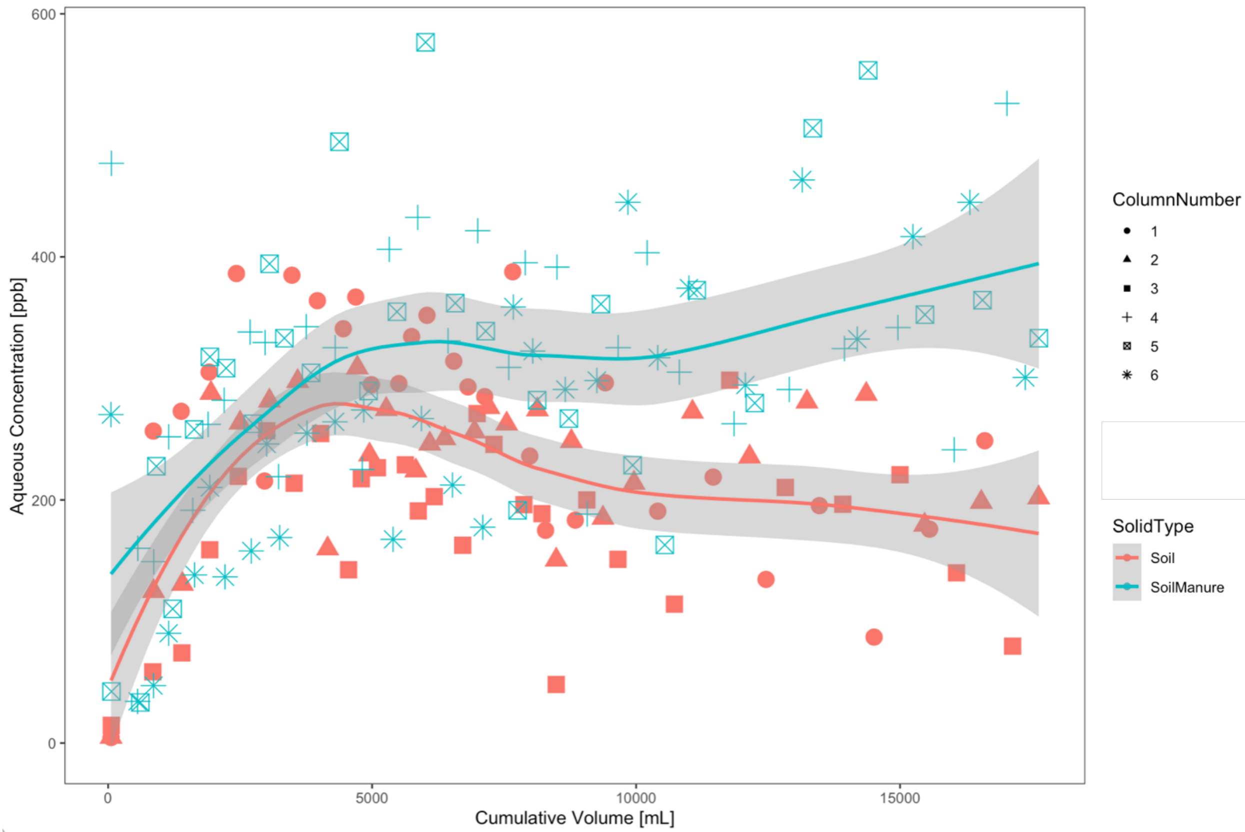

Figure 2.5: Aqueous concentrations of erythromycin after passage through S or SM columns. Unique shapes indicate samples from the same columns. Columns 1-3 were S, columns 4-6 were SM. Trend lines are fitted with a loess smoothing model.

4.0 Discussion

We found that erythromycin adsorbs to soil (S treatments), but in the presence of manure (SM treatments), erythromycin is characterized by an adsorption isotherm favoring its aqueous phase, leading to higher mobility and contamination risk. We found that adsorption equilibrium was established after approximately 72 hr in soil batch reactors, suggesting in-field adsorption after manure application may be lowest immediately following application, with high risk of water contamination if applied during saturated soil conditions or immediately preceding precipitation. Following the recommendation by Menz et al. (2018), this work defines the soil-erythromycin adsorption interaction to better understand residue movement in the environment, especially under application conditions of manure application, one likely source of erythromycin contamination in the environment.

Despite the organic carbon partitioning coefficient predicted by EPI Suite™ and previous soil adsorption experiments indicating increased erythromycin sorption to soil with organic matter presence (Kruger, 1961), we found that erythromycin became more mobile when interacting with organic carbon in manure. These results suggest that adsorption sites previously taken by erythromycin are instead occupied by a component from the manure or that the manure alters the form of erythromycin to make it less likely to sorb to the soil. The specific mechanisms of these possible explanations were not investigated. It is possible that erythromycin—DOM-manure complexes formed, which were small enough to pass through the filter, and unable to sorb to the soil. These complexes could have effectively made the contaminant more mobile and would be expected given the propensity of this compound to partition into organic carbon. We did not explicitly test this mechanism. DOM has been shown to influence contaminant adsorption to minerals by transforming contaminants through hydrolysis and redox reactions, competing for adsorption sites, and complexation reactions yielding compounds with higher or lower affinities for adsorption sites (Polubesova & Chefez, 2014). Clarithromycin and roxithromycin, two related macrolide antibiotics, have both showed similarly reduced adsorption to minerals in the presence of DOM. Clarithromycin and roxithromycin were both transformed by humic acid-DOM via hydrolysis, reducing their adsorption to manganese oxide and ferrihydrite minerals (Feitosa-Felizzola et al., 2009), a possible mechanism that may similarly influence erythromycin. To the authors knowledge, this is the first-time manure has been shown to inhibit adsorption of erythromycin to soils. In comparison to sorption to pure clays, our experiments in both S and SM treatments produced Freundlich model parameters (k_f, *n)* within the range tested for erythromycin adsorption to montmorillonite ($k_f \in (10^{-1}, 10^{1})$, $n \in (0,1)$)and kaolinite ($k_f \in (10^{-20}, 10^{0})$, $n \in (1, 10)$) when exposed to various exchangeable cations (Kim et al, 2004a).

Antibiotics have been previously displayed biphasic adsorption, as seen in our column experiments. Tetracycline was observed to have a biphasic adsorption to a highly porous human-hair-based substrate, filling external adsorption sites prior to less accessible internal adsorption sites (Ahmed et al., 2017). Biphasic mineralization of erythromycin has been observed and associated with desorption of the compound from sediments (Kim et al, 2004b), but no prior studies have noted a biphasic adsorption of the erythromycin to soils to the authors knowledge.

4.1 Study implications

We found that erythromycin was more mobile in SM treatments in comparison with S treatment environments. This conclusion held across adsorption isotherm experiments and column experiments. Therefore, erythromycin contamination sourced from agricultural manure application may pose a higher risk of entering surface and ground water supplies than previously concluded from S experiments (Pan & Chun, 2016). To assess risk of antibiotic contamination in other antibiotic classes and with other dominant functional groups, manure and SM interactions should be investigated similar to and beyond those preformed here to describe mechanisms of adsorption. Reducing transport, and, more broadly antibiotic environmental impact, requires a deeper understanding than our current knowledge of the controls of sorption of erythromycin to environmentally relevant compounds and soil surfaces.

Because erythromycin sorbs readily to soils, best management practices (BMPs) that reduce sediment transport may also reduce erythromycin transport. However, because of continuous flushing of sediments in riparian zones and other frequently saturated areas (such as sediment control BMPs), conditions may favor the aqueous phase of the compound, increasing mobility and reducing removal of erythromycin during high flow conditions. Control of erythromycin residue transport may be more effective on the manure management level, e.g., breaking down this compound through heat treatment (Oliver et al., 2020) or biological degradation prior to soil application prior to manure field application. Kim et al. (2004b) found natural biological degradation of erythromycin was controlled by desorption of erythromycin from stream sediments, suggesting microbial degradation could be enhanced if a system was designed to allow degradation in manure storage prior to field application.

5.0 Conclusion

We found that erythromycin adsorbs more strongly to agricultural soil in the absence of manure, remaining more mobile in the presence of manure. We found that the characteristic adsorption isotherms that fit both environments were Langmuir isotherms, with the nonlinear regression fitting the soil-only (S) adsorption isotherm best and a linearization of the Langmuir fitting the SM condition best. In our column experiment we observed more passage of erythromycin in the SM condition, as would be expected from the differences in their isotherms, and possible biphasic adsorption breakthrough curve. If we are to reduce the transport of compounds such as erythromycin, we must consider these physical and chemical processes alongside biological utilization and human decision-making pathways to predict impact and risk of transport.

6.0 Acknowledgements

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CHAPTER 3 -
FARMER PERCEPTIONS OF DAIRY FARM ANTIBIOTIC USE AND TRANSPORT PATHWAYS AS DETERMINANTS OF CONTAMINANT LOADS TO THE ENVIRONMENT [2]

Abstract

Agricultural antibiotic contamination into milk and beef products has been considered extensively, but antibiotic transport into soil and water environments is less regulated and studied. Farmer perceptions of these transport processes are critical to understanding how antibiotics reach soils and surface waters and what management strategies can be implemented to reduce environmental antibiotic loads. We have conducted semi-structured interviews with twenty-seven dairy farmers in central New York to understand farmer perceptions of environmental transport of antibiotics and decisions that reduce environmental antibiotic loads. Interviews were qualitatively analyzed and coded using thematic analysis. We found that farmers extensively considered transport of antibiotics into milk and beef, while consideration of antibiotic transport into manure was less common, and no farmers discussed antibiotic transport from carcasses into soil from on-farm animal mortality. Farmers highlighted decisions that reduce antibiotic environmental loads through disease prevention actions, usage of non-antibiotic treatments, and culturing bacterial samples before antibiotic treatment. Farmers did not cite reduction of environmental antibiotic loads as a driver of their waste

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management decisions. Farmers perceived antibiotic usage was already minimized on farms in the region, suggesting future environmental antibiotic contamination mitigation strategies should focus on waste management pathways.

1.0 Introduction

Understanding farmer perceptions of transport of antibiotic residues is a critical step to reducing negative environmental impacts of these pharmaceuticals and other contaminants of emerging concern and the spread of antimicrobial resistance (AMR). Antibiotic residues are harmful water and soil pollutants because of their toxic and inhibitory interactions with aquatic organisms (Välitalo et al., 2017) and impacts on soil microbial communities' structure and genetic composition (Guo et al., 2016; Kumar et al., 2005). Agriculture accounted for six million kg of medically important and 5.5 million kg of non-medically important antibiotics sold and distributed annually in the United States in 2018 (U.S. FDA, 2019A), where these compounds were used to treat and prevent diseases and promote livestock growth and productivity (McEwen & Fedorka-Cray, 2002). Many antibiotics are poorly absorbed by their target animals, leading to excretion rates of 50-90% of ingested and injected compounds depending on antibiotic type (Kim et al., 2011; Alcock, 1999; Feinman & Maheson, 1978). Because adsorption and soil-water partitioning vary widely between antibiotics (e.g. Tolls, 2001; Davis et al, 2006), transport depends on both the specific antibiotics used due to differing biotic and abiotic break down of residues and the physical manure management system in place (Oliver et al, 2019).

A refined understanding of farmer perceptions can expose the various pressures felt and rationales underlying decisions regarding antibiotic usage. This knowledge can, in turn, direct efficient management changes to reduce contaminant transport. Friedman et al. (2007) and Wemette et al. (2020) assessed perceptions of prudent antibiotic usage and potential to impact human AMR, while Schneider et al., (2018) analyzed knowledge pathways and information sources about AMR. Veterinarians' impact on antibiotic usage has been studied through possible overuse resulting from veterinarian absence at time of treatment, lack of written records, extent of treatment completion (Sawant et al., 2005), and barriers to changing veterinarians' antibiotic prescribing behavior (Golding et al., 2019, McDougal et al., 2017). To asses some drivers of antibiotic usage, Cardoso et al. (2019) described characteristics of ideal farms and how those perceptions influence antibiotic usage, while Swinkles et al. (2015) assess social influences on antibiotic treatment duration, and Jones et al. (2015) address drivers motivating farmers to reduce antibiotic usage. Wemette et al. (2020) found differing antibiotic usage perceptions amongst New York State (NYS) farmers across management practices (i.e. conventional and organic). Redding et al. (2020) underscored the importance of considering the underlying values of prescribers and antimicrobial users in modifying behavior. Though antibiotic residues, antibiotic resistant bacteria, and resistance gene coding sequences have been found in areas where manure has been spread (Han et al., 2018; Walczak & Xu, 2011; Kumar et al., 2005), and farmer knowledge of antibiotic interactions with soil has been in some cases found to be minimal (Phares et al., 2020), farmer perceptions of antibiotic transport with manure and environmental presence have not been previously studied, to the authors' knowledge. Understanding farmer perceptions of antibiotic transport and decisions leading to increased or decreased environmental loads will allow us to develop mitigation strategies that are environmentally beneficial while accounting for on-farm systems and decision-making processes.

Farmer perceptions of environmental antibiotic transport pathways through dairy cows into beef, milk, and manure constitutes a knowledge gap in our understanding of farmer insights into antibiotic residue transport and how those perceptions influence decision making. Oliver et al. (2020) suggest a gap in the literature for community (i.e. farmer) engaged research to ensure relatability of results to on-farm management systems, specifically concerning AMR. Dairy farms are a large industry in NYS, with about 4,000 farms constituting 625,000 dairy cows (NYS Dept. Ag & Markets, 2018). Medically important antibiotics intended for use in cattle (both dairy and beef) account for forty-two percent by weight of medically important agricultural antibiotics distributed in the United States annually (U.S. FDA, 2019A). Given this industry's size and antibiotic usage, effective manure and contaminant management is critical to protect natural resources and ecosystems. Our study investigated farmer perceptions of antibiotic transport and decisions that reduced environmental residue loads to direct future AMR mitigation efforts. We qualitatively analyzed interviews of NYS dairy farmers on both conventional and organic farms that are characteristic of the region.

2.0 Materials and Methods

2.1 Study Population

The first author interviewed dairy farmers in central New York. Twenty-seven dairy farms were included in this study: eight farms were organically managed, according to USDA organic certification standards, and nineteen were conventionally managed (**Table 3.1**). In order to participate in the study, farms were required to have dairy cows. However participants were not limited to farms that relied exclusively upon dairy income and some farms had other livestock. Throughout this study, 'conventional' is used to reference non-organic farms. Farmers reported management practice (i.e. organic or conventional), number of milking cows, acreage, and farm generation; the authors assigned farmer age groups after interviews (**Table 3.1**). Interviewees were farm owners, farm family members, herd managers, and/or farm managers, hereafter referred to as farmers or interviewees. Ten interviewees were female and twenty-four were male. Three interviews were with one female interviewee and seventeen interviews were with one male interviewee. Seven interviews had more than one interviewee present, all of which consisted of at least one male and one female in the same age group. One interview involved three interviewees, one female and male in the same age group and one male in an older age group; we used the primary respondent's age to categorize the farmer age for this interview. Interviewees were recruited via email and telephone. Ninety-five farms were contacted with a 28% positive response rate.

2.2 Data Collection

Authors established farm contacts using a combination of snowball sampling (Biernacki, & Waldorf, 1981), online county databases, and co-author recommendations. This study design and interview guide received exemption from full review by the Institutional Review Board at Cornell University. Interviews were semi-structured (see **Table A2.2** for interview guide), lasting from 21 to 87 mins. Nine of our thirty-seven interview questions were taken from the interview guide used by Wemette et al, (2020). All interviewees were given a $10 honorarium for their time. All interviews were performed in-person and all but one were conducted on-farm. A farm walk accompanied some interviews. Second or third parties interrupted some interviews and asked unrelated questions of the interviewees; these interruptions are omitted from analysis and, in some cases, were not transcribed. All interviews were recorded, and the interviewer took handwritten notes during the interview. All audio recordings were professionally transcribed by The Cornell Institute for Survey and Economic Research (CISER, Ithaca, NY).

2.3 Data Analysis

First and second authors read and coded interview transcripts for themes. Final coding analysis was performed in ATLAS.ti (version 8.4.4 for Mac). Responses were compared between agricultural practices (i.e. organic and conventional operations), number of milking cows, farmer age group, and farm generation (**Table 3.1**).

Qualitative data analysis and theme generation were conducted using the method outlined by Braun & Clarke (2006). Emergent themes within each topic are discussed categorically. After initial coding, themes were manually clustered based on similarity and transcripts searched for key words for thorough inclusion (for search words and associated codes see **Table A2.3**). Qualitative statements were accompanied by quantifiers where appropriate, as recommended by Maxwell (2010). Interviewee responses were categorized into topics related to antibiotic pathways outlined in **Figure 3.1**: disease prevention actions, treatment perspectives, antibiotic residue transport perceptions, waste management perspectives, and environmental impact of residues. Trusted and avoided information sources do not appear in **Figure 3.1**, but are included in this study to holistically represent antibiotic discussions on farms. Disease preventions were discussed in conjunction with non-antibiotic treatment methods, discussed here as alternatives to antibiotic usage. Antibiotic transport was discussed in the context of physical residues through biological and environmental pathways. Not included in this analysis, though sometimes discussed were topics of on-farm antibiotic container storage and disposal of antibiotic containers that may contain residues.

Table 3.1: Farm and farmer demographics. Percentages represent farms interviewed within the management category of conventional or organic across the category of number of milking cows, farmer age, or farm generation. The number of farms within each category appears in parentheses under the percentage.

		Management	
		Conventional	Organic
Number of Milking Cows	Small *(0-50 cows)*	15.8% *(3)*	50.0% *(4)*
	Medium-small *(51-100 cows)*	15.8% *(3)*	25.0% *(2)*
	Medium *(101-500 cows)*	15.8% *(3)*	25.0% *(2)*
	Medium-large *(501-1000 cows)*	26.3% *(5)*	0.0% *(0)*
	Large *(>1001 cows)*	26.3% *(5)*	0.0% *(0)*
Farmer Age	Millennial *Born 1981-1996*	36.8% *(7)*	25.0% *(2)*
	Gen X *Born 1965-1980*	36.8% *(7)*	12.5% *(1)*
	Baby Boomer *Born 1946-1964*	26.3% *(5)*	62.5% *(5)*
Farm Generation	1st	0.0% *(0)*	37.5% *(3)*
	2nd	78.9% *(15)*	37.5% *(3)*
	>3rd	21.1% *(4)*	25.0% *(2)*
Total Number of Farms		*(19)*	*(8)*

3.0 Results

The interviews assessed farmer perceptions of their role in and decisions leading to increased and/or reduced environmental presence and transport of antibiotics. **Figure 3.1** links farmer decisions and physical antibiotic pathways that influence the environmental presence of these emerging contaminants. The results follow the order set forth by **Figure 3.1** (sections A through E). Farmers make antibiotic-relevant decisions based on many factors other than minimizing their environmental presence, however we discuss factors here in the context of reducing agricultural antibiotic environmental presence. Because the dairy industry is linked with the beef industry through selling of bull calves and animals culled for beef, many perspectives have a beef focus.

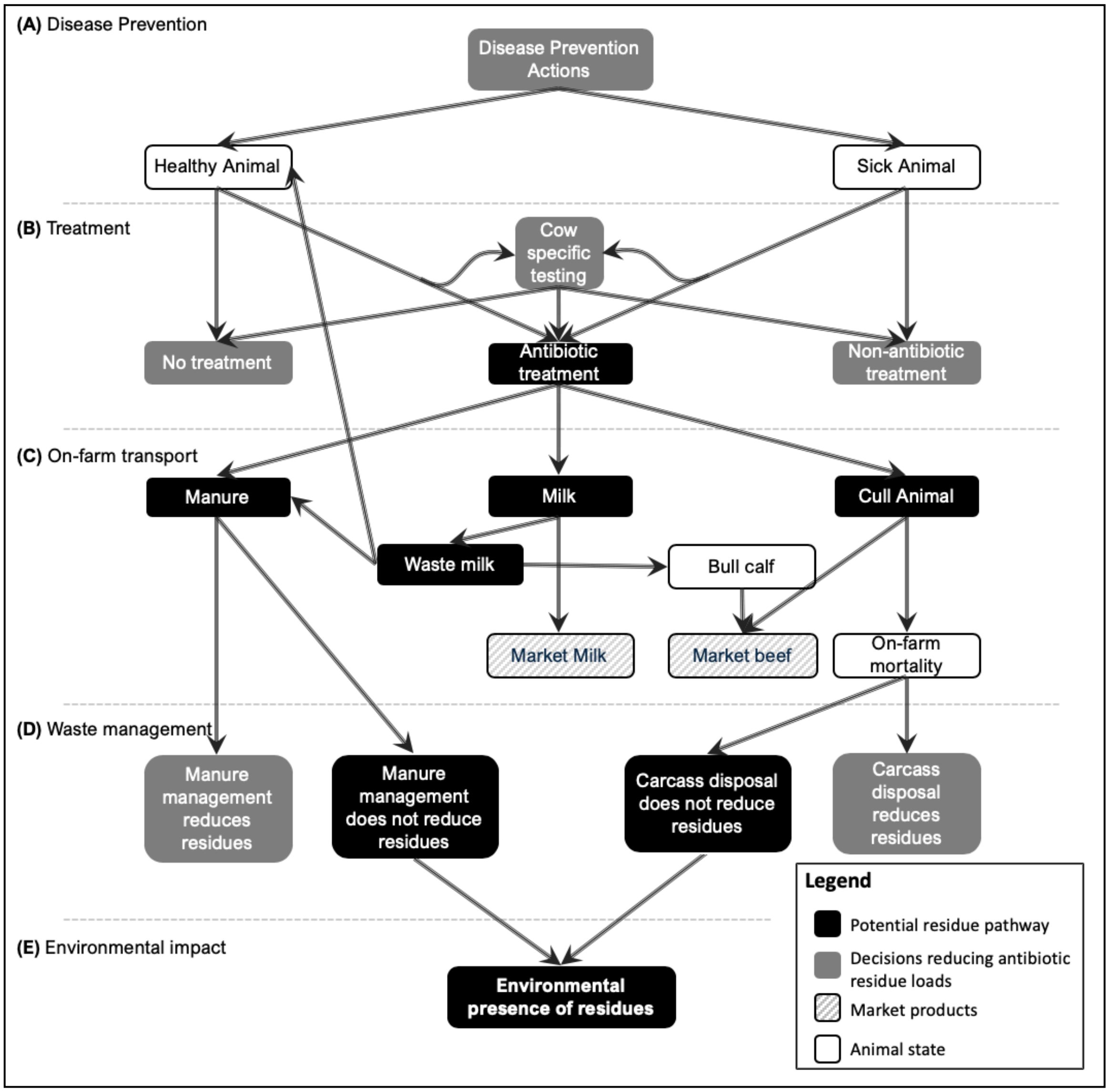

Figure 3.1: Simplified antibiotic environmental residue pathway with associated dairy farmer decisions that reduce antibiotic environmental loads. Some connections have been omitted for simplicity. Black boxes indicate direct movement of potential antibiotic residues into the environment. Grey boxes indicate decisions made that reduce eventual environmental antibiotic residue loads. Hashed boxes indicate potential residue movement into human food systems. White boxes indicate animal health conditions. Waste milk is connected to manure to reflect disposal of treated or non-saleable milk, and to healthy animal/bull calf to reflect the practice of feeding waste milk to calves and heifers on-farm. Both bull calves and heifers can then be sold for beef. Letters A-E are used to locate perspectives and decisions in the pathway to environmental residue presence throughout the results.

3.1 Disease prevention actions

Disease prevention (**Figure 3.1A**) reduces antibiotic usage, thereby reducing potential environmental antibiotic loads. The primary methods for disease prevention discussed were use of vaccines, attention to cow comfort and facility conditions, and attention to life-stage dependent nutrition.

3.1.1 Vaccines

In the dairy context, vaccinations are used to prevent disease from both viral and bacterial infections. The interviewer did not differentiate between viral and bacterial vaccinations during the interviews. Farms across all categories (**Table 3.1**) referenced using vaccination for disease prevention. Ten of nineteen conventional farms and five of eight organic farms referenced preventative vaccination. One farm chose not to vaccinate because animals did not enter and leave their herd frequently. Twelve of the twenty-eight farms interviewed did not mention vaccination. When discussed, vaccinations were seen as one of the strongest disease prevention tools, leading to reduced antibiotic usage. A Millennial organic farmer noted *"we try to find, do everything possibly under the sun, to prevent… That's the key, how to prevent all these issues,"* (interview 6) noting that in the organic management style, there are many more opportunities to prevent than to treat illness. A Millennial large conventional farmer noted the reduction in antibiotic usage as the farm grew, as a result of intensive farm-specific vaccination protocols developed jointly by veterinarians and farmers, *"when we were 100 cow farm, we would probably treat more cows with that than we ever do now. It was, in years past, it was more of a reactive thing. Cows are sick, now the bigger* *your farm gets, you tend to be more … proactive…. So you tend to worry about your vaccines, and making sure they're all in line"* (interview 18). A Baby Boomer conventional farmer noted the urgency of helping newborn calves gain their immunity before they reach the appropriate age for vaccination, and the importance of feeding colostrum, the antibody rich secretion from the mammary gland after birth, *"a baby is going to get her first couple of months of immunity from that colostrum. If you collect it right and you give it to them in a timely fashion, and you give them enough… they're going to get their immunities from there because you can't really vaccinate a calf and have their immune system ready to respond to that vaccine until three/four months of age"* (interview 22). Feeding colostrum was similarly referenced across farm categories (**Table 3.1**).

3.1.2 Cow comfort & facility conditions

All farm categories mentioned cow comfort as an important factor in maintaining good animal health. Aggregated into this theme were several actions perceived to increase cow comfort, such as barn design, floor material, cow stress levels, and exercise. *"Give them the best you can give them to eat and keep them comfortable with good air"* (interview 4), was a perception shared frequently around cow comfort. Baby Boomers cited stress as an important factor, while farmers in other age categories did not explicitly refer to cow stress. One Baby Boomer related cow stress to human stress, *"you know, it's kind of like people. You hear about people getting sick and their doctor says, 'You need to quit your job. There's too much stress.' And then they just start feeling better. Well I think it's the same with cows. When you're not pushing them for* *production"* (interview 1). A Gen X farmer similarly discussed cow comfort, *"we don't push them for production so much. So in that way, they're probably not as likely to get mastitis. …We just like them to be cows. I hate doing anything to them that I don't have to do"* (interview 10). Millennial farmers were more likely to cite pasture grazing as improving cow comfort and health and exercise, *"we do rotational graze…and you know, it gets the cows outside. It'll improve your herd health, reproduction"* (interview 13).

Farmers highlighted facility conditions to improve cow comfort. First generation farmers stressed facility structures, *"making sure they're in the healthiest environment we can have them in depending on the weather…We have some air quality issues in our calf barn and if we could spend the money tomorrow to build them a new facility, we would. Unfortunately we can't do that, so we just have to make sure they have the driest bedding"* (interview 21). Second and third generation farmers commented on cleanliness of the cows' living and milking spaces; *"our cows are extremely clean. They [the stalls] are constantly scraped back. We put a lot of bedding under them"* (interview 10). Second and third generation farmers similarly reflected, *"if we have a lot of mastitis issues… [I will ask] is everything clean? Is there an issue in the equipment? And then in the barn where the cows lay in the stalls, liming the stalls, keeping everything nice and clean and dry"* (interview 25). Environmental cleanliness and ventilation were the main focal points of improving cow environmental conditions to prevent disease.

3.1.3 Nutrition

Farmers referenced both general nutrition and nutrition to maintain metabolic health as disease reduction methods. Across farm categories, general nutrition focused on diet and diet modifications for specific life stages while metabolic nutrition focused on fresh cow dietary supplements (fresh cows refer to cows that have recently calved). General nutrition was often referenced as ensuring cows have *"good air and a clean bed and good feed and water all the time. If they got all the stuff they need, they tend to stay healthy"* (interview 16). Farmers across categories referenced fresh cows as a life stage requiring more attention, with increased antibiotic and non-antibiotic treatment needs as compared to the rest of lactation. For fresh cow metabolic nutrition, practices such as giving a calcium bolus, drenching, and ingestion or injection of vitamins (e.g. vitamin B) were practiced across management scales, though conventional farmers were more likely to mention it as an alternative to antibiotics than organic farmers. Drenching is the practice of filling a cow's rumen with a solution rich in minerals, vitamins, and high energy molecules. Some of these actions, like drenching and vitamin B injections, can also be employed as a responsive treatment when indications of stress or abnormality appear. Probiotics were referenced as a metabolic nutrient supplement for fresh cows, and seem to be widely used across the industry.

In addition to fresh cows, calves were also frequently refenced in treatment and disease prevention decisions. All farm categories were concerned about calf nutrition and health, with no thematic differences across categories. The perception that "*the calf stuff has been a really big deal in terms of preventative stuff, and in terms of treatment*" (interview 26) was prevalent. Farmers discussed improving calf immunity through feeding probiotics and colostrum, and, once old enough, vaccination. Probiotics were referenced both for general calf nutrition and for treatment of calf scours. Emphasizing their farm's attention to feeding colostrum, one farmer stated "*every calf gets colostrum. If it's born at 11 o'clock at night, I stay up with that damn calf, and it gets colostrum*" (interview 26). Feeding colostrum is essential in transferring immunity as it is the most efficient means of gaining immunity and preventing disease in newborn calves when given in a timely fashion (e.g. Godden, 2008).

3.2 Antibiotic usage perceptions

Decisions around antibiotic usage begin the pathway of physical antibiotic transport on farms (**Figure 3.1B**). According to USDA organic certification standards, organic animals cannot be treated with antibiotics and remain organic animals. However, organic farmers can administer antibiotics under the condition that treated animals' milk as well as those animals culled for beef are not sold with organic branding. Perceptions of antibiotic usage included a discussion of the effectiveness and necessity of antibiotics in general from both organic and conventional farms. Themes emerged around perceptions of other farms' antibiotic usage, the desire to keep a cow alive (on farm), and the opinion that dairy farmers were already minimizing antibiotic usage.

<u>3.2.1 Usage by others</u>

The two categories that presented an 'us versus them' paradigm were small versus large farms, and conventional versus organic farms. Small farm farmers tended to state "*when you have a smaller farm, you are able to make [treatment] decisions like this a little* *easier…you put your hand on just about every animal every day…you can catch things a little easier than if you're running around a 1000 head free-stall*" (interview 6). This perspective suggests farmers who interact with each animal every day allows for more informed antibiotic decisions than farmers who have less direct animal contact. However, large farms tended to rely on extensive data collection to inform antibiotic usage decisions. One large conventionally managed farmer emphasized formal data collection, stating "*a lot of farms don't do this, the way that we do it, but we take blood from the cows [to test]. Sometimes with the fresh cows usually up to 65 days, I look at their milk rates, I go out and look at them. If even one milking is off, I will go test them*" (interview 12), implying that, despite the large number of animals, managers made case-by-case decisions with the assistance of targeted and widespread data collection. This data driven versus observation driven knowledge generation was observed across other farm types in central New York (Menzies Pluer, 2019).

The 'us versus them' paradigm was readily visible between management classifications in in the antibiotic usage context. An organic farmer felt conventional farmers managed around the idea that *"you have an antibiotic, so why bother doing anything else? That's the magic silver bullet, so they don't…necessarily do all the other things that you could do to help supplement, because they're like, 'Well, they're on an antibiotic. What more do they need?' When I feel like it's all the other things that we do, that almost you don't need the antibiotic if you're doing those other things"* (interview 14). Conversely, a farmer helping manage both conventional and organic dairies commented "*sometimes it will make me cringe that…I wasn't able to treat that cow. But she gets through it. But I think she would have gotten through it better…with* *some sort of treatment"* (interview 17). Three conventional farmers shared the perception that organic cows are not as healthy as conventional cows, exemplified by, "*I wouldn't want to be an organic cow…I'll go into an organic herd and 80% will be three-quartered, like they've had mastitis and lost a quarter"* (interview 26)('three-quartered' references losing one of the four independently functioning mammary glands that comprise the cow's mammary system). Conversely, organic farmers who transitioned from conventional practices argued "*we just don't have the issues that we used to…I think that relates back to that we don't push the cows for production so they're less stressed*" (interview 1). The opportunity for a strong 'us versus them' paradigm may exist in part because of differences in production methods and market structure across conventional and organic dairy production.

3.2.2 Keep cows alive

Preventing on-farm death was an important motivator across agricultural practice and farm size. The decision to treat or sell an animal for beef complicated this driver and added nuance to the decision-making pathways, but all types of farms identified using antibiotics as a last resort. A conventional farmer identified using non-antibiotic treatments such as *"udder mint or something to see if the cow can…fight it herself. But certainly if the cow's health…is at risk then we'll definitely utilize antibiotic use"* (interview 13). Udder mint is a commonly used non-antibiotic topical cream applied when mastitis symptoms appear. Similarly, an organic farmer stated**,** "*if it's going to save her life, we've exhausted organic methods, by all means, we use antibiotics…then* *she's got to leave the herd"* (interview 6). However, these decisions were interwoven with decisions to cull rather than treat animals.

3.2.3 Minimize antibiotic usage

Farmers minimize antibiotic usage for many reasons and reflected upon longitudinal shifts to more judicious antibiotic usage. These shifts may have been byproducts of actions intended to minimize disease. Organic farmers tended to align with the ideology of contaminant reduction, such as "*well, now there is an argument whether organics is good or bad, right? Whether chemicals are good or bad, whether spray is good or bad, whether antibiotics are or are not in our soil or in our food or not, right? We agree that there's a problem? Well there isn't on my farm because I don't use it…I'm convinced I'm better off without it"* (interview 4). Large conventional farms tended to have the perception that "*antibiotics managed right is a good tool. It's no different than our children"* (interview 9), yet another larger conventional farm operator stated *"we firmly believe that there are too many [antibiotics] being used. And, so we've implemented a lot of changes with that. We use antibiotics as a last resort"* (interview 12). Smaller conventional farms tended to identify alternative treatments as a regular method of dealing with illnesses such as mastitis. One farmer exemplified, "*if we get a cow with a hard quarter we're more likely to hand strip it and put udder mint on it than we are to shove a tube of antibiotics up there. Nine times out of ten they take care of it themselves anyway"* (interview 10). Several identified economic pressures driving their antibiotic usage decisions. One small conventional farm stated, *"vets are expensive, drugs are* *expensive. You want to minimize that as much as possible"* (interview 20). Most farmers thought their usage had improved in the recent past and wanted to reduce antibiotic usage on their farms in general. Several farmers reflected *"I use a lot less than my dad ever did…I remember when I was a kid we used to milk cows that were pretty nasty, I mean, they treat them, and treat them, and treat them and they never cleared up"* (interview 19). As expected, these perceptions were prevalent with second and third or greater farm generation farmers.

Some farms also discussed testing bacteria (**Figure 3.1B**) to determine appropriate antibiotics to use. Extent of employing this practice was not overwhelmingly discussed (6 of 19 conventional farms, 1 of 8 organic farms), though it appeared in each farm and farmer category (**Table 3.1**). One conventional farmer reflected upon their culture and treatment method, *"so if we have a cow that shows signs of mastitis, we'll take a sample, it gets sent to lab, and then once we get it, we'll get that resolved within 24 hours. If it shows a no growth, or it's a gram negative [bacteria], we don't treat that with antibiotics, because it's not going to help you. If it's a gram positive [bacteria]…then we know that we can utilize antibiotics on that particular animal"* (interview 17). Some farms incubated bacteria on farm, while others sent samples to labs, both in reference to minimizing antibiotic usage through testing, especially in the context of mastitis.

3.3 Non-antibiotic treatment

We defined non-antibiotic treatments (**Figure 3.1B**) as responsive actions to disease that farmers choose over antibiotic treatments. The two main actions discussed were usage of herbals to treat sick or high-risk animals and decisions to cull based on animal health histories.

3.3.1 Herbals

Conventional farmers tended to avoid herbal therapies while organic farmers used them. A common response to herbal treatment usage by conventional farms (seven of nineteen conventional farmers) was *"we have [used them] in the past. I had very limited results with it… I'm still undecided. We've tried it on and off at times. We do use a lot of the udder creams. Try to help circulation and stuff like that on a mastitis cow. That's about as far as we can go now. I have tried some other herbal stuff. I didn't think it worked"* (interview 9). Udder creams (generally mint based) were the only herbal therapy highly referenced by conventional farmers (seven of nineteen conventional farmers). Two conventional farmers also referenced probiotic treatments, *"no herbal stuff…we feed probiotics. I like probiotics. I feed that to all my baby calves"* (interview 26). Organic farmers tended to embrace the herbal treatments, though showed interest in prioritizing preventative measures. One organic farmer stated *"I make my own garlic tincture. That's probably our biggest antibiotic. And echinacea tincture I make too. I buy…licorice root, barberry, astragalus"* (interview 7). Two organic farmers also commented on the presence of antibiotic active ingredients in garlic and other organic herbal treatments, though this observation was not prevalent.

3.3.2 Culling cows for medical reasons

The decision to cull an animal, or sell it for beef, due to its disease history was one of the most impactful ways of managing antibiotic usage on farms across categories. Extent of culling practices varied with farm size, but across categories, farmers relied on culling to improve their long-term herd health. One large farm stated, *"we always have a lot of heifers coming in…most of the time we can actually [make a] cull decision based on the cow's past history"* (interview 9). Another large farm stated *"we're tired of diseases on the farm, we don't have pneumonia issues, your primary disease would be mastitis. And if they treat it more than twice, the third time they get reevaluated, and most of those don't get treated a third time, they end up going to slaughter"* (interview 27). One medium-small farm reflected, *"I use a lot less [antibiotics] than my dad ever did…I cull a lot harder than he did"* (interview 19). Another medium-small farm stated *"I hate dealing with down and dying animals. I prefer to cull them from the herd before they get to that point"* (interview 14). Farms that focused on breeding genetics were outliers from this farm-size cull decision perspective. A medium sized farm, with a focus on breeding genetics, reflected on a cow with recurring treatment requirements, *"that cow's gone…at a big farm. But we're going to give her a shot, because yeah, she's my screen saver [on my computer], so it's kind of hard to kill the cow that's your* *screen"* (interview 26). Though culling thresholds differed across farms sizes and marketing strategies, it was a critical component of reducing antibiotic usage.

3.4 Antibiotic transport perceptions

We found that farmer perceptions and valuations of antibiotic transport with manure, milk, and beef influenced on-farm decisions (see **Figure 3.1C** for location within the decision tree). Milk and beef antibiotic residue content is regulated nationwide by the USDA Food Safety and Inspection Service, and therefore ranks highly in dairy farm decision making. Antibiotic treatments have labeled beef and milk withhold times. Withhold times are established through animal studies specific to each drug (e.g. Durel et al., 2019; Seymore et al., 1988). The main pathways identified by farmers were through marketable milk and beef, waste milk fed to calves or disposed of on farm, and manure (differences between manure and urine transport pathways were not discussed as most manure management systems conglomerate the two). No farmers (conventional nor organic) identified transport of antibiotics with deceased animals. Transport through the milk, beef, and waste milk streams center on human food systems, while transport through the manure and treated-animal mortality interact primarily with the environment.

3.4.1 Market Milk

Antibiotic presence in market milk is a concern across the dairy industry and carefully monitored by the U.S Food and Drug Administration (0.009% bulk milk tank tests were positive for one of the three antibiotic classes tested 2019 fiscal year in the United States, U.S.FDA, 2019B). All farmers acknowledged potential passage of antibiotic residues into milk. Several organic farmers cited concern about antibiotic presence in their milk as a reason for switching to organic management practices. One small organic farmer noted, *"Well we don't have to worry about contaminating our milk and our beef. We don't have to watch withholding times and so for that, that's a big thing. And mistakes happen*" (interview 1). Similarly, another organic farmer noted concern about presence of antibiotics in milk, stating *"I think there's three antibiotic [classes] that got tested for in our milk. Right? But how many antibiotics…are there, that we can use? So do I worry? Yeah, [I'm] worried"* (interview 4).

Conventional farmers varied highly in their perceptions of risk of antibiotics in milk. Perceptions trended with farmer age across the scale of Millennial (born 1981-1996), Gen X (born 1965-1980), and Baby Boomer age groups (born 1946-1964) (**Table 3.1**). Baby Boomers tended to have the perception that *"there's no antibiotic that goes into the food stream via milk"* (interview 17) because all milk is tested, and a farmer is required to buy and dump the entire tank or truck of contaminated milk. Gen X farmers tended to be very concerned about antibiotic presence in milk, and stressed the animal tracking systems they used to reduce the risk of contaminating their products (three of seven Gen X conventional farmers referenced antibiotic administration tracking systems). One Gen X farmer stated that *"one of the things we're super sensitive to is making sure we stay on top of [documenting antibiotic usage]. I created a book with anytime an animal gets treated with anything that has a withhold. So we put it in here. Anytime an animal gets sold or moved, we make sure that we know exactly what's been* *in them. I am very organized"* (interview 12). Millennial farmers tended to emphasize on-farm testing of their milk to ensure risk of contamination was minimized. The practice of *"always test[ing] it here until its negative"* (interview 15) was common across Millennial farmers (three of seven Millennial conventional farmers referenced testing milk) (on-farm testing is frequently completed using rapid one-step assay lateral flow tests). Millennial conventional farmers were also more likely to have the opinion *"well, there is a level of antibiotics in milk, you know. It's just whether it's met that [testing] threshold"* (interview 15). As a result of judicious usage and detailed treatment tracking, conventional farmers across age categories were confident that their milk had a low risk of antibiotic residue contamination.

3.4.2 Market beef

Across conventional farms, all age groups and farm sizes agreed that the highest risk antibiotic contamination was through beef. Motivations to prevent beef contamination varied between economic and social pressures. Two farmers shared the perception that *"the biggest route that I see, is leaving in…marketed animals for slaughter. You sure…don't want a residue to come up in something like that"* (interview 9). Economic pressures heavily impacted culling practices. One farmer noted *"even your cull animals, you can't market anything with any, any antibiotics showing up in them… So, all the way from your cull animals right through to the milk that you ship, everything is tested. And, so it's, it's economic pressure, is the great one there. But, at the same time,* *conversely, these medicines are expensive, so you want to use it at a minimum"* (interview 22).

Other farmers cited social pressures of producing antibiotic free beef. The perception that there was *"a lot of social pressure, not in terms of me not using it, but just the understanding that you have to be really careful with, like I said, withholdings. And they're checked more than ever, your milk and meat"* (interview 25). Organic farmers tended to discuss beef contamination less than milk contamination (one organic farmer discussed beef contamination). Similar to concern for residue contamination in milk, farmers were highly concerned about risk of transport into beef, in some cases due to economic and social pressures.

3.4.3 Waste milk

Feeding waste milk to calves was widespread across management practices. Waste milk includes milk from cows treated with antibiotics, high somatic cell count milk, and milk that cannot be sold for any reason, and is generated on both organic and conventional dairy farms. Several farmers commented on pasteurization protocols implemented before feeding waste milk to calves. Across farm size and farmer age, perceptions that both displayed concern over feeding waste milk to calves and that demonstrated less concern existed, with no thematic patterns. Some farmers attributed their low concern to residue dilution during feeding, stating "*I'm not concerned about the level of antibiotics that would be in the waste milk, because we dilute that anyways with untreated milk"* (interview 27). Others posed the questions, *"is there a resistance? Does* *that pose a resistance for that calf through the rest of her life?"* (interview 17), suggesting that affirmative answers to these questions have not been supported by their experiences.

Other farmers indicated a more nuanced approach, deciding if waste milk was appropriate for feeding on a case by case basis: *"it all depends on how heavily we dose her too…if she's sick, we do not feed it to the calves…if she's on the upswing, then yeah, and we haven't given her any antibiotics in a couple days…we'll start feeding the calves"* (interview 19). Others still modified their waste milk feeding protocols to avoid feeding it to animals intended for sale, recognizing that waste milk *"does have some [antibiotic] residue in it. So you can't use that milk for calves that we plan on selling"* (interview 18). Similar to antibiotic transport into milk and beef , market and regulatory pressure may have influenced feeding waste milk perceptions as bull calves are tested for antibiotic residues when sold.

3.4.4 Manure

Antibiotic transport into manure, and subsequently the environment, was the least considered antibiotic transport pathway discussed by farmers (six farmers had considered it while all farmers considered residue transport into milk and beef when prompted). Antibiotic presence in manure was more often acknowledged by organic farmers than conventional farmers. Organic farmers were more likely to state *"it is in our manure…you give whatever to an animal, it comes out somewhere. It wasn't until [we went] organic that I realized about all the microscopic activity in a handful of* *soil"* (interview 4), suggesting a connection between manure contaminants and soil health impacts. Some organic farmers also showed hesitance bringing in manure from other farms, indicating that they *"have thought about [antibiotics in manure] because we've thought about using…conventional farms' manure and we have leaned away from that because, maybe not just antibiotics…we just don't know what's in that manure"* (interview 21). Conventional farmers were more likely to state "*it's probably in the urine …whether there is or isn't any effect on anything, I don't know"* (interview 8) or *"in terms of in the manure, I know it happens…I guess I don't consume any time with it because I don't know how you could control it at this point"* (interview 3).

There were also farmers of both conventional and organic management practices who had not previously thought about antibiotics in manure (thirteen of the twenty-eight total farms interviewed had not previously considered it). Some attributed their lack of concern to their antibiotic usage patterns, *"because it breaks down when it gets [to the manure]…I don't use much…if we had tons of cows on it, I would be worried"* (interview 19). No farmers stated that they managed manure in order to reduce antibiotic residue transport, many suggesting that the idea was novel, *"I'd never heard of anyone ever separating manure based on whether it's a treated animal or not"* (interview 14).

3.4.5 Treated-animal on-farm mortality

No farmers discussed antibiotic transport into soil and water via treated-animal mortality and the interviewer did not seed conversation with a prompt. Farmers employ a variety of practices to deal with on-farm animal mortality, such as on-farm burial, composting, and rendering (the farm pays for animal disposal). Rendering in central New York has become a less common practice for on-farm animal disposal due to high cost and difficulty hiring a renderer while extension has begun emphasizing mortality-composting (author experience). Additionally, farmers referenced the desire to cull animals (see section 3.3.2) over managing dying animals.

3.5 Manure management

Manure management systems, barriers to change, and drivers behind current systems were considered to understand antibiotics' role in waste management decisions (**Figure 3.1D**). Funding as a barrier to changing systems and nutrient management considerations were the key drivers of manure management systems. Antibiotic residue reduction through manure management was not a driver on any of the farms interviewed.

3.5.1 Funding as a barrier to changing manure systems

Money was a barrier to changing manure management systems across farm categories. However, Baby Boomer farmers were more likely than other groups to reference it as hindering their manure management modifications. This was especially true on farms without multiple interested generations. One Baby Boomer organic farmer stated, *"we just haven't made the investment in a storage facility. Unless they require me, I'm going to get through to retirement without out it. We'll see. At times it would be nice to have it. But it's a major investment. And obviously there's nobody interested in taking the* *farm over. You know, I don't see the point in making that investment"* (interview 1). Younger farmers cited capital costs of modifying manure systems as the major barrier.

Similarly, many farmers cited convenience of using their current systems, with equipment and technology installed. This perspective was present across manure management of daily manure spreading or storage systems, farmers repeatedly stated, *"It's the system we have…to be totally truthful, that's the one. That's the biggest one. That's what we have, so it's what we use"* (interview 9).

Large and medium-large farms expressed interest in new systems, were financial barriers overcome, such as *"if there was a way to process the manure and it came out in these nice little pellets, and you're able to concentrate the nutrients into a nice pellet, it could just be spread onto the land, [that] would be awesome"* (interview 17). Smaller farms that used daily spreading manure management strategies showed interest in manure storage for both liquid and solid manures. Specifically, smaller farms indicated an interest in composting systems, *"we'd like to make more compost, but we need to have a pad or a bunk or something"* (interview 7), whereas larger farms did not discuss compost. This discrepancy may be in part because smaller farms tended to have solid manure over liquid manure management systems, which eases transitioning to composting systems. Not all farms desired changes to their manure systems.

<u>3.5.2 Nutrient management considerations in manure management</u>

Farms across categories identified nutrient management as a key driver in their manure management systems. An organically managed, medium sized farm reflected that *"the* *liquid manure pit has been…a great asset for us in that it allows me not to daily spread and waste as many nutrients… or to conserve as many nutrients as possible in…timing with our corn planting"* (interview 6). Two large and small conventional farms similarly cited their motivations to efficiently utilize nutrients, the large farm stating, *"we do a lot of, it's called drag lining, and injecting into the ground…We do as much of that as possible. Obviously, you get more efficiency from it. It's efficient for a utilization of nutrients. It's efficient from the utilization of labor and time. And it's also a lot better for the environment"* (interview 3), while the small farm commented that *"the biggest thing [is nutrient management] I was just at a meeting here a couple weeks ago about the effects that nitrogen, phosphorus, and sediment is having in the [watershed]"* (interview 13).

Large farms are required to comply with state and federal manure management regulations. Some mentioned working with agencies, such as Soil and Water Conservation Districts, to ensure compliance, *"we work with Soil and Water, use the standards and regulations, and they help us come up with protocols in place so then we can spread whatever we can spread, how much we can spread"* (interview 18). Smaller farms are not inspected for compliance under state and federal regulations and did not comment on regulations being a driver of their manure management strategy.

3.5.3 Manure management decisions around antibiotics

No farmers mentioned reduction of antibiotic residues as a driver of manure management strategies on their farms or any other farm. One organic Baby Boomer farmer remarked on managing manure with antibiotics that they *"wouldn't go to an extent where we need to have a law, [that] you can't use antibiotics or all manure now has got to be treated, separated, tested, and you can't put it on the fields"* (interview 4), while another organic Baby Boomer farmer responded that if they were aware of manure management practices to reduce antibiotic transport, *"Oh, I think, yeah, I think [I would do it], if I was still using [antibiotics]. You're responsible to everybody on the planet, not just yourself*" (interview 7).

3.6 Environmental presence of antibiotics

Perceptions of environmental residue presence (**Figure 3.1E)**, and the subsequent impacts on soil microbiology was highly stratified by management practice. All five farmers who shared the perception "*so you drop some antibiotics in there… I'm not saying it kills soil, but you know what, the soil's better off without it*" (interview 4) managed their farms organically. No conventional farmers mentioned interactions between soil health and antibiotics. Conventional farmers were more likely to mention anthropogenic antibiotics over agricultural antibiotics in the environment through sewage and septic leachate (two conventional farmers shared this perception, and two organic farmers also mentioned septic leachate). Three organic farmers were concerned about environmental residue presence, specifically though food and water systems. These concerns focused on human health, with transport of antibiotics into *"milk, to the meat, to the what goes out the back end, it goes back on the ground, goes back in the soil, it goes back in the food system, it goes into the water"* (interview 6). Organic farmers' perceptions of environmental antibiotic residue presence further suggested their alignment with the contaminant reduction ideology mentioned in reasons farmers minimize antibiotic usage.

3.7 AMR perceptions

AMR, the broader reaching impact of antibiotic usage and residue environmental presence (**Figure 3.1E**), was discussed to assess the perception of risk of antibiotic usage on farms. AMR was discussed both in the context of livestock and human health impacts. Farmers were either: concerned about AMR (either on their farm or more generally), not concerned about AMR, or concerned about AMR with respect to human health.

3.7.1 Concerned about AMR

Farmers concerned about AMR fell into two categories: those concerned about AMR on their farm and those concerned in general, though not necessarily on their farm. For those farms concerned about AMR on their farm, farm generation appeared to be an important predictor. Second generation farmers tended to report, *"you start to see [AMR] a little, and you just rotate your medicine to see what works better"* (interview 23). Knowledge passage from one generation to the next may have made these farmers more aware of changes in antibiotic effectiveness. About one third of 1st and >3rd generation farms held this perception (1 of 3 and 2 of 6, respectively) while more than half (10 of 18) 2nd generation farmers were concerned about AMR on their farms (note:

all 1st generation farms interviewed were organic, **Table 3.1**). One organic farmer was concerned about impact of resistant bacteria on their operation, *"because I'm going to have the same bug as everybody else that's already resistant to it. Even though, if I choose not to use [antibiotics], I'm going to have the same issue. But hopefully, we're building them better immune systems, a less stressed animal, to be able to deal with whatever's thrown at them"* (interview 4). This perspective acknowledges AMR concerns extend beyond conventional farms.

Several farms within each farmer age, farm size, management, and generation category shared the perception that, while they were concerned about AMR in general, they were currently not concerned about it on their farms. One conventional, Gen X farmer responded, *"Concerned? No. Because we don't use a lot of antibiotics. Do I think that's a real possibility? It probably is. Maybe not so much here, but if you use more antibiotics I could definitely see where that could become an issue, especially in the future with a lot of use"* (interview 9). Organic farmers were less concerned about resistance on their farm, and more concerned about resistance in general.

3.7.2 Not concerned about AMR

Some farmers expressed no concern about AMR in the dairy industry in general or on their farms. All farmers with this perception were conventionally managed. Some responses suggested lack of knowledge on AMR, one interviewee questioning, *"has anyone got any research on cows to figure out whether or not they have any bacterial resistance?"* (interview 3) while another suggested, *"I've never heard that topic ever* *mentioned"* (interview 2). Others suggested that their personal experiences had not indicated that AMR was a problem, stating that *"when we do use [antibiotics], we have good luck with it…we usually don't run into problems with using something and not having a result from it"* (interview 13). Farmer age appeared to predict who held these perceptions: 3 of 5 conventional Baby Boomer farmers held this opinion, while only 2 of 7 for both conventional Gen X and conventional Millennial farmers agreed.

3.7.3 Concerned about AMR with respect to human health

No farmers related agricultural antibiotic usage to human antibiotic resistance. Rather, human resistance was attributed to human antibiotic usage. One Gen X conventional farmer stated, *"I've done research on humans and the impact of overuse of antibiotics, you know, even just in my children, I know that it impacts their gut health and they tend to take a while to recover just from the antibiotic usage. And, with cows, I can only imagine it's the same thing. And, creating superbugs and all this. I worry about that. I worry about just on our farm, you overusing antibiotics, what is that doing on our, on farms scale, but also in the world? It's quite scary to think that one day the antibiotics won't be beneficial at all because people overuse them"* (interview 12). All farm categories (**Table 3.1**) expressed thinking about human antibiotic resistance.

3.8 Information reliance

Information sources were investigated to better understand influences on dairy farmers' decisions. We asked all interviewees for their trusted and actively avoided information sources about antibiotic and alternative treatments. Trusted information sources for non-antibiotic treatments were more highly variable between personal contacts/experiences, trade publications (e.g. Hoard's Dairyman, Progressive Dairy, electronic and physical newsletters), and through trainings/meetings (**Table 2**). Antibiotic information sources were more focused on veterinarian and personal contacts/experience than any other sources **(Table 3.2)**.

When asked about avoided information sources, farmers stated they were open to most information, but noted that all information should be taken "*with a grain of salt*" (interviews 14, 15 & 24). A few farmers cited the internet as an untrustworthy information source (1 respondent for alternative information and 2 respondents for antibiotic information), 3 farmers cited magazines as untrustworthy for antibiotic information, and 5 farmers cited pharmaceutical companies as untrustworthy for antibiotic information.

One organic farmer noted difficulty in gaining alternative treatment advice from veterinarians, stating *"our local vets…when they go to the vet schools…don't take a class on organic treatments because, most of them, it wasn't popular back then. And I don't know if it's even available now. It should be, but…so it's hard to get advice from them"* (interview 1), suggesting a reason why veterinarians may rank lower on the alternative information scale. A conventional farmer noted that they were *"not against [alternative treatments], just don't really have any knowledge or experience. It's not something typically you hear a lot about from, you know, vets and professionals"* (interview 3).

Table 3.2: Number of farmers (n=27) who cited each media source on trusted information for antibiotic and non-antibiotic treatments.

Source	Non-antibiotic Treatment Information	Antibiotic Treatment Information
Veterinarians	4	20
Personal Contact/ Experience	14	13
Trade Publications	8	3
Product Companies	6	6
Trainings/Meetings	7	2
Milk Processors/shippers	4	1
Internet	4	4
Cooperative Extension*	2	2
Books	3	1
Product Label	0	4

**Some meetings/trainings, trade publications, and personal contacts referenced were likely organized by Cooperative Extension and Cooperative Extension Educators. Unless extension was explicitly referenced, those sources of information are not counted under the extension category.*

4.0 Discussion

Farmer perceptions are instrumental in understanding antibiotic transport and better estimating risk of antibiotic environmental presence. Perceptions around antibiotic usage and decisions concerning disease treatment vary between farm and farmer demographics, and should not be generalized across categories of differing management practices, farm sizes, farmer ages, and farm generations (**Table 3.1**). This study highlights NYS dairy farmer perceptions, recognizing that perceptions may vary across other livestock operations and regions. Organic farmers tended to see antibiotics more as contaminants of emerging concern than conventional farmers, though conventional farms varied significantly on their perceptions of antibiotic impact. Wemette et al., (2020) similarly found organic farmers were more concerned about antibiotic resistance and impacts on human health than conventional farmers, and conventional farmers held the perception that they judiciously used antibiotics. Wemette et al., (2020) did not investigate farmer perceptions of transport of antibiotic residues or possible variance of perceptions across farm sizes or famer ages. Manure management methods outlined by Oliver et al. (2019), such as well-managed composting and high temperature anaerobic digesters, that reduce antibiotic residue and resistant bacteria transmission are not currently widely used and residue and resistant bacteria reduction are not drivers of manure management changes on the dairy farms in this study. Non-antibiotic treatments and preventative actions varied between farm categories, most dramatically between conventional and organic management practices with organic farmers heavily relying on non-antibiotic treatments. Antibiotic usage perceptions varied between management practice, emphasizing the strength of the 'us versus them' paradigm present between organic and conventional farmers. Farm size highlighted the data management differences outlined by Menzies Pluer (2019) between data driven and observation driven knowledge acquisition in the context tracking animal health and making antibiotic usage decisions.

Disease prevention actions were the most highly referenced practices to reduce antibiotic environmental presence. Wemette et al. (2020) also found that herd health management was the key preventative action taken by farmers leading to reduced antibiotic usage. To a lesser extent, farms employed practices such as culturing bacteria to assess effectiveness of antibiotic treatment. The perception that dairy farmers minimize antibiotic usage despite high masses of antibiotics sold for agriculture in the US suggests a possible discrepancy between actual and perceived usage. Feed-through antibiotics (i.e., those not injected) and non-medically important antibiotics may not be considered by farmers in antibiotic usage discussions, due to usage in preventative rather than therapeutic treatment. This study differentiate between antibiotics in interviews. No farmers mentioned that they make manure management or carcass disposal decisions to reduce environmental antibiotic presence. Waste management decisions reducing environmental antibiotic presence appear to be underutilized contamination reduction pathways. Two routes of waste management that should be considered are through manure management and on-farm animal mortality (**Figure 3.1D)**. As outlined by Redding et al (2020), evoking behavioral change in antibiotic end users (i.e. farmers, herd managers) is most effective with insight into farmer values. This study highlights the perceptions that stem from those underlying values, which should be further studied to direct future communication and management change recommendations.

4.1 Impact of regulations on antibiotic residue transport perceptions in market products

With USDA regulations strictly enforcing antibiotic concentration in market milk and beef, dairy farmers have economic incentives to limit antibiotic transport into these products. Antibiotic transport into marketable products dominates antibiotic transport perceptions. One step removed from this economically driven decision-making, antibiotic transport into waste milk is frequently considered, likely due to feeding waste milk to claves sold for beef. The farther from market regulation influence, the less influence antibiotic transport has in decision making. Some dairy farmers considered transport of antibiotic residues with manure, though this number was drastically smaller compared to those who considered transport into market products. Farther from the marketable product pathway, antibiotic transport with animal mortality was not discussed.

4.2 Binary classification of organic and conventional management practices

The binary classification of conventional and organic systems lacks flexibility to document nuances amongst conventional farms. Some conventional dairy farmers embrace other marketable practices (such as grass-fed, rBST free, or A2-A2 milk, but many positive environmental practices that conventional farmers employ, that may not result in an economic gain, are lost on the consumer. Some farmers generate value-added products or bottle on-farm to market products with greater specificity to farm practices. One medium sized conventional farmer noted, *"we're kind of in the middle… there's things that I don't like about organic, that is why I won't go the whole way. But there's things that I do like about it, and that's why we do no-till, so we're starting to use natural cover crops to depress weeds, so you don't have to spray…we're not organic, but we do follow a lot of their practices"* (interview 15). The organic and conventional binning system generates a strong 'us versus them' paradigm, especially in antibiotic and non-antibiotic treatment discussions. A sliding scale between conventional and organic practices rather than a binary binning system would capture some of this nuance, but would complicate milk marketing and processing.

4.3 Study implications

This study outlines the divergent perceptions around antibiotic usage and residue transport on farms in central NYS. These perceptions highlight decision points where human behaviors alter environmental antibiotic residue loads, influencing soil and water systems. Incorporating these decision points, such as choices to treat or cull animals, to treat with antibiotic or non-antibiotic therapies, and between waste management practices, with likelihoods that the actions are taken into fate and transport models of contaminant transport will allow for more accurate prediction of downstream impacts. Probability that certain load reducing actions are taken may be predictable based on dairy farm or farmer characteristics, but should be informed buy a more widely distributed survey. Additionally, a survey across production systems to understand differences in perceptions between various livestock farming systems would allow for more effective identification of management points to reduce environmental antibiotic loads.

Linking antibiotic transport and usage perceptions directs future value-based management changes to reduce agricultural antibiotic environmental presence. Trusted antibiotic information sources were primarily personal contacts and veterinarians, as found by Jones et al. (2015). Non-antibiotic treatment information sources varied more, suggesting multiple effective communication avenues. Collaboration between scientists, extension educators, and farmers more thoroughly describes the state of antibiotic usage and transport perceptions, and increases industry impact. Though each of these fields utilize different vocabularies and tools with different end goals, collaboration is critical to ensure all perspectives are voiced and research and outreach appropriately directed. To facilitate collaboration across disciplines, initiatives to reduce antibiotic usage and provide antibiotic resistance education (e.g. I (AM)Responsible, University of Nebraska-Lincoln, USA; Ecoantibio, France) have begun important communication campaigns and policy implementations.

5.0 Conclusions

In this study we have identified farmer perceptions of antibiotic environmental transport, and decisions that can reduce environmental antibiotic loads. We found that farmer perceptions of antibiotic transport vary across management practices, farmer age, and farm size. Farmer decisions resulting in reduced environmental antibiotic loads vary across the same categories. Incorporation of waste management technologies (e.g. heat-treated systems, separating antibiotic treated from untreated manure) that reduce transport of antibiotic residues and resistant bacteria with manure is a waste management strategy not ubiquitously adopted in NYS. Reducing these loads aids efforts to reduce the spread of AMR through interactions with soil and water organisms. Informing farm management through collaborative research between scientists, extension educators, veterinarians and farmers is critical to ensuring all parties are aware of other groups' drivers. The dairy industry is particularly averse to additional regulations, but initiatives such as Ecoantibio that are incentive rather than regulation motivated may be more positively received. Additionally, communicating new information through trusted sources, as outlined in this study, could ease adoption obstacles associated with new practices.

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CONCLUSIONS

Contaminant transport is controlled by both biochemical interactions and human decisions. To fully address the problems posed by contaminants at both local and global scales, we must integrate conclusions and knowledge from both of those control points to improve environmental conditions and reduce contaminant impact.

We found that cattle exclusion riparian buffers could be effectively used to reduce stream total phosphorous and sediment loads. We also found that soluble reactive phosphorous (SRP) showed less change over the same period, potentially attributed to desorption of SRP from sediments in the buffer zone. We posit that the water quality gains of the buffer could have been improved beyond what we observed had the point sources associated with new flow paths, tile drains, and settling ponds been addressed in a more effective way. We recommend a three staged approach to increase water quality benefits of future riparian buffers: (1) identify and address point sources, (2) include saturated and likely-to-be-saturated areas in the buffer region, and (3) modify buffer widths as new saturated areas and preferential flow paths develop.

In our adsorption experiments, we found that erythromycin sorbs to agricultural soils, but sorbs less to soils when manure is present. We found the equilibration time of erythromycin adsorption to soil was approximately 72 hours in the small batches we tested. We expect this equilibration to be faster in-field with more adsorbent present. We observed a shifted isotherm between soil with and without manure, which can be expected to shift further in favor of aqueous over sorbed erythromycin with additional manure. We found that two Langmuir isotherms were the best fit models for the adsorption isotherms. In our column experiments, we found from a mass balance perspective that more erythromycin remained in the soil-only column, representing more adsorption. The difference between the soil-only and soil + manure columns is consistent with differences in their respective adsorption isotherms. Our results suggest that erythromycin may be more mobile in the presence of manure reducing removal efficiency of this contaminant in sediment control structures. We recommend mitigating erythromycin contamination prior to manure land application.

In our interview study, we found that farmers primary consideration related to antibiotic transport was contamination of market products. Farmers referenced managing antibiotic use to reduce transport via this pathway. Antibiotic transport with waste products, such as waste milk, manure, and mortality, were considered considerably less frequently, with stronger divides between farmer groups. Transport of antibiotics with this milk was highly considered by some farmers, while not considered influential by others. Transport with manure was more frequently considered by organic farmers than conventional farmers. However, several organic farmers expressed a desire to avoid additional regulation on contaminants in waste. No farmers mentioned antibiotic transport with treated animal carcasses from on-farm mortality. We found a strong 'us versus them' paradigm present between management practices of organic and conventional, and small and large farms, especially in antibiotic usage and ways of collecting data. We also found that conventional farmers tended to rely on fewer information sources than organic farmers, and all farmers prioritized trusted individuals over other forms of information collection. To further reduce antibiotic environmental contamination from dairy farms, we believe that mitigation programs would be most effective when targeting reductions in waste streams.

1.0 Recommendations for future research directions

The studies that comprise this dissertation generated a series of unanswered questions that provide opportunities for new research trajectories. The following are some potential follow up questions to this dissertation.

The cattle exclusion riparian buffer was assessed one year after implementation, and specific farm-scale cattle and manure management strategies were not included in this study. The following questions would be logical next steps from this work:

[1] Does the effectiveness of a cattle exclusion riparian buffer change over time as phosphorus legacy desorption in the buffer area equilibrates with new conditions?

[2] Do farm scale management changes in grazing patterns (inside and outside of the buffer) impact buffer lifespan?

[3] What drives the management decisions farmers make regarding construction and maintenance of riparian buffers and how can these drivers be used to install more effective buffer regions?

The erythromycin adsorption experiments all occurred in the controlled environment of the laboratory, using sterilized soil and manure solids. We assessed only erythromycin aqueous concentration and did not investigate biological activity or contaminant adsorption outside of these conditions. The following questions would be logical next steps from this work:

[1] What are the specific adsorption mechanisms of erythromycin to soil and how are those mechanisms influenced by presence of dissolved organic matter from manure? How do those mechanisms change across soil types?

[2] At the field scale, does erythromycin pass through sediment control structures or nutrient management practices? How can existing structures be modified to reduce antibiotic transport?

[3] How does erythromycin interact with bacteria with and without the presence of manure-based dissolved organic matter?

[4] Is erythromycin desorption from soil solids influenced by presence or absence of manure?

[5] How is adsorption and desportion of erythromycin to soil influenced by the presence of other antibiotics? Do antibiotics' functional groups predict differences?

The interview study included 27 central New York dairy farmers across management, size, and farmer age classifications. The following questions would be logical next steps from this work:

[1] How do farmer perceptions of antibiotic transport change over time?

[2] How do antibiotic transport perceptions from central New York Dairy farmers of specific farm size, management, and farmer age categories compare with farmers perceptions in the same categories in other regions of the United States?

[3] What are the most effective ways to invoke behavioral change in farmers that reduces antibiotic environmental contamination?

Integrating biophysical and social data through paired surveys and water sampling would link farmer perceptions with water quality metrics, allowing for unique connections between behavior and water quality outcomes.

APPENDICES

Appendix 1: Chapter 2 supplementary material

Table A1.1. Effect of autoclaving solids on bulk characteristics. 'AO' represents samples that were autoclaved then oven dried while 'O' represents samples that were only oven dried. All experiments used AO samples. All manure samples were from Farm 2.

Characteristic	Soil-AO	Soil-O	Manure-AO	Manure-O
Bulk Density(g/cm^3)	0.872	0.876	0.167	0.183
SWC (g water/g dry sample)	0.4752	0.4700	5.253	4.761
pH	4.37	4.93	6.39	7.2
Nanoparticle Diameter (nm)	1,216	1,169	11,428	13,742
Electrical conductivity (µScm)	370	190	4.42	3.35
OM loss on ignition (%)	9.81	10.23	76.00	70.50
Surface Area (m^2/g)	4.93	5.13	0.52	0.44
Pore Diameter (nm)	14.93	15.64	28.83	26.56
Zeta potential (mV)	-13.11	-13.91	-13.20	-11.77

Table A1.2. Manure solid characteristics from each farm. Farms 1-4 were averaged and reported in **Chapter 2, Table 1** along with soil characteristic data.

Characteristic	Farm 1	Farm 2	Farm 3	Farm 4
Bulk Density(g/cm^3)	0.1711	0.1665	0.1354	0.1216
SWC (g water/g dry sample)	5.17	5.25	6.14	7.41
pH	6.74	6.39	6.67	7.25
Particle Diameter (nm)	12,402	11,429	19,343	14,011
Electrical conductivity (µScm)	4.800	4.425	4.100	8.025
OM loss on ignition (%)	83.66	90.84	90.92	84.78
Surface Area (m^2/g)	0.484	0.525	0.310	0.428
Pore Diameter (nm)	32.98	28.83	39.04	33.66
Zeta Potenital (mV)	-2.80	-13.2	-2.81	-4.67

Table A1.3: Adsorption isotherm model coefficients and associated SSE values. Non-linear regressions (Eq 1, 3) and linearized models (Eq. 2,4,5) are displayed below. ‘S’ indicates soil-only isotherm coefficients while ‘SM’ indicates soil + manure isotherm coefficients. Parameter p-values were computed from t-statistics. Statistical significance is marked by an asterisk ‘*’. S adsorption isotherm was best fit by Eq. 3. SM adsorption isotherm was best fit by Eq. 4.

Eq.	S model parameters	S model parameter p-values	S-SSE	SM model parameters	SM-parameter p-values	SM- SSE
Eq.1	$k_f = 2.169 \times 10^{-4}$	7.7×10^{-4}*	3.65×10^{-7}	$k_f = 1.873 \times 10^{-15}$	0.9259	2.49×10^{-7}
	$n = 4.522 \times 10^{-1}$	7.8×10^{-6}*		$n = 5.975$	0.0341	
Eq.2	$a = -9.5738$	2.0×10^{-16}*	2.85×10^{-6}	$a = -11.51019$	2.38×10^{-10}*	3.67×10^{-7}
	$b = 0.8869$	2.0×10^{-13}*		$b = 1.00776$	3.33×10^{-6}*	
Eq. 3	$q_{max} = 1.5300 \times 10^{-3}$	1.0×10^{-7}*	1.93×10^{-7}	$q_{max} = 2.144e-4$	0.191	1.96×10^{-6}
	$K = 8.0144 \times 10^{-2}$	5.2×10^{-3}*		$K = -7.590$	0.222	
Eq.4	$a = 1.9503 \times 10^{4}$	0.87	5.49×10^{-6}	$a = -193.3$	0.985	3.50×10^{-7}
	$b = 1.2316 \times 10^{4}$	1.18×10^{-8}*		$b = 9.9659 \times 10^{4}$	3.91×10^{-10}*	
Eq. 5	$a = 1.0245 \times 10^{4}$	1.5×10^{-5}*	2.33×10^{-7}	$a = 1.0816 \times 10^{5}$	01.08×10^{-3}*	4.32×10^{-7}
	$b = 6.5397 \times 10^{2}$	1.77×10^{-6}*		$b = 21.58$	0.96354	

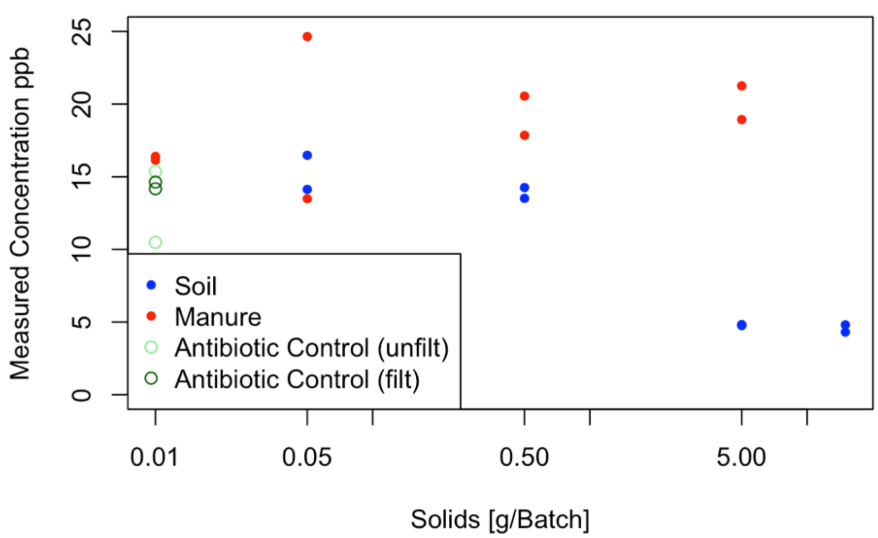

Figure A1.1: Solids ratios tested to determine adsorption detection within ELISA detection range for laboratory batch reactions. Following this test, 5.0000 g soil was used in each batch reactor (150 mL).

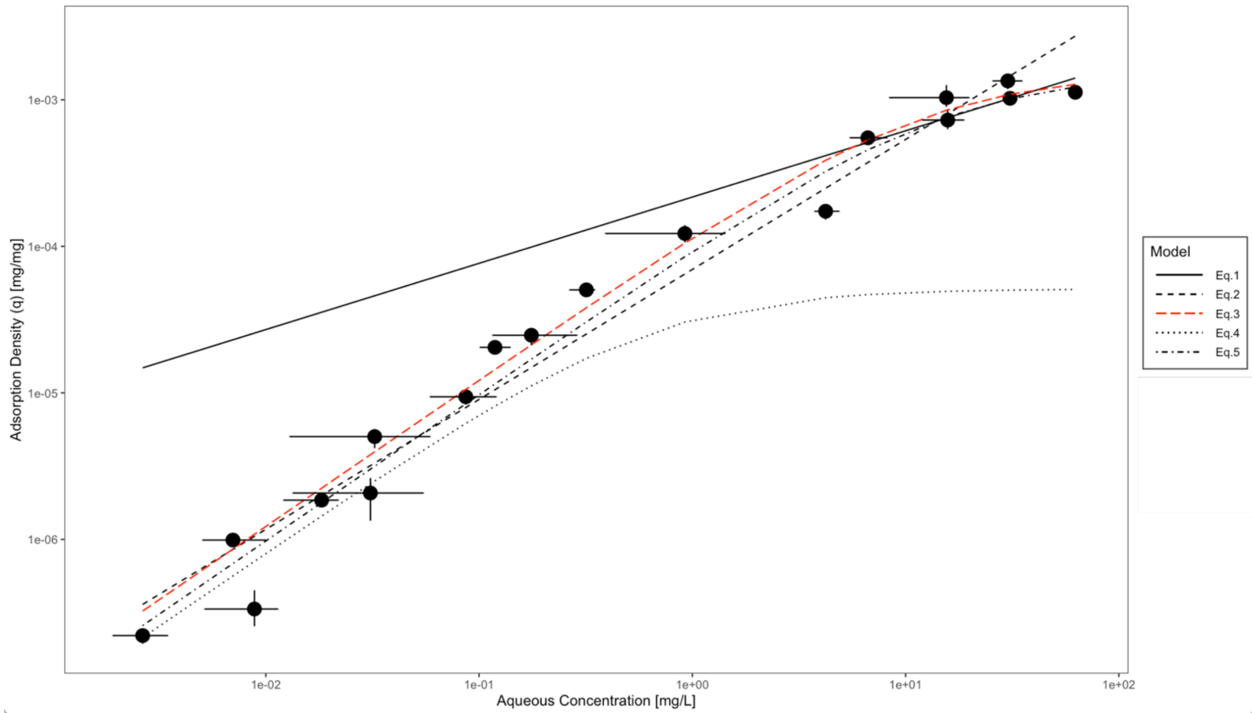

Figure A1.2: Soil-only (S) adsorption isotherm and fitted models. Red Langmuir non linear regression (Eq. 3) was the best fit model. Each data point is the mean of three replicates, with error bars representing the range of the replicates. Eq. 1-2 are Freundlich isotherms, while Eq.3-5 are Langmuir isotherms.

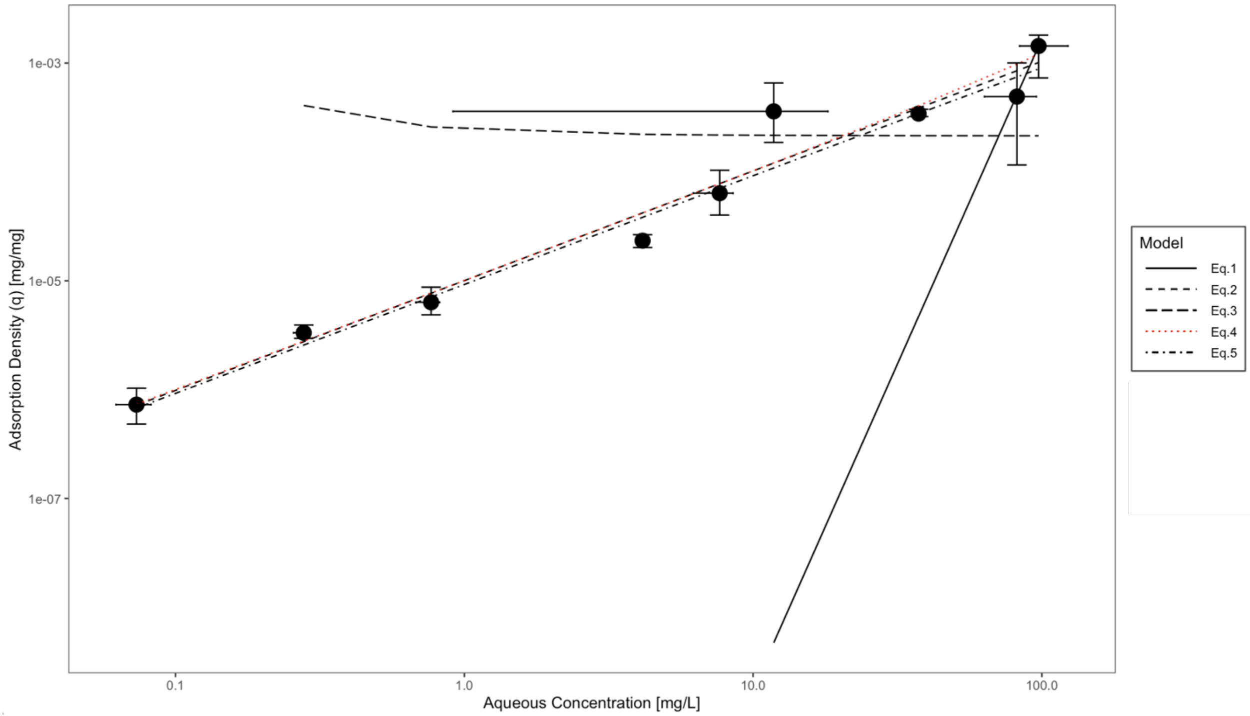

Figure A1.3: Soil + Manure (SM) adsorption isotherm and fitted models. Red Langmuir linearized model (Eq. 4) was the best fit model. Each data point is the mean of three replicates, with error bars representing the range of the replicates. Eq. 1-2 are Freundlich isotherms, while Eq.3-5 are Langmuir isotherms. .

Appendix 2: Chapter 3 supplementary material

Data in Brief Article

Dairy farmer perceptions of antibiotic transport and usage in animal agriculture dataset[3]

Abstract

These data were from semi-structured interviews with dairy farmers. The content of the interviews focused on antibiotic transport and usage on dairy farms. Twenty-seven interviews were conducted in Central New York in 2019. Interviews were recorded and subsequently transcribed for qualitative thematic analysis. Qualitative coding analysis was preformed using ATLAS.ti and content filtered to ensure farmer anonymity. The dataset includes direct quotations from dairy farmers paired with farm and farmer characteristics. Quotations are subdivided thematically into the themes of disease prevention, antibiotic usage, non-antibiotic treatments, antibiotic transport, and environmental residue presence impacts, as structured in Georgakakos et al [1]. Farm characteristics include management practice, farm size, and farm generation. Farm size was determined by number of lactating cows: small (0-50), medium-small (51-100), medium (101-500), medium-large (501-1000), and large (>1000). Farmer characteristics were farmer age categorized by birth year: Baby Boomer (1946-1964), Gen X (1965-1980), and Millennial (1981-1996). This dataset is particularly

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[3] Reproduced from *Data in Brief, 2021*. 35, 106785. DOI: 10.1016/j.dib.2021.106785

promising for longitudinal studies, incorporation of human behaviour into contaminant load models, or for recoding and analysis for themes other than those discussed by Georgakakos et al [1].

Keywords

Qualitative data, interview, contaminant loads, emerging contaminant, pharmaceutical

Table A2.1: Specifications Table

Subject	Environmental Science: Pollution
Specific subject area	Farmer perceptions of antibiotic residue transport and usage on dairy farms in upstate New York
Type of data	Table
How data were acquired	Semi-structured interviews were recorded on site using a cell phone, coded using ATLAS.ti software Coding software: ATLAS.ti Software version: ATLAS.ti 8.4.5 for mac
Data format	Raw Filtered
Parameters for data collection	Parameters for inclusion in the data set were: farms were located in central upstate New York, and farms produced a dairy product from dairy cows. All farms were in operation between January 2019 and July 2019.
Description of data collection	All interviews were conducted in person by the first author, recorded, and professionally transcribed by Cornell Institute of Survey and Economic Research. Interviews lasted between 21 and 87 min. Twenty seven interviews were conducted in total.
Data source location	Institution: Cornell University City/Town/Region: Ithaca, NY Country: USA
Data accessibility	http://dx.doi.org/10.17632/cf2wvytr5r.1
Related research article	C.B. Georgakakos, B.J. Hicks, M.T. Walter. Farmer perceptions of dairy farm antibiotic use and transport pathways as determinants of contaminant loads to the environment, *Journal of Environmental Management.* Volume 281,1 March 2021,11180. doi.org/10.1016/j.jenvman.2020.111880

Value of the Data

- Qualitative raw data are infrequently published. Antibiotic residue transport and spread antimicrobial resistance are of increasing concern, with a lack of both quantitative and qualitative data publicly available.
- These data can benefit future researchers in guiding new research directions, extension educators/outreach personnel to focus educational programs, and policy initiatives aimed to reduce spread of antimicrobial resistance.
- These data are useful for inclusion of human behaviours and drivers in contaminant models tracing pharmaceutical loads into soil and water systems from agriculture as well as for researchers building upon themes that emerged in these interviews to direct future surveys and data collection. Additionally, interviews could be recoded for novel themes.
- This dataset is especially useful in the case of a repetitive, longitudinal study to assess changes in antibiotic transport and usage perceptions over time and across differing policy environments.

Data Description

Dataset: Full interview transcripts have not been published for to ensure confidentiality of included farmers. Direct quotations used for analysis in Georgakakos et al. [1], have been sorted by interview number and theme. Descriptive data on each interview has been tabulated and included. Any identifying information included in these quote pulls were removed from the dataset and replaced with 'XXXX'. 'I:' indicates text from the interviewer, 'FR:' indicates text from a female respondent, and 'MR:' indicates text from a male respondent. Noncontinuous quotations are separated by '…'. The data included relate to antibiotic residue pathways through farm systems, and farmer decisions that intentional or unintentionally modify environmental antibiotic loading.

Supplementary Material: Table A.4: Interview Guide. This semi-structured interview guide was developed from the interview guide developed by Wemette et al. [2]. Nine of the 37 interview questions were taken from Wemette et al [2]. The questions that are consistent between Wemette et al, [2] and Georgakakos et al., [1] are predominantly background and introductory questions used to locate the type of operation each farm employs. Georgakakos et al. [1] focus heavily on antibiotic residue pathways into the environment, a direction not explored by Wemette et al. [2].

Supplementary Material: Table A.5: Code book. This table includes all associated codes, search words, and instances of occurrence throughout all interviews. All code words included in this table are not analyzed by Georgkakos et al. [1] and all associated quotations do not appear in the dataset.

Experimental Design, Materials and Methods

We conducted semi-structured interviews on dairy farms in central New York state. For inclusion in this dataset, farms were required to be located in central New York State, and produce a dairy product from cows. Farms were not required to rely entirely upon dairy income for inclusion. Eight farms were organically while nineteen farms were conventionally managed. Ten interviewees were female and twenty-four were male. Seven interviews interviewed two interviewees, all of which were with one male and one female interviewee in the same age group One interview involved three interviewees, spanning two age groups.

We employed a combination of snowball sampling [3], online database references, and co-author recommendations to form our sampling pool. Ninety-five farms were contacted with a 28% positive response rate. All interviewees were given a $10 honorarium. Farm size and management practice were the primary farm characteristics used to ensure thorough inclusion of perceptions. Farmer gender, age, and farm generation were recorded but not used to select farms.

Interviews were conducted across management practice, farm size, and farmer age distributions. Twenty-seven total interviews were completed. All interviews were conducted in-person, recorded using a cell phone, and professionally transcribed by the Cornell Institute for Survey and Economic Research. Some interviews were interrupted by third parties with unrelated content. These interruptions were omitted from analysis and largely not transcribed. Upon transcription, interviews were preliminarily coded into the themes by the first author, that appear in the headers of each column in the dataset. Within each of these categories, subthemes emerged and were documented by Georgakakos et al [1]. Coding and analysis were preformed using ATLAS.ti 8.4.5 for Mac following the thematic coding methods outlined by Braun & Clarke [4]. The ATLAS.ti software allows filtering of dataset by each theme, which was used to generate the tabulated dataset. The complete codebook code book used for this analysis is reported in supplementary Table A.5.

The institutional review board at Cornell University reviewed all study materials and exempt this study from full review. All interview responses have been deidentified, and all potentially identifying information that appeared in the text has been removed.

Acknowledgments

We would like to thank all farmers interviewed for their participation and willingness to share their perceptions and farm details. The Office of Engaged Initiatives supported this Engaged Cornell work by an Engaged Graduate Student Grant (https://engaged.cornell.edu/grant/engaged-graduate-student-grants/).

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[1] Georgakakos, C.B., Hicks, B.J., Walter, M.T., Farmer perceptions of dairy farm antibiotic use and transport pathways as determinants of contaminant loads to the environment, J. Env. Mgmt. In Press.

[2] Wemette, M., Safi, A. G., Beauvais, W., Ceres, K., Shapiro, M., Moroni, P., Francis L. Welcome, F.L., & Ivanek, R., 2020. New York State dairy farmers' perceptions of antibiotic use and resistance: A qualitative interview study. Plos one, 15(5), e0232937. https://doi.org/10.1371/journal.pone.0232937

[3] Biernacki, P., & Waldorf, D.,1981. Snowball sampling: Problems and techniques of chain referral sampling. Sociol Methods Res, 10(2):141-163. https://doi.org/10.1177/004912418101000205

[4] Braun, V., & Clarke, V., 2006. Using thematic analysis in psychology. Qual Res Psychol. 3(2):77-101.

Table A2.2. Interview guide.

Interview phase	Questions
Warm up	1) How did you get started farming? a. What is the biggest change you've experienced in the past several years in regard to operating your farm? b. Farm size: How many <u>*lactating cows*</u> do you have? Total animals? Acreage owned and rented? c. How many years have you been working with cattle? d. Do you plan on continuing to work with cattle for the foreseeable future? e. What step(s) in the dairy production line do you fill? f. Do you participate in any dairy or agricultural groups or committees? 2) Do you have non-dairy centered income? Sell any forage or grains? 3) Can you describe your method of milk production? *(i.e. conventional, organic, grass-fed, A2, etc.)*
Research Questions	4) How do you manage manure on your farm? a. Why do you manage manure the way you do? b. Would you like to change your system? If so, what barriers do you face? 5) Do you use antibiotics to treat your animals? a. How do you decide on whether to treat a cow with antibiotics? b. How do you decide which specific antibiotic to use for treatment? c. Where do you get information to make those decisions? d. Are there any sources you avoid for antibiotic information? e. How has your usage of antibiotics changed over the last 10-15 years? 6) Can you list alternative (to antibiotics) medical treatments you use? a. How do you decide on whether to treat a cow with alternative medicines? b. Where do you get information to make those decisions? c. Are there any sources you avoid for medicinal information? 7) How do you think of antibiotics moving physically around your/a farm? a. Entry points? b. Passage through animal? c. Movement with manure? d. Do you consider manure management strategies to reduce antibiotic transport/persistence? e. What barriers would you encounter changing manure management strategies? 8) Do you find some categories or life stages of animals require more treatment than others? What are they? 9) What influences your decisions to use/not use antibiotics on your farm in general? a. Have consumer opinions and preferences affected you? b. Is antibiotic use/resistance something you talk about with others you know in the business?

	c. Are you concerned about anything related to antibiotic resistance in your farm? d. Animal recovery? e. Economic reasons?
Additional Info	10) Is there anything we haven't discussed that you would like to include? 11) Do you know anyone else who might be interested in participating in this study?

Table A2.3. Code book used in generating themes, associated definitions of codes, search words used to assign codes after initial coding, and number of quotations that were tagged with each code. Bolded codes were further subdivided into codes listed within their subsections. Instances where no search words accompany codes indicate codes that were assigned without using search assistant in Atlas.ti.

Code	Definition	Search words	Number of quotations
Antibiotic usage		usage	74
Antibiotic usage by others	Perception of antibiotic usage by groups deemed to be the 'other'		13
Keep cows alive	Usage of antibiotics as a last case scenario, to keep an animal alive	Save her, alive	3
Minimize antibiotic usage	Farmer perspective that they minimize usage of antibiotics	Minimize;	7
Organic cow = sick cow	Perspective that organic cows tend to be sicker than conventional	Organic cow	3
Antibiotic Transport	Movement of antibiotic compounds around the farm, after an animal is treated	Transport	46
Antibiotics in manure	Transport of antibiotics broadly with manure	Manure	27
Antibiotics in manure – no	Farmer does not think about transport of antibiotic with manure	Manure	15
Antibiotics in manure – yes	Farmer thinks about transport of antibiotics with manure	Manure	7
Antibiotics in meat	Perspective on antibiotic presence in meat	Meat, beef	11
Dead animal disposal	Discussion of what is done with carcasses on farm	render, dead, mortality	
Market milk	Milk shipped for sale	Milk	25
Waste milk	Milk not sold but sometimes fed to calves	Waste milk, fed to calves	16
Alternatives to antibiotics	Therapies used or actions taken that can result in reduced antibiotic usage.	Alternative, preventative	62
Facility conditions	Reference to facility conditions of the cows	Facility, ventilation, building, cow comfort	19
Nutrition	Importance of feed in maintaining healthy cows	Nutrition, diet, feed*	
Calf Health & Nutrition	Considerations specific to calves for nutrition and supplements	calf, calves	33
Preventative action for sickness	Actions taken to reduce disease		30
Stress reductions	Stress reductions, generally around not pushing cows for production	stress	5
Vaccines	Usage of vaccines as a disease prevention	Vaccin*	24
Culling cows for medical reasons	Culling cows as related to reducing disease presence and antibiotic usage	Cull*, call+, cold+, sell for beef	17

Herbals	Supplements used as a therapy in place of an antibiotic	Herb*, garlic, mint	34
Minerals	Mineral supplements given to fresh cows	Mineral, calcium	12
Manure Management	Manure management system on the farm	Manure	46
Money is a barrier to changing the system	Funding as a barrier to implementing another management system	Manure, money	12
Nutrient management considerations	Nutrient management for pollution or usage on crops	Manure, nutrient	16
Regulations require this system	Regulations requiring farms to use specific manure management	Manure, regulation, CAFO	7
This is the system we have	Current system is used primarily out of convenience	Manure	11
Desired changes to manure management system	Any desired change to their manure management system	Manure	39
Manure management considerations around antibiotics	Manure management decisions drive by reducing antibiotic residue spread	Manure	10
Antimicrobial Resistance	Perspectives of antimicrobial resistance	Resistance	44
Concerned about AMR on this farm	Farmers expressed concern about AMR on their farm	Resistance	14
Concerned about AMR, but not on this farm	Farmers expressed concern in general about AMR, but do not think it is a problem on their farm	Resistance	7
Concerned about human resistance	Farmers expressed concern about human antimicrobial resistance, related or not to usage of antibiotics in agriculture	Human, anthropogenic, resistance	8
Not concerned about AMR	Farmer did not express concern about AMR specifically or in general	Resistance	7
Alternative Information	Sources trusted or relied on for alternative information	Info*	46
Books, positive		Book, info	4
Extension, positive	,	extension, info	1
Internet, positive		internet, info	5
Lack of knowledge	Identification of a lack of information on alternatives as a suggestion to why they may not be used		1
Magazines, positive		magazine, newsletter, publication, info	7
Milk shippers, positive		organic valley, co-op, coop, info	4
People, positive		personal experience, info	17
Product companies, positive		compan*, info	5

Sources avoided	Information sources actively avoided or not trusted	avoid, info	13
Trainings, positive		training, meeting	7
Vets, positive		vet*, info	3
Antibiotic information	Sources trusted or relied on for antibiotic information	Info*	55
Books, positive		Book, info	1
Companies, positive		compan*, info	6
Extension, positive		extension, info	2
Internet, Negative		internet, info	3
Internet, positive		internet, info	4
Lack of knowledge			1
Magazines, negative		magazine, newsletter, publication, info	3
Magazines, positive		magazine, newsletter, publication, info	3
Milk shippers, positive		organic valley, co-op, coop, info	1
Open to all	Identification that the farmer is open to all sources, once investigated		1
People, positive		personal experience, info	14
Pharmaceutical companies, negative		drug, compan*, info	5
Product Label, positive		label, bottle, info	4
Sources avoid		avoid, info	17
Trainings, positive		train*, meeting	2
Vet, positive		vet*, info	29

* Search word roots that were used to be inclusive of more than one word
[+] Term that was incorrectly transcribed in place of ‘cull’.