

A LONGITUDINAL, LANDSCAPE-SCALE FIELD STUDY ASSESSING THE EFFECTS OF
COMMERCIAL HOG OPERATIONS ON MICROBIAL QUALITY OF SURFACE WATERS
IN NORTH CAROLINA, USA

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environmental Sciences and Engineering in the Gillings School of Global Public Health.

Chapel Hill
2019

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ABSTRACT

Elizabeth Christenson: A longitudinal, landscape-scale field study assessing the effects of commercial hog operations on microbial quality of surface waters in North Carolina, USA
(Under the direction of Jill Stewart)

North Carolina is one of the leading states in the USA for swine production on commercial hog operations (CHOs). Swine manure from CHOs contains bacteria and antibiotic resistance elements (AREs) and there is concern that CHOs act as sources of fecal bacteria and AREs to surface water through precipitation-driven runoff or leaching. While research has found high concentrations of bacteria and AREs downstream of CHOs, this work did not adequately take into account other fecal sources. Additionally, few studies have appropriately controlled for background levels of resistance so that the effects of CHOs on dissemination of AREs in the environment is difficult to assess.

A longitudinal, landscape-scale field study was designed to determine whether there were effects of CHOs on microbial water quality while addressing concerns of bias and confounding between observational groups. This work compared similar, small, agricultural watersheds with (n=13) and without (n=9) CHOs over one year and found higher measures of *E. coli*, swine-associated gene marker, pig-2-bac, and antibiotic resistant *E. coli* in watersheds with CHOs compared to those without. Resistance to highest priority antibiotics was only observed in sites with CHOs. A multiple linear model was constructed to determine whether higher concentrations of *E. coli* in sites with CHOs were a result of differences in environmental

conditions or exposure to confounding fecal sources. Modeling showed that even when controlling for large effects from precipitation and effects from confounding fecal sources, CHOs contributed *E. coli* to surface water and had a larger effect compared to human and wildlife sources.

Results suggest that microbial water quality is poorer with increasing CHO size and proximity to surface water and that some CHOs may act as sources for human pathogens and AREs in surface water. These results have implications for state and federal policy, suggesting a need to recognize and regulate the discharge from CHOs during dry and wet conditions through discharge permits and/or CHO-specific management plans. Mitigation strategies that should be considered include improvements to CHO manure and land management practices aimed to reduce loading of fecal bacteria to surface water and to limit bacterial transport.

All praise and glory be to the Father, and to the Son, and to the Holy Spirit.
“Do unto those downstream as you would have those upstream do unto you.”
--Wendell Berry

ACKNOWLEDGEMENTS

We all stand on the shoulders of those that have come before. I want to thank the people that encouraged me to become a graduate student, whether they knew it or not:

To Mrs. Shari Mudd, my high school Environmental Science AP teacher, for teaching me that the love of plants and animals could be rooted in scientific theory and methods.

To Dr. Greg Gangi, for encouraging me to take math courses in undergraduate and for organizing a beautiful study abroad in the Sierra Nevada, cementing reason and wonder forever in my mind.

To Dr. William Grey, for mentoring me and encouraging me in the non-linear path that brought me to complete a PhD at UNC. Thank you for teaching me to think about processes and for thinking critically about your teaching philosophy. Grading your students' papers was always a pleasure.

To Ovik Banerjee, for reminding me that I could do this when I didn't know if I wanted to and for inviting me to write a paper with you to keep my interest kindled.

I want to thank the many advisors and committee members at and affiliated with UNC who have advised and directed me throughout my graduate studies:

To Dr. Jamie Bartram, for working with me before I was a graduate student and always providing good, critical commentary on papers. Thank you for reliably providing substantive comments that identified interesting and more significant conclusions and implications when I

was stuck in the weeds and bored of my work. I'm appreciative of the mentorship you've given for how to write strong reviews of peer-reviewed literature and am thankful for the support you gave to me especially earlier in my graduate career to explore spatial relationships in the WaSH sector. Thank you, especially, for your willingness to be on my committee even as my defense and dissertation submission co-occurred with your retirement.

To Dr. Megan Jacob— thank you for bringing your expertise and valuable perspective from the veterinary school at NCSU and for being an encouragement to this work from the start. Thank you for your help determining what *E. coli* genes to test and for sharing your laboratory space with me. Working with you has given me a much appreciated perspective that I would not have had otherwise.

To Dr. Rachel Noble — your laboratory has the highest of standards and when I hear approval from you with respect to an analysis I've conducted, I know that I did something not just well, but exceptionally well. Thank you for pushing me past “good” and towards “excellent” in my laboratory work and also in my conclusions and interpretations.

To Dr. Marc Serre — thank you for always thinking of new ways to explore the data. Thank you for the hours you've spent meeting with me, teaching and explaining methods for calculating exposure variables, implementing statistical analyses, and for helping me find interesting ways to slice the data. Your enthusiasm is contagious.

To Dr. Jill Stewart — it has been a joy working with you these past four years and I am thankful for you patient advising, allowing me to be independent in this multi-faceted dissertation project, and yet also being available whenever I needed to ask a question. Most importantly I want to thank you for facilitating a supportive laboratory environment—that doesn't happen by accident and I am convinced that this originates from the top-down.

To Dr. Steve Wing for believing in me, for always asking good questions, and advocating for community groups by finding funding for community group time spent organizing and planning studies, spending time listening to and observing community concerns, and making the time to report back to the community in ways that are easily understandable. Thank you for modeling a good example of what community-based-participatory research looks like.

I want to thank my co-workers, peers, and other students who helped me implement this dissertation. Research is never independent:

To Sarah Rhodes, Ph.D., you taught me microbiology. And you are similarly of Dr. Noble's ilk in that you always push past "good" and towards "excellence." Thank you for teaching me how to use microbiology as a tool to understand the questions I wanted to ask. I am so grateful that we worked so closely together for so long. You kept me sane and excited and our motivation helped us both along. You're the best lab partner, a powerful woman, and I'm so grateful to call you a dear friend.

To KD Brown --Thank for pushing me to think bigger and for your encouragement and affirmation. You so easily see why something can be important and can verbalize this. I am grateful for your friendship and for how much fun and rest you bring. Also, thank you for giving me the idea to test for ESBL and ACBL in this work!

To David Holcomb, Ph.D. we had a similar timeline and shared a lot of laughs and cries about our respective work. Thank you for sharing food, walks, and being a true friend throughout graduate school.

To Kristen Downs, for bringing life and light to the department and my life. Thank you for your tireless devotion to detail and your willingness to help with food, with presentations, and with life.

Laboratory assistants Rachel Lempp, Ryan Leighton, and Lindsay Wickersham -- Thank you for spending hours out of your lives to drive a big van through rural NC collecting samples, for plating hundreds of *E. coli* isolates, and learning so many new protocols. I was continually impressed with how much you managed as full-time undergraduate students with a full course load in addition to helping me with this work and in addition to implementing your own independent projects.

To the individuals in the Stewart lab before my time and during my time, thank you for always having a friendly, encouraging, and supportive co-working environment.

To Oksana Kharaboro—Thank you for teaching me ddPCR and allowing me to use the laboratory space upstairs.

To Anna Rogers-- Thank you for teaching me PCR assays to assess beta-lactamase production genes in *E. coli* at NCSU.

To all my friends outside of school and family, it truly takes a village.

To all my housemates in Carrboro, Chapel Hill, and Durham—while you may not have been able to see the defense, you've heard an earful about my work. Thank you for listening and being so patient! Especially to Jessica Eaddy, Melissa Ballard with Luna, and Sloane and Nathan Tilley with Levin and Penelope.

To Grace Community and Oak Churches - Those who try to find their validation in their work, in affirmation from supervisors and advisors will be ill-equipped to flourish in a PhD, but

you all gave me a support network outside of work that made doing a PhD bearable. Thank you for reminding me that I am made in the image of God and that is my value.

To Steve and Jeannie Cox, for your food, conversation, garden, and living room escape.

To Lydia Kiefer, for your constant companionship through and through.

To my family for listening endlessly to troubleshooting and research design throughout graduate school. You know a lot more about hogs and bacteria than you ever thought you'd know.

To Nujumi, for being so endlessly full of joy.

And to Bryan Diver, for loving me so well and trying to understand this marathon.

أحبك إلى الأبد

I would like to thank my funding sources:

The NIH Training Grant T32ES007018 and the NSF grant 1316318 of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program. I particularly want to thank the graduate student WRRRI- NC Sea Grant R/MG-1619 which facilitated this specific research by awarding funding for the objectives of this dissertation.

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LIST OF ABBREVIATIONS

ACBL	AmpC beta-lactamase
AIC	Akaike information
ARE	antibiotic resistance element
ARG	antibiotic resistance gene
BD	below the limit of detection
CAFO	Concentrated Animal Feeding Operation
CDC	Centers for Disease Control and Prevention
CDL	Cropland Data Layer
CFU	colony forming units
CHO	commercial hog operation
CI	confidence interval
CLSI	Clinical Laboratory Standards Institute
ddPCR	droplet digital polymerase chain reaction
DEQ	Department of Environmental Quality
<i>E. coli</i>	<i>Escherichia coli</i>
EPA	Environmental Protection Agency
ESBL	extended spectrum beta-lactamase
Euc	Euclidean
FIB	fecal indicator bacteria
GAO	Government Accountability Office
Grav	gravity
HSC	hydrological soil class

IQR	interquartile range
LLD	lower limit of detection
LLQ	lower limit of quantification
MPN	Most Probable Number
MST	microbial source tracking
NARMS	National Antimicrobial Resistance Monitoring System
NC	North Carolina
NHD	National Hydrography Dataset
NMP	Nutrient Management Plan
NPDES	National Pollution Discharge Elimination System
PCR	polymerase chain reaction
RR	relative risk
SED	sum of exponential decay
SED-int	sum of exponential decay with interaction
SF	surface water flow
TNTC	too numerous to count
ULD	upper limit of detection
ULQ	upper limit of quantification
VIF	variance inflation factor
WHO	World Health Organization
UNC	University of North Carolina
US	United States

CHAPTER 1: INTRODUCTION AND RESEARCH OBJECTIVES

A shift towards high-density food production in commercial hog operations (CHOs) occurred in the United States (US) beginning in the 1980s. In North Carolina (NC), the number of swine farms decreased by 62% while the number of hogs produced increased by 109% between 1982 and 1992 [1], which moved NC to one of the leading states in the nation for food animal production. Swine production is a \$4 billion industry, and in NC has the highest density of swine production in the country [2] with almost 10,000,000 permitted swine on more than 2,100 CHOs primarily located in eastern NC [3]. High-density food animal production yields large volumes of manure. Swine waste management in NC on over 99% of permitted CHOs consists of the lagoon-sprayfield system where liquid swine effluent is stored in open-air lagoons for anaerobic treatment and then sprayed onto fields to fertilize crops.

However there are concerns regarding the environmental and health effects of the CHO lagoon-sprayfield waste management system since swine manure contains fecal bacteria, human pathogens, and antibiotic resistance elements (AREs) [4–6]. The US Environmental Protection Agency (EPA) identifies pathogens as the largest cause of impairment to US rivers and streams and attributes most surface water impairment to agriculture [7]. Additionally, antibiotic resistance is a global public health concern and international agencies, scientific experts, and US federal agencies have called for increased surveillance of food animal production environments to better understand their influence on the dissemination of antibiotic resistant bacteria [8–10]. Most research on the whether there are effects of CHOs on quality of surface water has focused

on transport of nutrients or bacteria from land-applied swine manure. However the influence of CHOs on surface water quality is not well understood.

Monitoring fecal indicator bacteria (FIB) has been the basis for assessing microbial water quality for many decades. FIB, such as fecal coliforms, *E. coli* and *Enterococcus*, originate in the guts of warm-bodied animals and are considered indicators of recent fecal contamination [11]. Pathogens from feces can be more difficult to culture than FIB and are found in lower concentrations in feces such that FIB concentration is currently used as a regulatory tool as a proxy for public health risk. FIB and human pathogens have been found in swine waste, stored waste, land-applied soil, and plants on land-applied fields [5] and plot studies have identified that microbial loads of FIB from land-applied fields can be transported to surface waters especially after precipitation [12]. Swine manure also contains antibiotic residues and multi-drug resistant bacteria [13,14] and studies have found similar antibiotic resistance patterns in swine manure or lagoon effluent as in the on-farm environment [13], in soils with manure application [15], in effluent from tile-drains and ditches [6,15,16], and in receiving surface waters [17].

Additionally, AREs have been assessed along lagoon effluent treatment systems, including in anaerobic digesters and constructed wetland treatment, and have found that while treatment reduced FIB concentrations and the concentration of AREs, AREs are still identified in effluent transported to soil and surface water [15,18]. Thus, while it is clear that high concentrations of FIB and AREs have been detected in swine manure, in the on-farm environment, and in drainage or surface water proximal to CHOs, the effects of CHOs compared to ambient and other sources of FIB and AREs have not been well-studied on a landscape scale.

The few larger field studies that have been conducted to assess the effects of CHOs on FIB or AREs in surface water have inconsistent results, with some studies reporting strong

associations of FIB and AREs with the presence of CHOs, and other studies not finding an effect from CHOs. Some studies additionally assessed microbial source tracking (MST) markers targeting genes in fecal material of swine to better determine source of FIB [19–21]. However, these field-scale studies assessing whether there were effects of CHOs on microbial quality of surface water did not incorporate controls without influence from CHOs and also did not control for confounding sources of FIB and AREs, such as from wildlife, humans, and ambient or background sources. Thus, among studies which identify that CHOs have effects on FIB concentrations [20–22] and AREs [15,22,23] in surface water, it is not clearly demonstrated whether the effect is truly from CHOs or from other sources. And similarly, among studies which identify that CHOs do not have effects on FIB concentrations [23–26] and AREs [25,26], it is not clear whether CHOs truly do not affect microbial quality of surface water when compared to other sources or whether the effects of CHOs are masked due to confounding sources of FIB and AREs.

This research presents the results of a longitudinal, landscape-scale field study designed to reduce bias and confounding between observational groups so that the effect of CHOs on microbial water quality can be assessed. This field study had a higher number of sampling locations than prior work assessing the effects from CHOs on microbial quality of surface water and incorporates control sites without point sources of fecal bacteria or other CHOs. This work compares similar, small, agricultural watersheds with (n=13) and without (n=9) CHOs over one year to assess the effect size of CHO contribution to FIB and AREs to surface water. The dissertation has three specific objectives described below.

Objective 1: To determine whether there is an effect of commercial hog operations (CHOs) on concentrations of fecal indicator bacteria in surface waters in an area of high density hog farming

Approach: Conduct a longitudinal, landscape-scale monitoring study to assess concentrations of *E. coli*, human and swine-associated microbial source tracking markers in similar, small, agricultural watersheds with and without CHOs

Objective 2: To determine whether there is an effect of commercial hog operations on antimicrobial resistance in surface waters in an area of high density hog farming

Approach: Conduct a longitudinal, landscape-scale monitoring study to assess antimicrobial resistance of *E. coli*, including Centers for Disease Control and Prevention (CDC) priority pathogen beta-lactamase producing *E. coli*, in similar, small, agricultural watersheds with and without CHOs

Objective 3: To model effects of human fecal sources and commercial hog operations on microbial water quality while controlling for confounding variables using multiple linear regression

Approach: Predict *E. coli* concentration using a multiple linear regression incorporating land use, precipitation, and hydrological variables in addition to variables that approximate exposure to human septic, CHO lagoons, and CHO sprayfields. Additionally, different methods for approximating exposure are compared. The model controls for confounding fecal sources by approximating exposure from confounding sources and also controls for varied influence from CHOs by approximating exposure based on CHO size and proximity to surface water.

CHAPTER 2: BACKGROUND, LITERATURE REVIEWS, AND SIGNIFICANCE

Background

The basis for monitoring microbial water quality is protecting public health. Monitoring microbial water quality includes assessing concentrations of fecal indicator bacteria (FIB) and pathogens of concern as well as antibiotic resistance elements and using study design and other tools to determine source of microbial contamination. The following sections describe three indicators used in microbial water quality studies and provide an overview of the current research regarding the mechanisms of transport of these indicators from commercial hog operations (CHOs).

Fecal Indicator Bacteria

Quantification of FIB has been the basis for assessing microbial water quality for many decades. FIB, such as fecal coliforms, *E. coli* and *Enterococcus*, originate in the guts of warm-bodied animals and are considered indicators of recent fecal contamination [11]. Pathogens from feces can be more difficult to culture than FIB and it is impractical to measure all potential pathogens associated with fecal contamination. Therefore, FIB concentration is currently used as a regulatory tool as a proxy for public health risk. For example, in 1986 the US EPA began using *E. coli* concentration as a regulatory tool to determine microbial recreational water quality.

Sources of FIB to surface water include fecal sources such as wastewater treatment plants, agricultural runoff, wildlife scat, runoff from urban areas, and household sewer and septic leaks[27]. Factors that contribute to the transport of FIB from fecal sources to surface waters include soil porosity and moisture, precipitation events which can flush bacteria into surface water, and percolation through the soil into groundwater. Other factors affecting survival and persistence of FIB in surface waters include UV, temperature, and competition from other bacteria [28].

While measuring FIB concentrations provide a low-cost and more practical alternative to monitoring the many waterborne pathogens known to cause human illness, the limitations of using FIB concentration as an indicator for public health are two-fold. The first is that FIB concentration does not consistently correlate to human risk of gastrointestinal illness or pathogen concentration, especially from mixed and diffuse fecal sources [29,30], however, FIB may correlate with point-source contamination such as from wastewater treatment sewage [29,31]. Correlation of FIB concentration to specific pathogens such as viruses are particularly low[32].

The second limitation of using FIB concentrations for monitoring risk to human health is that the source of FIB is not readily determined by measurement of FIB concentration alone [32]. The second microbial water quality indicator considered here is the use of microbial source tracking markers.

Microbial Source Tracking markers

The purpose of microbial source tracking (MST) is to determine the fecal source of FIB or waterborne pathogens by matching phenotypic or genotypic characteristics of bacteria from a host organism with those from an environmental matrix. MST methods are varied and have

included testing of antibiotic resistance profiles of bacteria, DNA fingerprinting techniques, testing of chemical indicators (e.g. caffeine, pesticides), and use of molecular methods targeting host-specific viruses or bacterial genetic markers [32]. MST markers are assessed and validated such that they have a high sensitivity (i.e. true positives in host material) and high specificity (i.e. true negatives in non-host material). Additionally, prior to implementing, MST marker specificity should be confirmed in host-samples from the geographic region of interest[33]. The most rigorous assessment of MST markers incorporates blind sample testing and methods comparisons.

We review two host-specific PCR-based MST markers used for identification of human and swine fecal waste. The most widely used human marker targets a 16S rRNA from *Bacteroides*, HF183, and is associated primarily with human sewage with a wide geographical distribution. A multi-laboratory performance evaluation found that HF183 exhibited high sensitivity and specificity (>80%) when data were considered quantitatively (Boehm 2013) and a meta-analysis found HF183 had 83.1% sensitivity and 94.6% specificity [34]. Lower sensitivity has been found in individual human feces when compared to combined septic or wastewater effluent[34]. Other studies have also found high sensitivity and specificity (84% and 89% respectively) using this marker [35]. Some cross-reactivity with dogs, deer, chickens, and turkeys has been sometimes observed and a second human-specific fecal marker, such as HumM2 or *Methanobrevibacter smithii* (nifH), has been suggested for confirmation of human source [33,34,36,37].

The most widely used swine marker is pig-2-bac, which targets a 16S rRNA from *Bacteroidales*. The marker was developed for a surface water study in France [38] and has been validated for swine lagoon effluent in NC [21]. Validation studies reported 100% sensitivity

[38,39] with reported specificity as 100% [35,38]. A separate multi-laboratory study assessing performance of MST markers found reduced, but over 80%, specificity of pig-2-bac with some cross-reactivity with dog and septage [39].

Marker degradation rate affects detection of the gene in the environment. While quantification of MST markers can be determined through PCR-based methods, determination of relative contribution of fecal sources depends on knowing the survival and persistence of each marker under different environmental conditions as well as the initial concentration of the markers [32]. Additionally, detection of a marker does not identify when the gene was released into the environment. Research on the MST genetic marker decay and persistence in the environment have focused primarily on temperature, salinity, predation, and UV light exposure variables [29,35]. A recent study compared the fate of HF183 to pig-2-bac in laboratory and field conditions with varied temperature and light exposure finding that the markers decayed at a similar rate and that light exposure was more important for marker decay rate than temperature difference[35].

Limitations to the use of MST markers are that there are currently no regulatory guidelines for MST markers and the relationship of MST markers to human health risk is not clear. The correlation of host-specific genes used as MST markers to FIB or pathogen concentrations is currently not well-understood [29,32] and the relationship to human health is difficult to assess as a result. Another limitation is that while MST markers can provide evidence as to a probable source of fecal bacteria, multiple lines of evidence are needed to demonstrate reasonable proof of the sources of fecal bacteria in surface water [29]. There has been some criticism of MST studies to move beyond monitoring and to incorporate quantification of exposure when assessing concentrations of MST markers. This criticism has

suggested that monitoring studies are not designed to assess a source contributions of a source contaminant but only to determine what exposures can be detected [40].

Antibiotic Resistance Elements

The dissemination of antibiotic resistance is a distinct public health threat and current recommendations from the *Review on Antimicrobial Resistance* call for decreasing the demand for antibiotics and secondly to find new antimicrobials to treat antibiotic-resistant infections [9]. Decreasing demand requires a OneHealth framework assessing antibiotic-resistance emergence, dissemination, and persistence in human reservoirs such as in hospitals, in food animal production such as CHOs, and in environmental reservoirs.

The primary hypothesis regarding the dissemination and persistence of antibiotic resistance in any environment is that selective pressure from antibiotics must be maintained to promote the growth and out-competition of antibiotic-resistant bacteria compared to susceptible bacteria [41]. Selection pressure may also contribute to the emergence of new antibiotic resistance genes [42]. ARGs confer resistance to antibiotic classes, each with a different resistance mechanism [43,44]. Mechanisms for resistance include ARGs that confer resistance to antibiotic classes by pumping out the antibiotic from the cell through efflux pumps, inactivating the drug (e.g. beta-lactamases), or changing the target protein to inhibit antibiotic binding[43,44]. ARGs can be transferred between bacteria of the same species or different species primarily through horizontal gene transfer via plasmids [44]. Additionally, an ARG may confer resistance to multiple classes of antibiotics or ARGs may co-occur indicating that selection pressure for one antibiotic may actually select for resistance to multiple antibiotics [45].

For example, resistance to some heavy metals co-selects for resistance to some antibiotics as well [6].

As a transport mechanism, the environment can play a role in dissemination and persistence of antibiotic-resistant bacteria from human and animal sources via wind and water [41]. Of note, however, is that naturally-occurring resistance has also been reported without antibiotics as a driving force. For example, some ARGs also encode for other proteins in the cell and ARGs in bacteria may select for decreased susceptibility to toxins in the environment [43]. Resistant organisms have been found in the environment and in remote people groups without influence from antibiotic selection pressure [43]. While some research has suggested that carrying ARGs is a burden for bacteria, contrasting research indicates that, in some cases, ARGs persist despite lack of selection pressure [41]. As such, research assessing the effects of antibiotic use in humans or animals on the dissemination of antibiotic-resistant bacteria must carefully consider research design to control for natural environmental reservoirs of antibiotic resistance.

Antibiotic use in humans and animals have been implicated in contributing to increased antibiotic resistance [10]. Antibiotics are routinely given in food-animal production for treatment of disease, and only recently in 2014, did the US Food and Drug Administration (FDA) ban antibiotic use for growth promotion in food-producing animals in the US [46]. Of medically important antibiotics used to treat human disease, 70% of them are sold for use in food-producing animals [47,48]. There is concern the use of antibiotics in veterinary medicine and food animal production will provide selective pressure for the dissemination of antibiotic-resistant bacteria from commercial animal operations into the environment [41]. The US Centers for Disease Control and Prevention (CDC) National Antimicrobial Resistance Monitoring

System (NARMS) tracks antibiotic resistance in potential food-borne pathogens and the CDC supports increased capacity to track foodborne disease when discovered at the farm-scale [49]. Similarly, the US Government Accountability Office (GAO) and *Review on Antimicrobial Resistance* call for increased surveillance of food animal production environment and off-site transport from CHOs and other food animal production operations to better understand the influence of the animal production reservoir on antibiotic resistance and human health [8,9]. Sources of antibiotic resistance from animal reservoirs can be transferred to humans through antibiotic-resistant bacteria from contaminated food and also through the environment from land application of manure [6,49]. Additionally occupational exposure in animal operations can be a risk factor for human-animal transmission of antibiotic-resistant bacteria such as *E. coli*, *Enterococcus*, and *S. aureus* [41,50].

There are many antibiotic-resistant organisms and genes to assess and so priority parameters for monitoring and preventing dissemination of AREs are reported in the CDC [49] and the World Health Organization (WHO) [51] reports on prioritization of resistant pathogens and antibiotics for risk management, which include last-resort extended-spectrum antibiotics. Highest priority antibiotics include 3rd-5th generation cephalosporins and fluoroquinolones [51], which are considered last -resort antibiotics and were developed in addition to carbapenems to combat the prevalent beta-lactam resistance in gram negative bacteria [52]. These extended-spectrum antibiotics are effective even in the presence of many beta-lactamases. However, some beta-lactamases are still able to inactivate these extended-spectrum antibiotics including extended-spectrum beta-lactamases (ESBLs) and ampC beta-lactamases (ACBLs). As such, CDC indicates that urgent and serious threats include carbapenem-resistant *E. coli* and ESBL-producing *E. coli* [49].

Current gaps in knowledge regarding the dissemination of antibiotic-resistant bacteria include low spatial and temporal scales of surveillance, lack of knowledge about basic horizontal gene transfer rates, and inability to link antibiotic-resistant bacteria with human health risk [42,53]. While the CDC has provided estimates on human infections from antibiotic-resistant bacteria, they stress that their estimates are lower bounds and are unable to comment on their knowledge about infections acquired from the community and the environment due to low spatial and temporal scale of surveillance, identification, and reporting[49]. A recent review of the dissemination of ARGs in the environment indicates that few studies have appropriately controlled for other sources of antibiotics and that effect size of sources and reservoirs on dissemination of ARGs in the environment is difficult to assess as a result [40]. Managing antibiotics use and monitoring antibiotic-resistant bacteria in the food animal production setting has remained difficult in the United States[8]. Even while antibiotic use is higher in food animal production compared to human consumption, assessment of ARGs in food animal production environments is more challenging compared to assessing human sources such as from wastewater treatment plant effluent [40]. While access to wastewater treatment plant influent, effluent, and along the biological treatment process has been accessible to most researchers, access to on-site sampling at CHOs is more challenging. As highlighted in the 2017 US GAO report on antibiotic resistance in food animals, even federal access to CHOs when investigating foodborne outbreaks is restricted as they must obtain consent from the food-animal producer for access to a CHO[8]. CHO access is restricted to protect health and biosecurity of the animals. Additionally, as the GAO report highlights, there is minimal transparency in antibiotics consumed at specific CHOs with data only publically available for antibiotics sales for food-animal production aggregated at the national level[54].

Literature Reviews

The background section summarized microbial water quality indicators important for public health and their relationship to CHOs as a source of fecal bacteria. The following sections include literature reviews identifying research assessing whether there are effects of CHOs on FIB concentrations, MST markers, and AREs in surface water.

Fecal Indicator Bacteria Effects from CHOs: Literature Review

Current manure application practices in CHOs can contribute FIB and pathogens to surface water through overland runoff [28] or movement along subsurface tile drains regardless of manure application or environmental conditions [55]. FIB and bacterial pathogens have been found in swine waste, stored waste, land-applied soil, and plants on land-applied fields [5]. Plot studies have identified that microbial loads of FIB from land-applied fields can be transported to surface waters especially after precipitation [12]. Manure management conditions that reduced, but did not eliminate, bacterial transport included long-term and high-temperature waste storage and composting [28,55]. The environmental variables most influential for bacterial transport along subsurface tile drains were increased soil moisture and precipitation up to three weeks after application [28,55]. Other protective factors to reduce FIB transport from CHOs may include increasing flow resistance through vegetation and river buffers and longer overland distance to surface waters from CHOs [28].

Research assessing fecal contamination from CHOs has assessed water quality after an extreme event such as a lagoon spill [56,57] and has demonstrated that fecal contamination can be transported from manure application fields to surface water [12,58]. Fewer studies have measured fecal bacteria concentrations proximal to CHOs compared to a control site. Table 1

identifies eight studies that have been conducted to assess FIB concentrations in surface waters near CHOs during routine monitoring compared to at least one control site, rather than sampling after an extreme event such as a lagoon spill or hurricane. These studies are incomplete in their ability to assess CHO effect on surface water quality due to their low spatial and temporal monitoring scales, lack of effect size reporting, and/or not controlling for confounding sources of fecal bacteria inputs. Epidemiological studies use measures of effect to determine causal rather than correlating outcomes between two distributions of exposure. These effect measures include relative risks or mean differences with confidence intervals, however do not include measures of correlation such as correlation coefficients, p-values, and X^2 statistics [40,59]. Measures of effect size provide useful tools to compare effects of CHOs on surface water quality to wastewater treatment plants, human septic systems, and other fecal sources that are more commonly studied.

Among the studies assessing whether there were effects of CHOs on fecal bacteria in surface water, effect size is not usually calculated and the studies do not control for confounding fecal sources such as human septic, wastewater, or wildlife (Table 1). Among these monitoring studies assessing fecal water quality proximal to CHOs, one included a downstream control and found chronic, precipitation-independent, fecal contamination in watersheds with high CHO density, but did not quantify this relationship nor provide correlation or effect measures relative to the downstream control site [60]. Other research assessing whether there were effects of CHOs on surface water quality have compared upstream to downstream of CHOs or have compared watersheds with varied effects from CHOs.

Results from studies comparing upstream to downstream of CHOs (Table 1) have identified that concentrations of fecal coliforms, *E. coli*, and *Enterococcus* [21,22] were, on

average, higher at downstream sites (maximum n=6) compared to upstream sites (maximum n=3). However, only one study found a significant difference ($p < 0.01$) at the one CHO site evaluated [22]. A second study provided a causal measure of effect for fecal coliforms, *E. coli* and *Enterococcus* concentrations above the state limit but did not find significant differences (overlapping 1.0 in odds ratio) [21]. Another study [23] found that almost all upstream and downstream of CHO sites were above the state fecal coliform density (400 CFU/100mL) after precipitation but found no difference between high concentrations of fecal coliforms in upstream compared to downstream of CHO sites. While these studies found evidence of CHO effects on FIB concentrations, these studies did not quantify CHO effect because they did not characterize confounding sources of fecal sources in upstream sites such as land application sites of swine manure or human fecal sources, nor did they compare to background and ambient concentrations of FIB without influence from CHOs.

In studies comparing watersheds with CHOs to watersheds with fewer CHOs (see Table 1), fecal coliform and *Enterococcus* concentrations [20] were positively correlated with total animal manure units upstream, but in a separate study, *E. coli* concentration [24] was negatively correlated with number of animal operations and number of animal units upstream. Both studies did not specifically target CHOs but quantified a measure of animal operation exposure from, e.g., swine, cattle, and poultry, for each watershed. Neither study quantified other fecal sources nor compared to background concentrations of FIB [20]. Brendel and Soupir [24] recognize that although they found that animal operations are negatively associated with *E. coli* concentration, their findings may be reflect the negative correlation of area with *E. coli* concentration. Both studies introduced bias with large watershed areas. Bias can be introduced into studies with larger watershed areas when fecal sources are not characterized because fecal sources become

more disperse when far from the sampling location[61]. Watersheds in one study[20] were relatively similar ranging between 1800 and 2500 square miles while the other[24] watersheds had a large range in area from under one square mile to over 5,000 square miles. Finally, two studies[25,26] incorporated well-defined controls comparing watersheds with CHOs to an out-of-basin control, but the effect of CHOs in these studies are less evident. One study reports similar concentrations of fecal coliforms and *E. coli* between CHO sites and an out-of-basin control watershed [25] but higher concentrations of *Enterococcus* [25] in the out-of-basin control watersheds compared to watersheds with CHOs. This study did not assess animal operation density or other fecal sources upstream of CHO sites, however the control site was assessed for similarity with respect to land use and soil characteristics and was close (<80 km) to the CHO sites. The second study [26] was conducted among small watersheds under 12 square miles with and without CHOs finding that *E. coli* and *Enterococcus* concentration were, on average, higher in the out-of-basin controls compared to CHO watersheds. However, while this study had smaller watersheds, sites were distributed in multiple states and site comparability was not discussed. These studies similarly did not evaluate confounding fecal sources for out-of-basin controls nor for watersheds with CHOs.

Table 1 - Reviewing study design, results, and analysis of prior research on the effects of commercial hog operations on fecal bacteria in surface water

Reference		Study Design						Results
First Author	Year	Control Type	Measured Concentration: Bacteria	n Control Sites	n Swine Sites	n Sample Times	Range	Results, significance, measure of effect
Mallin	2015	downstream of control	Fecal coliforms	1	6	10	2 months	No significant difference between rain and non-rain events indicating chronic pollution; no comparison between control and swine sites
Sapkota	2007	upstream of CHO compared to downstream	Fecal coliforms, <i>E. coli</i> , <i>Enterococcus</i>	1	1	6	1.3 years	Significantly (p <0.01) higher downstream compared to upstream for all three outcomes measured
West	2011		Fecal coliforms	3	3	5	3 months	Fecal coliforms were over state limit (400 CFU/100mL) for almost all upstream and downstream sites after precipitation; no difference identified between control and swine sites
Heaney	2015		Fecal coliforms, <i>E. coli</i> , <i>Enterococcus</i>	3	6	~ 23	1 year	Mean values for all three outcomes were higher (not significant) at downstream sites. Effect measure calculated using the odds ratio with 95% confidence interval of concentration above the state standard. Fecal coliforms: 1.86 (0.96-3.62); <i>E. coli</i> : 1.73 (0.79-3.78), <i>Enterococcus</i> : 1.49 (0.77- 2.88). Rainfall was associated with higher concentrations of fecal indicators.
Jokinen	2012	Watershed comparisons	Fecal coliforms, <i>E. coli</i> , <i>Enterococcus</i>	5	4	38	3 years	Fecal coliforms and <i>Enterococcus</i> significantly (p<0.05) positively associated with total animal manure units upstream
Brendel ¹	2017		<i>E. coli</i> concentration	10	20	21	3 years	Regression results estimated that geometric mean <i>E. coli</i> concentration was significantly negatively associated with number of animal units and number of animal operations in watershed
Givens	2016		Fecal coliforms, <i>E. coli</i> , <i>Enterococcus</i>	1	5	4	6 months	Mean fecal coliform and <i>E. coli</i> concentrations were similar , however <i>Enterococcus</i> concentration was significantly (p=0.04) higher in the control basin; all three concentrations were significantly higher post-manure compared to pre-manure
Haack	2016		Fecal coliforms, <i>E. coli</i> , <i>Enterococcus</i>	4	5	2	2 weeks	Mean <i>E. coli</i> and <i>Enterococcus</i> concentrations were higher (not significantly) in control sites compared to swine sites; fecal coliform concentration was not different between control and swine sites; effect of precipitation on FIB concentration not calculated

¹Not a field-based study

Microbial Source Tracking Effects from CHOs: Literature Review

While a handful of studies have targeted swine-specific MST markers to monitor sources of fecal contamination [38] in surface waters or even used as a biomarker to provide evidence of health risks from CHOs [62,63], few studies have systematically studied CHO contribution of swine MST markers in surface water. Of the studies identified in Table 1, three assessed swine-specific fecal markers in addition to fecal bacteria concentrations from CHOs and two had comparison groups [19–21]. The study designs and results of these studies are summarized in Table 2. The prevalence of detecting swine-specific MST markers, pig-1-bac and pig-2-bac, was positively associated with downstream of CHO sites compared to upstream sites. The odds of detecting pig-1-bac downstream of CHOs was 2.47 (95% confidence interval 1.03 – 5.94) times the odds of detection upstream of CHOs [21]. A second study also found that prevalence of a different swine MST marker, PF163, was more often found in watersheds with more CHOs compared to those with fewer (Jokinen). While these studies provide evidence that swine manure can be detected in surface water and has been transported off-site [19–21,38], they were not able to quantify marker concentrations. Additionally, PCR-based markers can be subject to cross-reactivity and so comparing MST marker prevalence to comparable background sites can provide an estimate of cross-reactivity in the study area if upstream exposure is definitively known. Finally, these studies are also limited in their study design, as discussed in the prior section pertaining to FIB concentration, by not providing an effect size or effect size and measures of correlation being confounded due to swine-fecal exposure in control sites with CHO effects.

Table 2 - Reviewing study design, results, and analysis of prior research on the effects of commercial hog operations on swine-specific microbial source tracking markers in surface water

Reference		Study Design						Results
First Author	Year	Control Type	Measured Outcome: Microbial Source Tracking Marker	n Control Sites	n Swine Sites	n Sample Times	Temporal Range	Results, Significance, Measure of effect
Arfken	2015	No control	Prevalence of detecting PF163	0	9	5	1 year	Swine marker observed in surface waters downstream of CHOs. No comparison between swine and control sites.
Heaney	2015	upstream of CHO compared to downstream	Prevalence of detecting pig-1-bac, pig-2-bac	3	6	~ 23	1 year	Prevalence of marker detection was higher at downstream sites. Effect measure calculated using the odds ratio with 95% confidence interval of marker prevalence. pig-1-bac: 2.47 (1.03-5.94), pig-2-bac: 2.30 (0.90 - 5.88). Rainfall was associated with higher concentration of MST marker.
Jokinen	2012	Watershed comparison	Prevalence of detecting PF163	0	9	38	3 years	Prevalence of marker detection was three times higher ($p < 0.05$) in sites with more CHOs compared to sites with fewer CHOs; effect measure not calculated

Antibiotic Resistance Element Effects from CHOs: Literature Review

Mechanisms of off-site transport of AREs from CHOs include runoff from land-applied fields and ditch or tile-drain transport to surface waters. Research has found that swine manure can contain antibiotic residues and high concentrations of multi-drug resistant bacteria [13,14] and have found similar antibiotic resistance patterns in swine manure or lagoon effluent in the on-farm environment [13], in soils with manure application [15], and in effluent from tile-drains and ditches [6,15,16]. One study found similar ESBL *E. coli* genes in swine manure and receiving surface waters [17]. Additionally, AREs have been assessed along lagoon effluent treatment systems, including in anaerobic digesters and wetland treatment, and have found that while treatment reduced FIB concentrations and thus also the concentration of AREs, AREs are still identified in effluent transported to soil and surface water [15,18]. A review on the fate and transport of AREs from land application of manure indicates that transport of AREs are similar to that of FIB identifying precipitation and soil conditions as drivers of ARE transport from field to surface water[6]. Unlike FIB fate and transport, however, AREs may persist by sharing resistance genes among bacteria through conjugation or transduction[6]

Research design considerations regarding selection of control sites with known fecal source confounders and high spatial and temporal variability are similar for monitoring antimicrobial resistance as for monitoring FIB concentrations. Of studies assessing AREs in surface waters proximal to CHOs, many parameters can be chosen to assess antimicrobial resistance including quantification of antimicrobial resistance genes, assessing phenotypic resistance of a model organism such as *E. coli* or *Enterococcus*, or measuring antibiotic residues in surface waters. As with research on CHO effects on FIB concentrations, there have been studies reporting evidence that AREs can be found proximal or downstream of due to runoff or

tile drain transport such as antibiotic residues [14], antibiotic-resistant *E. coli* [13], or ESBL *E. coli* genes [17].

Fewer studies have compared AREs in surface water proximal to CHOs to a control group. Table 3 identifies seven studies that have assessed AREs in surface waters proximal to CHOs with a comparison group. Significantly higher phenotypic resistance was observed among *Enterococcus* [22] and fecal coliforms [23] in studies comparing upstream to downstream of CHOs. These studies, however, did not assess ambient resistance or other potential sources of AREs in the environment such as from human or naturally occurring resistance that may contribute to surface waters and which limits ability to conclusively identify the CHO as the source for increased antibiotic resistance downstream.

Three additional studies incorporated control sites which were identified to not have swine fecal source inputs. Two studies prioritized two antibiotic resistance genes conferring resistance to vancomycin, which are important for public health. One did not find these genes in any sample [26] and the other study identified the genes infrequently in both the control and swine samples [25]. The third study [15] quantified 22 antibiotic resistance genes along the swine manure treatment system, manure-applied soils, effluent-receiving ditches, downstream surface waters, and an upstream reservoir as a control. This study concluded that while antibiotic resistance gene concentration is reduced between manure treatment and the receiving surface waters, the environment still receives discharge from the CHO and downstream surface waters have higher antibiotic resistance gene concentrations compared to the upstream reservoir.

While it is clear that AREs have been detected proximal to CHOs into surface waters and that it is probable that CHOs contribute to AREs in the environment, the effect size of the contribution of AREs from CHOs compared to background and other sources of AREs have not

been well-studied. The lack of effect size from CHOs is partially due to research design considerations and partially due to the the mixed methods (i.e. genotypic/phenotypic) and the variety of parameters that are possible to assess with some studies focusing on high impact public health parameters (such as vancomycin resistance) [25,26]), while others have focused on assessing a more complete resistance profile of the sample [15] or of phenotypic bacterial resistance [22,23].

Prior studies are criticized for not controlling for other sources of FIB or AREs, such as human septic or natural background levels of antibiotic resistance, in their study designs. Some have even suggested that human or ambient sources of resistance may contribute more AREs than CHOs [18] and identify that field studies have not proven the source of fecal contaminants[64]. A recent review of the dissemination of antibiotic resistant genes in the environment confirms that few studies have appropriately controlled for confounding sources of antibiotics from CHOs or from wastewater treatment plants, but clarifies that effect size in any direction of sources, whether ambient, human, or animal, on dissemination of ARGs in the environment is difficult to assess as a result [40,61].

Table 3 - Reviewing study design of prior research and proposed research on antimicrobial resistance elements in surface waters proximal to CHOs

Reference		Study Design						Results
First Author	Year	Control Description	Measured Outcome: Antimicrobial Resistance Element	n Control Sites	n Swine Sites	n Sample Times	Temporal Range	Results, Significance, Measure of effect
Sapkota	2007	Upstream of CHO compared to downstream	Prevalence of antibiotic-resistant <i>Enterococcus</i> for 5 antibiotics	1	1	6	1.3 years	Resistance to four of five antibiotics was observed more often resistant in downstream samples especially erythromycin (p=0.02) and tetracycline (p=0.06); effect measure not calculated.
West	2011		Prevalence of antibiotic-resistant fecal coliforms to 5 antibiotics	3	3	5	3 months	Resistance to two or more antibiotics was observed more often in 42% of isolates from downstream of CHO samples compared to 17% isolates upstream (p<0.001). Effect measure not calculated.
He	2016		Quantification of 22 antibiotic resistance genes	1	3	1	day	Identified that higher concentration of all antibiotics were identified at CHO discharge compared to downstream river water or upstream reservoir (control). Effect measure not calculated.
Rieke	2018		Quantification of two resistance genes	3	2	35-43	2 years	Concentrations of both resistance genes (<i>ermB</i> , <i>ermF</i>) were higher in drainage outlets compared to surface water controls (p<0.01). Effect measure not calculated.
Givens	2016	Watershed Comparison	Prevalence of <i>Enterococcus</i> with vancomycin resistant gene	1	5	4	6 months	Two vancomycin resistance genes (<i>vanA</i> , <i>vanB</i>) were detected infrequently at control and swine sites. Comparison not reported.
Haack	2016		Prevalence of <i>Enterococcus</i> with vancomycin resistant gene	4	3	2	3-8 weeks, before/after rain	Two vancomycin resistance genes (<i>vanA</i> , <i>vanB</i>) were not detected in swine or control watersheds.

Significance

This chapter identified that the scientific literature requires more targeted research on how CHOs affect microbial water quality. Our review of the literature identified that CHOs can affect water quality, especially after precipitation and post-manure application, or more extreme events such as lagoon spills, however results are not consistent across studies. Without rigorously quantifying fecal sources or controlling for confounding fecal sources, these studies cannot prove fecal contamination is from the CHO and must allow that CHOs may not affect microbial quality of surface water when compared to ambient concentrations of fecal bacteria [5]. Study design limitations are, in part, due to study questions assessing effect of manure application [25,26] and/or precipitation [26] on surface water quality from CHO rather than looking at effect of CHO compared to control site. Review articles have called for field studies assessing fecal source effects on environment to control for confounding sources of fecal contamination by design through land use analysis and to provide measures of effect by increasing spatial and temporal scales of sampling [61]. Measures of effect size would provide a useful metric to compare effects of CHOs on surface water quality to wastewater treatment plants, human septic, and other fecal sources that are more commonly studied.

This research contributes to the literature as the largest field study to-date with respect to sites with (n=13) and without (n=9) CHOs to assess whether there are effects of CHOs on microbial water quality. This research collects samples longitudinally to control for environmental and seasonal land-use variation. This research addresses site similarity by quantitatively assessing comparability between CHO and background sites to ensure similarity with respect to watershed area, soil type, and land use. Site watershed area is small to reduce bias of including unknown fecal sources. Confounding fecal source variables are also

quantitatively assessed for comparability with respect to human population and human population density and all sites are known to not have effects from wastewater treatment plants or other animal operations. Finally, swine exposure is well-defined such that CHO watersheds have known swine manure application sites and background sites are known to not have effects from CHOs. Chapter 3 quantifies whether there is an effect of CHOs on the fecal indicator *E. coli* concentration and is the first field study to compare human and swine MST marker concentration between CHO exposure groups. Chapter 4 quantifies the effect of CHOs on antibiotic resistance in *E. coli*. Finally, Chapter 5 determines effects from CHOs on *E. coli* concentrations of surface water by predicting *E. coli* concentration while controlling for land use, hydrological, and environmental variables in addition to other fecal sources through approximations of exposure. Methods for exposure approximation are compared using density metrics in addition to density paired with distance metrics.

CHAPTER 3: DO COMMERCIAL HOG OPERATIONS CONTRIBUTE FECAL BACTERIA TO SURFACE WATER?

Introduction

NC is one of the leading states in the nation for food animal production, including its \$4 billion swine production industry. Additionally, an area in eastern NC is home to the highest density of swine in the country [2]. With almost 10,000,000 permitted swine on more than 2,100 commercial hog operations in eastern NC [3], state regulation and scientific research has focused on swine waste management. Swine effluent in NC is typically stored in open-air lagoons and sprayed onto fields to fertilize crops and the regulatory structure is in the form of nutrient management plans focusing extensively on nutrients in surface water and, to a small degree, microbial water quality effects. While NC state requirements in nutrient management plans prohibit transport of swine waste by runoff, discharge, or land application to surface water [65], almost all CHOs in NC are regulated as non-discharge sources so monitoring is not required to assess off-site transport of nutrients or FIB. However there are concerns regarding whether there are effects of CHOs on nearby communities with respect to health and water quality.

High concentrations of nutrients and organics have been found in swine lagoon effluent and are implicated in fish kills, algal blooms, and odor complaints [4,66,67]. Heavy metals in manure can build up in soil and risks groundwater and surface water contamination [4], and presence of heavy metals can lead to increased antibiotic resistance in bacteria [6]. Crop yields can be affected by ion and salt accumulation from swine manure [4]. Additionally fecal bacteria

are exposed to pharmaceutically active products such as antibiotics and hormones in swine manure and CHOs are known to contribute to increased antibiotic resistance of microorganisms in manure [4,6,68]. Finally, bacteria in manure may be pathogenic or resistant to antibiotics posing risks to human health [4,5].

The basis for monitoring microbial water quality is protecting public health. Monitoring microbial water quality includes assessing FIB concentrations which is used as a regulatory tool as a proxy for public health risk. While lagoon spills and extreme events affect water quality [56,57], research suggests that current manure application practices in CHOs also contribute FIB and pathogens to surface water through overland runoff or movement along subsurface tile drains regardless of manure application or environmental conditions [12,28,55,58]. Field and case monitoring studies have found a positive association of higher nutrients [67] and FIB concentrations [21–23,60] with proximity to or downstream of CHOs.

Review articles have called for field studies assessing fecal source effects on environment to control for confounding sources of fecal contamination by design through land use analysis and to provide effect size by increasing spatial and temporal scales of sampling [61]. Measures of effect size provide useful tools to compare effects of CHOs on surface water quality to wastewater treatment plants, human septic, and other fecal sources that are more commonly studied. While studies have found that it is possible for CHOs to contribute FIB to surface waters, these studies did not provide measures of effect due to low spatial and temporal scale. Additionally, in studies assessing effects of CHOs on surface water, many did not identify the land use for control sites with respect to confounding sources of fecal contamination such as wastewater treatment plants or other animals [15,22,23], or have known swine influence at their control sites, upstream of sampling location [21,60]. Among field studies with out-of-basin

controls, the control was identified without CHOs, however other sources of fecal contamination were not identified or controlled for [25,26]. Studies assessing microbial source tracking markers of swine-feces-specific genes identify that swine manure is present in surface water but due to study design limitations are unable to determine effect measures due to low spatial and temporal scales or due to confounding fecal sources affecting effect measurements [19–21,38].

We designed a longitudinal, landscape-scale monitoring study in similar, small, agricultural watersheds with and without CHOs to address concerns of bias and confounding identified in the literature. To determine how CHOs affect microbial quality of surface waters, we compared concentrations of FIB *Escherichia coli* in watersheds with and without CHOs. Additionally, we compared concentrations of human-feces MST marker HF183 and swine-feces MST marker pig-2-bac to provide additional evidence human-feces and swine manure transport into surface water.

Materials and Methods

Site Selection

Nine background and thirteen swine sites were selected from a prior longitudinal, landscape-scale USGS report identifying CHO effects on nutrients in small watersheds in North Carolina [67]. Background sites were chosen as controls. Swine sites were defined as having a CHO in the watershed upstream of the sampling point. The study sites spanned four major river basins and eleven counties in eastern NC (Figure 1). All sites were selected to reduce confounding fecal sources and did not have wastewater treatment plants, National Pollution Discharge Elimination System (NPDES) points, and other types of commercial animal

operations. Watersheds were small and primarily agricultural land use. A t-test or Mann-Whitney U test was used to evaluate difference in mean for each watershed variable between background and swine groups. Statistical test was determined based on normality of the data, assessed using the Shapiro-Wilkes p-value. Watershed variables include watershed area, percent hydrologic soil class [69], 2016 percent cultivated, percent forested, and percent wetland land [70], percent land use within a 50m buffer of all surface water in a watershed, and 2010 census block population and population density [71]. Dual hydrological classes were grouped to assume the drained condition. Data were clipped by watershed area and areally weighted using ArcMap 10.1 [72]. Watershed area, population, and population density were \log_{10} -transformed prior to analysis. The mean and 95% confidence interval (CI) for watershed variables for background and swine groups are presented in Table 4 and mean values for all sites in Table 5. Site-specific maps are detailed in Appendix A and latitude and longitude are provided in Table 5. Mean values of watershed variables were not significantly different between background and swine groups (Table 4) indicating comparability.

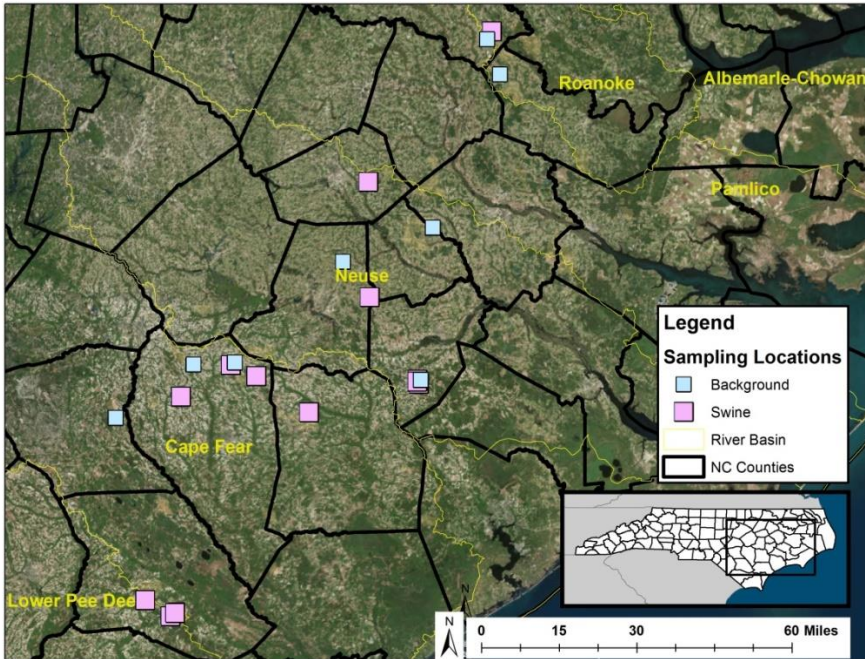


Figure 1 - Swine (pink) and background (blue) site distribution in eastern North Carolina among major water basins

Table 4 – Evaluation of comparability of watershed variables in background (n=9) and swine (n=13) sites

Watershed Variable	Background Mean (95% CI)	Swine Mean (95% CI)	p-value ¹	Shapiro-Wilkes
Watershed area (mi ²)†	4.5 (1.5 – 7.5)	3.2 (1.9 – 4.5)	0.95	0.81
% Wetland	20 (14 - 27)	18 (16 – 20)	0.50	0.68
% Forest	23 (15 – 31)	22 (16 – 29)	0.91	0.24
% Cultivated	38 (27 - 49)	37 (28 - 46)	0.87	0.85
% Wetland, 50m Buffer	36 (20 - 51)	49 (40 - 58)	0.18	0.52
% Forest, 50m Buffer†	12 (6 – 17)	14 (9 – 19)	0.39	0.72
% Cultivated, 50m Buffer	36 (15 – 57)	18 (8 – 27)	0.23	0.008*
% Hydrological Soil Class A	54 (35 – 72)	48 (36 – 61)	0.56	0.02*
% Hydrological Soil B	28 (14 – 41)	31 (21 – 41)	0.72	0.37
% Hydrological Soil C	14 (0 – 30)	13 (2 – 23)	0.52	< 0.001*
% Hydrological Soil B	2 (0 – 5)	3 (0 – 6)	0.85	< 0.001*
Population†	300 (2 – 599)	128 (81 – 174)	0.97	0.97
Population Density (Population/mi ²)	51 (30 - 71)	55 (24 – 87)	0.99	0.99
Address (SED, Euc)	2.2 (1.2 – 5.5)	4.1(3.1 – 11.3)	0.14	<0.001*
Address (SED-int) †	0.58 (0.7 – 1.9)	1.62 (1.4 – 4.7)	0.17	0.37

¹t-test or *Mann-Whitney rank sum test for non-normal distributions when Shapiro Wilkes p<0.1

† log₁₀-transformed for t-test

Table 5 - Watershed variables by site including watershed area, % land cover by wetland, forest and agriculture in 2016, % hydrological soil classes (HSC) A, B, C, and D, and 2010 census block population and population density

Site	Latitude (decimal degrees)	Longitude (decimal degrees)	Watershed Area (mi ²)	% Wetland	% Forest	% Agriculture	% HSC A	% HSC B	% HSC C	% HSC D	Pop.	Density (Pop./mi ²)
BK01U	36.087895	-77.387593	0.42	6%	11%	80%	0%	8%	79%	12%	7	17
BK03	35.988369	-77.348199	3.67	14%	30%	53%	49%	40%	11%	0%	24	7
BK05U	35.141297	-77.657042	0.76	11%	52%	31%	76%	0%	0%	1%	34	45
BK10U	35.20601	-78.289634	0.65	26%	25%	48%	78%	22%	0%	0%	25	38
BK12	35.479161	-77.909864	3.55	14%	21%	60%	54%	39%	4%	3%	305	86
BK14	35.566209	-77.598524	13.27	27%	21%	44%	50%	39%	8%	0%	1436	108
BK15	35.059708	-78.701025	7.42	37%	23%	37%	77%	12%	9%	2%	337	45
BK16	35.204286	-78.431314	9.34	22%	7%	64%	78%	20%	0%	0%	452	48
BK17U	34.516723	-78.511549	1.38	26%	17%	52%	19%	68%	12%	0%	83	60
SW01	36.10924	-77.370417	5.38	18%	10%	69%	8%	23%	54%	15%	139	26
SW04	35.377967	-77.82253	1.23	15%	24%	55%	75%	25%	0%	0%	284	231
SW05	35.14293	-77.669125	4.13	17%	7%	70%	62%	17%	0%	5%	245	59
SW05A	35.131303	-77.663667	1.02	20%	21%	53%	50%	21%	0%	11%	71	70
SW05C	35.136944	-77.668937	1.17	12%	24%	61%	67%	4%	0%	0%	53	45
SW07	35.700274	-77.81418	1.25	18%	37%	42%	78%	7%	9%	6%	111	89
SW09	35.114741	-78.476699	3.85	25%	16%	53%	67%	32%	0%	0%	98	25
SW10	35.199711	-78.303515	1.89	14%	8%	69%	68%	32%	0%	0%	51	27
SW11	35.16673	-78.217344	1.95	22%	15%	61%	56%	42%	0%	0%	26	13
SW13	35.06047	-78.041838	2	12%	17%	70%	27%	72%	0%	0%	34	17
SW16	34.547185	-78.614709	3.38	20%	40%	39%	30%	34%	36%	0%	237	70
SW17	34.500347	-78.531594	9.12	19%	38%	40%	24%	41%	35%	0%	175	19
SW17U	34.509015	-78.515195	5.16	21%	34%	43%	18%	51%	32%	0%	137	27

Sample Processing

Approximately 1 L of water was collected at each sampling event between August 2016 and August 2017 in sterile, plastic bottles that were rinsed with surface water immediately prior to sample collection and transferred on ice to the laboratory at the University of NC (UNC) at Chapel Hill. At the time of sample, a handheld YSI Pro Professional Plus meter was used to assess water temperature, specific conductance, dissolved oxygen, and pH of sample. Samples were processed at the UNC-Chapel Hill laboratory within 24 h of sample collection. Field blanks were included. Standard membrane filtration methods were used to quantify concentrations of thermotolerant *E. coli* from each water sample collected [73]. Volumes of 50 mL, 25 mL, 5 mL, and 1 mL sample were vacuum filtered through 0.45 μm , 47 mm mixed cellulose ester filters (MilliporeSigma, Burlington, MA) and aseptically placed onto selective M-TEC ChromoSelect agar (Sigma-Aldrich, St. Louis, MO). The plates were inverted and incubated at 37 °C for 2 h followed by 44°C for 22 h (+/- 2 h) then colonies with purple morphological characteristics of *E. coli* were counted.

To determine concentrations of colony forming units (CFUs) per 100 mL, dilution plates with *E. coli* counts between 20 and 80 colonies were normalized to 100 mL and averaged. Samples with all dilution plates below 20 colonies were considered to be at the lower limit of quantification (LLQ) and the plate with the highest colony count was used to determine CFU/100mL. Samples with all dilution plates with a count above 80 were considered to be at the upper limit of quantification (ULQ) and the plate with the lowest colony count was used to determine CFU/100mL. Samples with zero colonies identified were determined to be at the lower limit of detection (LLD) and set to one CFU/100mL. Samples with plates too numerous to

count (TNTC) were considered to be at the upper limit of detection (ULD) and samples set to the highest colony count observed per 100mL.

Microbial source tracking markers

During water sample processing, 100 mL of each sample was filtered through 0.4 µm Isopore® polycarbonate filters (MilliporeSigma, Burlington, MA) and saved in MO Bio PowerSoil DNA extraction tubes (MO Bio Laboratories, Carlsbad, CA) at -80 °C until DNA extraction. DNA extraction was conducted between June and September 2017 following manufacturer's protocol with the MO Bio Power Soil kit (MO Bio Laboratories, Carlsbad, CA) with one addendum that tubes were bead beaten for two minutes prior to extraction using the high velocity Mini Bead-Beater-16 (BioSpec, Burtlesville, OK). A negative extraction control (NEC) was prepared for each extraction date. Extracted DNA was frozen at -20 °C until droplet digital PCR (ddPCR) analysis between September and November 2017.

A ddPCR duplex assay was conducted on all DNA extracts targeting swine-specific *Bacteroidales* associated with swine fecal contamination, pig-2-bac [21,38], and *Bacteroides* HF183, associated with human fecal contamination, HF183 [33,74]. While HF183 has been optimized for ddPCR [75], pig-2-bac has not. As such, we optimized the pig-2-bac annealing temperature (Figure 2), assessed the range of quantification for both targets (Figure 3), and assessed the duplex assay for assay competition (Figure 4) following the procedure in Cao *et al.* 2015 [76]. To assess optimal temperature, we used the pig-2-bac standard, a lagoon water sample provided by a NC CHO, and surface water field sample. The lagoon water sample was extracted in the same method as surface water samples. The annealing temperature was varied and optimization parameters were droplet separation and mean fluorescence amplitude [76].

Inhibition is considered to be reduced in ddPCR technology compared to qPCR technology and, as such, inhibition controls are not included [76]. To assess assay range of quantification, five ten-fold serial dilutions of the standard for HF183 and pig-2-bac targets were assessed in duplicate in a ddPCR simplex. Ideally, concentration is linear among \log_{10} -concentrations in a ten-fold serial dilution. We note that at higher concentrations of target marker there is more variability among samples, consistent with ddPCR technology [77]. We found higher variability for pig-2-bac compared to HF183 at the highest concentrations measured. To assess assay competition, a ten-fold serial dilution of HF183 with an equal volume of a constant concentration of pig-2-bac was assessed, and vice versa. Samples were run as a duplex and assay competition assessed as in [76]. Due to lower concentration sensitivity at higher pig-2-bac concentrations, a four-fold dilution was assessed for the pig-2-bac duplex compared to a five-fold dilution for HF183. We found that target \log_{10} -concentrations of the serial dilution behaved linearly when in the presence of a constant concentration of a second target indicating that assay competition did not affect final concentration results.

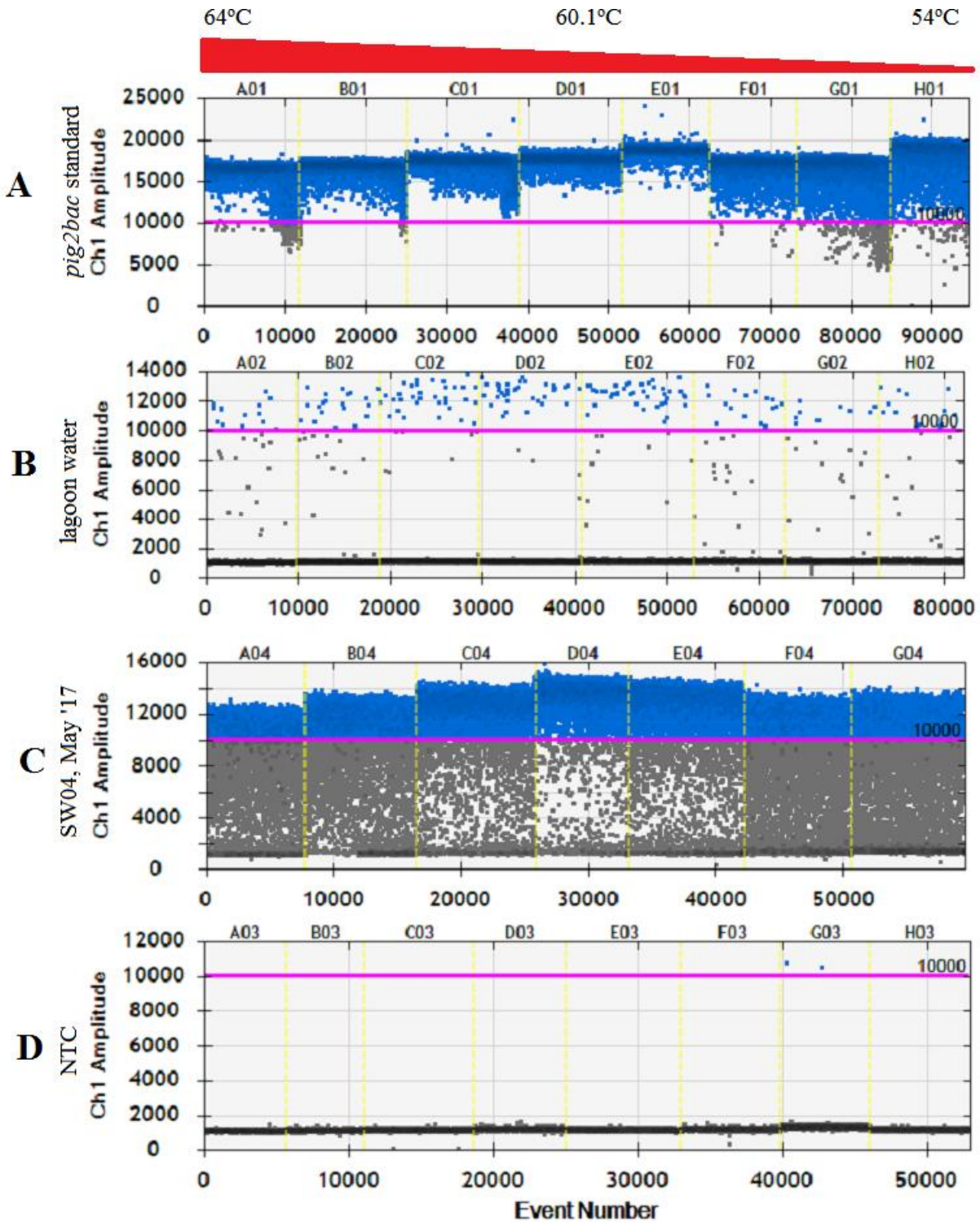


Figure 2 - ddPCR temperature optimization for pig-2-bac in pig-2-bac standard (A), lagoon water sample (B), possible lagoon discharge from sample SW04 May 2017 (C), and negative template control (D). Annealing temperature ranged from 64° C (left) to 54° C (right).

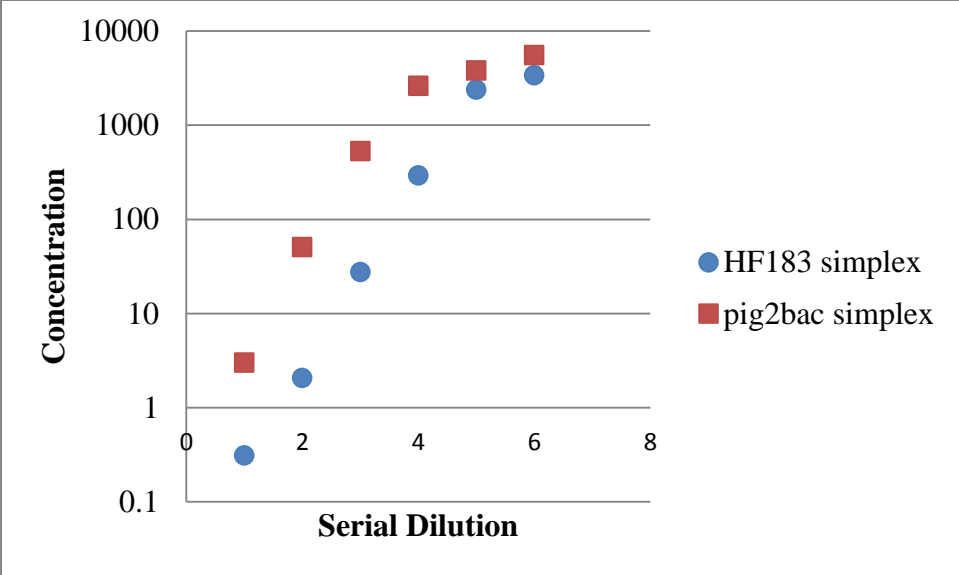


Figure 3 – simplex ddPCR assessment of range of concentration for microbial source targets HF183 and pig-2-bac

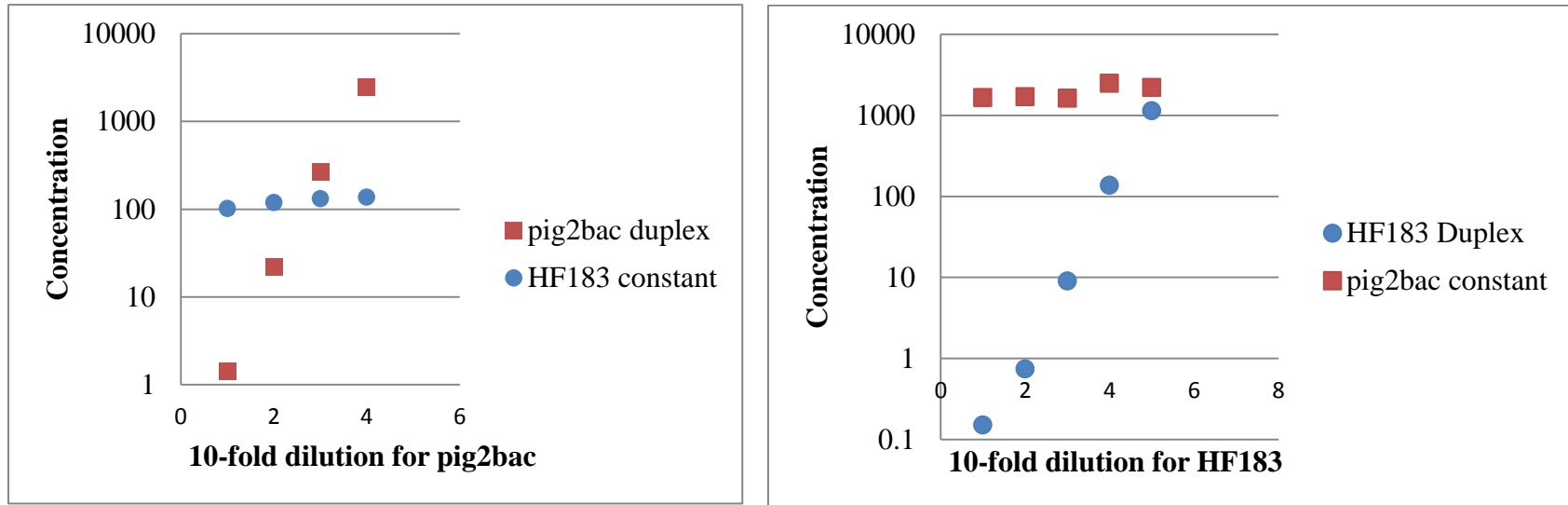


Figure 4 - duplex ddPCR assessment of assay competition with sample mixtures of constant concentration of HF183 with four-fold dilution series of pig-2-bac (left) and constant concentration of pig-2-bac with five-fold dilution series of HF183 (right).

We prepared a PCR mixture of master mix with 900 nM concentration of pig-2-bac and HF183 forward and reverse primers, 250 nM concentration of pig-2-bac and HF183 probes as suggested for ddPCR[77]. Each PCR well contained 0.16 μ L of nuclease-free water, 12 μ L of 2x ddPCR Supermix with no DUTP (BioRad), 2.16 μ L of each 10 μ M forward primer, 2.16 μ L of each 10 μ M reverse primer, 0.6 μ L of each 10 μ M probe and 2 μ L of extracted sample DNA for a total volume of 24 μ L per well. Samples, field blanks, and negative extraction controls were run in duplicate with positive and negative template controls included for each PCR plate. Positive controls consisted of a mixture of 22 μ L nuclease free water, 1 μ L 10^3 copies pig-2-bac standard [38] and 1 μ L 10^3 copies HF183 standard [33]. Primer, probe, and standard sequences are reported in Table 6.

Droplet generation was conducted on the Droplet Generator (Bio-Rad, Hercules, CA). We mixed 20 μ L of PCR mixture and 70 μ L droplet generation oil for each well and pipetted 40 μ L of droplets into a new 96-well plate, which was heat sealed with PX1 plate sealer (Bio-Rad) at 180 $^{\circ}$ C. PCR was conducted on Bio-Rad T100TM thermal cycler with the following optimized cycling conditions: denaturation for 10 minutes at 95 $^{\circ}$ C followed by 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 60 s with a 2 $^{\circ}$ C/s ramp, followed by 10 minutes at 98 $^{\circ}$ C. The QX200 Droplet Reader (Bio-Rad) determined concentration of both MST markers by measuring fluorescence of each MST probe in each droplet in each well using the absolute quantification setting.

Table 6 - Microbial source tracking primers, probes, and standards for pig-2-bac and HF183

Oligo ID or Accession No.	Description	Sequence (5' → 3')	Reference
HF183	HF183 Forward Primer	ATCATGAGTTCACATGTCCG	[74]
BacR287	HF183 Reverse Primer	CTTCCTCTCAGAACCCCTATCC	[33]
BacP234MGB	HF183 Probe	FAM-CTAATGGAACGCATCCC - MGB	[36]
AB242142.1	HF183 Standard	ATCATGAGTTCACATGTCCGCATG ATTAAAGGTATTTTCCGGTAGACG ATGGGGATGCGTTCCATTAGCTCA GATAGTAGGCGGGGTAACGGCCCA CCTAGTCAACGATGGATAGGGGTT CTGAGAGGAAGGTCCCCCACATTG GAACTGAGACACGGTCCAAACTCC TACG	[33]
Pig-2-Bac41F	pig-2-bac Forward Primer	GCATGAATTTAGCTTGCTAAATTT GAT	[38]
Pig-2-Bac163Rm	pig-2-bac Reverse Primer	ACCTCATACGGTATTAATCCGC	
Pig-2Bac113MGB	pig-2-bac Probe	6-FAM- TCCACGGGATAGCC -MGB	
HQ201815.1	pig-2-bac Standard	GCAGCATGAATTTAGCTTGCTAAA TTTGATGGCGACCGGCGCACGGGT GAGTAACGCGTATCCAACCTTCCC CTGTCCACGGGATAGCCCGTCGAA AGGCGGATTAATACCGTATGAGGT	

ddPCR Analyses

To quantify concentration of MST targets, a unique threshold value was determined for each target for each ddPCR run. Three thresholds were identified by first identifying the fluorescence range, defined as the mean negative fluorescence amplitude value from negative control wells subtracted from the mean positive fluorescence amplitude of positive standard wells. A high threshold was calculated as 20% of the fluorescence range subtracted from the mean positive fluorescence amplitude. A mean threshold was calculated as the mean of the mean positives and mean negative droplets. A low threshold was calculated as 20% of the

fluorescence range added to the mean negative fluorescence amplitude (see Figure 5). We present results using the high threshold value as a conservative estimate for marker concentration.

A droplet was considered positive if above the threshold value for the gene target. Duplicate sample wells were merged and a sample was considered positive if three or more droplets were considered positive among the two merged wells. Concentration is reported as copies per μL extracted DNA with 95% confidence intervals for merged data for the three threshold values in QuantaSoft software. Concentration is based on a Poisson-corrected proportion of droplets positive as reported by QuantaSoft software. Log_{10} copies per μL was calculated as $\log_{10}(c*24/2)$ since 2 μL sample DNA extract was added to a total well volume of 24 μL during PCR well preparation. Copies/ μL extracted DNA is analogous to copies/mL sample water because 100 mL of water was filtered and 100 μL DNA eluted from each sample during DNA extraction. Samples with less than three droplets positive were considered below the detection threshold (BD) and set to one half the limit of detection concentration equal to 0.7 copies/mL.

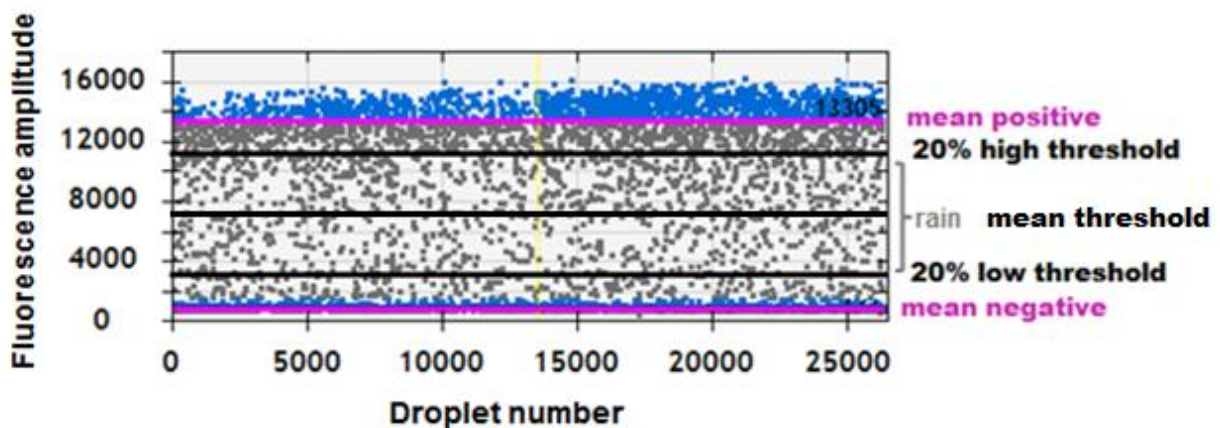


Figure 5 - Example output of ddPCR run displaying two wells of the positive standard targeting pig-2-bac and determination of high, mean, and low thresholds to calculate concentration

Data Analyses

The Shapiro-Wilkes test was used to assess normality of the concentrations and log₁₀ concentrations of *E. coli* and microbial source tracking markers among samples. Because untransformed and log-transformed data were not normally distributed (data not presented), mean differences in concentration are presented but were not used to evaluate differences between swine and background groups. Instead, the non-parametric Mann-Whitney U Test was used to evaluate the difference in mean ranks of physical water parameters, concentration of *E. coli*, concentrations of MST markers among all samples, and concentrations of MST markers among samples with detected MST markers between all swine and background samples. All mean values are presented with a 95% CI.

To evaluate the difference in prevalence outcomes between swine and background samples the test of equal proportions with Yates continuity correction was used. Prevalence outcomes include proportion of samples with *E. coli* concentration above EPA recommendation, and proportion positive samples and sites for MST markers. Additionally, a measure of effect size was included when comparing prevalence outcomes by calculating a relative risk (RR) with a 95% CI. Analysis includes comparisons at the isolate, sample, and site level where appropriate.

Results

Water Characteristics

Water samples were collected from small streams with mean watershed 3.7 square miles (Table 4). Samples generally had qualitatively high turbidity and were taken from slow to

stagnant water movement unless collected following a precipitation event. Average water temperature and dissolved oxygen content did not differ between groups, however average pH and specific conductance were significantly higher in swine sites compared to background sites (Table 7). These results match prior sampling in Harden 2015 finding higher pH and specific conductance at swine sites compared to background. The higher specific conductance was attributed to the higher median concentrations of measured dissolved magnesium, sodium, potassium, and chloride in sites with CHOs compared to background sites.

Table 7 - Physical water parameters in background and swine sites

	Background	Swine	P value¹
Mean Water Temperature in Celsius (95% CI)	18.0 (16.8 - 19.2)	18.0 (16.9 - 19.0)	0.92
Mean pH (95% CI)	5.6 (5.4 - 5.7)	5.9 (5.8 - 6.0)	<0.001
Mean % Dissolved Oxygen (95% CI)	62 (56 - 67)	65 (60 - 69)	0.59
Mean Specific Conductance in mS/cm (95% CI)	91 (85 - 98)	132 (122 - 143)	<0.001

¹non-parametric Mann-Whitney U test for difference in mean ranks

***E. coli* concentration**

Of 196 total sampling events, an *E. coli* concentration was determined for 177 events. Two samples were not included because the sites were dry at the time of sample collection, and seventeen samples were excluded due to a laboratory error in culturing *E. coli* at a lower temperature than required. Table 8 identifies the number of times a site was sampled and the number of times a concentration was determined per site. An *E. coli* concentration was determined a median of 8 times for both swine and background sites between August 2016 and August 2017. Appendix C details physical water characteristics and *E. coli* concentrations for every sampling event.

Mean rank of *E. coli* concentration was higher ($p < 0.001$) among swine samples (1,284 CFU/100 ml, CI: 625-1,944, $n=103$) compared to background samples (687 CFU/100 ml, CI: 263-1,111, $n=74$). Additionally swine samples exceeded the EPA recommendation for recreational waters of 126 CFU/100 mL more often than background samples (73% vs. 42%, relative risk: 1.74, CI: 1.30 – 2.33, $p < 0.001$). Likewise, using *E. coli* alone instead of all fecal coliforms, swine sites were also almost twice as likely to be above the NC state standard for fecal coliform geometric monthly mean of 200 CFU/100 mL (RR=1.86, CI: 1.26 – 2.75, $p < 0.01$) and swine sites were twice as likely to be above the NC state monthly maximum fecal coliform standard of 400 CFU/100 mL (RR=2.21, CI: 1.28 – 3.83, $p < 0.01$) compared to background sites. Figure 6 displays a boxplot of \log_{10} -transformed *E. coli* concentrations measured at all sites. Table 9 presents swine and background sample mean, geometric mean, and median concentrations as well as the percent of samples above the EPA recommendation of a geometric mean of 126 CFU *E. coli*/100 mL in recreational waters[78]. The maximum concentration was 25,400 CFU/100 mL at swine site SW04 and 9,700 CFU/100 mL at background site BK15. Figure 6 identifies that all sites had samples above the EPA recommendation however the geometric mean of background samples (114 CFU/100 mL, CI: 113-116) is below the EPA recommendation while the geometric mean of swine samples (298 CFU/100 mL, CI: 296-299) is above the EPA recommendation. We also note that mean *E. coli* concentration for background and swine samples as well as the median concentration of swine samples are above the NC state standard for fecal coliforms in recreational freshwater is geometric mean of 200 CFU/100 mL.

These data indicate that while there is site-specific variation with some background sites (e.g. BK17U) having higher median *E. coli* concentration and some swine concentrations (e.g.

SW11) having lower median *E. coli* concentration, swine sites were significantly more likely to be above the EPA recommended standard compared to background sites without a CHO.

Table 8 - Descriptive statistics summarizing sample collection for 22 sites between August 2016 and August 2017 including the number of sites sampled, number of sampling events per site, and number of times *E. coli* concentration was determined

Site	n event dates	n <i>E. coli</i> concentration determined
BK01U	9	9
BK03	9	9
BK05U	9	8
BK10U	9	8
BK12	9	9
BK14	9	9
BK15	9	8
BK16	9	8
BK17U	9	6
SW01	9	9
SW04	9	9
SW05	9	8
SW05A	9	7
SW05C	9	8
SW07	9	9
SW09	8	7
SW10	9	9
SW11	9	9
SW13	8	7
SW16	9	7
SW17	9	7
SW17U	9	7

Table 9 - Descriptive statistics for *E. coli* concentration and % samples above EPA standard comparing background and swine samples

	Background	Swine
n Samples	74	103
Mean CFU/100mL (95% CI)	687 (263-1111)	1284 (625-1944) ***
Median CFU/100mL	104	252
% > EPA standard	42%	73% ***

***p<0.001; Proportions were evaluated using the test of equal proportions with Yates continuity correction. Mean rank concentration differences were evaluated using the Mann-Whitney U test.

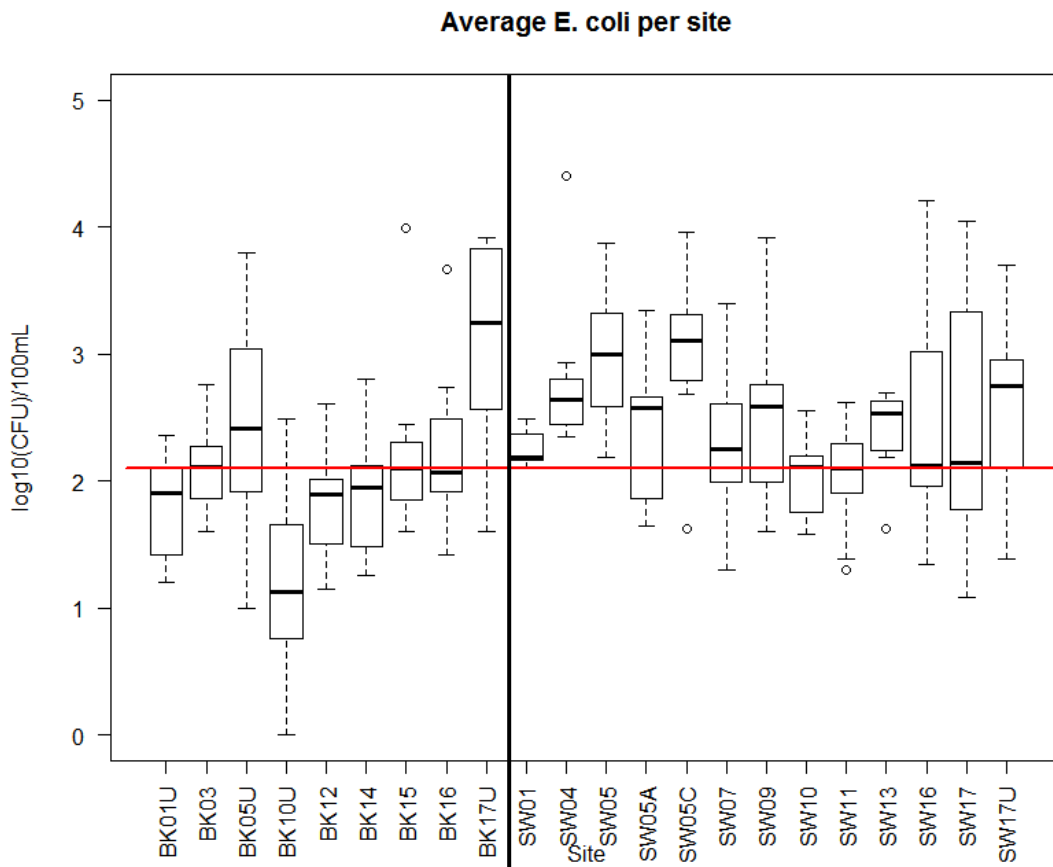


Figure 6 - Boxplot for log₁₀-transformed *E. coli* concentration for each site among all (n=177) events with background sites listed first. In each boxplot the thick black line represents the median value, the box represents the interquartile range between the 25th and 75th percentile, and the dotted lines extend up to 1.5 times the interquartile range. Circles represent statistical outliers. The red line represents the EPA recommended concentration of *E. coli* for recreational waters.

MST marker detection prevalence and concentration

This is the largest landscape-scale study to quantify swine-specific MST markers. Of 196 sampling events, 194 samples were analyzed for concentration of MST markers HF183 and pig-2-bac. Two samples were not analyzed because sites were dry at the time of sampling. Figure 7 presents boxplots of concentrations of HF183 and pig-2-bac for each site. Figure 8 displays events with detectable MST markers. We report prevalence of detection and difference in mean rank of MST markers HF183 and pig-2-bac with three thresholds defining detection. We compare prevalence and detection between swine and background samples. Accepted droplets per merged well ranged from 17,529 to 38,692 with a median of 33,042 for HF183 and pig-2-bac targets. The median for proportion positive droplets was below 0.005 for all detectable concentrations of HF183 and pig-2-bac with the exception of one high outlier for pig-2-bac concentration which had 89% positive droplets, possibly underestimating the concentration for this outlier.

The prevalence of detecting the human-feces-specific MST marker HF183 was 100% in swine sites (n=13) compared to 78%-100% of background sites (n=9) depending on definition of detection (high threshold, RR=1.3, CI: 0.91 – 1.8, p=0.30). HF183 was detected significantly more often in swine samples (n=115) compared to background samples (n=79) with a conservative definition of detection (high threshold: 34% vs. 20%, RR= 1.7, CI: 1.0-2.8, p=0.044). However this relationship did not hold true for mean and low thresholds of detection. While not all swine sites provided evidence of swine manure, the prevalence of detection for pig-2-bac was 77% in swine sites compared to 22% in background sites for all definitions of detection (RR=3.5, CI: 0.98-12, p=0.035). Furthermore, pig-2-bac was detected more often in

swine samples compared to background samples (19% vs. 4%, $R=5.2$, CI: 1.7- 17, $p<0.001$) with similar results across detection definition.

The mean difference in rank of HF183 concentration was significantly higher in swine compared to background samples only when using a conservative definition of detection with mean concentration of 1.6 vs. 1.5 copies HF183/mL, respectively ($p=0.046$). However, the mean difference in rank of pig-2-bac concentration was significantly higher in swine compared to background samples across all three definitions (Table 10). Mean concentrations of pig-2-bac compared to HF183 was 283 vs. 0.76 copies/mL respectively at the high threshold ($p=0.0016$). When comparing concentration of pig-2-bac to HF183 across all samples, we found that pig-2-bac was detected at significantly higher concentrations than HF183 across all detection definitions. Across all samples, mean pig-2-bac compared to HF183 concentration was 168 vs. 1.5 copies/mL ($p<0.001$) at the high threshold, 188 vs. 2.6 copies/mL at the mean threshold ($p<0.001$), and 200 vs. 5.7 copies/mL at the low threshold ($p<0.001$).

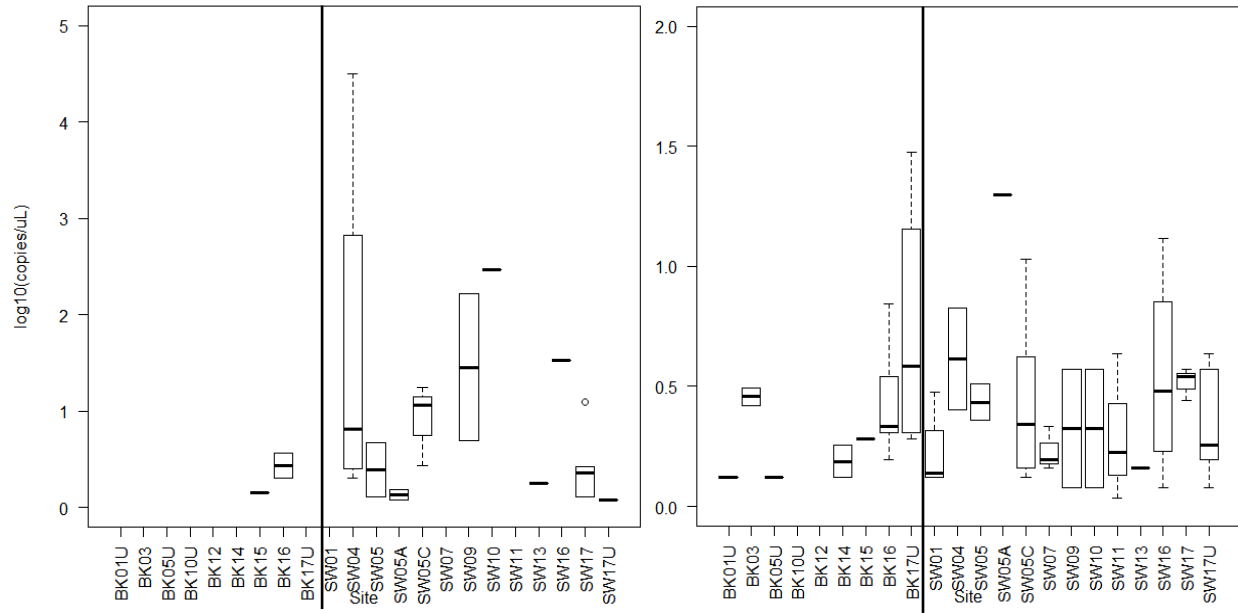


Figure 7 - Boxplots of log₁₀ concentration of MST markers, pig-2-bac (left) and HF183 (right), by site among all (n=194) events with background sites listed first. Concentration is defined as the high threshold for detection. In each boxplot the thick black line represents the median value, the box represents the interquartile range between the 25th and 75th percentile, and the dotted lines extend up to 1.5 times the interquartile range. Circles represent statistical outliers.

Table 10 - Descriptive statistics for MST marker concentrations (gene copies/ mL sample water) using high, mean, and low threshold definitions for detection, from background (n=79) and swine (n=115) samples and background (n=9) and swine (n=13) sites. Mean concentration incorporates below detect values that were set to half the value of the detection limit of 1.4 gene copies/mL.

	Threshold	HF183			pig-2-bac		
		Background	Swine	Test ¹	Background	Swine	Test ¹
n (%) samples positive	High	16 (20%)	39 (34%)	1.7 (1.0 – 2.8) *	3 (4%)	22 (19%)	5.2 (1.6 – 17) **
	Mean	35 (44%)	62 (54%)	1.2 (0.93 - 1.7)	3 (4%)	22 (19%)	5.2 (1.6 – 17) **
	Low	59 (75%)	87 (76%)	1.0 (0.88 - 1.2)	3 (4%)	24 (21%)	5.6 (1.8 - 18) **
n (%) sites positive	High	7 (78%)	13 (100%)	1.3 (0.91 - 1.8)	2 (22%)	10 (77%)	3.5 (0.98 - 12)*
	Mean	8 (89%)	13 (100%)	1.1 (0.89 - 1.4)	2 (22%)	10 (77%)	3.5 (0.98 - 12)*
	Low	9 (100%)	13 (100%)	1.0 (1.0 - 1.0)	2 (22%)	10 (77%)	3.5 (0.98 - 12)*
Mean Concentration (copies/mL)	High	1.5	1.6	0.046 *	0.76	283	0.0016 **
	Mean	2.4	2.7	0.11	0.76	317	0.0016 **
	Low	6.2	5.4	0.37	0.76	338	0.0011 **

¹ Proportions were evaluated using the test of equal proportions with Yates continuity correction and effect size estimated using relative risk with 95% confidence interval. Mean rank concentration differences were evaluated using the Mann-Whitney U test.
* p<0.05, **p<0.01

	2016					2017							
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
SW01					
SW04		.	.			/					.		.
SW05	
SW05A		
SW05C	/	/				.			/		.	.	
SW07		.	*		
SW09		.		.	/	.		.	/		.	.	
SW10	
SW11	
SW13		
SW16	/	
SW17	.			.	/						.	/	
SW17U	.				/	.			.				
BK01U	
BK03			
BK05U	
BK10U			
BK12	
BK14	
BK15	
BK16	.				/	.		.	/		.		
BK17U					

Legend	
	Not sampled
	Positive for pig-2-bac, below detect for HF183
	Positive for HF183, below detect for pig-2-bac
	Positive for both HF183 and pig-2-bac
.	Below detect for all outcomes listed above

Figure 8 - Sites and associated sampling events with detectable microbial source tracking markers using the high threshold HF183 (grey), pig-2-bac (red), or both (purple).

Discussion

Fecal Indicator Bacteria

This study found significantly higher ($p < 0.001$) *E. coli* concentrations in watersheds with CHOs compared to those without (1,284 CFU/100 ml, CI: 625-1,944 vs. 687 CFU/100 ml, CI: 263-1,111). Additionally swine samples exceeded the EPA recommendation for recreational waters of 126 CFU/100mL more often ($p < 0.001$) than background samples (73% vs. 42%, relative risk: 1.74, CI: 1.30 – 2.33). Our study found the strongest evidence to-date with respect to reported effect size and significance that watersheds with CHOs affect FIB concentrations in surface water more than background sites. Some other field studies have found higher FIB concentrations downstream compared to upstream of CHOs [20–22], however their measures of association or effect size were lower and had lower spatial sampling scale. Sapkota *et al.* found significantly higher ($p < 0.01$) downstream compared to upstream at one CHO site [22]. Heaney *et al.* found higher odds of FIB concentrations exceeding NC state standards downstream compared to upstream of CHOs at three sites One watershed study found that *E. coli* concentrations were positively associated ($p < 0.05$) with higher animal manure units upstream[20]. It is likely that these prior studies had lower measures of association or effect size than our study due to confounding fecal sources in upstream control sites because confounding fecal sources were not reported or there were known swine fecal sources upstream.

Some other field studies on whether there are effects of CHOs found conflicting results with either no difference or higher concentrations of FIB in out-of-basin control watersheds compared to watersheds with CHOs [25,26]. Bias can be introduced into studies with larger watershed areas when fecal sources are not characterized because fecal sources become more

disperse when far from the sampling location [61]. Additionally, as watershed area increases, surface waters receive larger runoff volumes and there is more uncertainty regarding FIB fate from fecal source to surface water. Of these studies, one had one out-of-basin control that was much smaller than the five sites with CHOs [25] while another [26] was conducted among small watersheds under 12 square miles that were distributed in multiple states. Both studies did not assess or identify other fecal sources such as human septic or wastewater treatment plants.

Microbial Source Tracking Markers

In studies assessing the effects of septic systems on small watersheds, Sowah *et al.* found septic density and septic distance to sampling but not sewer line density positively correlated with FIB concentration as well as HF183 marker yield [79]. They concluded that their study sites had pervasive septic rather than storm-related event effects especially in watersheds with higher septic density. HF183 was detected in 57% of their samples and its average concentration was between 6 and 18 copies/mL, with a maximum concentration of 501 copies/mL. Our study detected HF183 in 20-75% samples depending on detection definition with lower average HF183 concentration (1.5 – 6.2 copies/mL), and a lower maximum HF183 concentration of 30, 41, and 109 copies/mL at the high, mean, and low threshold definitions compared to Sowah *et al.* Since we do not have NC state data to assess septic density in swine and background watersheds, we consider that the lower HF183 concentrations in our study demonstrate lower density effects of septic or further distance to septic systems from sampling location in our study sites compared to Sowah *et al.*

We detected pig-2-bac significantly more often ($p < 0.01$) in swine samples compared to background samples (19% vs. 4%, relative risk: 5.2, CI: 1.6–17). Other landscape-scale studies

proximal to CHOs found similar or higher detection prevalence of swine-feces MST markers[20,21]. Heaney *et al.* detected similar prevalence of pig-2-bac in 21% of downstream samples with higher prevalence of 11% in upstream samples in NC (RR= 2.0, CI: 0.90-4.3). Jokinen *et al.* detected a different swine marker, PF163, in 29% of downstream samples compared to 8% of upstream samples ($p < 0.05$). These studies found somewhat lower measures of association which may be indicative of lower sampling scale or confounding swine fecal sources in upstream samples. These prior studies identify that their control sites were not pristine or not affected by swine fecal sources. Our study is the first to quantitatively assess swine MST markers in watersheds with and without CHOs.

Contextualization: Comparability to septic and wastewater treatment effluent

It is useful to consider *E. coli* and MST marker concentrations as indicators of microbial water quality and to compare them between regulated discharge (i.e. wastewater treatment plants) and other sources that are not regulated or considered non-discharge with respect to microbial effluent (i.e. septic systems, CHOs). Although lagoon effluent is land applied rather than discharged directly into surface water as with wastewater effluent, our study found that CHO sites had higher mean *E. coli* concentrations compared to treated wastewater effluent ($3.1 \log_{10}$ CFU/100 mL vs. $2.5 \log_{10}$ CFU/100mL [80]). We also note that background sites had average $2.8 \log_{10}$ CFU/100 mL, also higher than wastewater effluent, suggesting that effects from septic or wildlife in addition to CHOs may have contributed to high *E. coli* concentrations.

The highest *E. coli* concentration observed was at a presumptive lagoon discharge or irrigation leak ($4.4 \log_{10}$ CFU/100 mL), and is above concentrations found in lagoon waters or wastewater treatment effluent. *E. coli* concentration in lagoon effluent can be significantly

reduced through secondary treatment and/or the use of constructed wetlands. However, most CHOs (99.5%) in NC have only anaerobic treatment with reported *E. coli* concentrations as 5.3 log₁₀ CFU/100 mL[81] and 4.5-5.5 log₁₀ most probable number (MPN)/100 mL[18]. With constructed wetlands, *E. coli* concentrations can be reduced by up to 2 log₁₀, but effluent concentrations are still high at 3.4 log₁₀ CFU/100 mL[81] or between 1.5-3.5 MPN/100 mL [18]. Compared to wastewater effluent at 2.5 log₁₀ CFU/100 mL[80], *E. coli* concentrations in lagoon waters can be up to 3 log₁₀ above treated wastewater effluent. We note that NC state standards for water reuse for non-food crops is a daily maximum of 25 CFU *E. coli*/100 mL, which is well below lagoon water and surface water concentrations identified in this study and elsewhere [4].

Additionally our study assessed human and swine-associated MST markers to differentiate effects of septic from CHOs. Sowah *et al.* [79] quantified HF183 concentrations in comparable, small watersheds with varied septic density and reported that most concentrations of HF183 were between 0.75 and 1.25 log₁₀ copies HF183/mL with a maximum of 2.7 log₁₀ copies HF183/mL [82]. Our study found lower mean HF183 concentrations in our surface water samples (0.2 log₁₀, 0.41 log₁₀, and 0.76 log₁₀ copies/mL at the high, mean, and low threshold) compared to Sowah *et al.* suggesting that our sites may be affected by septic, but have, on average, lower concentrations of HF183 than Sowah *et al.*

We note that our most conservative (high threshold) reported mean pig-2-bac concentration (2.4 log₁₀ copies/mL) among swine sites is similar to the reported pig-2-bac concentration in lagoon waters which we report as 1.7 log₁₀ copies/mL (Figure 2) compared to 2.1 log₁₀ copies/mL reported elsewhere [38]. We identified three events at swine sites SW04, SW09, and SW10 with concentrations above 2.1 log₁₀ copies pig-2-bac/mL. The maximum pig-2-bac concentration of 31,872 copies/mL (4.5 log₁₀ copies/mL) was identified at what we

hypothesize to be a lagoon discharge in May 2017 at site SW04. This event was not after a precipitation event. The detected concentration of pig-2-bac from this event was over twice the concentration of reported pig-2-bac in lagoon waters and half the reported concentration detected in swine feces reported as 8.5 [38] and 10 log₁₀ copies/g [35]. Figure 2 shows ddPCR concentration output for this surface water event compared to lagoon waters. At this same event we also found the maximum concentration of *E. coli* (see outlier SW04 in Figure 6) and maximum specific conductance among all events in this study.

When comparing *E. coli* and MST marker concentrations to prior research on surface water effects from wastewater effluent and septic systems, we find that, on average, *E. coli* concentrations from swine and background sites are above wastewater treatment plant effluent concentrations. We also find that HF183 concentrations in this study are lower than other studies assessing effects of septic on surface water quality but that the maximum pig-2-bac concentration at a presumptive lagoon discharge are comparable to pig-2-bac concentration in lagoon effluent. We are unable to determine whether the maximum pig-2-bac concentrations were the result of a lagoon discharge, faulty irrigation, or the result of normal and compliant waste management practices. While our study is not representative to determine how often lagoon discharges occur, nor are we able to conclusively determine that a lagoon discharge occurred without access to on-farm records at this event, we were able to detect a probable lagoon discharge during our longitudinal study while only sampling surface water downstream from less than 2% (n=32) of CHOs in NC.

Limitations

One large limitation of this study is that we were not able to determine time of lagoon effluent land application. Prior work has found higher FIB concentrations post-manure compared to pre-manure concentrations in surface water[25]. As such, we cannot identify whether swine sites with high indicator concentrations are correlated to recent land applications, and it is unclear whether lower indicator concentrations are a result of not sampling after spray events or whether these facilities are sufficiently far away from streams with enough wetland or river buffer. Additionally, we did not know CHO-specific practices. Some manure management conditions can reduce, but not eliminate, FIB concentrations in lagoon effluent such as secondary treatment ponds and constructed wetlands in addition to long-term and high-temperature waste storage and composting [4,18,55,83] and different lagoon treatments reduce *E. coli* concentration. In this study CHO-specific manure management conditions for each facility in swine sites are not known and CHOs are assumed to apply effluent in accordance to nutrient management plan guidelines.

Additionally, we did not assess the effects of precipitation or fecal source density and distance on concentration of *E. coli* or MST markers. Microbial loads of FIB from land-applied fields can be transported to surface waters especially after precipitation [12], one of the most influential environmental variable for overland bacterial transport [28,55]. Although our study did not specifically target rain events, we had high enough temporal sampling scale to capture a range of seasonal conditions and captured some precipitation events at all sites. Protective factors to reduce FIB transport from CHOs may include increasing flow resistance through vegetation and river buffers and longer overland distance to surface waters from CHOs [28].

It is possible that *E. coli* concentration in some watersheds was affected by unmeasured parameters such as organic matter or type of wetland, whether constructed or natural, along rivers since nutrient availability and organic matter in soil can contribute to survival and growth of *E. coli* populations [55,84]. Other unmeasured variables that could affect *E. coli* growth and survival include competition from other microorganisms. This research assumes that organic matter and wetland type were not significantly different between watersheds with and without CHOs because watersheds were similar, small, agricultural, and from the same region in NC.

MST marker detection indicates fecal transport off-site, however age of fecal material cannot be determined from MST concentration and cross-reactivity with non-source hosts at low concentrations is a possibility. Detection of MST markers depends on MST marker decay from excretion to surface water sampling. For HF183 and pig-2-bac, 90% decay is about 2-3 days indicating that high concentrations of MST markers indicate recent fecal influent from the host source [35]. When comparing concentrations of MST markers, behavior of these markers should be known in order to quantify differential marker decay. Prior work has demonstrated environmental parameters dominate marker decay time, especially temperature and light. Field and lab studies indicate that pig-2-bac and HF183 behave similarly in varied temperature and light conditions, and that the 90% decay time is about 2-3 days in freshwater [35].

Host fecal sources contain high MST marker concentrations and high concentrations of MST markers in the environment are considered strong evidence for fecal material from the host-source. As such, samples with high concentrations of MST markers are not assumed to have high cross-reactivity. However, cross-reactivity can sometimes occur in low concentrations[39]. We consider that cross-reactivity for HF183 and pig-2-bac, if present, would have occurred similarly in both swine and background due to comparable land use and wildlife in the region.

Cross-reactivity would, then, bias results towards the null. We assume that it is not probable that HF183 had cross-reactions with swine feces in swine sites as cross-reactivity of HF183 in pig feces is low, with 98.6% specificity of HF183 among swine fecal samples in a meta-analysis[34]. Additionally, HF183 was targeted using ddPCR in one NC lagoon effluent sample in NC and was not detected (data not shown).

We present MST concentration results with three definitions of detection to better assess effects of potential cross-reactivity and inhibition. We found that threshold definition did not change measures of effect for pig-2-bac concentration between swine and background sites, however the threshold definition did change measures of effect for HF183 concentration. This suggests that cross-reactivity or inefficient assays affected the HF183 target more than pig-2-bac target. As fluorescence threshold lowers, more droplets are considered positive for detection and thus higher concentrations and higher prevalence of detection is expected as the threshold for detection is reduced. Low fluorescence or lack of droplet separation may indicate sub-optimal annealing temperature, correct binding of primer to target but with inhibition, or non-specific binding of primer (i.e. cross-reaction) resulting in some fluorescence [85]. To confirm whether inhibition or cross-reactivity occurred for HF183, a second human-specific fecal marker, HumM2, is recommended for confirmation of human source [33,36].

FIB concentrations correlate to some pathogens and is an indicator for human health risk, especially when assessing point-source contamination from wastewater treatment sewage [29,31]. However, correlation of MST markers to FIB or pathogen concentrations is currently not well-understood [29,32]. The use of MST markers provide evidence of fecal source in the surface water but should be used in conjunction with study design to reduce confounding sources of exposure.

Conclusion

The results demonstrate a larger effect size and difference between watersheds with and without CHOs compared to prior studies, which could be due to the study design's strengths including its spatial sampling scale in addition to the study design's control for confounding fecal sources. This study assessed that background and CHO sites were not significantly different with respect to agricultural land use, soil type, watershed area, and human population density. As such, the study reduced bias by having similar watershed areas and human population density between site types reduce confounding fecal sources from wildlife or human septic. While background sites were not pristine and were probably affected by fecal sources including septic and wildlife, the significantly higher *E. coli* concentrations and higher proportion of sites above EPA standards in swine sites can be attributed to the presence of CHOs because sites were assessed for similarity.

Not only did study design reduce confounding, but it also measured MST markers. Evidence was found that specifically swine-feces were in surface waters. Additionally, swine MST markers were found significantly more often in swine sites and at higher concentrations than human MST markers suggesting higher effects from swine feces compared to human fecal sources in these small, rural watersheds. While evidence was found of pervasive human fecal contamination at low concentrations, when swine fecal contamination was found, it was found at significantly higher concentrations than human fecal concentrations. The maximum swine concentration was higher than lagoon effluent concentrations. While the lack of a marker cannot demonstrate absence and presence of a marker in low concentrations may indicate cross-reactivity, the presence of a marker in high concentrations demonstrates recent fecal

contamination from the host-source. Whether swine feces were transported from swine lagoons, from sprayfields, faulty irrigation infrastructure is not known.

Not every swine site displayed high concentrations of *E. coli* or swine MST marker and not every background site displayed low concentrations of *E. coli*. Future work should incorporate environmental and spatial variables such as precipitation, manure density, and fecal source distances to determine differential exposure in watersheds with varied CHO and human septic geography. Additionally, while we are unable to assess human health risk from the microbial indicators assessed in this study, we are able to compare microbial water quality indicators and demonstrate significantly higher effects from CHOs compared to background sites such. Next steps in well-controlled studies should determine whether presence of CHOs contributes to higher incidence of bacteria pathogenic to humans found in swine feces and antimicrobial resistance elements. Prior studies have pointed to the inability to assess or compare the effects of wastewater treatment plants and industrial agriculture due to lack of well-designed studies. Future work should also attempt to understand relative effects of septic, wastewater treatment plants, and CHOs by comparing microbial indicators to comparable background sites without effects from confounding fecal sources.

CHAPTER 4: DO COMMERCIAL HOG OPERATIONS AFFECT PREVALENCE OF ANTIMICROBIAL RESISTANCE IN SURFACE WATERS?

Introduction

The basis for monitoring microbial water quality is protecting public health. Monitoring microbial water quality includes assessing AREs such as antibiotic resistant bacteria and ARGs from pathogens known to be found in human feces or animal manure. Increased antibiotic resistance has been implicated for an excess \$20 billion in US health costs with at least 2 million people with infections resistant to antibiotics needed for treatment [49] and antibiotic use in humans and animals have been implicated in contributing to increased antibiotic resistance (85). Antibiotics are routinely given in food-animal production for treatment of disease, and only recently in 2014, did the FDA ban antibiotic use for disease prevention in food-producing animals in the United States [46]. Of medically important antibiotics used to treat human disease, 70% of them are sold for use in food-producing animals [47,48]. There is concern the use of antibiotics in veterinary medicine and food animal production will provide selective pressure for the dissemination of antibiotic-resistant bacteria from commercial animal operations into the environment [41].

Of studies assessing AREs in surface waters proximal to CHOs, many parameters can be chosen to assess antimicrobial resistance including quantification of ARGs, assessing phenotypic resistance of a model organism such as *E. coli* or *Enterococcus*, or measuring antibiotic residues in surface waters. Priority parameters for monitoring and preventing dissemination of AREs are reported in the CDC [49] and the WHO [51] reports on prioritization of resistant pathogens and

antibiotics for risk management. Highest priority antibiotics include 3rd-5th generation cephalosporins and fluoroquinolones [51], which are considered last-resort antibiotics and were developed in addition to carbapenems to combat the prevalent beta-lactam resistance in gram negative bacteria [52]. These extended-spectrum antibiotics are effective even in the presence of many beta-lactamases. However, some beta-lactamases are still able to inactivate these extended-spectrum antibiotics including ESBLs and ACBLs. As such, CDC indicates that urgent and serious threats include carbapenem-resistant *E. coli* and ESBL-producing *E. coli* [49]. While the same active ingredients of these last-resort antibiotics are not approved for use in food-producing animals, a third-generation cephalosporin (ceftiofur) in addition to two fluoroquinolones with similar resistance mechanisms to active ingredients used for human medicine are approved for use in food-producing animals[48]. The US Government Accountability Office (GAO) and *Review on Antimicrobial Resistance* [9] call for increased surveillance of the food animal production environment and off-site transport from CHOs and other food animal production operations to better understand environmental microbial resistance from the animal production reservoir [8–10].

Mechanisms of off-site transport of AREs from CHOs include runoff from land-applied fields and ditch or tile-drain transport to surface waters. Research has found that swine manure contains antibiotic residues and concentrations of multi-drug resistant bacteria [13,14] and have found similar antibiotic resistance patterns in swine manure or lagoon effluent in the on-farm environment [13], in soils with manure application [15], and in effluent from tile-drains and ditches [6,15,16]. One study found similar ESBL genes in swine manure and receiving surface waters [17]. Additionally, AREs have been assessed along lagoon effluent treatment systems, including in anaerobic digesters and constructed wetland treatment, finding that while treatment

reduced fecal indicator bacteria (FIB) concentrations and thus also the concentration of AREs, AREs were still identified in effluent transported to soil and surface water [15,18]. A review on the fate and transport of AREs from land application of manure indicates that transport of AREs are driven by precipitation and soil conditions from field to surface water[6]. AREs may persist by sharing resistance genes among bacteria through conjugation or transduction [6]

A recent review of the dissemination of ARGs in the environment indicates that few studies have appropriately controlled for confounding sources of antibiotics and that effect size of CHOs or other sources on dissemination of ARGs in the environment is difficult to assess as a result[40]. Assessing baseline antibiotic resistance is important because naturally-occurring resistance has also been reported without the presence of antibiotics causing selective pressure [43]. As such, research assessing the effects of antibiotic use in humans or animals on the dissemination of antibiotic-resistant bacteria must carefully consider research design to control for natural environmental reservoirs of antibiotic resistance. Thus while comparing upstream to downstream of CHOs may provide evidence that CHOs contribute to AREs in the environment, the quantification of the effect size is difficult without assessing baseline AREs upstream and other contributing sources of AREs in the environment.

Furthermore, few studies have compared AREs in surface water proximal to CHOs to a control group. Significantly higher phenotypic resistance was observed among *Enterococcus* [22] and fecal coliforms [23] in studies comparing upstream to downstream of CHOs. Another study [15] quantified 22 antibiotic resistance genes along the swine manure treatment system, manure-applied soils, effluent-receiving ditches, downstream surface waters, and an upstream reservoir as a control. This study concluded that while antibiotic resistance gene concentration is reduced between manure treatment and the receiving surface waters, the environment still

receives discharge from the CHO and downstream surface waters have higher antibiotic resistance gene concentrations compared to the upstream reservoir. These studies, however, did not identify land use in upstream samples to determine the other sources of AREs such as from human or naturally occurring resistance that may contribute to surface waters. As such, this limits the ability to conclusively identify the CHO as the source for increased antibiotic resistance downstream.

While it is clear that AREs have been detected proximal to CHOs into surface waters and that it is probable that CHOs contribute to AREs in the environment, the effect size of the contribution of AREs from CHOs compared to background and other sources of AREs have not been well-studied. The lack of effect size from CHOs is partially due to the mixed methods (i.e. genotypic/phenotypic) and the variety of parameters that are possible to assess with some studies focusing on high impact public health parameters (such as vancomycin resistance) [25,26]), while others have focused on assessing a more complete resistance profile of the sample [15] or of phenotypic bacterial resistance [22,23].

NC is one of the leading states in the nation for food animal production, especially its \$4 billion swine production industry, and NC has the highest density of swine in the country[2]. While NC state requirements in nutrient management plans prohibit transport of swine waste by runoff , discharge, or land application to surface water [65], almost all NC CHOs are regulated as non-discharge sources so monitoring is not required to assess off-site transport AREs. We designed a longitudinal, landscape-scale monitoring study in similar, small, agricultural watersheds with and without CHOs to address concerns of bias and confounding identified in the literature. We assess the prevalence of antibiotic resistance among *E. coli*, including beta-lactamase producing *E. coli* identified by the CDC as a priority ARE parameter for public health,

to determine the effect size of the presence of CHOs on these indicators of water quality and human health risk.

Materials and Methods

Site Selection

The same sites identified in Chapter 3 were assessed. See Figure 1, Table 2, and Table 3 for site comparability with respect to watershed land use variables. Appendix A presents detailed maps for each sample site.

***E. coli* isolation**

Following sample collection and membrane filtration of surface water samples on selective media as described in Chapter 2, up to six presumptive *E. coli* colonies per sample were isolated, purified, and confirmed through biochemical testing including indole production using Kovac's reagent. Isolates were taken from different dilution plates when possible to reduce possibility of selecting clones.

All isolates were archived in a tryptic soy broth with 15% glycerol solution at -80 °C. Antimicrobial resistance testing was conducted on all archived confirmed *E. coli* isolates using standard Kirby-Bauer disc diffusion methods and following standard Clinical Laboratory Standards Institute (CLSI) guidelines [86]. Isolates were tested for resistance to eleven antibiotics comprising nine antibiotic classes as recommended by NARMS [87] and CLSI [86] including antibiotics used primarily in industrial agriculture [54] and antibiotics used primarily in human medicine [47] with risk assessment priority levels assigned based on WHO criteria [51]

(Table 11). Multi-drug resistance was defined as resistance to three or more classes of antibiotics.

Isolates were also screened for two types of beta-lactamase production: ACBL and ESBL production. For this study, a positive screen for ACBL production is resistance to cefoxitin [88], and for ESBL production is intermediate or complete resistance to ceftriaxone [86]. Isolates with a positive screen for ACBL production were confirmed through the disc approximation test [88] and isolates with a positive screen for ESBL production were confirmed using CLSI protocol [86]. *E. coli* with a positive screen for ACBL or ESBL production were tested for two genes encoding beta-lactamase genes, *bla_{CMY2}* and *bla_{TEM}*, using polymerase chain reaction at the NC State University Clinical Microbiology Laboratory [89].

Data Analyses

To evaluate the difference in prevalence outcomes between swine and background samples a test of equal proportions with Yates continuity correction was used. Prevalence outcomes include proportion of samples with proportion isolates and samples resistant to each antibiotic, proportion isolates and samples positive for beta-lactamase producing *E. coli*, proportion isolates and samples resistant to one, two, and more than two classes of antibiotics. Additionally, a measure of effect size was included when comparing prevalence outcomes by calculating a RR with a 95% CI. Analysis includes comparisons at the isolate, sample, and site level where appropriate.

Table 11 - Antibiotic, class, and concentration included in antimicrobial resistance testing of *E. coli* and their use in veterinary and/or human medicine, and World Health Organization (WHO) priority

Antibiotic	Antibiotic Class	Concentration (ug)	Veterinary Use	Human Use	WHO Priority
Amoxicillin-Clavulanate Acid	Penicillin	20/10	Yes	Yes	High Priority Critical
Ampicillin	Penicillin	10	Yes	Yes	High Priority Critical
Cefoxitin	Cephalosporin	30	No	Yes	Highly Important
Ceftriaxone	Cephalosporin III	30	No	Yes	Highest Priority Critical
Chloramphenicol	Amphenicol	30	Yes	Yes	Highly Important
Ciprofloxacin	Fluoroquinolones	5	No	Yes	Highest Priority Critical
Gentamicin	Aminoglycosides	10	Yes	Yes	High Priority Critical
Imipenem	Carbapenem	10	No	Yes	High Priority Critical
Levofloxacin	Fluoroquinolones	5	No	Yes	Highest Priority Critical
Sulfamethoxazole-Trimethoprim	Sulfas	24/1	No	Yes	Highly Important
Tetracycline	Tetracyclines	30	Yes	Yes	Highly Important

Results

Water Characteristics

Water characteristics are reported in Table 7 of Chapter 3. Appendix C identifies physical water characteristics for every sampling event.

Antibiotic Resistance

Of the 194 samples collected, *E. coli* isolates were confirmed and isolated from 193 events. One event was not included because indole-positive *E. coli* isolates were not identified.

In total, 912 confirmed *E. coli* were isolated from swine (n=556) and background (n=356) sites. A median of five *E. coli* isolates were archived and tested for antibiotic resistance for each sampling event, an average of 40 *E. coli* isolates were archived over the study period for each background site, and an average of 43 isolates archived for each swine site. Table 12 identifies the number of isolates tested per site for antibiotic resistance and the average number of *E. coli* isolates tested per sampling event per site to demonstrate that background and swine sites were sampled similarly. Fewer than three *E. coli* isolates were tested for antibiotic resistance from two swine events and five background events because isolated colonies were not confirmed indole producers and/or sample *E. coli* concentration was too low. Appendix B identifies antibiotic resistance profiles for every isolate.

Antimicrobial resistance to at least one antibiotic was observed in 19% of isolates collected from swine samples compared to 6% of isolates from background samples (RR=3.2, CI: 2.1 – 5.1, p<0.001) (Table 3). For every antibiotic with observed resistance, resistance was more often observed in isolates from swine sites compared to those from background sites. Tetracycline resistance was the most commonly observed with 17% of swine isolates compared to 5% of background isolates (RR=3.2, CI: 2.0 – 5.2, p<0.001) followed by ampicillin resistance in 5% swine isolates compared to 0.8% background isolates (RR=6.0, CI: 1.8 – 20, p<0.001). Intermediate resistance was included in the susceptible group for relative risk and significance tests. Intermediate resistance was observed to amoxicillin-clavulanate acid in two swine isolates, to ampicillin in four background and four swine isolates, to cefoxitin in two swine isolates, to ceftriaxone in one background and one swine isolate, to chloramphenicol in three swine isolates, and to sulfamethoxazole-trimethoprim in one background and one swine isolate.

Swine samples were more likely to be resistant to a higher number of antibiotic classes. Multi-drug resistance, defined as resistance to three or more classes of antibiotics, was observed among 2.5% of *E. coli* isolates from swine sites compared to 0.28% of *E. coli* from background sites (RR=9.0, CI:1.2-68, p<0.05) (Table 13, Figure 9). Multi-drug resistance was observed at four swine sites and once at one background site (RR=8.1, CI: 1.1 – 61, p<0.05) (Table 13, Figure 9). Antibiotic resistance profiles of multi-drug resistant isolates are identified in Table 14. Nine isolates from four swine sites and one isolate identified at a background site were confirmed to be beta-lactamase producing *E. coli* (Table 13, Figure 9). Confirmation of isolates positive for beta-lactamase production is detailed in Table 15. Figure 9 identifies sampling events in background and swine sites with positive outcomes for antibiotic resistance to one, two, or three classes of antibiotics, or positive for beta-lactamase production. Finally, although prevalence of resistance to individual antibiotics with WHO highest priority critical was low, resistance was only found among swine samples. When combined, swine samples were significantly (p<0.05) more likely to be resistant to highest priority critical antibiotics (i.e. fluoroquinolones and third generation cephalosporins combined) compared to background sites.

Table 12 - Descriptive statistics summarizing sample collection for 22 sites between August 2016 and August 2017 including the number of sites sampled, number of sampling events per site, number of times antibiotic resistance was determined, and number of *E. coli* isolates archive

Site	n event dates	n <i>E. coli</i> isolates	n events	Average n <i>E. coli</i> isolates per event
BK01U	9	47	9	5.2
BK03	9	45	9	5
BK05U	9	40	9	4.4
BK10U	9	24	7	3.4
BK12	9	38	9	4.2
BK14	9	43	9	4.8
BK15	9	43	9	4.8
BK16	9	41	9	4.6
BK17U	9	35	8	4.4
SW01	9	46	9	5.1
SW04	9	46	9	5.1
SW05	9	43	9	4.8
SW05A	9	43	9	4.8
SW05C	9	41	9	4.6
SW07	9	44	9	4.9
SW09	8	39	8	4.9
SW10	9	45	9	5.0
SW11	9	45	9	5.0
SW13	8	40	8	5.0
SW16	9	43	9	4.8
SW17	9	39	9	4.3
SW17U	9	42	9	4.7

Table 13 - Number, percent, and relative risk of *E. coli* isolates and samples with observed resistance to individual antibiotics, to number of antibiotic classes, as well as priority outcomes including beta-lactamase production from water samples collected from

	Antibiotic Resistance	Background Isolates n (%)	Swine Isolates n (%)	Relative risk (95% CI)	Background Samples n (%)	Swine Samples n (%)	Relative Risk (95% CI)
	n isolates or samples	356	556	n/a	78	115	n/a
Individual Antibiotic	Amoxicillin-clavulanate acid (AmC)	1 (0.3%)	4 (7%)	2.6 (0.29– 23)	1 (1.3%)	3 (2.6%)	2.0 (0.22 – 19)
	Ampicillin (AM)	3 (0.8%)	28 (5%)	6.0 (1.8– 20) **	3 (3.8%)	22 (19%)	5.0 (1.5 – 16) **
	Ceftriaxone (CRO)	0	7 (1%)	n/a	0	6 (5.2%)	n/a
	Chloramphenicol (C)	0	5 (0.9%)	n/a	0	5 (4.3%)	n/a
	Ciprofloxacin (CIP)	0	2 (0.4%)	n/a	0	2 (1.7%)	n/a
	Cefoxitin (FOX)	1 (0.3%)	4 (0.7%)	2.6 (0.29– 23)	1 (1.3%)	3 (2.6%)	2.0 (0.22 – 19)
	Gentamycin (GM)	0	2 (0.4%)	n/a	0	2 (1.7%)	n/a
	Imipenem (IPM)	0	0	n/a	0	0	n/a
	Levofloxacin (LVX)	0	2 (0.4%)	n/a	0	2 (1.7%)	n/a
	Sulfamethoxazole-trimethoprim (SXT)	0	7 (1%)	n/a	0	7 (6.1%)	n/a
	Tetracycline (TE)	19 (5%)	96 (17%)	3.2 (2.0 – 5.2)***	14 (18%)	47 (41%)	2.2 (1.3 – 3.8) **
Number of Antibiotic Classes	Resistance to at least 1 antibiotic class	21 (6%)	106 (19%)	3.2 (2.1 – 5.1)***	16 (21%)	51 (44%)	2.2 (1.3 – 3.5) **
	Resistance to at least 2 antibiotic classes	1 (0.3%)	25 (4.5%)	16 (2.2–118) ***	1 (1.3%)	19 (17%)	12 (1.8 – 94)**
	Resistance to at least 3 antibiotic classes ¹	1 (0.3%)	14 (2.5%)	9.0 (1.2 – 68)*	1 (1.3%)	12 (10%)	8.1 (1.1 – 61) *
Priority Outcomes	Beta-lactamase production ²	1 (0.3%)	9 (1.6%)	5.8 (0.7 – 45)	1 (1.3%)	8 (7.0%)	5.4 (0.69 – 43)
	Highest Priority Critical (CRO, CIP, or LVX)	0	9 (1.6%)	n/a *	0	8 (7.0%)	n/a *
	High Priority Critical (AmC,AM,GM,or IPM)	3 (0.84%)	30 (5.4%)	6.4 (2.0 – 21) ***	3(3.8%)	23 (20%)	5.2 (1.6 – 17) **
	Highly Important (C, FOX, SXT, or TE)	19 (5.3%)	97 (17%)	3.3 (2.0– 5.2)***	14 (18%)	48 (42%)	2.3 (1.4–3.9)***

¹Defined as multi-drug resistant, See Table 14 for isolate-specific resistance profiles

²Defined as confirmed AmpC or extended-spectrum beta-lactamase production, either by culture or PCR. See Table 15 for differentiation by isolate

*** test of equal proportions with Yates continuity correction p<0.001, ** p<0.01, * p<0.05

Table 14 - Antibiotic resistance profiles for multi-drug resistant *E. coli* isolates. R=resistant, S=Susceptible, I=Intermediate resistance

Isolate ID	Site	Event Date	AmC	AM	FOX	CRO	C	CIP	GM	IMP	LVX	TE	SXT
E559	BK15	3/23/2017	R	R	R	I	S	S	S	S	S	R	S
E187	SW04	10/7/2016	I	R	S	S	R	S	S	S	S	R	S
E532	SW04	2/20/2017	R	R	R	R	S	S	S	S	S	S	S
E756	SW04	5/9/2017	S	R	S	S	S	S	R	S	S	R	R
E226	SW05	11/7/2016	S	R	S	R	S	S	S	S	S	R	S
E360	SW05	12/14/2016	S	R	S	R	S	S	S	S	S	R	S
E590	SW05	3/23/2017	S	S	S	S	R	S	S	S	S	R	R
E682	SW05	4/24/2017	S	R	S	S	S	S	R	S	S	R	S
E905	SW05	7/18/2017	S	R	S	R	S	S	S	S	S	R	S
E687	SW05C	4/24/2017	S	R	S	S	R	R	S	S	R	R	S
E779	SW05C	6/5/2017	R	R	R	R	S	S	S	S	S	R	S
E781	SW05C	6/5/2017	R	R	R	R	S	S	S	S	S	R	S
E782	SW05C	6/5/2017	S	R	S	S	R	S	S	S	S	R	R
E899	SW05C	7/18/2017	S	R	S	S	R	S	S	S	S	R	R
E379	SW09	12/14/2016	S	R	S	S	S	R	S	S	R	R	R

Table 15 - Description of isolates confirmed as beta-lactamase producing *E. coli*, whether extended-spectrum beta-lactamase (ESBL) or AmpC beta-lactamase (ACBL).

Isolate ID	Site	Event Date	Positive Screen	Confirmed by Culture ESBL	Confirmed by Culture ACBL	<i>bla</i> _{TEM} Positive	<i>bla</i> _{CMY2} Positive
E559	BK15	3/23/2017	ESBL, ACBL	0	0	0	1
E087	SW01	9/5/2016	ACBL	n/a	1	0	1
E188	SW04	10/7/2016	ESBL	0	n/a	1	0
E532	SW04	2/20/2017	ESBL, ACBL	0	0	0	1
E014	SW05	8/9/2016	ESBL	1	n/a	0	0
E226	SW05	11/7/2016	ESBL	1	n/a	0	0
E360	SW05	12/14/2016	ESBL	1	n/a	0	0
E905	SW05	7/18/2017	ESBL	1	n/a	0	0
E779	SW05C	6/5/2017	ESBL, ACBL	Undetermined ¹	Undetermined ¹	0	1
E781	SW05C	6/5/2017	ESBL, ACBL	Undetermined ¹	Undetermined ¹	0	1

¹This isolate was resistant to the antibiotics used for differentiation purposes to confirm a positive screen for beta-lactamase production. Confirmation could not be assessed using these culture-based methods.

	2016					2017							
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
SW01		aβ	.	.		*
SW04		*	@a*β	.		.	@aβ		a*	@a*	a		.
SW05	a*β	a*		@*β	@*β	*		@*	@*		*	@*β	
SW05A	.	.		*	.	*		*	*		.	.	
SW05C	*	*		*	*	*		*	@*		@*β	@	
SW07		.	*
SW09		.		.	@a	
SW10		.	.	*		.	.		.	*	*		*
SW11		*
SW13		
SW16	*		.	*	
SW17	.	a*		*	*	.		*	a*		*	*	
SW17U	.	.		*	.	.		*	*		*	.	
BK01U		.	*	.		*
BK03		*		.
BK05U	.	.		.	*	.		.	*		.	.	
BK10U			
BK12		.	.	.		*
BK14		.	.	*		.	.		.	*	.		*
BK15		@β	*		.	.	
BK16	*		.	.	
BK17U		.		*	.	.		*	.		*	*	

Legend	
	Not sampled
	Resistance to highest priority antibiotics
@	Resistance to 3 classes of antibiotics
a	Resistance to 2 classes of antibiotics
*	Resistance to 1 class of antibiotics
β	Positive for beta-lactamase production
.	Below detect for all outcomes listed above

Figure 9 - Sites and associated sampling events with at least one isolate positive for resistance to at least one class of antibiotics (*), at least two classes of antibiotics (a), at least three classes of antibiotics/multi-drug resistant (@), and/or positive for beta-lactamase production (β)

Discussion

This study is the largest study with respect to spatial and temporal scales and number of classes tested for phenotypic resistance among studies assessing influence of CHOs on phenotypic resistance in fecal indicator bacteria in surface water. This study tested antibiotic resistance measures from 22 sites comprising 193 sampling events, followed by West *et al.*'s study assessing 6 sites with 30 sampling events in total. Additionally, this is the first study assessing influence of CHOs on AREs in surface water to have well-defined control sites with respect to swine and human fecal sources. The results indicate that while there is some variability within each group (e.g. multi-drug resistance observed in BK15, no resistance observed in SW13), background samples never had higher proportions of antibiotic resistance compared to swine samples. Sites with CHOs have higher proportion sites resistance to multiple classes of antibiotics, higher proportion samples resistant to WHO highest priority antibiotics, and higher proportion samples positive for beta-lactamase producing *E. coli* compared to background sites. While the confidence intervals are large demonstrating the intra-group variability, the relative risk estimates of effect size indicate that resistance to tetracycline, ampicillin, resistance to more than one, two, and three antibiotics are all at least twice as likely ($RR > 2.0$, $p < 0.05$) than at background sites. Additionally, although resistance to highest priority antibiotics was observed in low prevalence, their detection could signify a public health risk and steps should be taken by state and local regulatory agencies to better determine the source of the selective pressure, whether from CHOs, human septic, improperly disposed antibiotics, or in background natural sources.

While observational groups were assessed for comparability with respect to land use variables, it is possible that *E. coli* survival was affected by unmeasured parameters such as

organic matter or type of wetland. Nutrient availability and organic matter in soil can contribute to survival and growth of *E. coli* populations [55,84]. Other unmeasured variables that could affect *E. coli* growth and survival include competition from other microorganisms.

Prior Work: Antibiotic Resistant Bacteria

Significantly higher phenotypic resistance was observed among *Enterococcus* [22] and fecal coliforms [23] in studies comparing upstream to downstream of CHOs. These studies, however, did not assess ambient resistance or other potential sources of AREs in the environment such as from human or naturally occurring resistance that may contribute to surface waters and which limits ability to conclusively identify the CHO as the source for increased antibiotic resistance downstream. Without rigorously quantifying fecal sources or controlling for confounding fecal sources, these studies have lower evidence that fecal contamination is from the CHO [5].

Among studies assessing phenotypic antibiotic resistance proximal to CHOs, one study assessed resistance of FIB *Enterococcus* to 5 antibiotics and found higher prevalence of resistant bacteria to four antibiotics downstream compared to upstream of one CHO [22]. Another study assessed phenotypic resistance of fecal coliform isolates to five antibiotics and found higher multi-drug resistance in downstream (42%) isolates compared to upstream (17%) isolates at three CHO sites[23]. When multi-drug resistance is defined as resistance to three or more classes of antibiotics, this study finds 14% of isolates resistant in downstream samples compared to 1.5% isolates in upstream samples[23] (prevalence difference=12.5%). This study may have found lower multi-drug resistance prevalence (prevalence difference=8.3%) when compared to West *et al.* due to lower isolate density (five isolates per sample compared to thirty isolates per sample), our correction for potential clonality at the sample level, more samples assessed, and only

assessing resistance among *E. coli* rather than all fecal coliforms. Similarly to these studies, we found higher prevalence of antibiotic resistance downstream compared to upstream of CHOs. In addition to these studies, this study tested isolates to more antibiotics among more samples and also presented results at the sample level in addition to the isolate level to correct for clonality among isolates from each sample.

We identified ESBL or ACBL *E. coli*, a bacterium identified as a current antibiotic resistance threat by the CDC[49], in eight samples from swine sites and one sample from a background sample. Most, but not all isolates confirmed as ESBL were also multi-drug resistant. Because over 200 ESBL genes have been identified [90], it is possible that we have underestimated beta-lactamase production prevalence among our samples. Most beta-lactamase genes are shared by horizontal transmission via plasmid and can be shared between and within bacterial species [52]. This is of concern because swine feces can contain bacteria pathogenic to humans including *Campylobacter*, *Salmonella*, and *Yersinia*[28]. The most common gene families transferred by plasmids include *TEM*, *SHV*, and *CTX* for ESBL and *CMY* for ACBL [44,52]. While ESBL genes compared to ACBL genes may be more important for human health [91], plasmid-mediated *CMY* and *CTX* are most important for beta-lactam resistance among food animals and are candidates for zoonotic transmission so should be monitored. Similar plasmids encoding ESBL genes have been found in both food animals, humans, and the environment indicating transmission between food animals and humans and the surrounding environment [52,92,93]. We are not able to determine risk to human health due to the presence of ESBL or ACBL *E. coli* in surface water samples from this study.

While culture-based, phenotypic approaches are needed to determine that resistance in bacteria is expressed, it is unable to assess sample-level concentrations of antibiotic resistance

genes. In some instances, phenotypic results may be negative but molecular methods positive indicating that molecular methods may be more sensitive than phenotypic methods, that the gene is from a dead or non-culturable bacterium, or that the gene is present but not phenotypically expressed [91]. Our study did not assess the total ARE profile of the surface water samples collected. Additionally, because we tested, on average, five isolates from each sample, we likely somewhat oversampled resistance in background samples compared to resistance in swine samples since *E. coli* concentrations were much higher in swine compared to background samples (see Chapter 3). When testing multiple isolates per sample there is a possibility of assessing resistance among clonal isolates from the same sample. However we reduced this possibility by selecting isolates from different dilution plates and presenting relative risks of resistance at the isolate and sample event levels.

Conclusion

Because this study systematically compared watersheds with and without CHOs, this study provides effect measures for different phenotypic antibiotic resistance measures, unlike prior work. This is due to the study design's strengths including its spatial sampling scale in addition to the study design's control for confounding fecal sources and control for natural environmental reservoirs of antibiotic resistance. In contrast to other studies, our study assessed that background and CHO sites were not significantly different with respect to agricultural land use, soil type, watershed area, and human population density. As such, we reduced bias by having similar watershed areas and human population density between site types reduce confounding fecal sources from wildlife or human septic. While background sites were not pristine and were probably affected by fecal sources including septic and wildlife, the

significantly higher proportions of resistant samples in swine sites can be attributed to the presence of CHOs because sites were assessed for similarity. Other studies assessing antibiotic resistance measures in sites affected by CHOs have not done similar analysis when comparing to control sites.

Not every swine site had high proportion of *E. coli* isolates resistant to antibiotics and one background sites had multi-drug resistance. Future work should incorporate environmental and fecal exposure variables such as precipitation, manure density, and fecal source distances to determine differential exposure in watersheds with varied CHO and human septic geography.

Antibiotic resistance does not always correlate to presence of pathogens, however horizontal transfer of antibiotic resistance elements to pathogens is an important mechanism for determining human health risk. While we are unable to assess human health risk from the antibiotic resistance observed in this study, we are able to demonstrate significantly higher effects from CHOs compared to background sites. Prior studies have pointed to the inability to assess or compare the effects of wastewater treatment plants and industrial agriculture due to lack of well-designed studies [61]. As such we are not able to compare relative contribution of CHOs and wastewater treatment plants to AREs in surface waters. However because we controlled for human and wildlife sources of feces by sampling background sites comparable to swine sites, it is likely that the increased prevalence of antibiotic resistance in swine site samples is a result of the presence of CHOs and the antibiotic use practices in CHOs. We do not know whether increased resistance from CHOs is specifically from overland flow, from lagoon seepage, or from another transport mechanism.

Next steps in well-controlled studies should determine whether presence of CHOs contributes to higher incidence of bacteria pathogenic to humans found in swine feces and

consider conjugation studies as in West *et al.* (2011) to determine possibility of horizontal antibiotic resistance gene transfer.

CHAPTER 5: WHAT ARE THE FACTORS THAT CONTRIBUTE TO OR PROTECT FROM CHO EFFECTS ON MICROBIAL WATER QUALITY?

Introduction

Little research has been conducted modeling the influence of fecal sources on bacterial fecal contamination, such as concentrations of FIB, AREs such as ARGs, and MST markers, genetic markers specific to fecal sources. Even fewer studies are specifically related to the potential effects of CHOs and have incorporated exposure variables for CHOs to understand whether they have effects on microbial quality of surface waters. There has been some criticism of studies assessing FIB, ARGs, and MST markers to move beyond monitoring and to incorporate quantification of exposure when assessing effects from sources [61]

Most research assessing FIB contamination from CHOs has assessed upstream compared to downstream of CHO for concentration of FIB, however land use for upstream control sites are usually ill-defined [15,22,23] or have known influence from swine upstream [20,21,60]. Indeed, a review of environmental dissemination of ARGs noted that most research, whether studying CHOs or wastewater treatment plants, lacks proper controls to ascertain effects on ARG concentration from confounding sources [40]. This seems to hold true for the majority of research assessing CHOs as a source of fecal contamination as well.

Only two prior studies have incorporated land use variables to assess bacterial fecal contamination from CHOs in surface waters beyond comparing presence of a CHO to absence of a CHO [20,26]. Haack *et al.* 2016 [26] used discriminatory analysis to determine if FIB before rain or after rain was associated with land use variables (e.g. percent wetland or agriculture), or

exposure variables such as the number of animals upstream or the percent of watershed area receiving manure. Mean FIB concentrations were higher in control sites compared to swine sites and the only landscape variable significantly associated with any FIB concentration was soil runoff soil runoff potential.

The second study [20] used classification tree data analysis to determine whether land use, hydrological, and exposure variables affected concentrations of FIB downstream of CHOs. Exposure variables included land area for manure application from any CHO and CHO manure produced. Jokinen *et al.* found that higher concentrations of two types of FIB were significantly associated with higher livestock density and negatively correlated with higher native pasture. Additionally, while Jokinen *et al.* had higher temporal and spatial sampling scale than Haack *et al.*, Jokinen *et al.* did not incorporate a control without influence from swine production to assess baseline fecal contamination, while Haack *et al.* had comparable out-of-basin control sites for comparison.

While both studies incorporated land use and exposure variables, neither controlled for confounding sources of FIB, such as from humans, nor incorporated proximity in variables approximating exposure to fecal sources. Studies assessing effects of other fecal sources, such as from cattle concentrated animal feeding operations, wastewater treatment plants [94], or septic systems[82] have identified that not only density of source (i.e. number of animals or density of septic), but also proximity variables such as flow distance or overland flow are predictors for higher fecal contamination.

To date, only two studies have modeled CHO effects on microbial water quality using land use variables in conjunction with exposure variables to assess CHO effects on bacterial fecal contamination. Neither controlled for nor quantified exposure for confounding fecal

sources. Additionally, there has not yet been research modeling CHO effects on microbial quality of surface waters using CHO exposure variables incorporating proximity to surface water, using exposure variables for confounding fecal sources, and comparing CHO-affected surface water to control sites without CHOs. As such, this chapter models *E. coli* concentration using CHO exposure variables comprising density and proximity metrics, watershed land use, hydrological, and environmental variables to determine whether CHOs affect microbial quality of surface water. The models control for confounding fecal sources, and variables are identified that contribute to or protect from higher concentrations of *E. coli*. Models use *E. coli* concentration outcomes from a longitudinal, landscape-scale monitoring study in watersheds without and with varying effects of CHOs. Finally, this chapter compares different exposure metrics to determine whether density or proximity metrics for different fecal sources best explain concentration of *E. coli* in nearby surface water.

Materials and Methods

This section describes dependent variable data, independent variable and exposure variable construction, model development, and methods assessing model performance and effect of independent variables.

Dependent variable data

A longitudinal, landscape-scale monitoring study was conducted to compare *E. coli* concentrations among sites without and sites with varying effects from CHOs. Site selection and sampling methods are discussed in Chapter 3. This chapter models log₁₀ concentration of *E. coli* collected a median of 8 times from 9 background sites without CHOs and 13 swine sites with

CHOs in the upstream watershed (see Table 8, Chapter 3) for a total of 177 sampling events. Sampling locations represented watersheds with an average area of four square miles and were not significantly different with respect to land use types, watershed area, soil type, and human population metrics between observational groups (see Table 4, Chapter 3).

Independent variables

Independent variables are categorized as land use variables, measured hydrological variables, and exposure variables. Twenty-two non-exposure independent variables, x_n , are described in Table 16. Land use variables incorporate variables that are hypothesized to prevent or contribute to transport of bacteria via overland flow and are measurements describing the entire watershed. Land use variables include soil parameters from the SSURGO database [95] including hydrologic soil types A through D to model soil drainage capacity [26,67]. Additional watershed land use variables were compiled in ArcMap 10.1 [72] were taken from the 2016 Cropland Data Layer (CDL) [70] for percent land use (i.e. forest, wetland, cultivated). Finally land use variables related to river buffers along National Hydrography Dataset (NHD) flowlines [96] were created in ArcMap 10.1 [72] to determine if protective effects are observed [18]. Environmental variables incorporate variables that are time-dependent and affect transport of bacteria including precipitation metrics identified at sampling event latitude and longitude locations from the gridded precipitation data obtained from the National Weather Service [97]. Runoff events were defined as in Setty *et al.* [98] Finally *in situ* hydrological measurements include pH, dissolved oxygen, conductivity, and water temperature are included as well as microbial source tracking marker concentrations targeting swine-feces specific marker pig-2-bac and human-feces specific marker HF183. Methods for microbial source tracking concentrations

are outlined in Chapter 3. Missing hydrological variables were imputed with the average value across the dataset for water temperature (n=1), pH (n=2), conductivity (n=1), and dissolved oxygen (n=15).

Table 16 - Non-exposure independent variables, x_n, by variable type including watershed land use, environmental, and measured hydrological variables

Variable Type	n	Variable	Description	Source
Watershed Land Use	1	% Wetland in 100m and 50m buffer	Land use 2016 CDL wetland within 100m or 50m of all perennial streams within watershed	2016 CDL; NHD flowline
	2	% Forest in 100m and 50m buffer	Land use 2016 CDL forest within 100m or 50m of all perennial streams within watershed	2016 CDL; NHD flowline
	3	% Cultivated in 100m and 50m buffer	Land use 2016 CDL cultivated within 100m or 50m of all perennial streams within watershed	2016 CDL; NHD flowline
	4	% Wetland	% Watershed area with wetland (defined as herbaceous wetlands, woody wetlands, and wetlands)	2016 CDL
	5	% Forest	% Watershed area forest (defined as deciduous forest, evergreen forest, forest, mixed forest)	2016 CDL
	6	% Cultivated	% Watershed area cultivated	2016 CDL
	7	Soil type A	% Watershed area with hydrological soil class A	SSURGO
	8	Soil type B	% Watershed area with hydrological soil class B	SSURGO
	9	Soil type C	% Watershed area with hydrological soil class C	SSURGO
	10	Soil type D	% Watershed area with hydrological soil class D	SSURGO
Environmental	11	Inches precipitation – prior 24 hours	Precipitation determined for sampling location using gridded observed precipitation data for 24 hours prior to 7AM of sampling date	National Weather Service
	13	Inches precipitation-prior 48 hours	Aggregated precipitation determined for sampling location using gridded observed precipitation data for 48 hours prior to 7AM of sampling date	National Weather Service
	14	Precipitation – prior 7 days	Aggregated precipitation determined for sampling location using gridded observed precipitation data defined as precipitation for 7 days hours prior to 7AM of sampling date	National Weather Service
	15	Runoff event	Greater than 10mm rain within 48 hours prior to 7AM sampling date	National Weather Service
	16	First runoff event	Runoff event within 48 hours prior to 7AM of sampling date and no	National Weather Service

			runoff event prior to 48 hours of sampling event through 7 days prior to sampling event	
Measured Hydrological	17	Water pH	pH	Field measurement
	18	Water Temperature	Degrees Celsius	Field measurement
	19	Conductivity	uS/cm	Field measurement
	20	Dissolved Oxygen	mg/L	Field measurement
	21	pig-2-bac concentration	Gene copies/mL of swine-feces specific microbial source tracking marker	Laboratory measurement
	22	HF183 concentration	Gene copies/mL of human-feces specific microbial source tracking marker	Laboratory measurement

Exposure variable methods

Exposure variables were constructed to approximate exposure of surface water to *E. coli* from fecal sources due to overland flow/surface runoff and surface water flow transport from fecal sources. A tiered approach was used to create four exposure variables using increasingly complex methods to approximate exposure to each of the following fecal sources: human septic tanks, CHO lagoons, and CHO sprayfields.

Exposure variables were created in increasing complexity in equations (1) through (4). The homogenous method describes exposure as a density in equation (1), however equations (2) through (4) incorporate proximity where all distances are from sampling point, s_i , to fecal source location, j_m , upstream of s_i , that is, within the watershed.

(1) Homogenous

$$x_1(\mathbf{s}_i) = \sum_{j_m=1}^n c_{0j_m}$$

(2) Gravity (Grav)

$$x_1(\mathbf{s}_i) = \sum_{j_m=1}^n c_{0j_m} / d_{nij_m}^2$$

(3) Sum of exponential decay (SED)

$$x_1(\mathbf{s}_i) = \sum_{j_m=1}^n c_{0j_m} \exp(-3 d_{nij_m} / \alpha)$$

(4) Sum of exponential decay model with distance interaction (SED-int)

$$x_1(\mathbf{s}_i) = \sum_{j_m=1}^n c_{0j_m} \exp\left(\frac{-3 d_{3ij_m}}{\alpha}\right) \exp\left(\frac{-3 d_{4ij_m}}{\gamma}\right)$$

Distance variable, d_n , is defined in Table 17. Fecal source location, j_m , and corresponding intensity metric, c_{0j_m} are defined in Table 18. Equation (2) estimates 95% reduction in the dependent variable, i.e. concentration of *E. coli*, based on squared Euclidean distance or squared overland flow distance decay (see Table 17). Equations (3) and (4) require estimation of distance parameters α and γ . In equation (3), α is a distance parameter estimating the Euclidean overland flow distance between the sampling point, s_i , and fecal source, j , for which there is a 95% reduction concentration of *E. coli*. In equation (4), α is the distance parameter for overland flow from source, j , to nearest surface water and γ is the distance parameter for surface water flow from nearest surface water to sampling point, s_i , estimating distance for which there is 95% reduction in the concentration of *E. coli*. To estimate α in equation (3), R^2 was optimized for $x_1(\mathbf{s}_i)$ as the sole predictive variable for y while varying α . To estimate two distance parameters in the case of equation (4), α was varied while γ remained constant to optimize R^2 while predicting y in the univariate case. When α was optimized with respect to R^2 , α was kept constant and γ was varied to optimize R^2 . Both α and γ were thus iteratively varied, one at a time, to optimize R^2 .

The intensity metric, c_0 , approximates weights for individual fecal sources from locations sampled. Human sources are assumed to be septic systems [82] because all sampling locations were in rural areas and assumed to be disconnected from municipal sewerage. Exposure to human fecal sources were approximated as population density for the homogenous case weighted

by area from the 2010 census [71], and address locations [99] were used to approximate locations of septic systems in equations (2) through (4) with no differentiation in the intensity metric, c_0 , since septic functionality was not known. Thus septic functionality was assumed the same for each address. Wildlife fecal sources were considered to be random over the watershed area and only approximated using the homogenous method as watershed area. CHO fecal sources included sprayfields and lagoons and were identified through satellite imagery and nutrient management plans obtained from the NC DEQ following prior methods for delineation [100]. Intensity, c_0 , for CHO fecal sources in the homogenous case included total sprayfield acres and gallons manure produced per area, taken from NC DEQ Nutrient Management Plans (NMPs). For equations (2) through (4), c_0 for CHO fecal sources included gallons of manure produced for each CHO weighted proportionally by number of lagoons or sprayfield acreage for the same CHO.

Table 17 - Methods for distance calculation from sampling point to fecal source, d_{nijm}

n	Description
1	Euclidean overland flow distance from each upstream source location, j , to sampling point, s_i
2	Approximation of overland flow and surface water flow: Euclidean overland flow distance from each source location, j , within sampling point watershed to nearest surface water, i , added to the surface water flow distance from nearest surface water, i , for each source location, j , to sampling point, s_i
3	Used only in equation (4), this is the surface water flow distance from the nearest surface water, i , for each source location, j , to sampling point, s_i
4	Used only in equation (4), this is the Euclidean overland flow distance from each source location, j , within sampling point watershed to nearest surface water, i

Table 18 - Intensity metric c_{0j_m} for each fecal source location, H_m or j_m

j_m	Description	c_{0j_m}
j_{H_1}	Population Density	Population density determined by 2010 census block population weighted by watershed area within census block
j_{H_2}	Wildlife	Watershed area
j_{H_3}	Manure Density	Sum of manure produced for each CHO in watershed divided by area of the watershed
j_{H_4}	Sprayfield Acres	Total acres of sprayfields for all CHOs within watershed
j_1	CHO sprayfield edge	total permitted annual swine manure produced for each CHO in watershed and normalized by fraction of sprayfield area to total sprayfield area for a given CHO
j_2	Lagoon Centroid	total permitted annual swine manure produced for each CHO in watershed and normalized by number of lagoons for a given CHO
j_3	Human Septic	2014 addresses which are centroid of tax parcels in watershed and up to 50m outside watershed boundary; c_0 not differentiated and set to 1

Model Development

A multivariate general linear model was used to predict \log_{10} concentration of *E. coli* of the form $y = \beta_0 + \beta_1 x_{1i} + \dots + \beta_n x_{ni} + \epsilon_i$ where y is concentration of *E. coli* and β is the regression coefficient measuring effect size of independent variable, x_n . A multivariate general linear model was developed using a set of land use, measured hydrological, and environmental variables for following three methods of constructing exposure variables: homogenous (equation 1), gravity using Euclidean overland flow distance (equation 2), and sum of exponential decay with distance interaction (equation 4).

First, a univariate general linear model was conducted in turn for each independent variable to predict \log_{10} concentration of *E. coli*. Outputs of the univariate analysis included R^2 to assess strength of the relationship and β coefficient to determine effect size of the independent variable.

Groups of similar independent variables were assessed for collinearity using principal component analysis and independent variables were removed from the set of non-exposure variables assessed in the model when high collinearity was observed. Additionally, the variance inflation factor (VIF) was calculated for each independent variable included in a the model. VIF is a measure of variable collinearity. The variable with the highest VIF was iteratively removed until the model had a maximum VIF below 3 and with an average VIF near 2.

Model development resulted in three multivariate general linear models each with a set of predicting independent non-exposure variables and each with a different set of predicting exposure variables. Additionally, each of the three multivariate models were re-assessed with the same set of predictor variables using forward stepwise regression to improve model performance metrics R^2 and Akaike information criterion (AIC) resulting in six models predicting *E. coli* concentration.

Model and Variable Assessment

Model performance was assessed using adjusted R^2 and AIC for predictive capability and the Brown-Forsythe measure of equal variance between observational groups.

Association of each independent variable with *E. coli* concentration was assessed by weighting the variable's β coefficient with the variable's interquartile range (IQR). The IQR is a measure of the independent variable's range for the middle 50% of the data from the 25th to 75th percentile. Multiplying β with the IQR yields a standardized measure of the increase in \log_{10} *E. coli* concentration, allowing comparability among variables with different units. Additionally, multiplying β with the IQR yields an IQR ratio of the 75th percentile divided by the 25th

percentile of the independent variable to determine an IQR ratio for the percent increase in *E. coli* concentration with one increase in the independent variable's IQR.

Finally, a ten-fold cross validation was conducted for every independent variable to predict \log_{10} *E. coli* concentration in a univariate model. For each fold, a β coefficient and associated p-value were determined and IQR ratio calculated with 95% confidence intervals based on the 10 iterations.

Results

Exposure Variables

Distance Parameters

For SED methods to create exposure variables from equations (3) and (4), α and γ parameters were optimized to estimate the distance for which there would be a 95% reduction in *E. coli* concentration. Optimized Euclidean overland flow distance from source to sampling point, α , using the SED method, was found to be 1200m, 2600m, and 4000m for human septic, lagoon, and sprayfield exposures, respectively. Optimized Euclidean overland flow distance from source to nearest river, α , using the SED interaction method, was found to be 500m, 750m, and 1500m for human septic, lagoon, and sprayfield exposures, respectively. Optimized surface water flow or river distance from the nearest river of source to sampling point, γ , using the SED interaction method, was found to be 2400m, 3050m, and 4500m for human septic, lagoon, and sprayfield exposures, respectively. In all instances, sprayfields had effects spanning longer distances than lagoons, which had effects spanning longer distances than human septic.

Methods Comparison

Cross-validation results suggest that SED methods best approximate exposure with both the highest R^2 and the highest IQR ratio when compared to homogenous and gravity methods. Table 19 identifies univariate R^2 , IQR ratio, and p-value for exposure variables, and Figure 10 compares IQR ratio +/- 95% confidence interval among folds in the cross-validation.

Among fecal sources assessed, the predictive metric R^2 increased (Table 19) and effect measure IQR ratio increased (Figure 10) using SED methods compared to gravity or homogenous methods demonstrating that exposure variables with intensity and optimized distance metrics out-perform exposure variables using density metrics for predicting concentration of *E. coli* in surface water. . From the least complex homogenous method to the most complex SED interaction method, the R^2 for variables assessing exposure increased from 0.02 to 0.12 for human septic exposure, 0.05 to 0.10 for lagoon exposure, and 0.03 to 0.08 for sprayfield exposure. Additionally, p-values for β coefficients improved to $p < 0.001$ from homogenous to SED methods. Among SED methods, methods for human septic, lagoon, and sprayfield exposures were most predictive when incorporating distance decay for overland flow distance as well as surface water flow. The gravity method using Euclidean overland distance (rather than including surface water flow) performed similarly to or worse than the homogenous method. While the gravity method with surface water flow improved upon the homogenous method for sprayfield and lagoon exposures, human exposure had higher R^2 and lower p-values for the homogenous method compared to both gravity methods, while still lower than SED methods.

When assessing measures of effect using IQR ratios for exposure variables, lagoon exposure using the SED interaction method produced the highest R^2 and highest IQR ratio (see

Figure 10 and Table 19) among all exposure variables assessed for univariate prediction of \log_{10} *E. coli* concentration (IQR ratio=2.61, 10-fold 95% CI: 2.48-2.75), indicating that without controlling for other variables, one unit IQR increase in lagoon exposure is associated with a 2.6 times higher concentration of *E. coli*. Among human exposures, the SED interaction method also produced the highest R^2 and highest IQR ratio (IQR ratio=1.69: 10-fold 95% CI:1.66-1.71). For sprayfield exposure, homogenous (IQR ratio=1.56, 10-fold 95% CI: 1.51-1.62), SED (IQR ratio=1.68, 10-fold 95% CI: 1.62-1.73), and SED interaction (IQR ratio=1.60, 10-fold 95% CI:1.54-1.64) methods had similar IQR ratios. Appendix E displays IQR ratios and confidence intervals for all variables.

Lagoon exposure has better predictive power and higher IQR ratio than sprayfield and septic exposure perhaps due to the fact that spray application times were not known, septic tank functionality was not known, and lagoons represent a location where large volumes of fecal material are stored consistently across time. It may be that SED methods using only Euclidean overland distance and SED interaction method for sprayfield exposure are more similar than for lagoon and septic exposures since tile drains and cultivated fields may change slope and surface water runoff direction in the small watersheds sampled.

Table 19 - Univariate model performance predicting \log_{10} *E. coli* concentration and measures of effect for exposure variables using homogenous, gravity, and sum of exponential decay (SED) methods

Fecal Source	Exposure Variable	Method	Distance	Univariate R²	IQR	β	Univariate IQR Ratio	Univariate p
Wildlife	Watershed Area	Homogenous	none	0.00	3.93	-0.01	0.92	0.59
Human septic	Pop. Density	Homogenous	none	0.02	45.00	0.00	1.23	0.08
	Address exposure	Gravity	Euclidean	0.00	0.00	25.6	1.01	0.91
		Gravity	Surface Water Flow	0.01	0.00	-306	0.89	0.27
		SED	Euclidean	0.09	4.93	0.03	1.48	***
	SED with interaction	Euclidean and Surface Water Flow	0.12	2.16	0.11	1.69	***	
CHO lagoon	Manure Density	Homogenous	none	0.05	188235	0.00	1.51	**
	Lagoon exposure	Gravity	Euclidean	0.05	28.34	0.01	1.46	**
		Gravity	Surface Water Flow	0.07	19.54	0.01	1.74	***
		SED	Euclidean	0.07	6647193	0.00	1.84	***
		SED with interaction	Euclidean and Surface Water Flow	0.10	3577340	0.00	2.61	***
CHO sprayfield	Sprayfield Acres	Homogenous	none	0.03	176.72	0.00	1.56	*
	Sprayfield exposure	Gravity	Euclidean	0.02	37.20	0.00	1.18	*
		Gravity	Surface Water Flow	0.06	20.44	0.01	1.39	**
		SED	Euclidean	0.07	7128925	0.00	1.67	***
		SED with interaction	Euclidean and Surface Water Flow	0.08	4271849	0.00	1.59	***

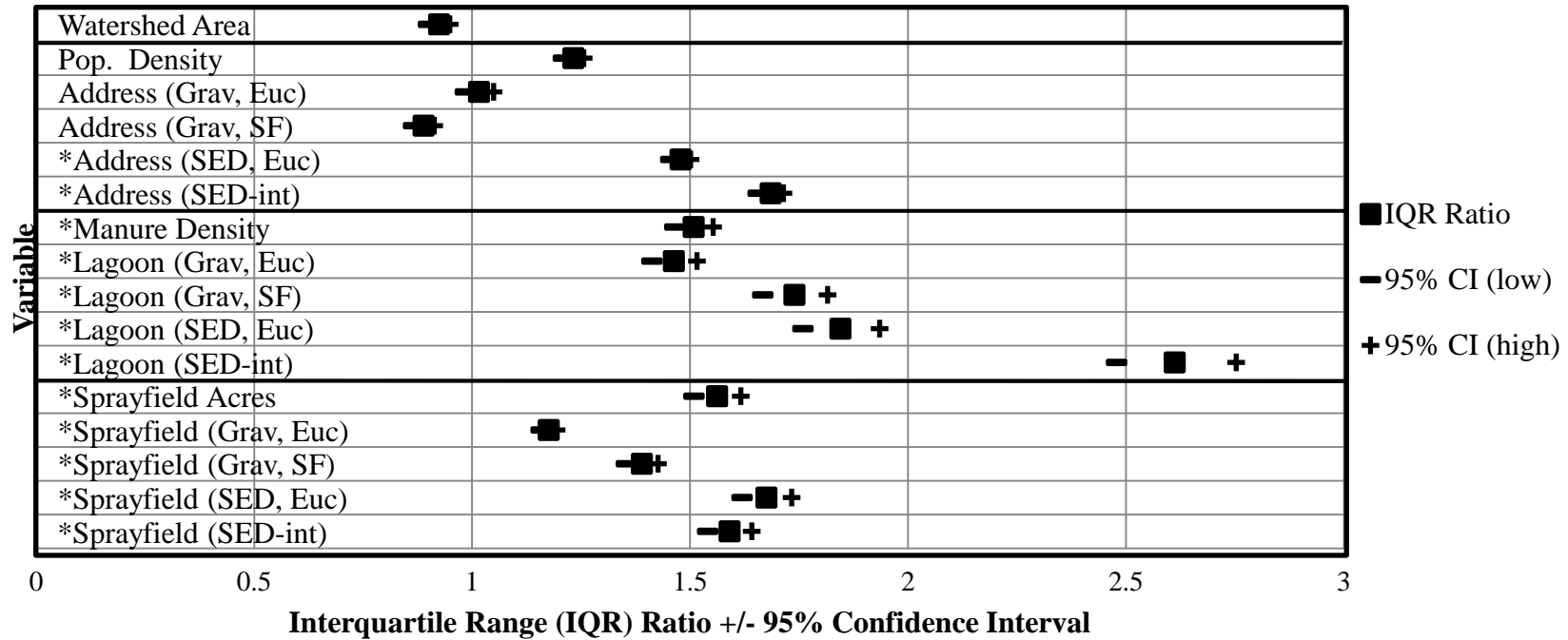


Figure 10 - Interquartile range ratio +/- 95% confidence interval in 10-fold cross validation, univariate prediction of \log_{10} concentration of *E. coli* for different methods of exposure to humans and CHO lagoons and sprayfields. * indicates that the majority of 10-fold iterations were significant ($p < 0.1$); Exposure variable methods include homogenous, gravity (Grav), sum of exponential decay (SED), and sum of exponential decay with interaction (SED-int) equations using Euclidean overland flow (Euc) and/or surface water flow (SF) distances.

Human exposure: Observational group comparison

When comparing observational groups for concentrations of fecal bacteria from a particular source, e.g. CHOs, exposure to confounding fecal sources, i.e. humans, should be similar or should be modeled to reduce bias in the results of the study. Table 20 indicates that the mean for homogenous exposure variables for human fecal input, that is population and population density, between observational groups (i.e. watersheds with and watersheds without CHOs) were statistically similar ($p=0.97$, $p=0.99$). However methods for exposure variables incorporating distance, especially the SED and SED-int methods, had more different mean exposures between background and swine sites ($p=0.14$, $p=0.17$). This demonstrates that exposure variables incorporating density metrics may hide differences in exposure to confounding sources between observational groups because SED methods, which incorporate distance, show more difference between observational groups than would otherwise be known when only using density. Table 20 demonstrates that the use of density metrics as approximation for exposure may not adequately assess similarity of observational groups with respect to exposure to fecal sources since exposure increases with closer proximity.

Table 20 - Evaluation of comparability of human exposure variables constructed using gravity (grav), SED (sum of exponential decay), and SED-interaction (SED-int) methods incorporating Euclidean overland flow distance (Euc) and/or surface water flow (SF) distance in background (n=9) and swine (n=13) sites

Human Exposure Variable	Background Sites (n=9) Mean (95% CI)	Swine Sites (n=13) Mean (95% CI)	p-value ¹	Shapiro-Wilkes p
Population [†]	300 (2 – 599)	128 (81 – 174)	0.97	0.97
Population Density (Population/mi ²)	51 (30 - 71)	55 (24 – 87)	0.99	0.99
Address (Grav, Euc)	1.9x10 ⁻⁴ (0– 3.9x10 ⁻⁴)	2.3x10 ⁻⁴ (9.6x10 ⁻⁵ – 3.7x10 ⁻⁴)	0.69	<0.001
Address (Grav, SF) [†]	1.5x10 ⁻⁴ (0–3.1x10 ⁻⁴)	1.5x10 ⁻⁴ (5.4x10 ⁻⁵ –2.5x10 ⁻⁴)	0.48	0.94
Address (SED, Euc)	2.2 (1.2 – 5.5)	4.1(3.1 – 11.3)	0.14	<0.001*
Address (SED-int) [†]	0.58 (0.7 – 1.9)	1.62 (1.4 – 4.7)	0.17	0.37

¹t-test for normal distributions or ^{*}Mann-Whitney rank sum test for non-normal distributions when Shapiro-Wilkes p<0.1

[†] log₁₀-transformed for t-test

Model Performance

Table 21 identifies model performance parameters, adjusted R² and AIC, in addition to the number of variables included in the model, two collinearity measures, maximum and average VIF, as well as the Brown-Forsythe statistic of equal variance. Figure 11 displays model results for the stepwise SED-int model (M6) plotting actual compared to predicted log₁₀ *E. coli* concentrations. Figure 11 also shows that model residuals for the stepwise SED-int model are not correlated. Model performance metrics improved among models using exposure variables incorporating proximity (M3, M4, M5, and M6) compared to homogenous models incorporating density metrics for exposure variables (M1 and M2) with mean adjusted R² of 0.41 compared to 0.37 (p=0.07), respectively, and with mean AIC of 319 compared to 330 (p=0.22), respectively. Stepwise regression increased model performance for each of the three exposure variable sets by removing variables that did not contribute to a better model, as measured by reduction in AIC. Stepwise models including exposure variables using gravity (M4) and SED-int (M6) methods

were similar, but both performed better than the stepwise model incorporating homogenous exposure variables (M2). Model results demonstrate that exposure variables incorporating proximity paired with intensity metrics improves model performance to predict *E. coli* concentration compared the use of a density metric for fecal source exposure.

Table 21 - Model descriptive and performance parameters including number of variables included, maximum and average variance inflation factor (VIF), adjusted R², AIC, and Brown-Forsythe statistic for six models predicting log₁₀ concentration of *E. coli* using homogenous, gravity, and sum of exponential decay with distance interaction (SED-int)

Model Parameter	M1 Homogenous	M2 Homogenous-Stepwise	M3 Gravity	M4 Gravity-Stepwise	M5 SED-int	M6 SED-int - Stepwise
n Variables	13	9	17	9	16	10
Maximum VIF	2.1	2.1	3.7	3.68	3.3	3.31
Average VIF	1.5	1.5	1.9	1.93	1.8	1.786
Adjusted R²	0.364	0.377	0.404	0.411	0.398	0.411
AIC	335	325	324	315	325	313
Brown-Forsythe	0.33	0.52	0.94	0.72	0.48	0.43

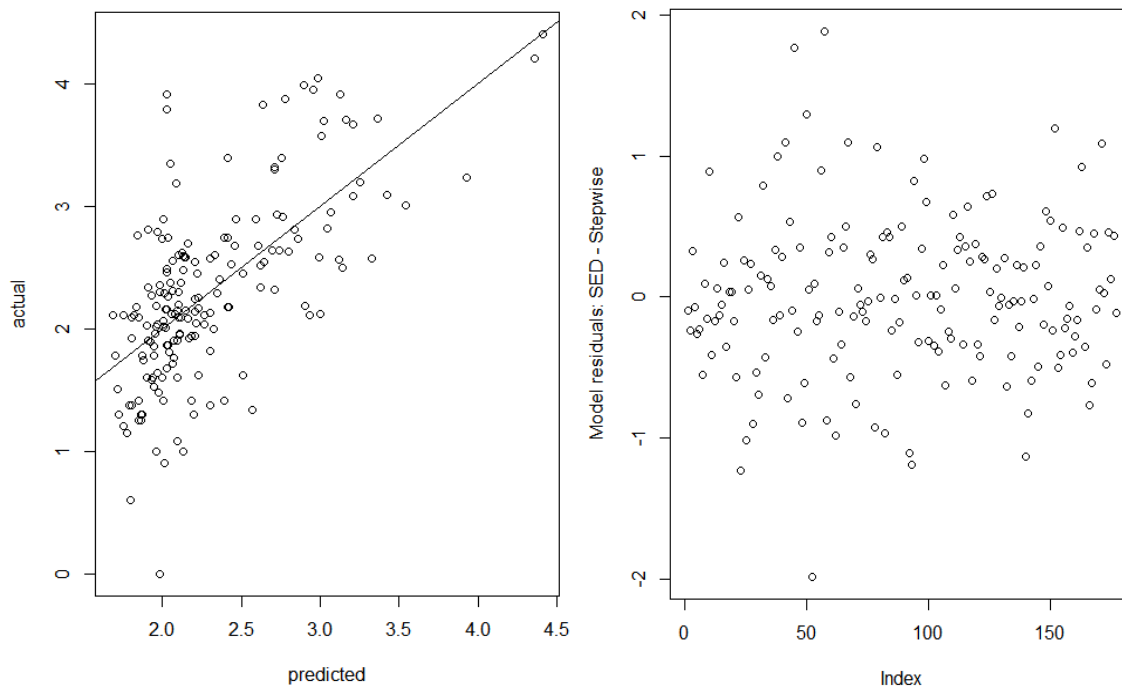


Figure 11 - Actual vs. predicted \log_{10} *E. coli* concentration for the stepwise regression model, M6, incorporating exposure variables using the SED-interaction method (Left); Model M6 residuals (Right)

Predictor variables

Interquartile Range Ratios

For each of the six models, Table 22 identifies variable effect as measured by IQR ratio and corresponding p-value on *E. coli* concentration, when controlling for other variables included in the same model. The same set of variables was not included for all six models.

Of precipitation variables included, when controlling for other variables in the model, first runoff events and prior 24 hour precipitation had significant and positive effect size ($p < 0.01$) in all six models. When a first runoff event occurred, there was a 4.6 to 5.3 times higher *E. coli* concentration compared to sampling events without first runoff events, depending on model. One interquartile range increase, corresponding to 0.05 inches, of prior 24 hour precipitation was associated with 5-6% increase in *E. coli* concentration.

Of land use variables included, when controlling for other variables in the model, none were selected in all models. Two models (M3, M4) selected percent wetland within a 50m buffer and identified that a one IQR increase (28%, $p < 0.001$) was associated with a 2.1-2.3 times higher *E. coli* concentration. One IQR increase (8%) in percent wetland in the watershed was associated with a 25-33% decrease ($p < 0.05$) in *E. coli* concentration in models M1 and M2. While the relationship was not as strong, i.e. a lower p-value, percent forest within a 50m buffer was similarly associated with higher *E. coli* concentrations while percent forest was associated with lower *E. coli* concentrations. Soil types were not significantly associated with *E. coli* concentration in any of the models. Other land use variables (e.g. percent cultivated land) were not included in models due to high collinearity with other variables.

Of measured variables, when controlling for other variables in the model, a one IQR increase (9.2 degree Celsius) in water temperature was associated ($p < 0.05$) with a 1.5 to 1.6 times

higher concentration of *E. coli* in five models. Other measured hydrological variables were either not selected to include in models (e.g. pH) or were not significantly associated with *E. coli* concentration (e.g. conductivity and dissolved oxygen). However, concentrations of microbial source tracking markers were significantly associated with higher concentrations of *E. coli*, with differing measures of effect. A one IQR increase (2.3 copies/mL) in HF183 concentration among detected samples was associated with 19-22% higher *E. coli* concentration ($p < 0.05$) in all models. While pig-2-bac was more significantly associated with *E. coli* concentration ($p < 0.01$) in all models, the measure of effect was minimal (IQR ratio=1.00) and upon further analysis, with the removal of one high outlier, pig-2-bac concentration no longer had significant IQR ratios nor was selected for stepwise models (data not shown). When the pig-2-bac outlier was removed from the analysis, IQR ratios did not change among other independent variables in Table 22 (data not shown).

Of exposure variables, when controlling for other variables in the model, fecal sources were significantly ($p < 0.05$) associated with higher *E. coli* concentrations. Wildlife sources approximated by watershed area was significantly ($p < 0.05$) negatively associated with *E. coli* concentration (IQR ratio=0.68) in one model (M3). Models including exposure variables using density metrics identified that a one IQR increase in population density (45 persons/mile²) and sprayfield acres (177 acres) was associated with a 1.2 and 1.7-1.9 times higher concentration of *E. coli*, respectively (M1, M2). Models including exposure variables using proximity metrics found that a one IQR increase in lagoon and human exposures (SED-int methods) found a 1.5 and 1.4 times ($p < 0.01$) the increase in *E. coli* concentration, respectively (M5, M6).

Cross-validation results

Cross-validation results support model results since most variables with significant β coefficients, and thus IQR ratios, included or selected in models from Table 22 also had consistently significant IQR ratios across folds. Cross-validation revealed that percent wetland, incorporated in two models, was not consistently significant across ten folds. IQR ratios, associated confidence intervals, and p-values among 10-fold IQR ratios are presented in Appendix E. Precipitation variables were consistently significant ($p < 0.1$) and positively associated with increased *E. coli* concentration among the ten folds. Additionally, as indicated in model results, percent wetland in a 50 and 100m buffer were significantly ($p < 0.1$) positively associated and percent cultivated in a 50 and 100m buffer were significantly ($p < 0.1$) negatively associated with *E. coli* concentration. While land use within a buffer was consistent in the cross-validation, percent land use over the watershed area was not consistently significant, suggesting models including significant associations of *E. coli* with percent wetland or percent forest are not as robust and have less confidence in the IQR ratio associated with these variables. Among measured variables, cross-validation results were similar to model results in that water temperature and microbial source tracking variables were consistently significant. Finally, among exposure variables, SED methods for exposure variables were always consistently significant across all fecal sources, while gravity and homogenous models were less robust among the ten folds for human exposures (see Figure 10 and Figure 12). Figure 12 displays ordered univariate IQR ratios from highest to lowest with 95% confidence intervals from a 10-fold cross-validation, and only including variables where the majority of folds ($n > 5$) had significant ($p < 0.1$) IQR ratios. Cross-validation demonstrated that stepwise models always, with one exception (i.e. percent wetland), selected variables that were consistently significant ($p < 0.1$)

among ten folds, and thus there is with more confidence in the IQR ratios from stepwise models (M2, M4, and M6).

Discussion

This work developed models to predict *E. coli* concentration in watersheds with and without CHOs by approximating exposure to CHOs in addition to confounding fecal sources. This work also compared methods for approximating exposure by comparing models with different exposure variable methods—those with density metrics as well as those incorporating intensity and distance metrics.

Model performance metrics improved among models using exposure variable methods incorporating proximity compared to exposure variable methods incorporating density metrics with mean adjusted R^2 of 0.41 compared to 0.37 ($p=0.07$), respectively. Forward stepwise regression increased model performance regardless of exposure variable method by removing variables that did not contribute to a better model, as measured by reduction in AIC. Cross-validation confirmed robust model results, especially among stepwise models, because all but one variable (percent wetland) selected in stepwise regression models were consistently (majority) significant ($p<0.1$) in the cross-validation, univariate prediction of *E. coli*.

This chapter showed that as exposure variable complexity increased, univariate R^2 increased, effect measure IQR ratio increased, and p-values for β coefficients improved to $p<0.001$ from homogenous to SED methods. Cross-validation results suggested that SED methods best approximate exposure with both the highest R^2 and the highest IQR ratio when compared to homogenous and gravity methods among human, lagoon, and sprayfield fecal source exposures. Among SED methods, lagoon exposure had the highest IQR ratio ($p<0.001$).

Table 22 - Interquartile range (IQR) ratios and associated p-values for variables included in full models and variables selected in stepwise models

Variable			Homogenous				Gravity				SED			
			M1 Full		M2 Stepwise		M3 Full		M4 Stepwise		M5 Full		M6 Stepwise	
Type	Description	IQR	IQR ratio	p (B)	IQR ratio	p	IQR ratio	p	IQR ratio	p	IQR ratio	p	IQR ratio	p
Precipitation	Prior 24 hours	0.05	1.06	***	1.06	***	1.05	***	1.05	***	1.05	**	1.05	***
	Runoff Event	1.00 ¹					1.10				1.23			
	First Runoff Event	1.00 ¹	4.67	***	5.04	***	4.64	**	5.32	***	4.07	**	5.02	***
Land Use Variables	% Wetland	0.08	0.67	*	0.75	*	0.81		0.76	<0.1	0.87			
	% Forest	0.16	0.85				0.71	*			0.83			
	%Wetland, 50m buffer	0.28					2.28	***	2.12	***				
	%Forest, 50m buffer	0.11	1.35	<0.1	1.29	<.1	0.97				1.05			
	Soil type B	0.23	0.86				0.70	<0.1						
	Soil type D	0.23	0.72				0.30	<0.1			1.30			
Measured	Water Temp.	9.20	1.53	*	1.59	**	1.53	*	1.60	**	1.45	<0.1	1.56	**
	Conductivity	61.40	0.83								0.78			
	Dissolved Oxygen	3.48	0.96				0.98				0.96			
	HF183	2.28 ²	1.22	*	1.21	*	1.20	*	1.19	*	1.20	*	1.21	*
	pig-2-bac	10.80 ²	1.00	**	1.00	**	1.00	**	1.00	**	1.00	***	1.00	**
Exposure Variables	Watershed Area	3.93	1.05				0.68	*	0.80		0.95			
	Pop. Density	45	1.22		1.23	*								
	Spray. Acres	177	1.89	**	1.66	**								
	Manure Density	188235	0.98											
	Lagoon (Grav)	28.34					1.20							
	Spray. (Grav)	37.20					1.03		1.09					
	Address (Grav)	0.00					0.87							
	Lagoon (SED)	3577340									1.35		1.52	*
	Spray. (SED)	4271849									1.16			
Address (SED)	2.16									1.41	**	1.39	**	

***p<0.001, **p<0.01, *p<0.05; ¹IQR for binary variables fixed at 1

²IQR for HF183 and pig-2-bac concentrations reflect the IQR among above the limit of detection data

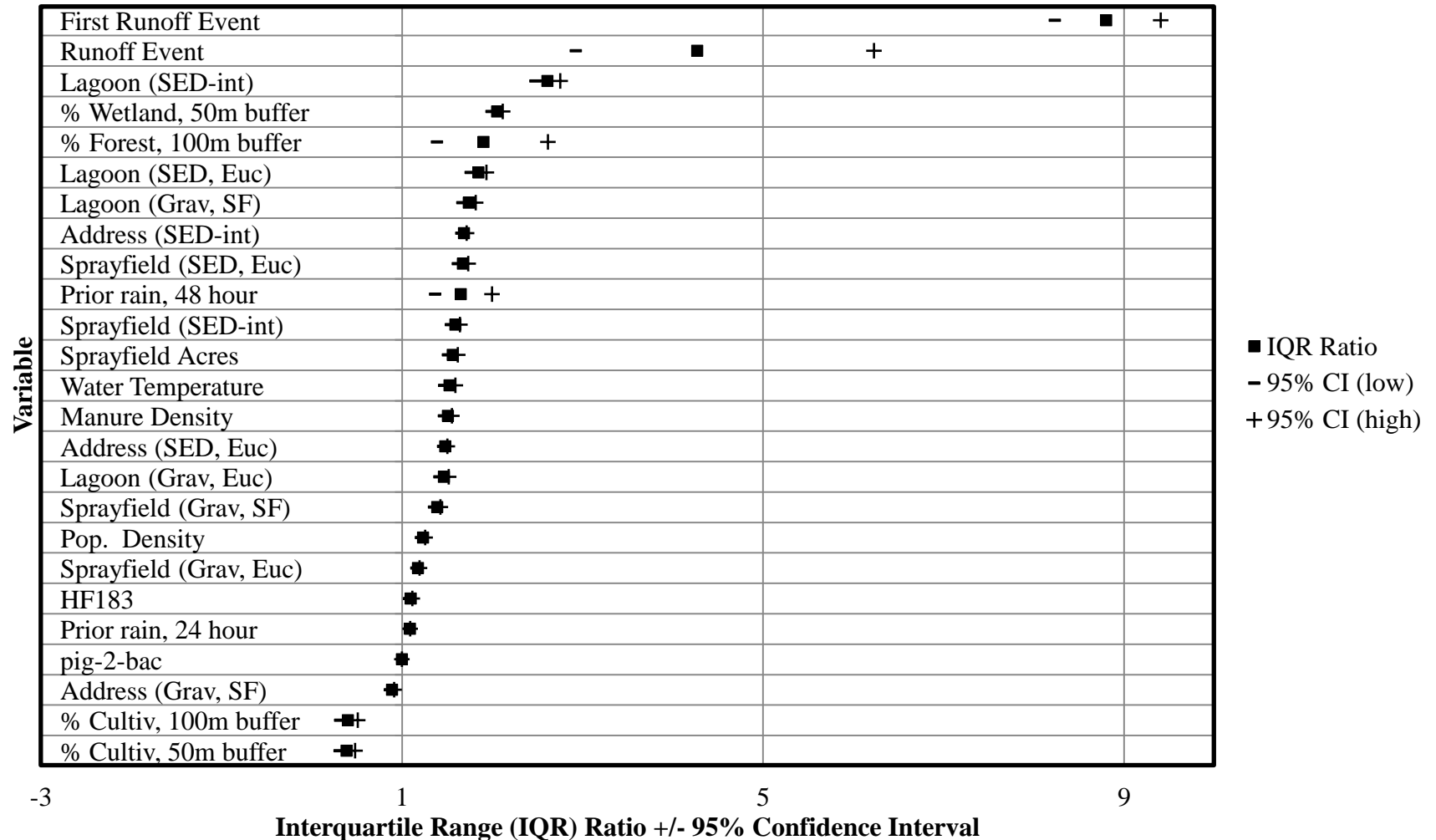


Figure 12 - Independent variables ordered by IQR ratio for univariate prediction of *E. coli*. with 95% confidence intervals representing the variability in IQR ratio among 10-fold cross validation results. Exposure variables include homogenous, gravity (Grav), sum of exponential decay (SED), and SED interaction (SED-int) methods incorporating Euclidean overland flow (Euc) and/or surface water flow (SF) distances

Improved univariate R^2 among exposure variable methods demonstrates that density metrics were less suitable than and out-performed by metrics incorporating intensity and distance metrics for predicting concentration of *E. coli* in surface water. Finally, this chapter found that exposure variables incorporating density metrics may mask differences in exposure to confounding sources between observational groups because SED methods, which incorporate distance, show more difference between observational groups than would otherwise be known when only using density metrics. Thus, the use of density metrics as an approximation for exposure may not adequately assess similarity of observational groups with respect to exposure to fecal sources since exposure increases with closer proximity.

IQR ratios from model results demonstrate that high concentrations of *E. coli* in surface water are predicated, in large part, by precipitation events 24-48 hours prior, and especially when precipitation is preceded by a dry period (i.e. a first runoff event). Plot studies have identified that microbial loads of FIB from land-applied fields can be transported to surface waters especially after precipitation [12]. Other studies have observed that fecal bacteria concentrations increase after precipitation events in both swine-affected and control sites[21,23]. Additionally, fecal bacteria concentrations may increase after initial precipitation, while further precipitation creates a dilution effect such that concentrations are reduced [28]. As such, we see a higher effect size from a first runoff event where it is known prior precipitation has not occurred, compared to amount of prior precipitation.

Land use effects on *E. coli* concentrations in surface waters are less clear from the modeling results. While prior work has found a protective effect from fecal coliforms [20] and nutrient [67] effects from CHOs with higher percent native pasture or wetland in watersheds, respectively, our results do not capture this. Other studies have also shown that protective

factors to reduce FIB transport from CHOs include increasing flow resistance through vegetation and river buffers and longer overland flow distance to surface waters from CHOs [28]. While our results show that exposure variables incorporating overland flow distance influence *E. coli* concentrations, our results did not find that wetland buffers of 50 or 100m reduced *E. coli* concentrations. Rather, our results indicate that a wetland river buffer of 50 or 100 m is positively associated (IQR ratio=2.1-2.3) with *E. coli* concentration. Bradford *et al.* identify that buffers and constructed wetlands can be used to remove FIB but there is still a knowledge gap in the understanding of FIB transport among soil, surface water, and subsurface flow that can limit efficacy of constructed wetlands [28]. It is possible that the wetland buffer distance chosen is not sufficient to absorb organic material and may also be the result of an interaction of precipitation with wetlands where with increased precipitation intensity, wetlands may not have the capacity to absorb high organic matter which may flow directly through the wetland buffer and into surface water. It is possible that inter-group variability was affected by unmeasured parameters such as organic matter or type of wetland, whether constructed or natural, along rivers since nutrient availability and organic matter in soil can contribute to survival and growth of *E. coli* populations [55,84].

Higher water temperature may also play a role in survival of *E. coli*. High temperatures (>30 °C) are generally associated with bacterial inactivation and are suggested for use in manure treatment while low temperatures (< 5 °C) are associated with reduced bacterial persistence in agricultural soils and surface water [20,28]. Water temperature generally fell between this range (5 – 30 °C). It is also possible that the positive association of water temperature with *E. coli* concentration is capturing seasonal differences in spray events with more spray events occurring in warmer temperatures when crops are grown.

Finally, results show that there is an effect by both CHOs and humans on concentration of *E. coli*. Exposure methods incorporating fecal source intensity (i.e. load) and proximity to surface water and sampling location are much better approximations for fecal source inputs than density metrics. No prior work has incorporated proximity metrics to assess CHO effects on microbial quality of surface waters. While septic functionality is not known for sampling locations, it is notable that concentrations of the human-feces specific genetic marker, HF183, were significantly associated with higher concentrations of *E. coli*. Sprayfield exposures were positively, though not always significantly, associated with *E. coli*, and this work was unable to identify spray events nor was it able to determine functionality of lagoon or sprayfield infrastructure. Even still, this work demonstrates an appreciable effect of CHOs on surface water *E. coli* concentrations while controlling for human fecal sources.

Variables not included in the models may have significant effects on *E. coli* concentrations as well. For example, swine life stage may affect concentrations of fecal bacteria in manure, manure management conditions such as long-term and high-temperature waste storage and composting can reduce bacterial survival [28,101]. Additionally, tile drains can change the drainage direction and flow of a cultivated field such that sprayfield drainage may be different in a localized context. Additionally, CHO sprayfield management may differ where some sprayfields may have infiltration ponds, galleries, riverbank and sand filtration systems to allow surface water runoff to infiltrate more slowly and filter FIB [28], while other sprayfields may pipe surface water runoff directly along road ditches into nearby streams. These local flow dynamics may be important for understanding differing effects of CHOs on microbial water quality in addition to the variables identified in this chapter's models. Finally, timing of spray events onto sprayfields was not known nor was septic functionality known for human exposure

approximations. As such, these fecal sources were considered constant sources rather than associated with timing of spray or timing of septic leak.

This work identified that even when controlling for precipitation and confounding fecal sources such as wildlife and human fecal sources, CHOs, as measured by lagoon exposure, had a measureable, significantly positive association with *E. coli* concentration. Exposure variables incorporating proximity should be used when assessing observational group comparability with respect to source of interest as well as confounding sources.

CHAPTER 6: DISSERTATION CONCLUSIONS AND IMPLICATIONS

This research has shown that CHOs in our study area have a substantive effect on microbial water quality according to multiple lines of evidence. This field study is the largest landscape-scale study with respect to sites with (n=13) and without (n=9) CHOs to assess whether there are effects of CHOs on fecal bacteria concentrations, presence of swine-specific MST markers, and number of phenotypic antibiotic classes assessed for resistance. This study found the strongest evidence to-date with respect to reported effect size and significance that watersheds with CHOs affect FIB concentrations in surface water more than background sites while implementing a study design that addresses concerns about seasonality, well-defined control sites, and confounding sources. This study also found swine MST marker in more swine sites than background sites and that background sites never had higher prevalence of any measure of antibiotic resistance tested compared to sites with CHOs. This was also the first study to model whether CHOs affect microbial water quality incorporating precipitation and also the first to incorporate distance-based metrics of exposure to multiple fecal sources. Taken together, this work demonstrates that, on average, CHOs contribute *E. coli* and antibiotic resistant *E. coli* to surface water. Results also suggest that microbial water quality is poorer with increasing CHO size and proximity to surface water. As such, there are implications that discharges from CHOs should be regulated for fecal bacteria by state and federal agencies.

Summary of Findings

Chapter 3

Chapter 3 found significantly higher ($p < 0.001$) mean concentrations of *E. coli* among 177 samples at swine sites (1,284 CFU/100 ml, 95% confidence interval (CI): 625-1,944) compared to background sites (687 CFU/100 ml, CI: 263-1,111) with swine samples exceeding the EPA recommendation for recreational waters more often than background samples (73% vs. 42%, RR= 1.74, CI: 1.30 – 2.33, $p < 0.001$). While not every swine site displayed high concentrations of *E. coli* and not every background site displayed low concentrations of *E. coli*, swine sites had, on average, higher concentrations of *E. coli* compared to background sites.

Chapter 3 also developed a duplex assay targeting two microbial source tracking (MST) markers HF183 and pig-2-bac and reported concentration using three thresholds for ddPCR positivity. For MST marker concentration using the most conservative threshold, HF183 was detected at all swine sites and 78% of background sites (RR=1.3, CI: 0.91 – 1.8) and found at low mean concentrations at swine sites (1.6 copies/mL) and background sites (1.5 copies/mL). However, pig-2-bac was found in most swine sites (77%) and in two background sites (4%) (RR=3.5, CI: 0.98 - 12, $p < 0.05$) and at significantly higher ($p = 0.003$) mean concentrations at swine sites (283 copies/mL) compared to background sites (0.76 copies/mL). MST results suggest that low concentrations of human fecal inputs are detectable across sampling sites in both observational groups, while swine fecal inputs are detectable more often and in higher concentrations at swine sites compared to background sites. While the lack of detection of an MST marker does not indicate that a fecal source of interest is not contributing to FIB in surface water, the detection of an MST marker provides supporting evidence for off-site transport of the source in question.

Chapter 4

For all samples processed through membrane filtration in chapter 3, up to six (median five) *E. coli* isolates were tested for antibiotic resistance (n=912) from 193 sampling events to 11 antibiotics from 9 antibiotic classes using the Kirby-Bauer disc diffusion method and resistance was classified using CLSI guidelines.

For every antibiotic with observed resistance, resistance was more often observed in isolates from sites with CHOs compared to those from background sites. Antimicrobial resistance was observed more often in swine sites compared to background sites and most commonly to tetracycline (RR=2.2, CI: 1.3 - 3.8, p<0.01) and ampicillin (RR=5.0, CI: 1.5 - 16, p<0.01). Additionally, resistance was observed to at least one (RR=2.2, CI: 1.3-3.5, p<0.01), two (RR=12, CI: 1.8- 94, p<0.01), and at least 3 (RR=8.1, CI: 1.1 – 61, p<0.05) classes of antibiotics more often in sites with CHOs. Some resistance outcomes were seen in too low prevalence to determine relative risk, however resistance was seen to WHO highest priority antibiotics ceftriaxone, ciprofloxacin, and levofloxacin only among isolates from swine samples (p<0.05). Additionally, beta-lactamase producing *E. coli*, a bacterium identified as a current antibiotic resistance threat by the CDC[49], was observed proximal to CHOs at eight sampling events compared to one sampling event from a background site (RR=5.4, CI: 0.69 – 43).

The results indicate that while there is some variability within observational groups, more sites with CHOs had phenotypic resistance to multiple classes of antibiotics compared to background sites. While the confidence intervals are large demonstrating high intra-group variability, the relative risk estimates of effect size indicate that resistance to tetracycline, ampicillin, and resistance to at least one, two, or three antibiotics are all at least twice as likely in swine sites (RR>2.0, p<0.05) compared to background sites.

Chapter 5

This work is the first to model CHO effects on microbial water quality incorporating exposure variables that are approximated using distance and this work quantifies the strength of determinants for dissemination of *E. coli* in surface water. To determine whether higher concentrations of *E. coli* in sites with CHOs were a result of differences in land use, environment, or exposure to confounding sources between observational groups, Chapter 5 uses the outputs from Chapter 3 to construct a multiple linear model taking into account precipitation, land use, and hydrological variables while also controlling for confounding fecal sources from human septic and wildlife. Chapter 5 also compared methods for approximation of exposure to fecal sources

Chapter 5 found that approximating exposure to fecal sources best predicted *E. coli* concentration when pairing intensity metrics and distance metrics rather than using density metrics such as population density. Analyzing methods for creating exposure variables, chapter 5 found that density and gravity methods were less useful for predicting *E. coli* concentrations from human and lagoon exposures compared to sum of exponential decay methods.

Model performance metrics identified that stepwise regression model using sum of exponential decay with interaction (SED-int) methods for approximation of exposure had best performance. Variables selected from the stepwise regression models using SED-int methods for exposure variables (i.e. M6) were first runoff events, prior 24 hour precipitation, water temperature, HF183 and pig-2-bac concentrations, and address and lagoon exposures. Models suggest that *E. coli* concentration in surface waters is primarily driven by precipitation events after a dry period suggesting precipitation-driven transport of accumulated fecal bacteria followed by a dilution effect from persistent rain. While a first runoff event was associated with

a 5.0 ($p < 0.001$) times higher *E. coli* concentration compared to sampling events without first runoff events, a smaller contribution of 1.05 ($p < 0.001$) times higher *E. coli* concentration was observed for one IQR increase in prior 24 hour precipitation. Of measured variables, when controlling for other variables in the model, a one IQR increase (9.2 degree Celsius) in water temperature was associated with a 1.6 ($p < 0.01$) times the concentration of *E. coli*. A one IQR increase (2.3 copies/mL) in concentration of HF183 among detectable samples was associated with 1.2 ($p < 0.05$) times the concentration of *E. coli* with a negligible IQR ratio for pig-2-bac. For exposure variables, when controlling for other variables, one increase in the IQR for lagoon and address exposure was associated with a 1.5 and 1.4 times ($p < 0.05$) higher concentration of *E. coli*, respectively. Modeling results identified that even when controlling for the large effects from precipitation, as well as effects from confounding fecal sources, CHOs were not only associated with higher *E. coli* concentrations in surface water, but had a larger effect compared to human and wildlife exposures. Modeling also identified that address locations representing human sources were also substantively associated with higher *E. coli* concentration.

Dissertation Strengths

This research is the largest field study to-date with respect to sites with ($n=13$) and without ($n=9$) CHOs to assess influence of CHOs on microbial quality of surface water. This research compares microbial water quality parameters between observational groups with well-defined exposure to fecal sources. Similarity was ensured between sites with and without CHOs with respect to watershed area, soil type, and land use and site watershed area was small to reduce bias of including unknown fecal sources. Confounding fecal source variables were also quantitatively assessed for comparability with respect to human population and human

population density and all sites were known to not have influences from wastewater treatment plants or other animal operations. Finally, swine exposure is well-defined such that CHO watersheds have known swine manure application sites and background sites are known to not have influences from CHOs. This study assessed that background and CHO sites were not significantly different with respect to agricultural land use, soil type, watershed area, and human population density. As such, the study reduced bias by having similar watershed areas and human population density between site types reduce confounding fecal sources from wildlife or human septic.

Dissertation Limitations

In chapters 3 and 4, observational groups were assessed for comparability of watersheds with respect to watershed land use and population density to ensure similarity of confounding fecal sources and found that, on average, sites with CHOs have higher *E. coli* concentrations and measures of antibiotic resistance compared to sites without CHOs. Chapter 5 found that exposure variables that do not incorporate proximity/distance (such as population density) may hide differences between observational groups, potentially biasing results. Thus, Chapter 3 and Chapter 4, which compared swine sites to background sites, swine sites had, by chance, about twice the mean exposure compared to background sites from human fecal sources using sum of exponential decay methods incorporating distance of human fecal sources to sampling location. This could be due to more households located near the sampling locations in swine sites compared to sampling locations in background sites. The comparisons in Chapters 3 and 4 may be biased towards increased *E. coli* concentration and increased antibiotic resistance in the swine watersheds due to higher exposure from human fecal sources. However, chapter 5 addresses

these concerns and controls for human exposure using variables that incorporate distance in both the background and swine sites and still finds that exposure to CHOs is significantly positively associated with appreciable effect size on *E. coli* concentrations in surface waters.

On the other hand, it is possible that background sites were affected by CHOs since bacterial transport by air from CHO barn fans or spray irrigation, transport by vectors including flies and birds, or river flooding is not limited by watershed boundaries. Additionally, river movement was consistently slow-moving to stagnant unless a precipitation event occurred suggesting that upstream river flow is possible in especially with a strong wind. CHO influence in background sites would bias results towards the null and so significant differences observed are despite possible CHO influence in background sites.

While watershed variables were comparable across observational groups, CHO-specific manure and land use management practices were not known, which could mitigate or contribute to *E. coli* or antibiotic resistant *E. coli* in surface waters. While modeling results demonstrated that proximity and size of CHO contributed to higher *E. coli* concentrations in surface water, it is possible that some sites with large CHOs near surface water do not similarly affect *E. coli* concentrations due to land management practices that slow *E. coli* transport and increase infiltration time. Similarly, while sprayfield exposures were positively, though not always significantly, associated with *E. coli*, this work was unable to identify spray events nor was it able to determine functionality of lagoon or sprayfield infrastructure. This work assumes that CHOs in swine sites had the same land and waste management practices and were in compliance with all regulations. It is possible that specific CHOs implemented more stringent land use and waste management practices compared to minimum requirements that reduced fecal bacterial load or inhibited transport of fecal bacteria, such as manure composting or incorporation of

wetland cells. At the same time, it is also possible that specific CHOs were not compliant with current waste and land management regulations. Waste and land management practices were not known, however the possibility that differences exist in CHO-specific waste and land use practices may contribute to the intra-group variability observed of *E. coli* concentration in swine sites suggesting that while not every CHO contributes fecal bacteria to surface water, on average, CHOs do. Understanding CHO-specific waste and land use practices that mitigate transport of *E. coli* to surface water could inform policy-making to better inform regulatory policies that can be feasible and are currently implemented among CHO operators.

There is evidence to suggest septic as a source of fecal bacteria in surface waters from this study, however more work is needed to determine whether septic is a fecal source in rural NC. High prevalence of HF183 detection was observed across observational groups and modeling results show that both HF183 concentration and distance-based metrics for household locations were significantly associated with higher concentrations of *E. coli*. While septic functionality in households is not known for sampling locations, it is notable that two lines of evidence suggest septic sources may contribute to higher concentrations of *E. coli*: presence of HF183 and modeling results. However, it is not clear whether low concentrations of HF183 are indicative of low concentrations of human feces, long-lasting persistence of the marker, or from cross-reactivity with other fecal sources. High concentrations of HF183 and pig-2-bac would indicate high probability of human and swine sources, respectively. While a few samples had high concentrations of HF183, most samples had low concentrations of HF183 in both background and swine sites. At the same time, HF183 has lower sensitivity than pig-2-bac, suggesting that HF183 target does not always detect when human feces are present, potentially underestimating the concentration of HF183 in surface waters. It is possible that cross-reactivity

could occur with MST markers, but it is not probable that cross-reactivity would occur differentially between background and swine sites because sites were in a similar geographic region and thus had similar fecal sources. As such, any significant differences identified would not be due to cross-reactivity. While the pig-2-bac marker has been detected in swine effluent from NC both in this dissertation and elsewhere [21], this work did not test septic tanks for the presence of HF183 so cannot confirm that the hypothesized source contains HF183. Even still, the modeling results imply that household locations are sources of *E. coli* in surface water, and septic in addition to other common household fecal sources such as pets or small-scale animal husbandry should be further investigated.

This work was not designed to assess human health risk to pathogens from any fecal source or to assess human health risk from *E. coli* and antibiotic resistant *E. coli* in surface water. While differences in prevalence of antibiotic resistant *E. coli* were consistently higher among swine sites compared to background sites, antibiotic use practices were not known for the CHOs or human population in sites sampled. CHO antibiotic use practices are only publically available aggregated at the national level.

Research Implications

The following section explores the possible implications of this body of work including how these results could influence future state and federal policy and regulatory structures as well as possible implications for future scientific research studies.

Regulatory Framework

Federal and state regulation and scientific research regarding environmental effects of CHOs has primarily focused on nutrients in surface water [67] and, to a lesser extent, on

microbial water quality. While the state of NC and the US EPA regulate fecal indicator bacteria concentration in surface waters for public health and require discharge permits for fecal sources defined as point sources under the NPDES, such as wastewater treatment plants, food animal production operations are not federally recognized as point sources for fecal bacteria or nutrients. The federal Clean Water Act of 1972 initially identified cattle feedlots as point sources requiring a NPDES permit that identified effluent limitation guidelines and standards for discharges to surface waters including nutrient and microbial water quality parameters. In 2003, all concentrated animal feeding operations (CAFOs) were required to obtain a NPDES permit as well as implement a nutrient management plan (NMP), however these rules were challenged in court and final rules adopted in 2012 only required CAFOs which “discharge or propose to discharge” to apply for a NPDES permit [102]. Additionally, these rules maintained the agricultural stormwater exemption which removes liability from CAFOs for discharges due to nature, such as from precipitation-driven runoff from sprayfields, as long as manure application is applied in accordance with nutrient management plans (NMPs) [103].

State regulation can be more strict than federal EPA regulation with some states, such as Michigan, requiring all CAFOs to obtain discharge permits unless CAFOs can prove they do not discharge [103]. In practice, NC CHOs are effectively regulated as non-discharge sources. For example, in NC, only 13 CHOs comprising 0.5% of CHO swine produced have discharge permits under the NPDES (i.e. point source) structure 2018 [3]. Non-discharge, general permits for CHOs are regulated in the NC State Administrative Code chapter 15A, subchapter 02T to limit the effects of non-discharge sources on the environment, however assessment, monitoring, and regulation of CHO microbial effects is absent. The state regulatory structure is in the form of NMPs, which are permitted by the NC DEQ, and focus extensively on nutrient balances

between receiving crops and applied manures on sprayfields. Other regulations for CHO waste management practices include requiring setbacks between CHO land application of manure to residences and surface waters, prohibiting land application of waste prior to and during precipitation events, and not exceeding agronomic rates for nitrogen application on crops. The only parameter specific to microbial water quality assessed in the State Administrative Code applies only to newly constructed or expanded CHOs stating that annual average fecal coliform concentrations in CHO final liquid effluent must be less than 7000 most probable number/100mL [104]. While NMPs indicate that “any discharge of waste which reaches surface water is prohibited,” monitoring is not required to assess off-site transport of nutrients or fecal bacteria.

At the same time, there are federal water quality standards for fecal bacteria, and the EPA identifies pathogens as the largest cause of impairment to US rivers and streams attributing most surface water impairment to agriculture [7]. NC DEQ has the authority to require monitoring requirements of surface water to determine effect of waste on surface and ground water [105], however recommendations or rules for monitoring have not been adopted for nutrients or for microbial water quality for CHOs. Past efforts to develop state rules in NC for monitoring CHOs included a 2007 petition to the NC Environmental Management Commission to implement rules to assess effects of CHOs on water quality. Initial proposed rules identified sampling parameters including fecal coliform concentration and various nutrient parameters [106], however final proposed rules in 2010 did not include fecal coliform parameters[107]. Public comments and DEQ response records indicate fecal coliforms were removed due to inability of proposed monitoring to determine source of fecal coliforms and the low temporal

sampling scale which would not allow enforcement under current US EPA federal sampling requirements of five samples within a thirty day period [108].

Thus, the effects by CHOs in NC have been not been assessed by the federal or state regulatory structures. While NC state requirements in NMPs prohibit transport of swine waste by runoff, discharge, or land application to surface water [65], CHOs in NC are almost always regulated as non-point sources so monitoring is not required to assess off-site transport of nutrients, FIB, or AREs.

Rationale for Regulation Under Current Policy

There are two rationales for recognizing CHOs as requiring a discharge permit under current regulatory policy. First, by federal regulation, the agricultural stormwater exemption for regulating discharge can only be upheld when CHOs are applying manure in accordance with CHO-specific, state-regulated NMPs. However NC NMPs have a required specification that “animal waste shall not reach surface waters of the state by runoff...during operation or land application” and that “any discharge of waste which reaches surface water is prohibited”[65]. This research provides three lines of evidence that swine waste from many CHOs reaches surface water, making these CHOs out of compliance with their NMP. First, the presence of swine-feces specific MST marker pig-2-bac in surface water suggests that swine feces have been transported off-site. Second, the presence of a CHO in swine sites is associated with almost twice the amount of samples above federal *E. coli* water quality metrics compared to background sites (RR=1.74, CI: 1.30 – 2.33, p<0.001) and, using *E. coli* alone instead of all fecal coliforms, swine sites are also almost twice as likely to be above the NC state standard for fecal coliform geometric monthly mean (RR=1.86, CI: 1.26 – 2.75, p<0.01). Likewise, sites with a CHO are twice as likely to be above the monthly maximum fecal coliform standard (RR=2.21, CI: 1.28 –

3.83, $p < 0.01$) compared to sites without a CHO. Third, modeling results suggest that distance and increased manure production capacity of CHOs are associated with increased *E. coli* concentrations even when controlling for human and wildlife sources of *E. coli*.

A second rationale for replacing CHO non-discharge permits with discharge permits under current law is that modeling results demonstrate that CHOs have a substantive effect on *E. coli* concentration even when accounting for the effects of precipitation and other possible sources of fecal bacteria (e.g. humans, wildlife). As such, precipitation-related contribution of fecal bacteria to surface water is probably not the only mechanism by which CHOs affect fecal bacteria concentrations in surface. Furthermore, although a runoff event (i.e. precipitation > 10 mm in a 48 h period) is significantly associated with non-compliance to the EPA *E. coli* standard in both swine and background sites, swine sites are more likely than background sites to be non-compliant when a runoff event has *not* occurred (RR=2.17, CI: 1.41 – 3.33, $p < 0.001$). This also suggests that while precipitation-driven transport is important, other transport mechanisms besides agricultural stormwater occur and contribute to microbial quality of surface water that is out of compliance with EPA regulatory standards.

Proposed Changes to Policy and Management

This research provides multiple lines of evidence that CHOs, on average, discharge fecal bacteria to surface waters indicating that federal and state regulatory policies should consider CHO lagoons and precipitation-driven transport of swine manure from CHO sprayfields as sources for discharge of pathogens and AREs in surface water. Policies should be implemented to regulate microbial discharge from CHOs and additional steps taken to reduce bacterial load from CHOs to surface water. One structure already in place is the NPDES which could designate CHOs as point sources with discharge permits. Additionally, NC DEQ has the

authority to implement monitoring requirements of surface water to determine effect of CHO waste on surface and ground water [105]. As such, the EPA and NC DEQ should give more consideration to regulating discharge from CHOs by requiring monitoring or requiring evidence that CHO-specific land management practices mitigate microbial transport, even during precipitation events. To determine whether off-site transport of swine manure has occurred, the detection of pig-2-bac could be used as a supporting metric to assess discharge from CHOs. This MST marker has very high sensitivity and specificity, making pig-2-bac a candidate for regulatory decision-making. Detection of pig-2-bac paired with measures of specific conductance and concentration of bacteria could be a first step towards determining efficacy of swine waste management and land use management strategies.

This work highlighted that precipitation, especially first runoff events where precipitation follows a dry period, is influential for *E. coli* transport to surface water. Regulatory policies should be implemented Modeling identified that precipitation is a significant transport mechanism of swine manure to surface water and while NC general statutes for surface water and wetland standards suggests that precipitation-driven effects of agricultural runoff are natural and from “uncontrollable nonpoint source pollution” and that CHOs do not have control over discharge caused by “natural conditions” (see subchapter 02B.0211, 02B.0205 [109]), there is a large body of research summarized below that suggests there are many waste management and land management techniques CHOs can use to mitigate effects of agricultural stormwater on microbial quality of surface water[28,101].

Effective waste and land use management practices can first be identified from prior scientific research and also from CHOs that demonstrate that they do not affect microbial water quality of surface water. These CHOs can provide important information regarding the manure

management and land use strategies needed to reduce transport of fecal bacteria whether by precipitation or other mechanisms. Waste treatment can reduce the effects of CHOs on fecal bacteria in surface water[55]. Waste management could be regulated by DEQ permits to reduce the fecal bacteria and pathogen concentrations in final effluent that is land-applied, potentially using pathogen management plans (PMPs) in addition to NMPs. Research has identified many technologies that can reduce fecal bacteria and pathogens in manure such as incorporating secondary or tertiary waste treatment systems and other technologies that inactivate pathogens better than anaerobic treatment such as long term storage, composting, and high temperature fermentation [28,55].

In addition to waste management practices, CHO-specific land use management decisions could be incorporated into NPDES permits and/or PMPs and NMPs by the NC DEQ. For example, land management decisions can increase infiltration time of runoff from normal precipitation events through the use of, for example., infiltration ponds, galleries, riverbank and sand filtration systems, and constructed or restored wetlands [28]. Wetlands can reduce bacterial transport to surface waters through physical (e.g. vegetative barriers), chemical (e.g. UV exposure), and biological (e.g. predation) means [18,83,110,111]. Regulatory structures could also be put in place to deter sprayfield drainage running directly to road ditches which can act as a pipe to transport runoff to nearby streams. These local flow dynamics may be important for understanding differing effects of CHOs on microbial quality of surface water. Finally, while land use treatment efficacy may reduce transport of bacteria to surface water, treatment is dependent on concentrations of influent bacteria[55]. Land use management should be paired with waste management since treatment does not always sufficiently reduce fecal bacteria or pathogen concentrations[18,83].

Calls for Monitoring Pathogens and Antibiotic Resistance Elements

Other bacteria or viruses in addition to fecal bacteria that can be found in swine feces may be a risk to human health such as influenza A, norovirus, Hepatitis E, *Salmonella*, *Yersina*, *Campylobacter* and others [64]. As such, monitoring should be conducted at CHOs not only to meet EPA water quality standards for fecal indicator bacteria but also for organisms of concern to public health. Especially considering that pathogens are generally found in lower concentrations than fecal indicator bacteria, even log-scale reductions in fecal bacteria concentrations during waste treatment may still allow transmission of pathogens into surface waters.

In addition to reducing pathogen discharge, reducing ARE discharge is also important. Increased antibiotic resistance has been implicated for an excess \$20 billion in US health costs with at least 2 million people with infections resistant to antibiotics needed for treatment [49] and antibiotic use in humans and animals have been implicated in contributing to increased antibiotic resistance (85). Currently there are no state or federal regulatory policies regarding ARE discharges to surface water. Ideally, regulatory agencies could regulate and monitor the sources of selective pressure from ARE sources for antimicrobial resistance to highest priority antibiotics, however research is needed to better identify ARE sources and metrics for monitoring. In the interim, regulatory agencies could provide more transparent antibiotics-use data at the CHO-scale to better inform research. Additionally, regulatory agencies such as the FDA, could remove antibiotics currently approved for use in food animal production that are in the same class as highest priority antibiotics for humans, such as third through fifth generation cephalosporins (e.g. ceftiofur [48]) and fluoroquinolones (e.g. enrofloxacin[48]) in addition to reducing antibiotics use generally, especially among the tetracycline class.

Research priorities to better inform regulatory policies should determine sources of ARE discharges to the environment. In this work, sites with observed resistance to highest priority antibiotics were in swine sites and it is possible that CHOs contributed. However of all sites assessed, sites with highest priority antibiotic resistance also had the highest measures of exposure to human septic sources using SED methods for approximating exposure. Without knowing antibiotics used on specific CHOs, which is not publically available information, and without septic tank or lagoon samples, it is difficult to know whether resistance observed to these high priority antibiotics is due to CHOs, due to septic disposal, or from another source entirely. However, because human or CHO fecal sources may contribute resistance to last-resort antibiotics into surface waters in rural NC, more work should be done to determine whether there is a public health risk. Additionally, it is clear that sites without CHOs have far fewer incidences of antibiotic resistant bacteria demonstrating that selective pressure in the CHO environment may be contributing to resistance. Only one event in a background sample had multi-drug resistance and that background site had the highest human exposure (SED methods) of all background sites. There is some evidence that CHOs also may contribute to horizontal gene transfer of resistance elements [23] and the possible additive effects from both CHOs and human septic have not been assessed on the contribution of multi-drug resistant bacteria in environmental media.

Other Fecal Sources

The presence of MST marker HF183 and results of modeling demonstrate that human source contamination may also have a measureable effect on surface water quality in rural areas in NC. Human fecal sources are more likely to carry human pathogens than other fecal sources and thus if septic sources are routinely contributing fecal bacteria to surface water, this would be

a public health concern. This work provides two lines of evidence that suggest the possibility of septic sources of fecal bacteria. At the same time the observed low concentrations of MST marker HF183 may be due cross-reactivity, and it is possible that higher concentrations of *E. coli* could be associated with distance of household due to presence of grazing livestock and/or pets proximal to households. NC DEQ and other scientific studies should determine whether septic is a source of *E. coli* in surface waters in order to determine whether septic regulation is needed.

Future Work

This work has shown that there is substantive evidence of off-site transport of swine waste to surface waters and that CHOs, on average, increase fecal bacteria concentrations in surface water. Increasing size and proximity of CHO is associated with higher concentrations of *E. coli*. There are policy implications of these results that EPA and NC DEQ should regulate the discharge from CHOs and should require regulation of CHO waste and land use management practices that reduce fecal bacteria load and reduce fecal bacterial transport from CHOs. This research has identified three key research priorities to inform regulatory policies and to better understand microbial quality of surface water.

First, future research about the effects of CHOs or any fecal source on the environment or human health should design well-controlled studies to ensure comparability of observational groups with respect to confounding fecal sources. Specifically related to studies addressing effects on AREs in surface water, the FDA and other federal agencies can support transparency to require that antibiotic-use data be publically available at the CHO-scale so that research determining whether there are effects of CHOs on the dissemination of antibiotic-resistant bacteria can identify sources of antibiotic-specific selective pressure. Alternatively, scientific

access to CHOs could be facilitated by industry officials to facilitate One Health studies that concurrently sample fecal sources and the environment.

Second, research should identify waste and land use management practices that reduce load and transport of FIB, ARE and pathogens from CHOs to inform and support regulatory actions, e.g. pathogen management plans. Research should identify metrics and methods for assessing ARE and pathogen survival and transport with respect to swine waste management and land use management. It will be important to determine whether higher FIB concentrations correlate with pathogens found in swine manure that are pathogenic to humans. This can be done through monitoring and screening for bacteria that are pathogenic to humans in swine waste and surface waters proximal to CHOs. Regulatory agencies should incorporate management plans to reduce ARE and pathogen loading and reduce ARE and pathogen transport to surface waters for all CHOs.

Third, this work identified that human fecal contamination may be another important fecal source contributing to surface waters in rural NC. To better understand whether septic or another human fecal source affects microbial quality of surface water, future work should incorporate study design elements that isolate the effect of septic and, if possible, concurrently sample septic tanks and surface water for human-associated markers. Determination of whether septic sources affect microbial quality of surface water is important for public health due to human pathogens present in human waste and the possibility of human source contribution to antibiotic resistance to highest priority antibiotics.

APPENDIX A: SITE MAPS

Appendix Figures

Figure A1 – Detail for control site BK03

Figure A2 - Detail for control site BK12

Figure A3 - Detail for control site BK14

Figure A4 - Detail for control site BK15

Figure A5 - Detail for control site BK16

Figure A6 - Detail for swine site SW04

Figure A7 - Detail for swine site SW07

Figure A8 - Detail for swine site SW09

Figure A9 - Detail for swine site SW11

Figure A10 - Detail for swine site SW13

Figure A11 - Detail for swine site SW16

Figure A12 - Detail for swine site SW01 and upstream control site BK01U

Figure A13 - Detail for swine sites SW05, SW05C, SW05A, and upstream control site BK05U

Figure A14 - Detail for swine site SW10 and upstream control site BK10U

Figure A15 - Detail for swine sites SW17 and SW17U and upstream control site BK17U

Site-specific maps

Figures A1 through A15 display site details for control sites (BK), swine sites (SW), and upstream control sites (BK-U). Detail for sites include location of sampling (blue square) commercial hog operation (CHO) sprayfield (hatched purple) and lagoon (pink circle), address point (yellow triangle), wetland (speckled green), surface water (linear blue), 100m river buffer and light blue triangles representative of river flow direction.

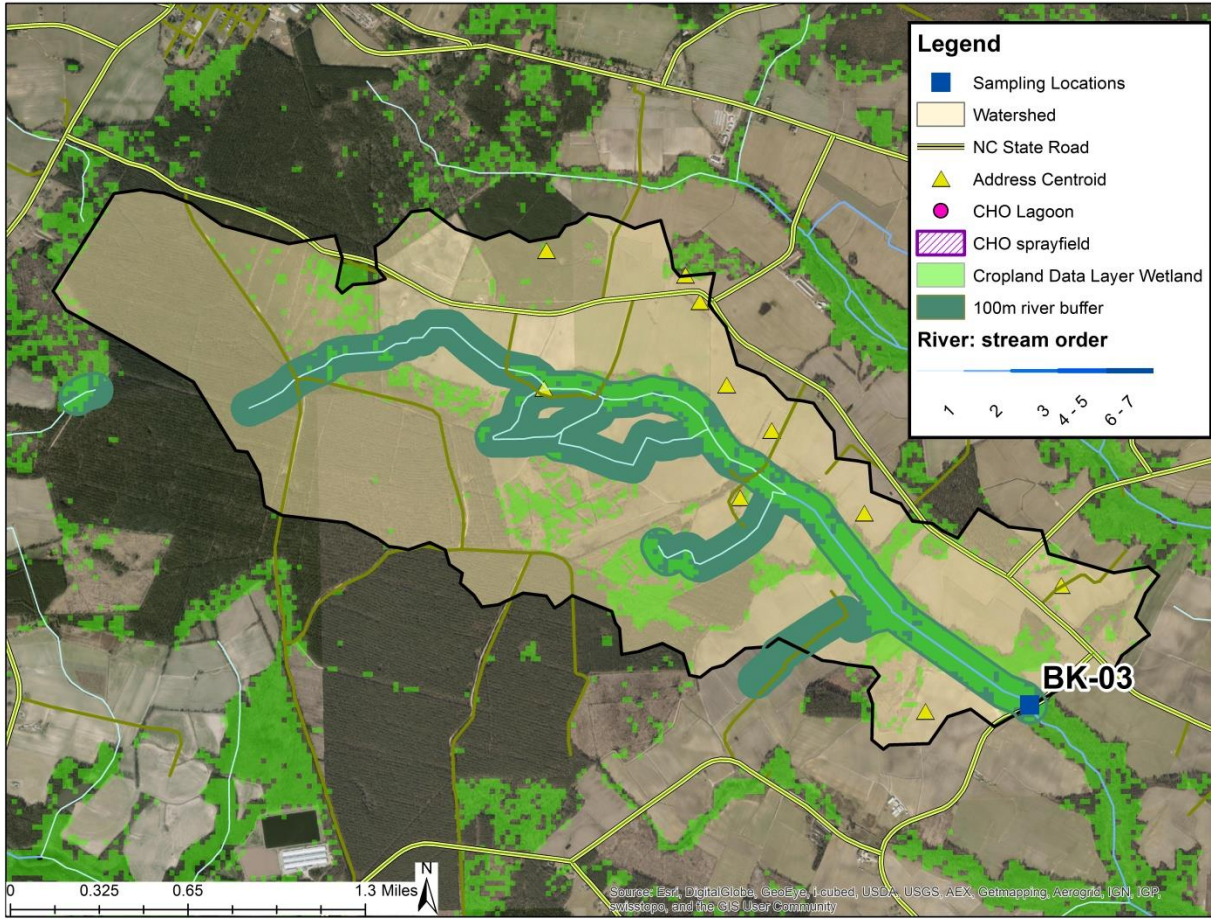


Figure A1 – Detail for control site BK03

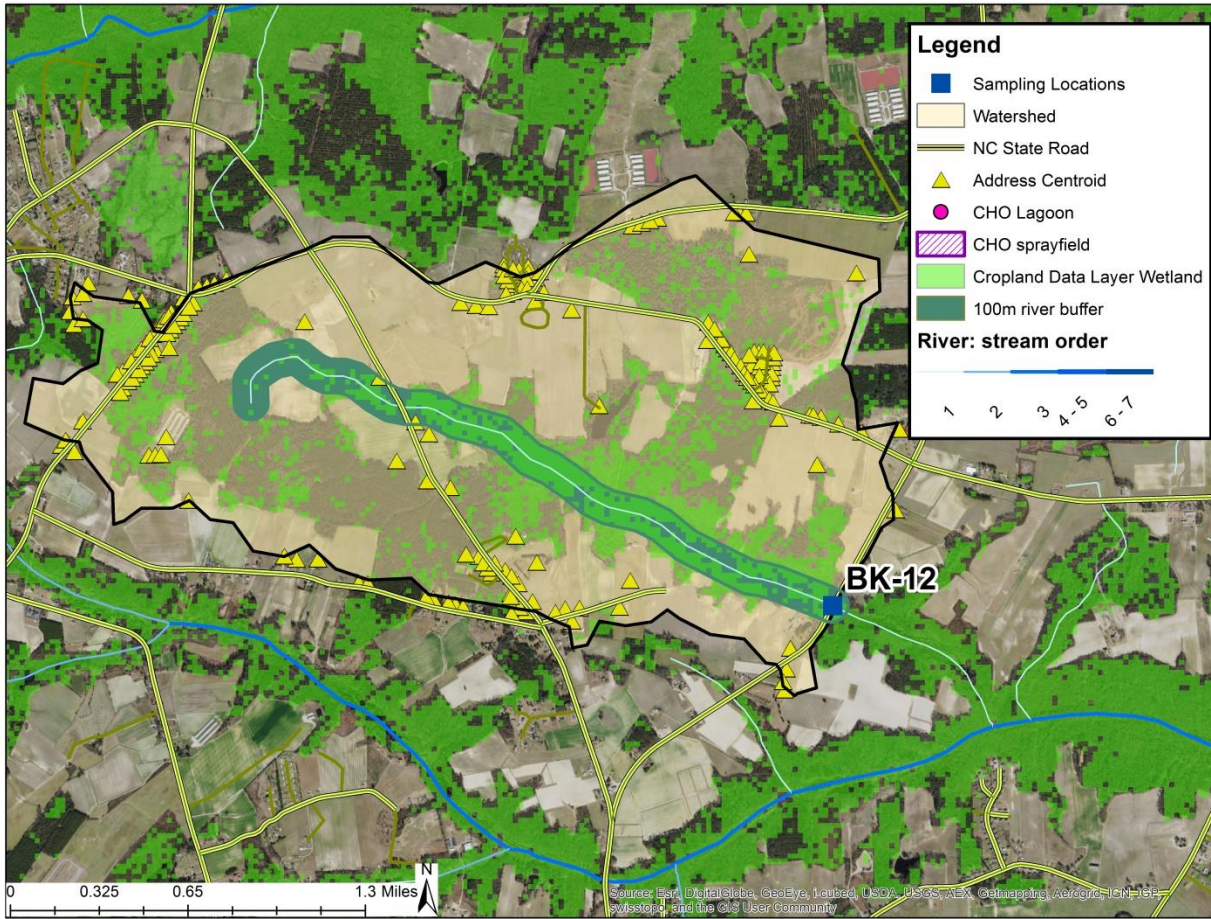


Figure A2 – Detail for control site BK12

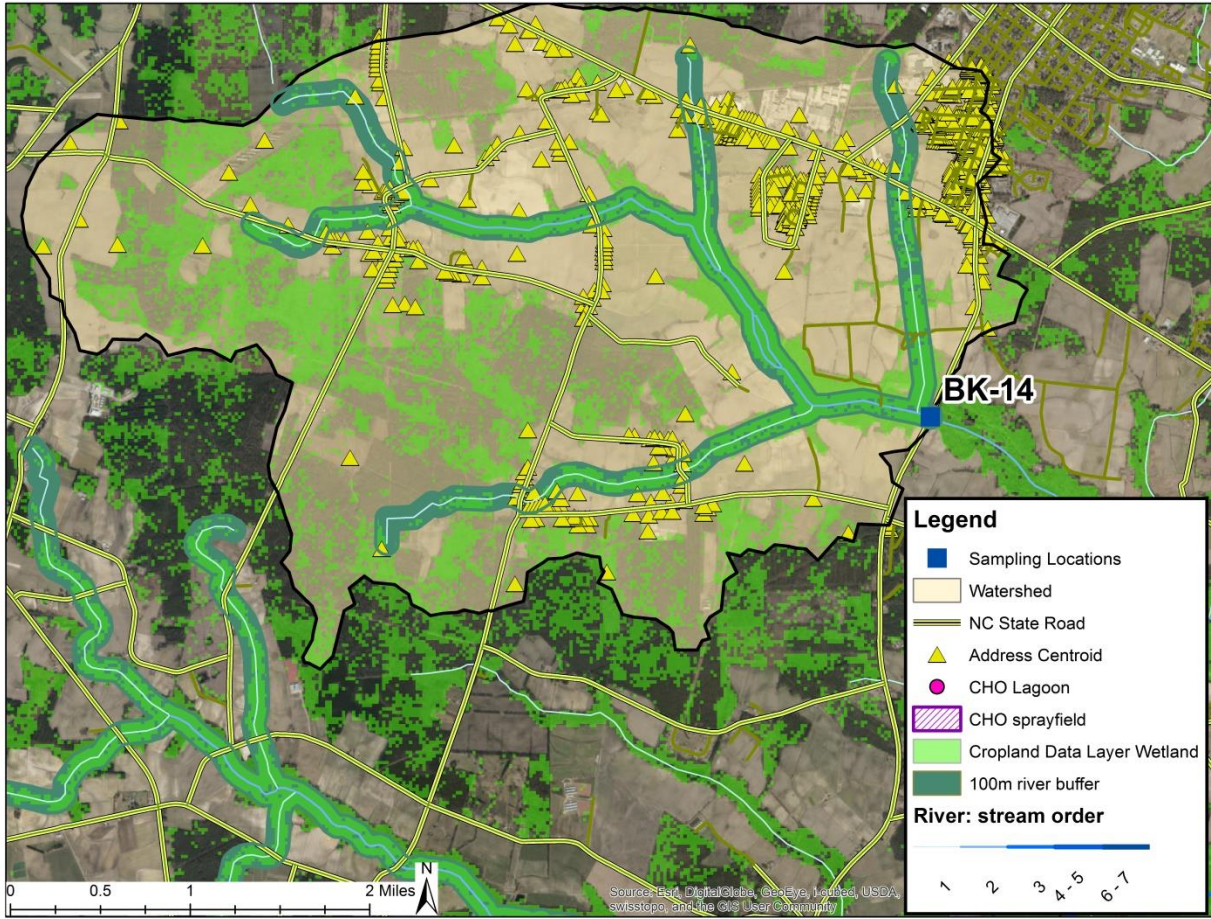


Figure A3 – Detail for control site BK14

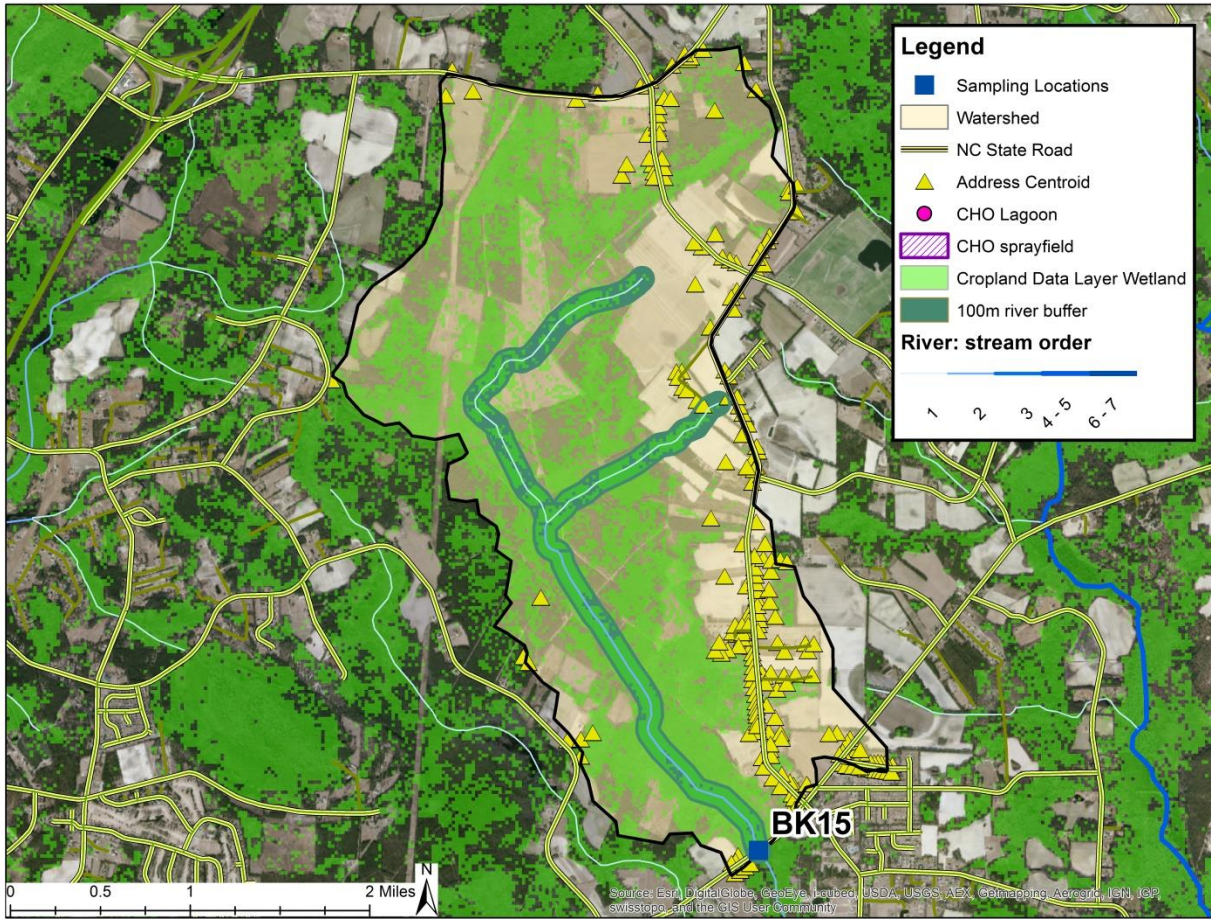


Figure A4 – Detail for control site BK15

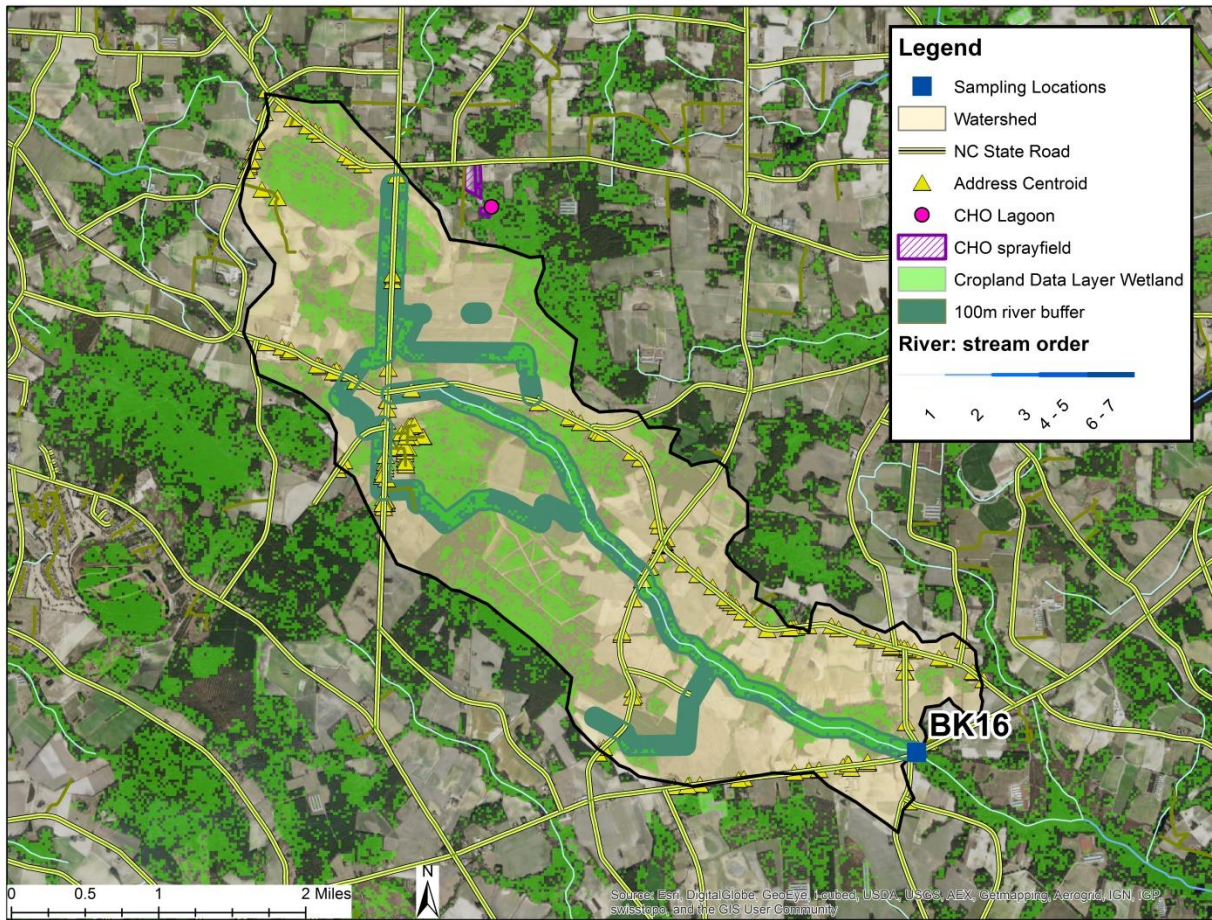


Figure A5 – Detail for control site BK16

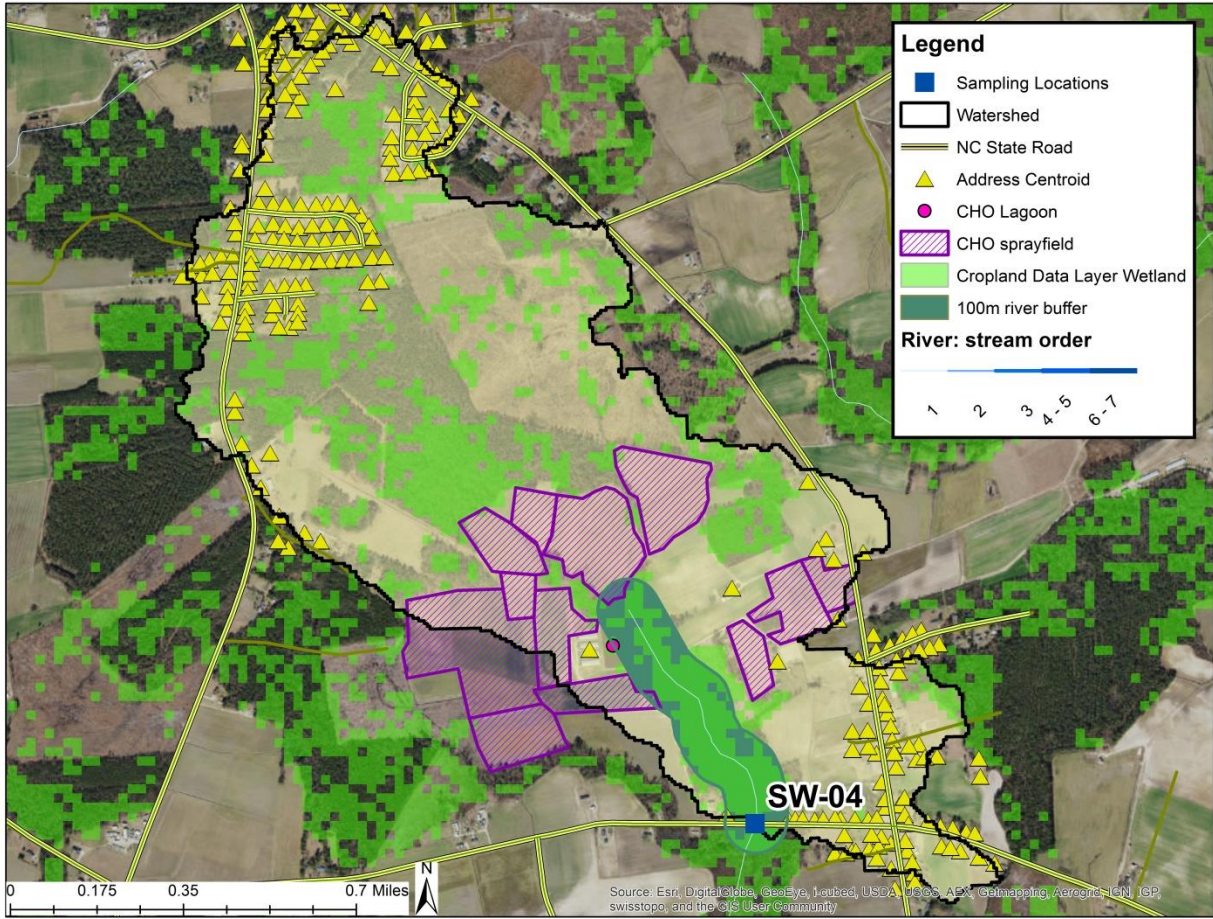


Figure A6 – Detail for swine site SW04

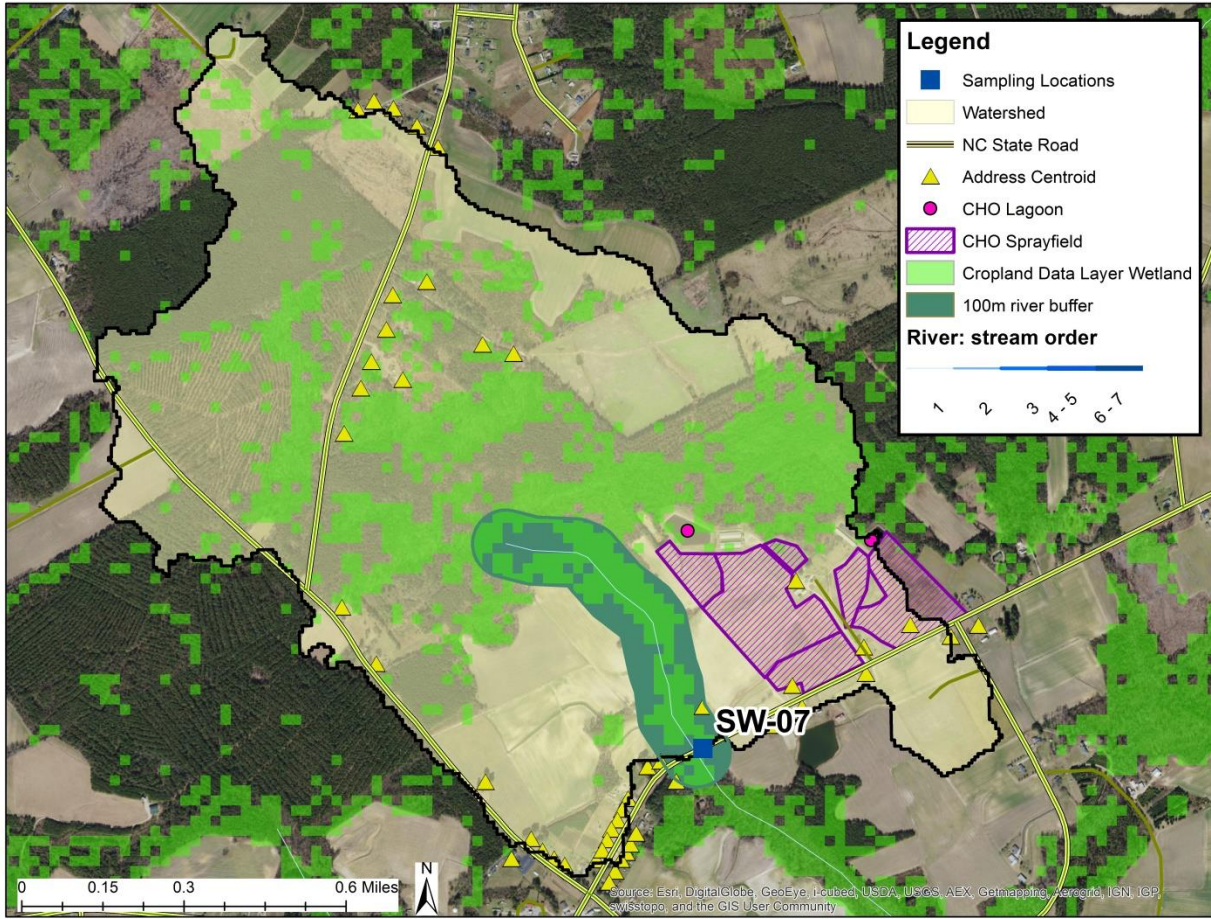


Figure A7 – Detail for swine site SW07

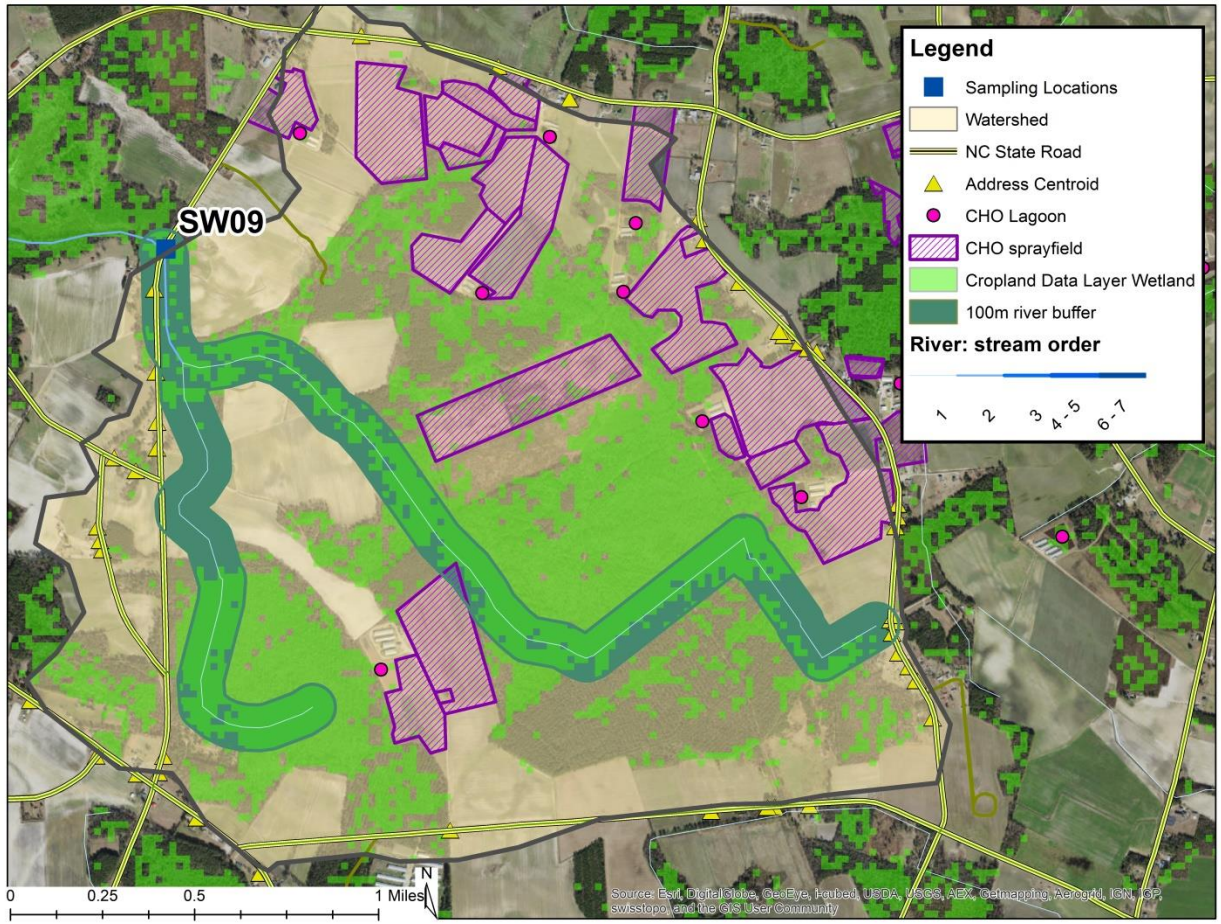


Figure A8 – Detail for swine site SW09

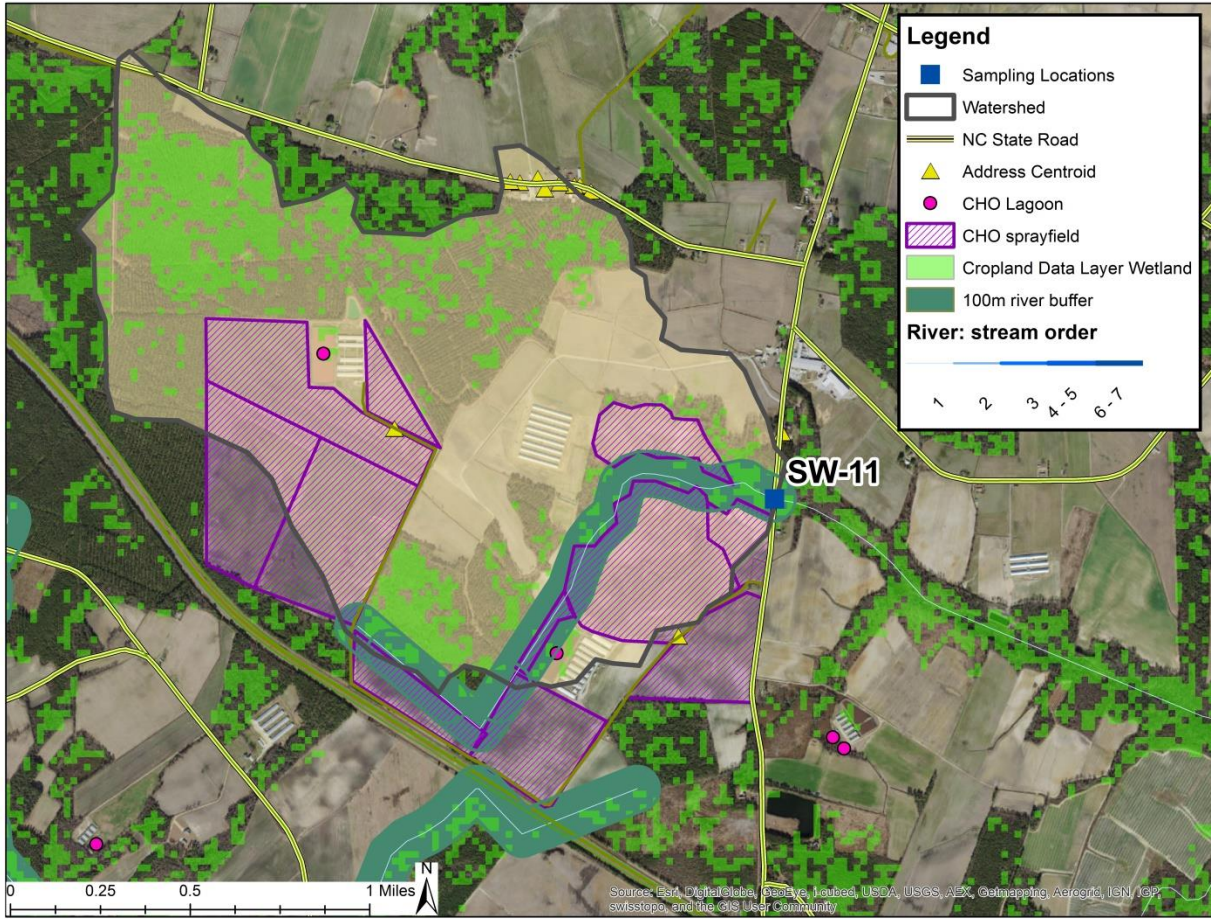


Figure A9 – Detail for swine site SW11

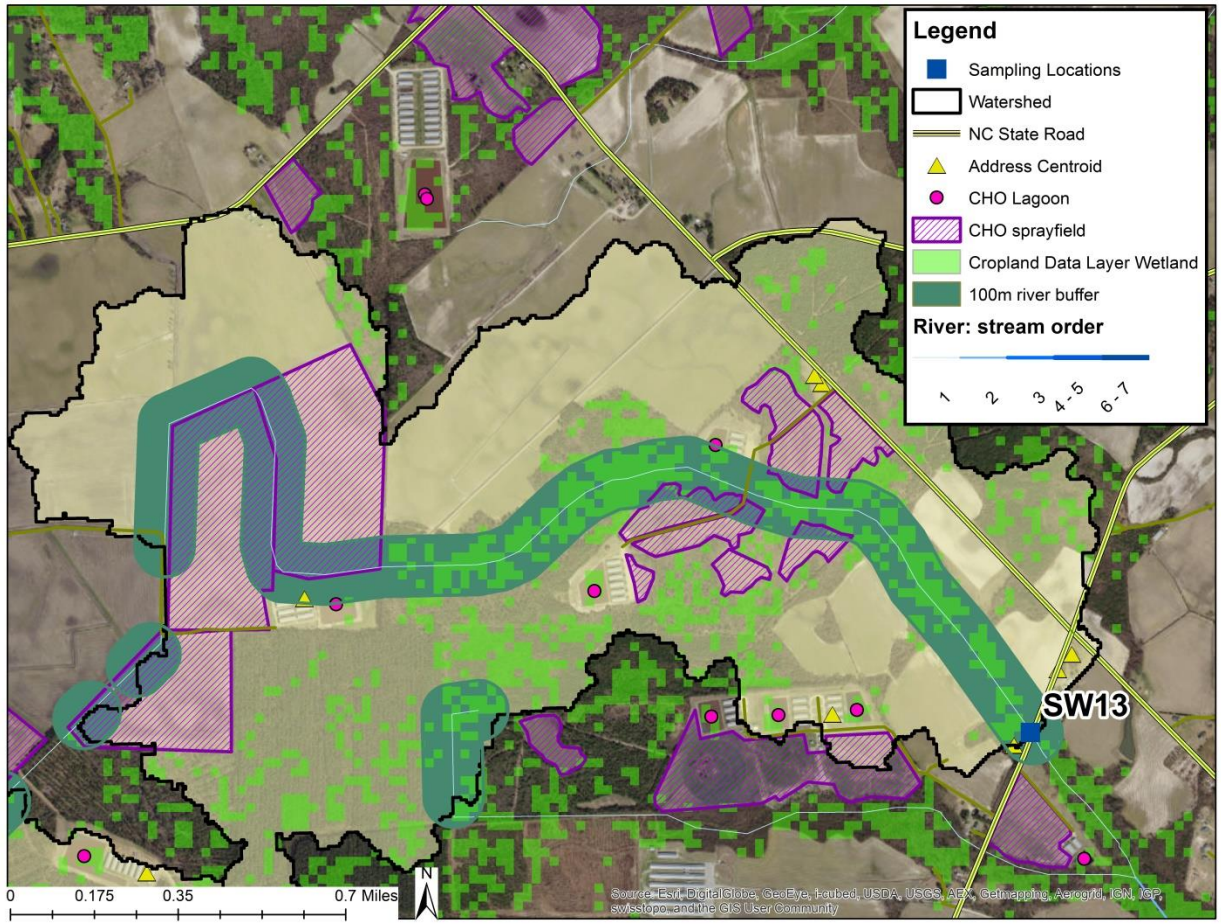


Figure A10 – Detail for swine site SW13

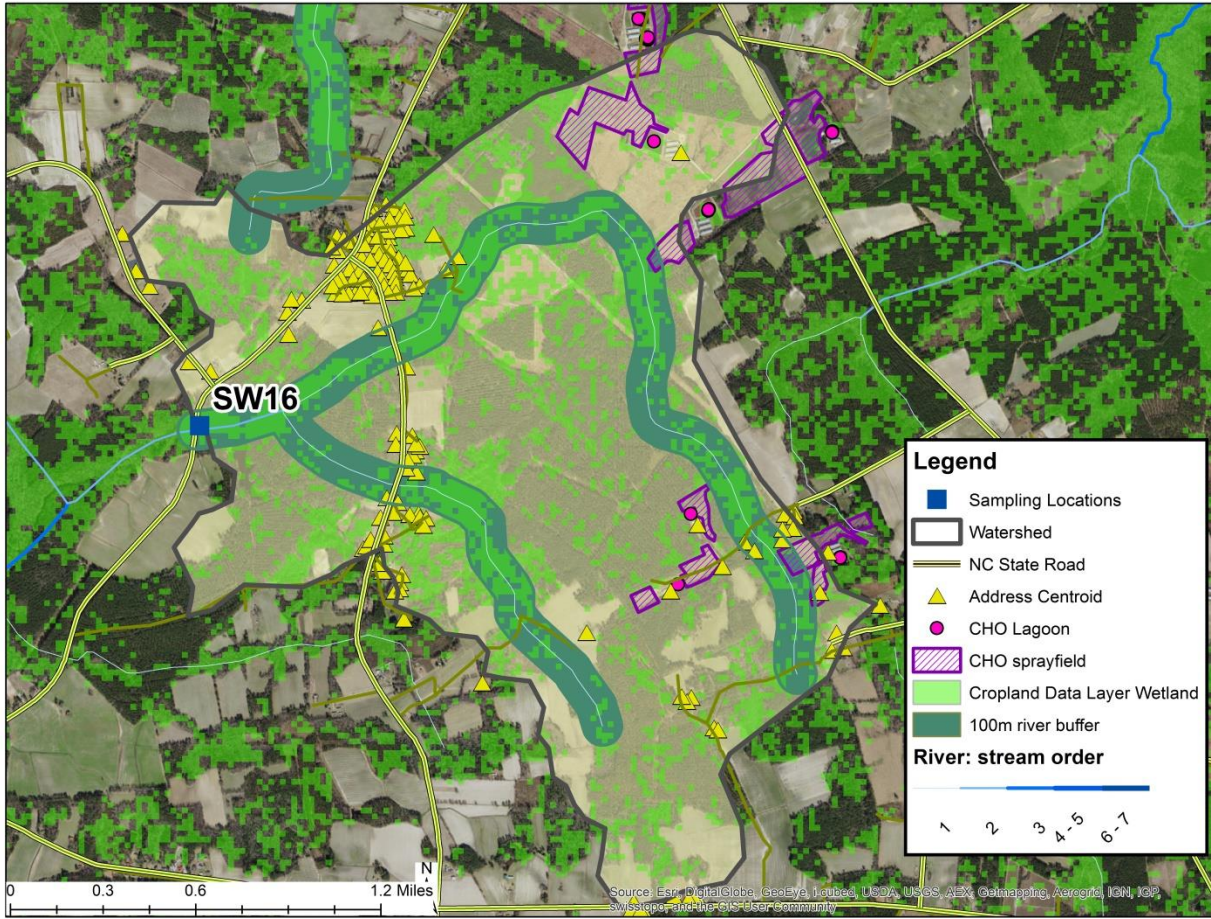


Figure A11 – Detail for swine site SW16

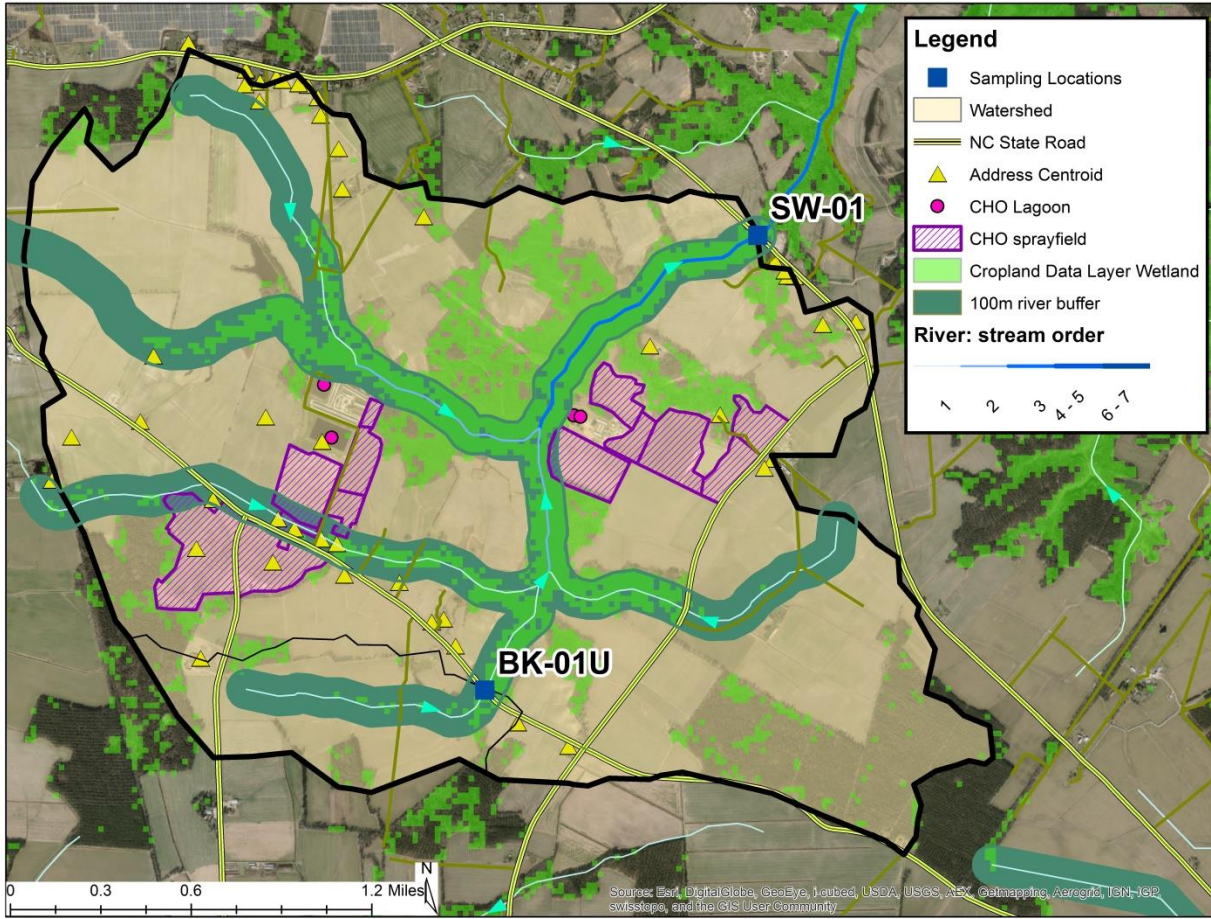


Figure A12 – Detail for swine site SW01 and upstream control site BK01U

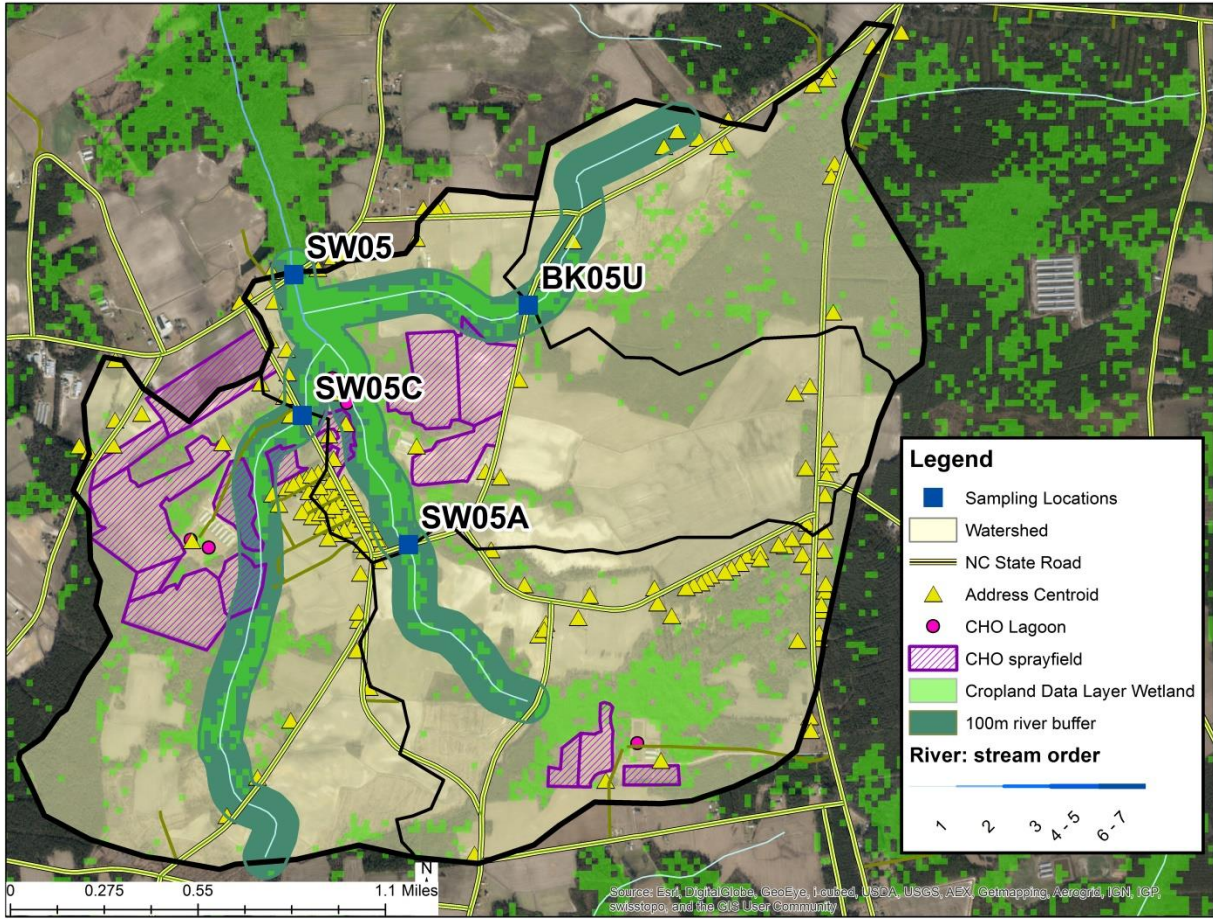


Figure A13 – Detail for swine sites SW05, SW05C, SW05A, and upstream control site BK05U

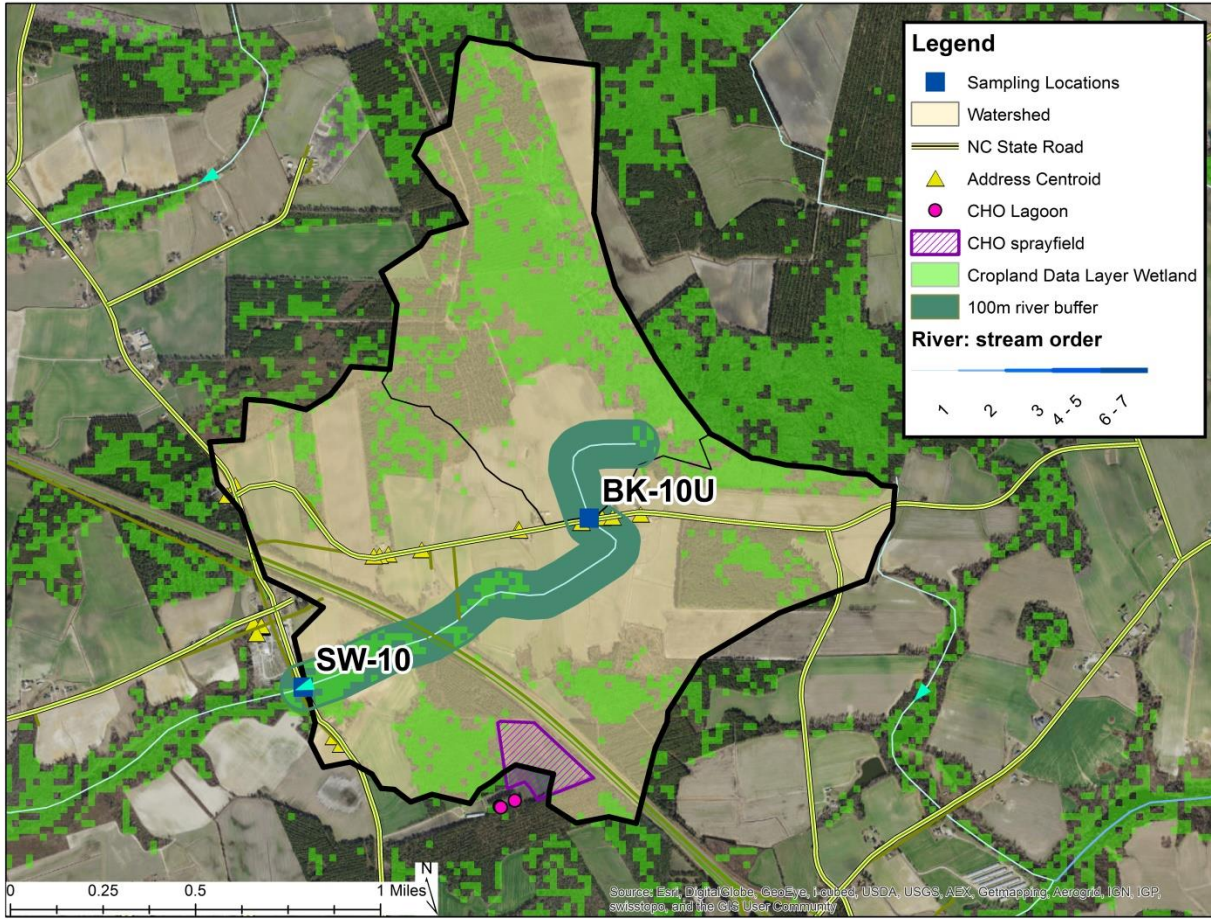


Figure A14 – Detail for swine site SW10 and upstream control site BK10U

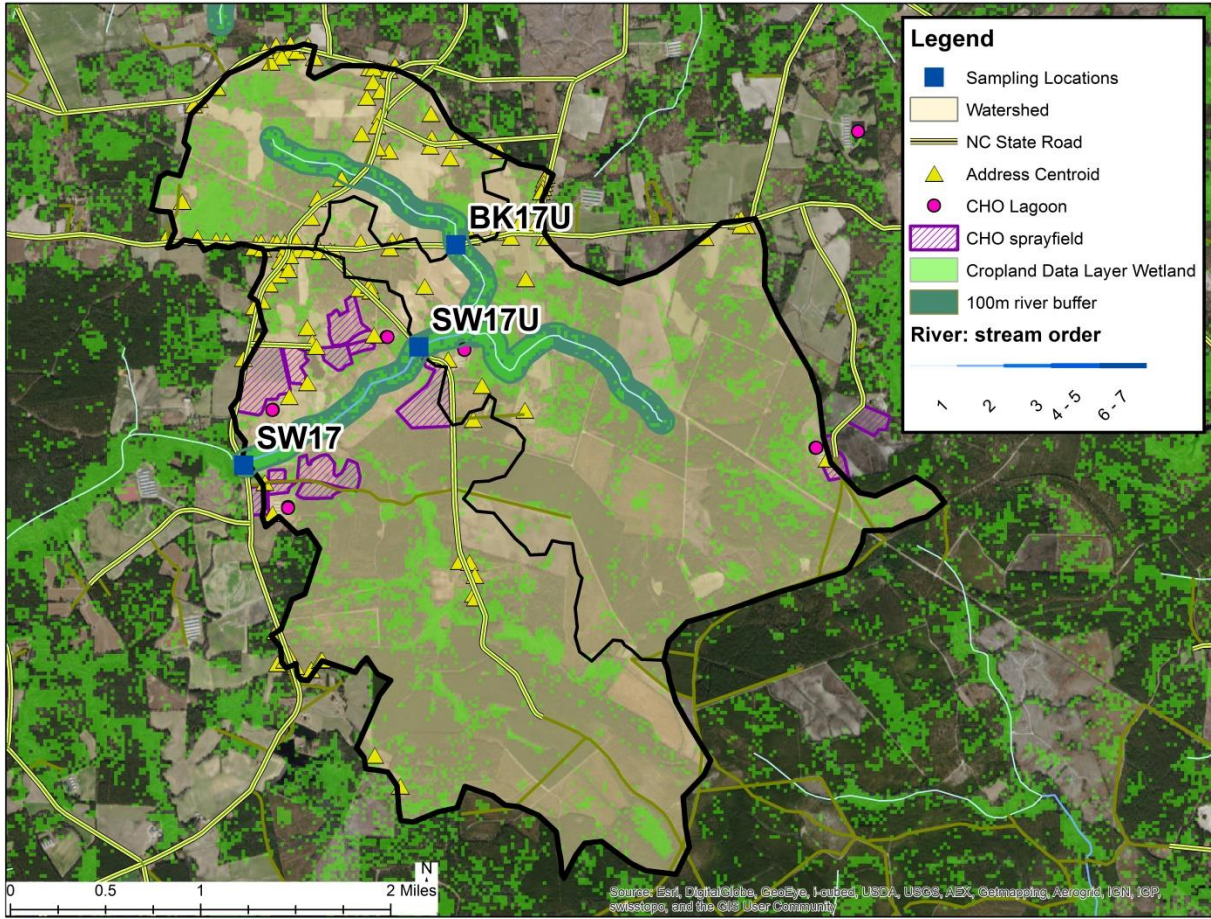


Figure A15 – Detail for swine sites SW17 and SW17U and upstream control site BK17U

APPENDIX B: *E. COLI* ANTIBIOTIC RESISTANCE DATA

Indole-positive *E. coli* isolate data are presented including sample site, sample date, and antibiotic resistance profiles of resistant (R), intermediate resistance (I), or susceptible to amoxicillin-clavulanate acid (AmC) at 20/10 ug, ampicillin (AM) at 10 ug, ceftiofloxacin (FOX) at 30 ug, ceftriaxone (CRO) at 30 ug, chloramphenicol (C) at 30 ug, ciprofloxacin (CIP) at 5 ug, gentamicin (GM) at 10 ug, imipenem at 10 ug, levofloxacin (LVX) at 5 ug, sulfamethoxazole-trimethoprim (SXT) at 24/1 ug, and tetracycline (TE) at 30 ug as determined through Kirby-Bauer disc diffusion in accordance with Clinical Laboratory Standards Institute methods.

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SXT
E001	BK16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E002	BK16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E003	BK16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E004	BK16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E005	BK15	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E006	BK15	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E007	BK15	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E009	SW16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E010	SW16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E011	SW16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E012	SW16	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E013	SW05	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E014	SW05	8/9/2016	S	R	S	R	S	S	S	S	S	S	S
E015	SW05	8/9/2016	S	S	S	S	S	S	S	S	S	R	S
E016	SW05	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E017	SW05A	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E018	SW05A	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E020	SW05A	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E021	SW05C	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E024	SW05C	8/9/2016	S	S	S	S	S	S	S	S	S	R	S
E025	SW17	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E026	SW17	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E029	SW17U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E030	SW17U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E033	BK05U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E034	BK05U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E035	BK05U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E036	BK05U	8/9/2016	S	S	S	S	S	S	S	S	S	S	S
E037	BK10U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E039	BK10U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E041	BK10U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E042	BK10U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E045	SW10	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E047	SW10	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E050	SW10	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E051	SW10	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E053	SW11	9/5/2016	S	S	S	S	S	S	S	S	S	R	S
E054	SW11	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E058	SW11	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E059	SW11	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E060	SW11	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E061	SW04	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E062	SW04	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E063	SW04	9/5/2016	S	S	S	S	S	S	S	S	S	R	S
E064	SW04	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E065	SW04	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E066	SW04	9/5/2016	S	R	S	S	S	S	S	S	S	S	S
E067	BK03	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E068	BK03	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E069	BK03	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E070	BK03	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E073	BK03	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E074	BK14	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E075	BK14	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E079	BK14	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E080	BK14	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E081	SW01	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E082	SW01	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E083	SW01	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E084	SW01	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E085	SW01	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E087	SW01	9/5/2016	R	S	R	S	S	S	S	S	S	S	S
E088	BK12	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E089	BK12	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E091	BK12	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E093	BK12	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E094	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E096	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E097	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E098	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E099	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E100	BK01U	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E101	SW07	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E102	SW07	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E104	SW07	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E105	SW07	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E106	SW07	9/5/2016	S	S	S	S	S	S	S	S	S	S	S
E108	SW13	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E109	SW13	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E110	SW13	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E111	SW13	9/26/2016	S	I	I	S	I	S	S	S	S	S	S
E112	SW13	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E113	BK05U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E115	BK05U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E116	BK05U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E117	BK05U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E118	SW05	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E119	SW05	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E120	SW05	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E121	SW05	9/26/2016	S	R	S	S	S	S	S	S	S	R	S
E122	SW05	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E123	SW05A	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E124	SW05A	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E125	SW05A	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E126	SW05A	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E127	SW05A	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E128	BK15	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E129	BK15	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E130	BK15	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E131	BK15	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E132	BK15	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E133	SW05C	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E134	SW05C	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E135	SW05C	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E136	SW05C	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E137	SW05C	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E138	SW09	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E139	SW09	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E140	SW09	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E141	SW09	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E142	SW09	9/26/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E143	BK17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E144	BK17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E145	BK17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E146	BK17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E148	SW17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E149	SW17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E150	SW17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E151	SW17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E152	SW17U	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E153	SW17	9/26/2016	S	I	S	S	S	S	S	S	S	R	R
E154	SW17	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E155	SW17	9/26/2016	S	S	S	S	S	S	S	S	S	R	S
E156	SW17	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E157	SW17	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E158	SW16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E159	SW16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E160	SW16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E161	SW16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E162	SW16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E163	BK16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E164	BK16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E165	BK16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E166	BK16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E167	BK16	9/26/2016	S	S	S	S	S	S	S	S	S	S	S
E168	SW11	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E169	SW11	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E170	SW11	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E171	SW11	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E172	SW11	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E173	BK10U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E174	BK10U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E175	BK10U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E176	BK10U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E177	BK10U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E178	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E179	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E180	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E181	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E183	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E184	SW10	10/7/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E185	SW04	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E186	SW04	10/7/2016	S	S	S	S	S	S	S	S	S	R	S
E187	SW04	10/7/2016	I	R	S	S	R	S	S	S	S	R	S
E188	SW04	10/7/2016	S	R	S	I	S	S	S	S	S	R	S
E189	SW04	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E190	BK12	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E191	BK12	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E192	BK12	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E193	BK12	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E195	BK14	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E196	BK14	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E197	BK14	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E198	BK14	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E199	BK14	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E200	SW01	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E201	SW01	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E202	SW01	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E203	SW01	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E204	SW01	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E205	BK01U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E206	BK01U	10/7/2016	S	I	S	S	S	S	S	S	S	S	S
E207	BK01U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E208	BK01U	10/7/2016	S	S	S	S	S	S	S	S	S	R	S
E209	BK01U	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E211	SW07	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E212	SW07	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E213	SW07	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E214	SW07	10/7/2016	S	R	S	S	S	S	S	S	S	S	S
E215	BK03	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E216	BK03	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E217	BK03	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E218	BK03	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E219	BK03	10/7/2016	S	S	S	S	S	S	S	S	S	S	S
E220	BK05U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E221	BK05U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E222	BK05U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E223	BK05U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E224	BK05U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E225	SW05	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E226	SW05	11/7/2016	S	R	S	R	S	S	S	S	S	R	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E227	SW05	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E228	SW05	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E229	SW05	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E230	BK15	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E231	BK15	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E232	BK15	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E233	BK15	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E234	BK15	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E235	SW05C	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E236	SW05C	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E237	SW05C	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E238	SW05C	11/7/2016	S	S	S	S	S	S	S	S	S	R	I
E239	SW05C	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E240	SW17	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E241	SW17	11/7/2016	S	R	S	S	S	S	S	S	S	S	S
E242	SW17	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E243	SW17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E244	SW17U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E245	SW17U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E246	SW17U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E247	SW17U	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E248	SW09	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E249	SW09	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E250	SW09	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E251	SW09	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E252	SW09	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E253	BK16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E254	BK16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E255	BK16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E256	BK16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E257	BK16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E258	SW05A	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E259	SW05A	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E260	SW05A	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E261	SW05A	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E262	SW05A	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E263	SW13	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E264	SW13	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E265	SW13	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E266	SW13	11/7/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E267	SW13	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E268	SW16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E269	SW16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E270	SW16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E271	SW16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E272	SW16	11/7/2016	S	S	S	S	S	S	S	S	S	S	S
E273	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E274	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E275	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E276	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E277	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E278	BK17U	11/7/2016	S	S	S	S	S	S	S	S	S	R	S
E279	BK12	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E280	BK12	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E281	BK12	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E282	BK12	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E283	BK12	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E284	SW11	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E285	SW11	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E286	SW11	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E287	SW11	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E288	SW11	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E289	BK10U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E290	SW10	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E291	SW10	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E292	SW10	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E293	SW10	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E294	SW10	11/28/2016	S	S	S	S	S	S	S	S	S	R	S
E295	SW04	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E296	SW04	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E297	SW04	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E298	SW04	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E299	SW04	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E300	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E301	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E302	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E303	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E304	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E305	BK01U	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E306	BK03	11/28/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E307	BK03	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E308	BK03	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E309	BK03	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E310	BK03	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E311	SW01	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E312	SW01	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E313	SW01	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E314	SW01	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E315	SW01	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E316	BK14	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E317	BK14	11/28/2016	S	R	S	S	S	S	S	S	S	S	S
E318	BK14	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E319	BK14	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E320	BK14	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E321	SW07	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E322	SW07	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E323	SW07	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E324	SW07	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E325	SW07	11/28/2016	S	S	S	S	S	S	S	S	S	S	S
E326	SW17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E327	SW17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E328	SW17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E329	SW17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E330	SW17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E331	SW05A	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E332	SW05A	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E333	SW05A	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E334	SW05A	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E335	SW05A	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E336	SW17	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E337	SW17	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E338	SW17	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E339	SW17	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E340	SW17	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E341	BK15	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E342	BK15	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E343	BK15	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E344	BK15	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E345	BK15	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E346	SW13	12/14/2016	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E347	SW13	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E348	SW13	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E349	SW13	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E350	SW13	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E351	BK05U	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E352	BK05U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E353	BK05U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E354	BK05U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E355	BK05U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E356	SW05	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E357	SW05	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E358	SW05	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E359	SW05	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E360	SW05	12/14/2016	S	R	S	R	S	S	S	S	S	R	S
E361	SW05C	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E362	SW05C	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E363	SW05C	12/14/2016	S	R	S	S	S	S	S	S	S	S	S
E364	SW05C	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E365	SW05C	12/14/2016	S	S	S	S	S	S	S	S	S	R	S
E366	SW16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E367	SW16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E368	SW16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E369	SW16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E370	SW16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E372	BK16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E373	BK16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E374	BK16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E375	BK16	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E376	SW09	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E377	SW09	12/14/2016	S	R	S	S	S	S	S	S	S	R	S
E378	SW09	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E379	SW09	12/14/2016	S	R	S	S	S	S	R	S	R	R	R
E380	SW09	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E381	BK17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E382	BK17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E383	BK17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E384	BK17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E385	BK17U	12/14/2016	S	S	S	S	S	S	S	S	S	S	S
E386	BK10U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E390	BK10U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E391	BK03	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E392	BK03	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E393	BK03	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E394	BK03	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E395	BK03	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E396	SW10	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E397	SW10	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E398	SW10	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E399	SW10	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E400	SW10	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E401	SW11	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E402	SW11	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E403	SW11	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E404	SW11	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E405	SW11	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E406	SW01	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E407	SW01	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E408	SW01	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E409	SW01	1/16/2017	S	S	S	S	S	S	S	S	S	R	S
E410	SW01	1/16/2017	S	S	S	S	S	S	S	S	S	R	S
E411	BK14	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E412	BK14	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E413	BK14	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E414	BK14	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E415	BK14	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E416	BK12	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E417	BK12	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E418	BK12	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E419	BK12	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E420	BK12	1/16/2017	S	S	S	S	S	S	S	S	S	R	S
E421	SW04	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E422	SW04	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E423	SW04	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E424	SW04	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E425	SW04	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E426	SW07	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E427	SW07	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E428	SW07	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E429	SW07	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E430	SW07	1/16/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E431	BK01U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E432	BK01U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E433	BK01U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E434	BK01U	1/16/2017	S	S	S	S	S	S	S	S	S	R	S
E435	BK01U	1/16/2017	S	S	S	S	S	S	S	S	S	S	S
E437	BK05U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E438	BK05U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E439	BK05U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E441	SW05C	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E442	SW05C	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E443	SW05C	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E444	SW05C	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E446	SW05A	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E447	SW05A	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E448	SW05A	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E449	SW05A	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E450	SW05A	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E451	SW05	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E452	SW05	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E453	SW05	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E454	SW05	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E455	SW05	1/30/2017	S	S	S	S	S	S	S	S	S	R	S
E456	BK16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E457	BK16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E458	BK16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E459	BK16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E461	BK15	1/30/2017	S	I	S	S	S	S	S	S	S	S	S
E462	BK15	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E463	BK15	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E464	BK15	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E465	BK15	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E466	SW09	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E468	SW09	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E469	SW09	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E470	SW09	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E471	SW16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E472	SW16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E473	SW16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E474	SW16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E475	SW16	1/30/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E476	SW13	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E477	SW13	1/30/2017	S	I	I	S	I	S	S	S	S	S	S
E478	SW13	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E479	SW13	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E480	SW13	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E481	SW17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E482	SW17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E483	SW17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E484	SW17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E485	SW17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E486	SW17	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E487	SW17	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E488	SW17	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E489	SW17	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E490	SW17	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E492	BK17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E493	BK17U	1/30/2017	S	S	S	S	S	S	S	S	S	S	S
E496	BK01U	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E497	BK01U	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E498	BK01U	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E499	BK01U	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E500	BK01U	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E501	SW01	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E502	SW01	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E503	SW01	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E504	SW01	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E505	SW01	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E506	SW11	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E507	SW11	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E508	SW11	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E509	SW11	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E510	SW11	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E511	SW07	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E512	SW07	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E513	SW07	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E514	SW07	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E515	SW07	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E517	BK12	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E518	SW10	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E519	SW10	2/20/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E520	SW10	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E521	SW10	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E522	SW10	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E523	BK14	2/20/2017	S	I	S	S	S	S	S	S	S	S	S
E524	BK14	2/20/2017	S	I	S	S	S	S	S	S	S	S	S
E525	BK14	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E526	BK14	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E527	BK14	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E528	SW04	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E529	SW04	2/20/2017	S	I	S	S	S	S	S	S	S	R	R
E530	SW04	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E531	SW04	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E532	SW04	2/20/2017	R	R	R	R	S	S	S	S	S	S	S
E533	BK03	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E534	BK03	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E535	BK03	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E536	BK03	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E537	BK03	2/20/2017	S	S	S	S	S	S	S	S	S	S	S
E538	SW13	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E539	SW13	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E540	SW13	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E541	SW13	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E542	SW13	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E543	SW05C	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E544	SW05C	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E545	SW05C	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E546	SW05C	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E547	SW05C	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E548	BK05U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E549	BK05U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E550	BK05U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E551	BK05U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E552	BK05U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E553	SW09	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E554	SW09	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E555	SW09	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E556	SW09	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E557	SW09	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E558	BK15	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E559	BK15	3/23/2017	R	R	R	I	S	S	S	S	S	R	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E560	BK15	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E561	BK15	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E562	BK15	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E563	SW16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E564	SW16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E565	SW16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E566	SW16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E567	SW16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E569	BK16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E570	BK16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E571	BK16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E572	BK16	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E573	SW17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E574	SW17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E575	SW17U	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E576	SW17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E577	SW17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E578	SW17	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E579	SW17	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E580	SW17	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E581	SW17	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E582	SW17	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E583	BK17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E584	BK17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E585	BK17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E586	BK17U	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E587	BK17U	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E588	SW05	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E589	SW05	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E590	SW05	3/23/2017	S	S	S	S	R	S	S	S	S	R	R
E591	SW05	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E592	SW05	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E593	SW05A	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E594	SW05A	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E595	SW05A	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E596	SW05A	3/23/2017	S	S	S	S	S	S	S	S	S	R	S
E597	SW05A	3/23/2017	S	S	S	S	S	S	S	S	S	S	S
E598	SW01	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E599	SW01	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E600	SW01	4/3/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E601	SW01	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E602	SW01	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E603	BK01U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E604	BK01U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E605	BK01U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E606	BK01U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E607	BK01U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E608	SW10	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E609	SW10	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E610	SW10	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E611	SW10	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E612	SW10	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E613	BK10U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E614	BK10U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E615	BK10U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E616	BK10U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E617	BK10U	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E618	SW11	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E619	SW11	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E620	SW11	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E621	SW11	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E622	SW11	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E623	SW04	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E624	SW04	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E625	SW04	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E626	SW04	4/3/2017	S	S	S	S	S	S	S	S	S	R	S
E627	SW04	4/3/2017	I	R	S	S	S	S	S	S	S	R	S
E628	SW07	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E629	SW07	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E630	SW07	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E631	SW07	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E632	SW07	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E633	BK12	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E634	BK12	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E635	BK12	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E636	BK12	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E637	BK12	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E638	BK03	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E639	BK03	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E640	BK03	4/3/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E641	BK03	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E642	BK03	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E643	BK14	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E644	BK14	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E645	BK14	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E646	BK14	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E647	BK14	4/3/2017	S	S	S	S	S	S	S	S	S	S	S
E648	SW13	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E649	SW13	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E650	SW13	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E651	SW13	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E652	SW13	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E653	BK16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E654	BK16	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E655	BK16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E656	BK16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E657	BK16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E658	BK05U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E659	BK05U	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E660	BK05U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E661	BK05U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E662	BK05U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E663	SW16	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E664	SW16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E665	SW16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E666	SW16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E667	SW16	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E668	BK17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E669	BK17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E671	BK17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E672	BK17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E673	SW09	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E674	SW09	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E675	SW09	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E676	SW09	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E677	SW09	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E678	SW05	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E679	SW05	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E681	SW05	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E682	SW05	4/24/2017	S	R	S	S	S	S	S	R	S	R	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E683	SW05C	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E684	SW05C	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E685	SW05C	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E686	SW05C	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E687	SW05C	4/24/2017	S	R	S	S	R	S	R	S	R	R	S
E688	SW05A	4/24/2017	S	R	S	S	S	S	S	S	S	S	S
E689	SW05A	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E690	SW05A	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E691	SW05A	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E692	SW05A	4/24/2017	S	R	S	S	S	S	S	S	S	S	S
E693	SW17U	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E694	SW17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E695	SW17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E696	SW17U	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E697	SW17U	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E698	BK15	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E699	BK15	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E700	BK15	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E701	BK15	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E702	BK15	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E703	SW17	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E704	SW17	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E705	SW17	4/24/2017	S	S	S	S	S	S	S	S	S	S	S
E706	SW17	4/24/2017	S	R	S	S	S	S	S	S	S	R	S
E707	SW17	4/24/2017	S	S	S	S	S	S	S	S	S	R	S
E708	SW11	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E709	SW11	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E710	SW11	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E711	SW11	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E712	SW11	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E713	SW01	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E714	SW01	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E715	SW01	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E716	SW01	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E717	SW01	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E718	SW07	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E719	SW07	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E720	SW07	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E721	SW07	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E722	SW07	5/9/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E723	SW10	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E724	SW10	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E725	SW10	5/9/2017	S	S	S	S	I	S	S	S	S	S	S
E726	SW10	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E727	SW10	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E728	BK10U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E729	BK10U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E731	BK10U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E733	BK14	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E735	BK14	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E736	BK14	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E737	BK14	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E738	BK03	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E739	BK03	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E740	BK03	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E741	BK03	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E742	BK03	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E743	BK01U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E744	BK01U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E745	BK01U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E746	BK01U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E747	BK01U	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E748	BK12	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E749	BK12	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E750	BK12	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E751	BK12	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E752	BK12	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E753	SW04	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E754	SW04	5/9/2017	S	S	S	S	S	S	S	S	S	R	S
E755	SW04	5/9/2017	S	R	S	S	S	S	S	S	S	R	S
E756	SW04	5/9/2017	S	R	S	S	S	S	S	R	S	R	R
E757	SW04	5/9/2017	S	S	S	S	S	S	S	S	S	S	S
E758	SW09	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E759	SW09	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E760	SW09	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E761	SW09	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E762	SW09	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E763	BK15	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E764	BK15	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E765	BK15	6/5/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E766	BK15	6/5/2017	S	S	S	S	S	S	S	S	S	S	I
E767	BK15	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E768	BK16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E769	BK16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E770	BK16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E771	BK16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E772	BK16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E773	BK05U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E774	BK05U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E776	BK05U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E777	BK05U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E778	SW05C	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E779	SW05C	6/5/2017	R	R	R	R	S	S	S	S	S	R	S
E780	SW05C	6/5/2017	S	S	S	S	S	S	S	S	S	R	S
E781	SW05C	6/5/2017	R	R	R	R	S	S	S	S	S	R	S
E782	SW05C	6/5/2017	S	R	S	S	R	S	S	S	S	R	R
E783	SW05	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E784	SW05	6/5/2017	S	S	S	S	S	S	S	S	S	R	S
E785	SW05	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E786	SW05	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E787	SW05	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E788	SW13	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E789	SW13	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E790	SW13	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E791	SW13	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E792	SW13	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E793	SW17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E794	SW17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E795	SW17U	6/5/2017	S	S	S	S	S	S	S	S	S	R	S
E796	SW17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E797	SW17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E799	SW17	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E800	SW17	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E801	SW17	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E802	SW17	6/5/2017	S	S	S	S	S	S	S	S	S	R	S
E804	BK17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E805	BK17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E806	BK17U	6/5/2017	S	S	S	S	S	S	S	S	S	R	S
E807	BK17U	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E808	SW05A	6/5/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E809	SW05A	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E810	SW05A	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E811	SW05A	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E812	SW05A	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E813	SW16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E814	SW16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E815	SW16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E816	SW16	6/5/2017	S	S	S	S	S	S	S	S	S	S	S
E818	BK03	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E819	BK03	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E820	BK03	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E821	BK03	6/26/2017	S	R	S	S	S	S	S	S	S	S	S
E822	BK03	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E823	BK10U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E824	BK10U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E826	BK10U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E827	BK10U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E828	SW10	6/26/2017	S	R	S	S	S	S	S	S	S	S	S
E829	SW10	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E830	SW10	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E831	SW10	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E832	SW10	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E833	BK12	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E834	BK12	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E835	BK12	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E836	BK12	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E838	SW01	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E839	SW01	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E840	SW01	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E841	SW01	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E842	SW01	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E843	SW07	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E844	SW07	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E845	SW07	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E846	SW07	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E847	SW07	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E848	SW04	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E849	SW04	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E850	SW04	6/26/2017	S	R	S	S	S	S	S	S	S	R	S
E851	SW04	6/26/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E852	SW04	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E853	BK14	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E854	BK14	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E855	BK14	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E856	BK14	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E857	BK14	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E858	BK01U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E859	BK01U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E860	BK01U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E861	BK01U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E862	BK01U	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E863	SW11	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E864	SW11	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E865	SW11	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E866	SW11	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E867	SW11	6/26/2017	S	S	S	S	S	S	S	S	S	S	S
E868	SW16	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E869	SW16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E870	SW16	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E871	SW16	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E872	SW16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E873	BK17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E874	BK17U	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E875	BK17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E876	BK17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E877	BK17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E878	SW17U	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E879	SW17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E880	SW17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E881	SW17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E882	SW17U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E883	SW17	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E884	SW17	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E885	SW17	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E886	SW17	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E887	SW17	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E888	BK05U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E889	BK05U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E890	BK05U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E891	BK05U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E892	BK05U	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E893	SW05A	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E894	SW05A	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E895	SW05A	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E896	SW05A	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E897	SW05A	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E898	SW05C	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E899	SW05C	7/18/2017	S	R	S	S	R	S	S	S	S	R	R
E900	SW05C	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E901	SW05C	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E902	SW05C	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E903	SW05	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E904	SW05	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E905	SW05	7/18/2017	S	R	S	R	S	S	S	S	S	R	S
E906	SW05	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E907	SW05	7/18/2017	S	S	S	S	S	S	S	S	S	R	S
E908	SW09	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E909	SW09	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E910	SW09	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E911	SW09	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E912	SW09	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E913	SW13	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E914	SW13	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E915	SW13	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E916	SW13	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E917	SW13	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E918	BK16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E919	BK16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E920	BK16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E921	BK16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E922	BK16	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E923	BK15	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E924	BK15	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E925	BK15	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E926	BK15	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E927	BK15	7/18/2017	S	S	S	S	S	S	S	S	S	S	S
E928	SW10	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E929	SW10	8/14/2017	S	S	S	S	S	S	S	S	S	R	S
E930	SW10	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E931	SW10	8/14/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E932	SW10	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E933	BK14	8/14/2017	S	S	S	S	S	S	S	S	S	R	S
E934	BK14	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E935	BK14	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E936	BK14	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E937	BK14	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E938	SW11	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E939	SW11	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E940	SW11	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E941	SW11	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E942	SW11	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E943	SW07	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E944	SW07	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E945	SW07	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E946	SW07	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E947	SW07	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E948	SW04	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E949	SW04	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E950	SW04	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E951	SW04	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E952	SW04	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E953	BK12	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E954	BK12	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E955	BK12	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E956	BK12	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E957	BK12	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E958	SW01	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E959	SW01	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E960	SW01	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E961	SW01	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E962	SW01	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E963	BK03	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E964	BK03	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E965	BK03	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E966	BK03	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E967	BK03	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E968	BK01U	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E969	BK01U	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E970	BK01U	8/14/2017	S	S	S	S	S	S	S	S	S	S	S
E971	BK01U	8/14/2017	S	S	S	S	S	S	S	S	S	S	S

Isolate ID	Sample ID	Sample Date	AmC	AM	FOX	CRO	C	IPM	CIP	GM	LVX	TE	SX T
E972	BK01U	8/14/2017	S	S	S	S	S	S	S	S	S	S	S

APPENDIX C: *E. COLI* CONCENTRATION AND WATER SAMPLE DATA

Sample data including site, sample date, air temperature, water temperature, pH, conductivity, percent dissolved oxygen, raw *E. coli* counts for dilution series, and final concentration. Concentration was calculated as described in the methods. -999 values indicate missing data in physical water parameters. TNTC values in *E. coli* counts indicate colonies were too numerous to count.

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
BK05U	8/9/2016	27.4	20.1	5.94	78.5	65	83	39	24		318
BK15	8/9/2016	29.8	24.8	4.71	46.5	50	46	32	6		110
BK16	8/9/2016	26.9	27.1	6.2	103.7	12	13	4	2		26
BK17U	8/9/2016		DRY								
SW05	8/9/2016	28.5	25.5	6.05	155.5	54	TNTC	TNTC	86		1720
SW05A	8/9/2016	28.1	26.4	5.72	136.5	21.8	TNTC	91	19		380
SW05C	8/9/2016	28.3	24.6	6.3	235	46	TNTC	TNTC	1		1016
SW16	8/9/2016	27.6	26.3	6.1	122.9	14.6	11	5	2		22
SW17	8/9/2016	28	27.6	6.2	69.6	34.5	13	6	2		26
SW17U	8/9/2016	28.5	27.1	6.2	103	80	75	19	2		150
BK01U	9/5/2016	24.1	22	6.41	94.9	75.5	TNTC		10	4	200
BK03	9/5/2016	24.3	21.7	5.73	120.2	50.2	54		9	1	108
BK10U	9/5/2016	21.8	22.2	4.72	71.6	69	TNTC		4	2	80
BK12	9/5/2016	23.7	23.2	5.95	74	22	51		10	9	102
BK14	9/5/2016	24.5	22.7	5.95	90.4	44	TNTC		10	4	200
SW01	9/5/2016	24.1	20.7	6.43	111.7	85.1	71		10	6	142
SW04	9/5/2016	26.2	22	5.73	104.6	52.2	106		22	4	440
SW07	9/5/2016	24	21.7	5.85	218.3	48	TNTC		20	6	400
SW10	9/5/2016	23.4	22.3	5.55	78.4	43.8	79		10	5	158
SW11	9/5/2016	22.8	21.7	5.92	175	64	57		23	3	287
BK05U	9/26/2016	23.3	19.8	5.56	74	73		16	6	1	64

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
BK15	9/26/2016	23.6	22.5	4.74	58.2	41.5		21	3	3	84
BK16	9/26/2016	22.5	23.8	6.01	90.5	62.8		44	13	1	176
BK17U	9/26/2016	23.8	22.4	4.62	67.7	13.7		64	24	5	368
SW05	9/26/2016	22.2	21.7	6.28	140.7	68.4		TNTC	93	25	2500
SW05A	9/26/2016	22.5	21.7	5.56	98.9	28.5		109	28	5	560
SW05C	9/26/2016	23	21.1	6.07	228.2	72		TNTC	93	20	2000
SW09	9/26/2016	23.7	22.4	5.76	124.2	60		33	7	2	132
SW13	9/26/2016	23	23.7	6.57	224.4	5.45		49	10	2	196
SW16	9/26/2016	25.2	23.5	5.95	94.3	12.2		32	6	1	128
SW17	9/26/2016	25.1	23.8	5.46	84	31		34	8	2	136
SW17U	9/26/2016	24.4	24.2	4.73	65.2	53		103	28	6	560
BK01U	10/17/2016	22	18.6	6.07	123.3	76		20	7	2	80
BK03	10/17/2016	23.1	20	5.86	82.1	40		47	15	4	188
BK10U	10/17/2016	22.7	20.2	4.3	88.7	82.2		53	20	3	306
BK12	10/17/2016	22.4	22.4	5.92	64.4	100		26	11	2	104
BK14	10/17/2016	23	19.5	5.85	100.5	22.7		33	7	0	132
SW01	10/17/2016	21.2	17.3	6.23	130.5	85		36	10	1	144
SW04	10/17/2016	24.4	18.6	5.89	147.5	14.5		55	9	4	220
SW07	10/17/2016	22.4	19.1	5.76	135.2	47.2		86	20	7	372
SW10	10/17/2016	22.6	19.6	5.79	98.4	72.9		79	20	4	358
SW11	10/17/2016	22.5	20.4	5.94	144.9	72.2		49	14	11	196
BK05U	11/7/2016	13.5	16.2	5.57	75.4	62.2		26	8	3	104
BK15	11/7/2016	10	12.1	4.84	56	63		15	3	0	60
BK16	11/7/2016	15	15.5	5.82	106.8	79.9		26	5	2	104
BK17U	11/7/2016	11.6	10.6	5.28	52.7	54.8		TNTC	246	82	8200
SW05	11/7/2016	13.1	13.9	6.31	150	75		120	39	6	780
SW05A	11/7/2016	15.4	14	6.02	187	11		15	2	1	60
SW05C	11/7/2016	13.4	13.2	6.15	242	54.7		123	39	11	780

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
SW09	11/7/2016	23	13.8	5.5	108.5	70		10	2	0	40
SW13	11/7/2016	13.2	15.1	6.4	181.7	86		126	25	1	500
SW16	11/7/2016	14	13.7	5.7	96.2	31.9		51	13	4	204
SW17	11/7/2016	13.8	13.7	4.94	50.7	33.3		3	0	0	12
SW17U	11/7/2016	12.6	13.9	4.6	51.4	34.5		27	3	0	108
BK01U	11/28/2016	11.1	11.2	6.18	128	67.7		4	2	0	16
BK03	11/28/2016	13.5	8.2	5.93	110	32.9		32	2	0	128
BK10U	11/28/2016	10.5	9.1	4.49	213.3	82.5		1	0	0	4
BK12	11/28/2016	11	8.8	5.95	83.2	28.7		8	1	0	32
BK14	11/28/2016	11.3	8.7	6.06	148.3	32.6		6	0	0	24
SW01	11/28/2016	13.3	9.1	6.23	134	88		31	7	2	124
SW04	11/28/2016	13.5	6.3	6.18	159.1	34		63	10	4	252
SW07	11/28/2016	12.9	9.1	5.88	143.2	12.8		31	1	2	124
SW10	11/28/2016	12.6	8.4	5.26	149.5	80		21	1	1	84
SW11	11/28/2016	11.4	9.4	6.02	168.4	70.4		31	5	1	124
BK05U	12/14/2016	11.1	12.6	5.22	93.1	71		52	11	3	208
BK15	12/14/2016	9.9	9.6	4.75	58.8	80		36	13	0	144
BK16	12/14/2016	10.3	11	5.82	112	82		23	7	0	92
BK17U	12/14/2016	10.6	12.2	4.73	70.9	51.6	TNTC	244	41	11	820
SW05	12/14/2016	10.7	11.3	6.09	147.5	85.3		222	62	12	1240
SW05A	12/14/2016	11.1	11.8	5.6	121	85.8	159	94	16	8	376
SW05C	12/14/2016	10.9	11.4	5.78	184.6	81	TNTC	252	79	12	1580
SW09	12/14/2016	10.1	11.8	5.74	134	90.8	252	151	31	6	620
SW13	12/14/2016	11.9	10	6.35	237	92	184	95	24	6	380
SW16	12/14/2016	11.2	11.6	5.42	73	78	69	32	7	0	133
SW17	12/14/2016	13.1	11.4	5.23	92.3	69.8		160	45	9	900
SW17U	12/14/2016	11.7	11.8	4.65	65.2	78.2		151	33	7	660
BK01U	1/16/2017	9.9	9.8	6.31	105.1	91.7	1	5	1	0	20

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
BK03	1/16/2017	9.8	8.9	6.1	111.5	103.6		15	6	0	60
BK10U	1/16/2017	8.3	10.8	4.34	101.5	95.6	9	7	0	1	18
BK12	1/16/2017	10.3	10.1	6.1	82.3	91.3	7	1	1	0	14
BK14	1/16/2017	10.8	10.5	6.08	122.4	74.4	9	3	1	1	18
SW01	1/16/2017	9	9.4	6.27	143.6	91.4		38	7	2	152
SW04	1/16/2017	12.4	9.8	5.98	133	95.2		70	6	3	280
SW07	1/16/2017	10.5	10.4	5.92	125.6	89.9		23	8	1	92
SW10	1/16/2017	11	10.4	5.76	134.2	87.6	28	8	1	1	56
SW11	1/16/2017	9.9	10	5.95	230.5	101.1		6	7	0	24
BK05U	1/30/2017	7.7	10.5	5.18	82.2	64.7					culture error
BK15	1/30/2017	6.3	7.3	4.78	57.2	85.8					culture error
BK16	1/30/2017	4.3	8.6	6.01	92	94.7					culture error
BK17U	1/30/2017	9.1	9.7	4.85	51.5	74.8					culture error
SW05	1/30/2017	4.5	8.3	6.17	137.8	97.6					culture error
SW05A	1/30/2017	5.9	8.3	5.38	109.7	70.5					culture error
SW05C	1/30/2017	5.5	9	5.7	202	85.5					culture error
SW09	1/30/2017	6.6	9.3	5.48	110.6	99.4					culture error
SW13	1/30/2017	6.1	8.5	6.35	212.7	94.4					culture error
SW16	1/30/2017	6.3	9.5	5.95	69.5	125					culture

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
											error
SW17	1/30/2017	7	8.3	5.1	52.8	92					culture error
SW17U	1/30/2017	6.7	9.5	4.81	48.8	103.6					culture error
BK01U	2/20/2017	14	14.3	6.28	122.1	87	32	48	3		128
BK03	2/20/2017	17	16	6.13	102	79	20	18	1		40
BK10U	2/20/2017	14.8	18	4.54	140	102	0	0	0		1
BK12	2/20/2017	17.3	15.9	6.18	82.5	76.5	0	1	1		20
BK14	2/20/2017	15.1	13.7	6.18	107.3	71	TNTC	140	32		640
SW01	2/20/2017	15.5	12.9	6.3	130	91.3	103	54	13		216
SW04	2/20/2017	16.4	13.9	6.1	125	94.1	155	106	22		440
SW07	2/20/2017	16.9	15.3	5.86	101.5	68.3	10	5	0		20
SW10	2/20/2017	17.7	14.3	5.89	150	81.4	19	8	3		38
SW11	2/20/2017	21.5	14.1	6.2	217	100	10	6	3		20
BK05U	3/27/2017	21.4	16.2	5.38	81.8	80.2	5	0	0		10
BK15	3/27/2017	21.8	19.1	4.76	53.1	73.3	20	5	2		40
BK16	3/27/2017	19.2	20.4	6.05	82.4	98.3	35	19	5		73
BK17U	3/27/2017	22	19.6	4.77	47	104	20	11	2		40
SW05	3/27/2017	18.8	16.4	6.21	128	121.6	121	87	13	7	348
SW05A	3/27/2017	18.8	18.4	5.73	102	82	2	11	0		44
SW05C	3/27/2017	19	16.5	5.98	197.5	81	21	11	1		42
SW09	3/27/2017	20.5	19.7	5.7	109.2	112	27	23	2		73
SW13	3/27/2017	19.4	16.6	6.42	194.6	70.5	21	13	0	1	42
SW16	3/27/2017	20.7	22.1	6.14	68.3	134	33	11	1		66
SW17	3/27/2017	20.2	19.2	5.03	53.2	95.5	72	33	9		138
SW17U	3/27/2017	20.5	21	4.86	49.3	113	12	2	0		24
BK01U	4/3/2017	18.9	17	6.09	112.1	77.3	53	18	3		106

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
BK03	4/3/2017	20.3	20.7	6.19	85.4	103	32	20	1		72
BK10U	4/3/2017	19.7	19	4.68	103.7	86.3	13	6	2		26
BK12	4/3/2017	21	19.8	6.01	80.5	53.3	17	8	1		34
BK14	4/3/2017	21.1	19.2	5.9	94.1	43.8	26	11	1		52
SW01	4/3/2017	19.3	15.7	6.12	125.7	78.7	97	59	12		236
SW04	4/3/2017	21.4	18.5	5.82	103.8	79.2	129	82	15		328
SW07	4/3/2017	20.5	20	5.51	94.8	62.6	47	26	13		99
SW10	4/3/2017	20.4	19.2	5.56	118	78.6	68	37	7		142
SW11	4/3/2017	20	18.7	6.13	205.8	82.1	40	20	4		80
BK05U	4/24/2017	17.3	16.3	5.34	87.4	59.2	TNTC	TNTC	212	62	6200
BK15	4/24/2017	-999	16.5	5.22	45	64	TNTC	TNTC	212	97	9700
BK16	4/24/2017	-999	16.8	5.77	50	89.1	TNTC	TNTC	191	47	4700
BK17U	4/24/2017	18	17.9	5.3	118.5	54.6	TNTC	TNTC	96	37.5	3750
SW05	4/24/2017	17.7	17.1	6.35	121.8	65.7	TNTC	TNTC	235	75	7500
SW05A	4/24/2017	17.3	18.2	6.38	174	33	TNTC	TNTC	71	30	2210
SW05C	4/24/2017	17.3	17.3	6.43	173	64.5	TNTC	TNTC	TNTC	90	9000
SW09	4/24/2017	-999	16.5	5.6	81	71.6	TNTC	TNTC	254	82	8200
SW13	4/24/2017	17.9	18.1	6.5	195.2	31.9	168	88	17	1	340
SW16	4/24/2017	17.1	17.7	5.61	43.25	75.7	TNTC	TNTC	222	52	5200
SW17	4/24/2017	18	17.4	5.3	57.7	60.1	TNTC	TNTC	TNTC	na	5080
SW17U	4/24/2017	17.9	17.8	5.62	46.6	73	TNTC	TNTC	95	12	1200
BK01U	5/9/2017	14.2	15.8	6.16	123	71.8	13	3	1	0	26
BK03	5/9/2017	14.8	15.7	6.04	95	52.5	182	116	29	6	580
BK10U	5/9/2017	15.6	17.7	4.41	106.4	90.6	4	0	1	0	8
BK12	5/9/2017	15.6	18.5	5.95	64.5	17.6	39	15	3	1	78
BK14	5/9/2017	15.9	16.8	5.91	97.9	32.7	15	10	2	0	30
SW01	5/9/2017	14.8	15.4	6.28	138.1	75.8	77	39	6	2	155
SW04	5/9/2017	15.7	14.7	6.69	400.5	54.6	TNTC	TNTC	TNTC	TNTC	25400

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
SW07	5/9/2017	15.3	16.8	5.75	84.5	65	85	45	8	2	180
SW10	5/9/2017	16.4	17.4	5.56	106.5	67.8	11	12	2	0	48
SW11	5/9/2017	15.7	19.3	6.34	170.3	85	53	31	6	1	115
BK05U	6/5/2017	25	18	5.63	77.8	69.1	TNTC	146	39	9	780
BK15	6/5/2017	25.5	23.9	4.99	55.2	36.1	108	70	12.5	0	280
BK17U	6/5/2017	-999	23.3	4.74	61	34.3					culture error
SW05	6/5/2017	-999	23.6	6.56	177.5	52.2	191	98	21.5	11	430
SW05A	6/5/2017	24.1	25	6.38	158.3	31.36					culture error
SW05C	6/5/2017	24.2	22.1	6.47	209.4	40.9	TNTC	114	24	3	480
SW09	6/5/2017	-999	24.6	5.53	96.2	74.2	96	68	25	4	386
SW13	6/5/2017	23.9	26.9	6.76	219.5	67.7	126	38	9	1	152
SW16	6/5/2017	23.6	23.9	5.85	71	53.5					culture error
SW17	6/5/2017	23.1	24.3	5.23	67.3	42.7					culture error
SW17U	6/5/2017	25.1	24.9	5.29	56	45.8					culture error
BK01U	6/26/2017	22.8	26.3	6.45	99.4	78.6	29	17	8	1	58
BK03	6/26/2017	23.5	24.8	6.2	109.8	16.8	81	46	10	1	184
BK10U	6/26/2017	22	25.1	4.65	190	92.1	5	2	1	1	10
BK12	6/26/2017	21.4	27.1	5.85	72.3	11.1	124	96	20	1	400
BK14	6/26/2017	21	25.7	6.13	111.6	39.8	54	33	2	2	120
SW01	6/26/2017	22.7	22.8	6.3	144.8	79.6	115	59	10	2	236
SW04	6/26/2017	23.1	23.3	5.78	152.5	56.8	TNTC	219	43	12	860
SW07	6/26/2017	21.4	25.7	6.06	129.4	55.5	TNTC	TNTC	110	25	2500
SW10	6/26/2017	26.1	5.95	-999	43.4	3.48	53	38	10	1	129

Site	Sample Date	Air Temp (° C)	Water Temp (° C)	pH	Conductivity (SPC)	Dissolved Oxygen (%)	Count (50mL)	Count (25mL)	Count (5mL)	Count (1mL)	Concentration (CFU/100 mL)
SW11	6/26/2017	23.9	25.3	6.39	221.5	82.3	na	31	4	3	124
BK05U	7/18/2017	24.4	19.1	5.53	76.2	58.8	TNTC		77	18	1540
BK15	7/18/2017	26.8	25.3	4.85	51.1	21.4	58	44	6	2	146
BK16	7/18/2017	24.8	27.4	6.16	87.3	84.5	67	31	5	1	129
BK17U	7/18/2017	25.9	23.9	4.48	32.1	47.5	TNTC	TNTC	TNTC	68	6800
SW05	7/18/2017	24.5	25.5	6.47	158.5	29.5	66	44	5	2	154
SW05A	7/18/2017	23.8	26.1	6.29	126.1	23.4	na	22	4	3	88
SW05C	7/18/2017	-999	23.6	6.42	209	57.3	TNTC	TNTC	92	21	2100
SW09	7/18/2017	24.8	26	5.76	102.1	60.2	TNTC	122	27	7	540
SW13	7/18/2017	25	25.7	6.65	174.1	72.1	TNTC	83	24	5	480
SW16	7/18/2017	-999	23.4	4.67	55.1	59	TNTC	TNTC	TNTC	162	16200
SW17	7/18/2017	25.6	24.9	5.41	60.5	52.6	TNTC	TNTC	TNTC	112	11200
SW17U	7/18/2017	25.6	24.7	4.94	40.6	40.1	TNTC	TNTC	TNTC	50	5000
BK01U	8/14/2017	26.6	22	6	112.9	71.3	81	57	10	3	228
BK10U	8/14/2017		DRY								
BK03	8/14/2017	27.8	24.8	5.83	99.5	48.5	TNTC	80	23	4	390
BK12	8/14/2017	27.9	27.1	5.69	79.2	14.6	45	15	3	0	90
BK14	8/14/2017	27.9	26.3	5.93	127.3	20	TNTC	22	5	1	88
SW01	8/14/2017	28	23.7	6.16	118.8	70.9	TNTC	76	14	5	304
SW04	8/14/2017	28.2	26.9	6.19	144	52.5	TNTC	144	32	2	640
SW07	8/14/2017	26.9	25.5	5.22	115.6	59.3	TNTC	107	28	10	560
SW10	8/14/2017	26.2	27.2	6.01	122.1	23.4	TNTC	74	20	5	348
SW11	8/14/2017	28.2	25.9	6.43	148.2	33.3	TNTC	111	21	5	420

APPENDIX D: DROPLET DIGITAL PCR DATA

Table D1: ddPCR validation metrics are presented in the following table for the ten highest concentrations of MST markers *HF183* and *pig-2-bac* displaying the mean, median, minimum, and maximum accepted droplets per merged well in addition to the proportion droplets positive in the reactions

	<i>HF183</i>		<i>pig-2-bac</i>	
	Accepted Droplets/merged well	Proportion droplets positive	Accepted Droplets/merged well	Proportion droplets positive
Mean	29919.55	0.000712	32331.1	0.08499
Median	30655	0.000482	31595	0.000981
Minimum	18125	0.000276	26778	0.000256
Maximum	37382	0.00211	38126	0.895394

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Table D2: Sample data are presented for ddPCR results targeting microbial source tracking markers *HF183* and *pig-2-bac*. Concentrations (Conc.) are presented for three definitions of detection defined in the methods section.

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
BK05U	8/9/2016	21486	0	0	1.32	2	7.2	11	0	0	0	0	0	0
BK15	8/9/2016	20522	0	0	0	0	0.72	1	0	0	0	0	0	0
BK16	8/9/2016	22291	0	0	0	0	0.6	1	0	0	0	0	0	0
SW05	8/9/2016	21765	0	0	0	0	0	0	0	0	0	0	0	0
SW05A	8/9/2016	30227	0.48	1	0.96	2	2.76	6	1.44	3	1.44	3	1.44	3

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
SW05C	8/9/2016	28938	1.44	3	2.4	5	8.28	17	11.28	23	12.24	25	12.24	25
SW16	8/9/2016	35099	0	0	0	0	0	0	0	0	0	0	0	0
SW17	8/9/2016	32949	0	0	0	0	0.84	2	0	0	0	0	0	0
SW17U	8/9/2016	26362	0	0	0	0	0.48	1	0	0	0	0	0	0
BK01U	9/5/2016	28294	0.96	2	2.04	4	3	6	0	0	0	0	0	0
BK03	9/5/2016	30192	0.48	1	0.48	1	0.48	1	0	0	0	0	0	0
BK10U	9/5/2016	32662	0	0	0.48	1	0.48	1	0	0	0	0	0	0
BK12	9/5/2016	29133	0.48	1	0.96	2	2.88	6	0	0	0	0	0	0
BK14	9/5/2016	28802	0	0	3	6	6.84	14	0	0	0	0	0	0
SW01	9/5/2016	32118	1.32	3	3.12	7	7.92	18	0	0	0	0	0	0
SW04	9/5/2016	31817	0.48	1	0.48	1	0.48	1	0	0	0	0	0.48	1
SW07	9/5/2016	32233	0	0	0	0	0.84	2	0	0	0	0	0	0
SW10	9/5/2016	27229	0	0	0.48	1	5.76	11	0	0	0	0	0	0
SW11	9/5/2016	31246	0.48	1	1.8	4	3.6	8	0	0	0	0	0	0
BK05U	9/26/2016	32159	0	0	0.84	2	2.64	6	0	0	0	0	0	0
BK15	9/26/2016	31205	0	0	0	0	0.48	1	0	0	0	0	0	0
BK16	9/26/2016	32531	3.48	8	7.32	17	14.28	33	0	0	0	0	0	0
BK17U	9/26/2016	32706	30	69	40.8	93	48	111	0	0	0	0	0	0
SW05	9/26/2016	31124	0.48	1	1.8	4	2.28	5	4.56	10	4.56	10	5.4	12
SW05A	9/26/2016	35093	0.84	2	2.04	5	3.24	8	0	0	0	0	0	0
SW05C	9/26/2016	30655	4.2	9	8.28	18	13.32	29	17.52	38	18	39	18.84	41
SW09	9/26/2016	34887	0.84	2	4.8	12	9.72	24	0	0	0	0	0	0
SW13	9/26/2016	36609	0	0	0.36	1	1.56	4	0	0	0	0	0.36	1
SW16	9/26/2016	35648	0.36	1	0.36	1	1.56	4	0	0	0	0	0	0
SW17	9/26/2016	34430	0.36	1	1.68	4	2.52	6	2.52	6	2.52	6	2.52	6
SW17U	9/26/2016	34759	1.68	4	1.68	4	2.4	6	0.36	1	0.36	1	0.36	1

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
BK01U	10/17/2016	32489	0	0	0.48	1	16.56	38	0	0	0	0	0	0
BK03	10/17/2016	31806	0.48	1	2.16	5	15.12	34	0	0	0	0	0	0
BK10U	10/17/2016	30855	0	0	0	0	25.2	55	0	0	0	0	0	0
BK12	10/17/2016	31692	0.48	1	1.32	3	8.88	20	0	0	0	0	0	0
BK14	10/17/2016	31873	1.32	3	4.92	11	9.36	21	0	0	0	0	0	0
SW01	10/17/2016	33243	1.32	3	2.52	6	8.04	19	0	0	0.48	1	0.48	1
SW04	10/17/2016	32283	0.84	2	1.8	4	4.32	10	0	0	0.48	1	0.48	1
SW07	10/17/2016	30581	1.44	3	1.44	3	3.24	7	0	0	0	0	0	0
SW10	10/17/2016	35013	1.2	3	4.8	12	11.28	28	0	0	0	0	0	0
SW11	10/17/2016	38173	1.08	3	1.44	4	7.08	19	0	0	0	0	0	0
BK05U	11/7/2016	37618	0.72	2	2.28	6	3	8	0	0	0	0	0	0
BK15	11/7/2016	33806	0.36	1	0.84	2	1.68	4	0	0	0	0	0	0
BK16	11/7/2016	35661	1.56	4	4.8	12	8.28	21	0	0	0	0	0	0
BK17U	11/7/2016	37382	6.84	18	9.84	26	15.48	41	0	0	0	0	0	0
SW05	11/7/2016	37933	0	0	0	0	1.44	4	0.36	1	0.36	1	0.36	1
SW05A	11/7/2016	38692	0	0	0	0	0	0	1.08	3	1.44	4	1.8	5
SW05C	11/7/2016	36288	1.92	5	3.48	9	7.8	20	0	0	0	0	0	0
SW09	11/7/2016	37700	0.72	2	1.44	4	1.92	5	0	0	0	0	0	0
SW13	11/7/2016	35798	0.36	1	1.56	4	3.96	10	0	0	0	0	0	0
SW16	11/7/2016	37319	0.72	2	1.92	5	2.64	7	0	0	0	0	0	0
SW17	11/7/2016	31003	0	0	0	0	1.32	3	0	0	0	0	0	0
SW17U	11/7/2016	25807	4.32	8	9.36	17	21.6	40	0	0	0.6	1	0.6	1
BK01U	11/28/2016	28565	0.48	1	0.96	2	1.92	4	0	0	0	0	0	0
BK03	11/28/2016	29932	0	0	1.44	3	3.72	8	0	0	0	0	0	0
BK10U	11/28/2016	27864	0	0	0	0	0	0	0	0	0	0	0	0
BK12	11/28/2016	28596	0	0	0.48	1	1.44	3	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
BK14	11/28/2016	28259	0.48	1	1.44	3	3.48	7	0	0	0	0	0	0
SW01	11/28/2016	29004	1.44	3	3.84	8	11.16	23	0.48	1	0.48	1	0.48	1
SW04	11/28/2016	29967	0.96	2	0.96	2	1.44	3	1.92	4	1.92	4	2.4	5
SW07	11/28/2016	32034	2.16	5	4.8	11	9.72	22	0	0	0	0	0.48	1
SW10	11/28/2016	34638	0.36	1	3.24	8	6.12	15	0	0	0	0	0	0
SW11	11/28/2016	34475	1.68	4	2.04	5	4.92	12	0	0	0	0	0	0
BK05U	12/14/2016	33375	1.32	3	1.32	3	1.68	4	0	0	0	0	0	0
BK15	12/14/2016	29732	1.92	4	2.88	6	3.84	8	0	0	0	0	0	0
BK16	12/14/2016	35145	2.04	5	8.88	22	13.68	34	3.6	9	3.96	10	3.96	10
BK17U	12/14/2016	35886	0.84	2	2.76	7	5.88	15	0.36	1	0.36	1	0.36	1
SW05	12/14/2016	34286	3.24	8	3.72	9	4.56	11	0	0	0	0	0	0
SW05A	12/14/2016	32581	19.92	46	25.2	58	27.6	64	0.48	1	0.84	2	0.84	2
SW05C	12/14/2016	33431	2.52	6	3.84	9	4.68	11	0	0	0	0	0	0
SW09	12/14/2016	35148	1.2	3	2.76	7	3.6	9	165.6	409	172.8	428	174	432
SW13	12/14/2016	33960	0	0	6.24	15	11.64	28	1.68	4	1.68	4	1.68	4
SW16	12/14/2016	35156	1.2	3	2.04	5	2.4	6	0	0	0	0	0	0
SW17	12/14/2016	37086	3.48	9	5.76	15	7.56	20	1.2	3	1.2	3	1.2	3
SW17U	12/14/2016	37216	1.56	4	1.92	5	3.36	9	1.08	3	1.08	3	1.08	3
BK01U	1/16/2017	33748	0	0	0.36	1	1.2	3	0	0	0	0	0	0
BK03	1/16/2017	34382	0.36	1	2.88	7	4.92	12	0	0	0	0	0	0
BK10U	1/16/2017	36881	0.36	1	2.28	6	2.64	7	0	0	0	0	0	0
BK12	1/16/2017	37864	0.36	1	1.44	4	3.72	10	0.36	1	0.36	1	0.36	1
BK14	1/16/2017	34479	0.84	2	0.84	2	1.68	4	0	0	0	0	0	0
SW01	1/16/2017	31009	0.48	1	3.24	7	15.96	35	0	0	0	0	0.48	1
SW04	1/16/2017	33040	2.52	6	3	7	5.52	13	3	7	3	7	3	7
SW07	1/16/2017	35376	0	0	0.36	1	4.44	11	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
SW10	1/16/2017	29402	0	0	0	0	0	0	294	607	326.4	673	337.2	694
SW11	1/16/2017	34292	0	0	2.04	5	4.08	10	0	0	0	0	0	0
BK05U	1/30/2017	35133	0.36	1	0.36	1	2.04	5	0	0	0	0	0	0
BK15	1/30/2017	37676	0.36	1	0.72	2	0.72	2	0	0	0	0	0	0
BK16	1/30/2017	33663	0	0	0	0	1.68	4	0	0	0	0	0	0
BK17U	1/30/2017	35958	0.36	1	1.2	3	1.92	5	0	0	0	0	0	0
SW05	1/30/2017	36620	0.36	1	0.36	1	2.64	7	1.2	3	1.2	3	1.2	3
SW05A	1/30/2017	36051	0	0	0.36	1	1.56	4	0.36	1	0.36	1	0.36	1
SW05C	1/30/2017	34296	0	0	0.36	1	0.36	1	0.36	1	0.36	1	0.36	1
SW09	1/30/2017	33043	0	0	0	0	0.84	2	0.84	2	0.84	2	0.84	2
SW13	1/30/2017	33700	0	0	0.84	2	0.84	2	0	0	0	0	0	0
SW16	1/30/2017	33301	0	0	0	0	0	0	0	0	0	0	0	0
SW17	1/30/2017	30926	2.76	6	4.08	9	16.92	37	0	0	0	0	0	0
SW17U	1/30/2017	31461	0	0	0	0	1.8	4	0	0	0	0	0	0
BK01U	2/20/2017	31417	0.48	1	0.84	2	0.84	2	0	0	0	0	0	0
BK03	2/20/2017	31966	3.12	7	8.88	20	21.6	49	0	0	0	0	0	0
BK10U	2/20/2017	29202	0	0	0	0	0.48	1	0.48	1	0.48	1	0.48	1
BK12	2/20/2017	31570	0	0	0.48	1	109.2	243	0	0	0	0	0	0
BK14	2/20/2017	31375	0	0	0.48	1	1.32	3	0	0	0	0	0	0
SW01	2/20/2017	34601	0.84	2	1.68	4	6.48	16	0	0	0	0	0	0
SW04	2/20/2017	31602	6.72	15	10.32	23	14.28	32	0	0	0	0	0	0
SW07	2/20/2017	33725	0	0	0.36	1	0.84	2	0	0	0	0	0	0
SW10	2/20/2017	31547	0.48	1	2.64	6	6.72	15	0.84	2	0.84	2	1.32	3
SW11	2/20/2017	33016	0	0	0	0	0	0	0	0	0	0	0	0
BK05U	3/27/2017	34107	0.84	2	1.2	3	1.68	4	0	0	0	0	0	0
BK15	3/27/2017	36117	0.84	2	1.2	3	3.48	9	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
BK16	3/27/2017	33930	0.84	2	2.52	6	6.24	15	0	0	0	0	0	0
BK17U	3/27/2017	32717	0	0	0	0	0.84	2	0	0	0	0	0	0
SW05	3/27/2017	36073	0.36	1	2.76	7	4.32	11	0	0	0	0	0	0
SW05A	3/27/2017	35507	0.36	1	3.24	8	7.2	18	0.36	1	0.36	1	0.36	1
SW05C	3/27/2017	32393	1.32	3	1.8	4	3.48	8	0	0	0	0	0.48	1
SW09	3/27/2017	33124	0	0	1.68	4	7.68	18	0	0	0	0	0	0
SW13	3/27/2017	30487	1.44	3	1.44	3	2.28	5	0	0	0	0	0	0
SW16	3/27/2017	34645	2.4	6	4.44	11	8.16	20	0	0	0	0	0	0
SW17	3/27/2017	36817	0.72	2	1.2	3	2.64	7	1.2	3	1.2	3	1.2	3
SW17U	3/27/2017	34380	1.2	3	2.52	6	3.24	8	0	0	0	0	0	0
BK01U	4/3/2017	32845	1.32	3	2.16	5	2.52	6	0	0	0	0	0	0
BK03	4/3/2017	28836	0.48	1	1.44	3	4.92	10	0	0	0	0	0	0
BK10U	4/3/2017	30868	0	0	0.48	1	0.48	1	0	0	0	0	0	0
BK12	4/3/2017	34281	0.36	1	0.84	2	1.2	3	0	0	0	0	0	0
BK14	4/3/2017	32042	1.8	4	2.64	6	5.28	12	0	0	0	0	0	0
SW01	4/3/2017	33126	3	7	12.36	29	25.2	60	0	0	0	0	0	0
SW04	4/3/2017	31595	0.84	2	2.64	6	8.04	18	13.8	31	14.28	32	14.76	33
SW07	4/3/2017	28112	1.56	3	1.56	3	3.48	7	0	0	0	0	0	0
SW10	4/3/2017	26788	3.72	7	8.4	16	14.76	28	0	0	0	0	0	0
SW11	4/3/2017	29754	4.32	9	11.88	25	20.4	44	0	0	0	0	0	0
BK05U	4/24/2017	32990	0	0	0.84	2	4.32	10	0.84	2	0.84	2	0.84	2
BK15	4/24/2017	31624	0.48	1	2.64	6	4.44	10	1.32	3	1.32	3	1.32	3
BK16	4/24/2017	28555	6.96	14	9.84	20	16.8	34	1.92	4	1.92	4	1.92	4
BK17U	4/24/2017	32849	2.16	5	4.32	10	8.16	19	0	0	0	0	0	0
SW05	4/24/2017	31127	2.28	5	6.84	15	12.24	27	0	0	0	0	0	0
SW05A	4/24/2017	29660	0.96	2	1.44	3	4.32	9	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
SW05C	4/24/2017	26376	10.68	20	16.56	31	27.6	51	2.64	5	2.64	5	2.64	5
SW09	4/24/2017	26778	3.72	7	5.76	11	16.92	32	4.8	9	4.8	9	5.76	11
SW13	4/24/2017	25996	0.6	1	0.6	1	4.92	9	0	0	0	0	0	0
SW16	4/24/2017	18125	3.84	5	5.4	7	8.52	11	0	0	0.72	1	0.72	1
SW17	4/24/2017	19366	0	0	0.72	1	1.44	2	2.16	3	2.16	3	2.88	4
SW17U	4/24/2017	17597	0	0	0	0	3.96	5	0	0	0	0	0	0
BK01U	5/9/2017	17529	0	0	0	0	0	0	0	0	0	0	0	0
BK03	5/9/2017	31647	0.48	1	0.84	2	5.76	13	0	0	0	0	0	0
BK10U	5/9/2017	30817	0	0	0	0	0.96	2	0.48	1	0.48	1	0.48	1
BK12	5/9/2017	32451	0	0	0.48	1	1.32	3	0	0	0.48	1	0.48	1
BK14	5/9/2017	32326	0.48	1	0.48	1	2.64	6	0	0	0	0	0	0
SW01	5/9/2017	34491	0	0	0.36	1	6.6	16	0	0	0	0	0	0
SW04	5/9/2017	35973	0	0	0.84	2	1.92	5	31872	32210	35712	33107	38124	33556
SW07	5/9/2017	36186	0	0	0.84	2	3.96	10	0.36	1	0.84	2	0.84	2
SW10	5/9/2017	34001	0.36	1	0.36	1	1.68	4	0	0	0	0	0	0
SW11	5/9/2017	33897	0.84	2	3.36	8	10.44	25	0	0	0.36	1	0.36	1
BK05U	6/5/2017	35193	0	0	0	0	0.36	1	0	0	0	0	0	0
BK15	6/5/2017	35860	0	0	0.36	1	1.2	3	0	0	0	0	0	0
BK16	6/5/2017	33667	0.84	2	1.2	3	2.52	6	0	0	0	0	0	0
BK17U	6/5/2017	35695	1.92	5	2.4	6	3.12	8	0	0	0	0	0	0
SW05	6/5/2017	37548	0	0	0	0	0.72	2	0	0	0	0	0	0
SW05A	6/5/2017	35825	0	0	0	0	2.4	6	0	0	0	0	0	0
SW05C	6/5/2017	33589	0.48	1	0.48	1	2.16	5	0	0	0	0	0	0
SW09	6/5/2017	35653	0	0	0.36	1	0.84	2	0	0	0	0	0	0
SW13	6/5/2017	36971	0	0	0.36	1	4.2	11	0	0	0	0	0	0
SW16	6/5/2017	37406	0	0	0	0	1.08	3	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
SW17	6/5/2017	34194	0	0	0.36	1	0.84	2	0	0	0	0	0	0
SW17U	6/5/2017	37080	1.92	5	4.2	11	5.76	15	0	0	0	0	0	0
BK01U	6/26/2017	25616	0	0	0	0	0.6	1	0	0	0	0	0	0
BK03	6/26/2017	25388	0	0	0	0	0.6	1	0	0	0	0	0	0
BK10U	6/26/2017	24373	0	0	0	0	0	0	0	0	0	0	0	0
BK12	6/26/2017	35302	0	0	0	0	2.76	7	0	0	0	0	0	0
BK14	6/26/2017	34889	0	0	0.36	1	4.8	12	0	0	0	0	0	0
SW01	6/26/2017	36937	0.36	1	0.36	1	0.36	1	0	0	0	0	0	0
SW04	6/26/2017	32735	0	0	0.48	1	0.48	1	0	0	0	0	0.48	1
SW07	6/26/2017	28361	0	0	0	0	3	6	0.48	1	0.48	1	0.48	1
SW10	6/26/2017	28643	0	0	0.48	1	3	6	0	0	0	0	0	0
SW11	6/26/2017	29869	0	0	0	0	0.48	1	0	0	0	0	0	0
BK05U	7/18/2017	25825	0	0	0	0	0	0	0	0	0	0	0	0
BK15	7/18/2017	29763	0	0	0	0	0.96	2	0	0	0	0	0.48	1
BK16	7/18/2017	32441	2.16	5	3.96	9	4.8	11	0	0	0	0	0	0
BK17U	7/18/2017	33964	0.84	2	3.72	9	3.72	9	0	0	0	0	0	0
SW05	7/18/2017	32750	0	0	0	0	0.48	1	0	0	0	0	0	0
SW05A	7/18/2017	34689	0	0	0.84	2	2.04	5	0	0	0	0	0	0
SW05C	7/18/2017	37193	0.72	2	1.08	3	2.64	7	0	0	0	0	0	0
SW09	7/18/2017	36826	0	0	0	0	0	0	0	0	0	0	0	0
SW13	7/18/2017	31679	0	0	0.48	1	0.84	2	0	0	0	0	0	0
SW16	7/18/2017	35572	13.08	33	17.88	45	24	59	33.6	86	36	92	38.4	97
SW17	7/18/2017	38126	3.72	10	5.52	15	7.8	21	12.24	33	12.24	33	12.24	33
SW17U	7/18/2017	37677	3.72	10	5.28	14	6.36	17	0	0	0	0	0	0
BK01U	8/14/2017	35001	0	0	0	0	0	0	0	0	0	0	0	0
BK03	8/14/2017	36830	2.64	7	3.48	9	8.4	22	0	0	0	0	0	0

Sample Event		Accepted Droplets	<i>HF183</i> High Threshold		<i>HF183</i> Mean Threshold		<i>HF183</i> Low Threshold		<i>pig-2-bac</i> High Threshold		<i>pig-2-bac</i> Mean Threshold		<i>pig-2-bac</i> Low Threshold	
Site	Time		Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets	Conc. (copies/mL)	n positive droplets
BK12	8/14/2017	38531	0	0	0	0	1.08	3	0	0	0	0	0	0
BK14	8/14/2017	37611	0	0	0	0	1.92	5	0	0	0	0	0	0
SW01	8/14/2017	35731	0	0	0.36	1	0.36	1	0	0	0	0	0	0
SW04	8/14/2017	37279	0	0	0	0	1.08	3	0.72	2	0.72	2	0.72	2
SW07	8/14/2017	36559	0	0	0	0	0	0	0	0	0	0	0	0
SW10	8/14/2017	35943	0	0	0	0	0.36	1	0	0	0	0	0	0
SW11	8/14/2017	32374	0	0	0	0	0.84	2	0	0	0	0	0	0

APPENDIX E: CROSS-VALIDATION DATA

Independent variable metrics including interquartile range (IQR), R², β , IQR ratio, p-value are presented for univariate prediction and 10-fold cross validation for log₁₀ *E. coli* concentration

Variable			Univariate Performance				Cross-Validation Performance			
Type	Description	IQR	R ²	β	IQR Ratio	p	IQR Ratio	CI (low)	CI (high)	n p<0.1
Precipitation	Flush Event	1.00 ¹	0.15	0.95	1.00	***	8.83	8.23	9.41	9
	Prior rain, 24 hour	0.05	0.18	0.70	1.09	***	1.09	1.082	1.09	10
	Prior rain, 48 hour	0.39	0.14	0.50	1.57	***	1.57	1.367	2.00	6
	Runoff Event	1.00	0.13	0.60	3.99	***	3.99	2.926	6.23	8
Land Use	% Cultivated	0.22	0.01	-0.36	0.83	0.28	0.83	0.446	1.26	3
	% Cultivated, 100m Buffer	0.34	0.11	-1.01	0.45	***	0.45	0.315	0.51	6
	% Cultivated, 50m Buffer	0.28	0.11	-0.98	0.53	***	0.44	1.991	2.12	10
	% Forest	0.16	0.00	0.38	1.14	0.44	1.14	1.122	1.19	0
	% Forest, 100 m Buffer	0.09	0.06	2.27	1.60	**	1.60	1.386	2.62	5
	% Forest, 50m Buffer	0.36	0.05	2.04	5.41	**	1.68	0.309	0.48	6
	% Wetland	0.08	0.00	-0.46	0.92	0.59	0.92	0.881	0.96	0
	% Wetland, 100m Buffer	0.25	0.07	1.16	1.95	***	1.95	1.711	3.03	4
	% Wetland, 50m Buffer	0.11	0.09	1.11	1.33	***	2.05	1.358	2.33	3
	Soil Type A	0.48	0.00	-0.05	0.95	0.84	0.95	0.889	1.01	0
	Soil Type B	0.23	0.00	0.04	1.02	0.89	1.02	0.985	1.06	0
	Soil Type C	0.13	-0.01	1.05	1.36		1.34	0.908	0.95	0
Soil Type D	0.23	0.00	-0.90	0.62	0.46	0.95	0.537	0.73	0	
Measured	<i>HF183</i>		0.06	0.06		***				10
	<i>pig-2-bac</i>		0.05	0.00		**				7
	Conductivity	61.40	0.00	0.00	1.08	0.60	1.08	1.039	1.13	0
	Dissolved Oxygen	3.48	0.01	-0.01	0.93	0.25	0.93	0.918	0.94	0
	Water Temperature	9.20	0.02	0.02	1.53	*	1.53	1.468	1.59	9
Exposure	Manure Density	188235	0.05	0.00	1.51	**	1.51	1.466	1.55	10
	Pop. Density	45.00	0.02	0.00	1.23	0.08	1.23	1.21	1.26	7
	Sprayfield Acres	176.72	0.03	0.00	1.56	*	1.56	1.509	1.62	10

Variable			Univariate Performance				Cross-Validation Performance			
Type	Description	IQR	R ²	β	IQR Ratio	p	IQR Ratio	CI (low)	CI (high)	n p<0.1
Exposure	Watershed Area	3.93	0.00	-0.01	0.92	0.59	0.92	0.9	0.95	0
	Address (Gravity, Euc)	0.00	0.00	25.58	1.01	0.91	1.01	0.984	1.05	0
	Address (SED, OF)	2.16	0.12	0.11	1.69	***	1.69	1.657	1.71	10
	Lagoon (Gravity, Euc)	28.34	0.05	0.01	1.46	**	1.46	1.413	1.52	10
	Lagoon (SED, OF)	3577340	0.10	0.00	2.61	***	2.61	2.479	2.75	10
	Sprayfield (Gravity, Euc.)	37.20	0.02	0.00	1.18	*	1.18	1.158	1.19	9
	Sprayfield (SED, OF)	4271849	0.08	0.00	1.59	***	1.59	1.541	1.64	10

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