ESTIMATING ASSOCIATED HUMAN HEALTH RISK FROM RECREATIONAL EXPOSURES IN FRESH WATER BODIES IMPACTED BY MULTIPLE FECAL SOURCES

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2019

Major Subject: Biological and Agricultural Engineering

ABSTRACT

Pathogens are the leading cause of impairment for rivers and streams in the United States. Microbial contamination in recreational water bodies was the cause of 510 waterborne disease outbreaks in the United States. Water quality standards should prevent these outbreaks from occurring, however, in 93% of outbreaks, where water quality information was available, the water body was meeting water quality standards at the time of the outbreak.

The probability of gastrointestinal (GI) illness from recreational exposure to human, cattle, and wildlife fecal contamination was calculated in three water bodies impaired by microbial contamination by applying Quantitative Microbial Risk Assessment (QMRA) and Microbial Source Tracking (MST). Six reference pathogens were used to estimate the probability of GI illness: *Campylobacter*, *Cryptosporidium*, *E. coli* O157:H7, *Giardia*, norovirus, and *Salmonella*. The largest contributor of fecal contamination within the water bodies (wildlife) had the least significant impact on human health in all three water bodies. Whereas, human fecal sources had the most significant impact on the probability of GI illness, especially through the reference pathogen norovirus.

Non-point source fecal loads were spatially estimated within the Lampasas River Watershed using SELECT from three general sources: cattle, human, and non-avian wildlife. SWAT was used to simulate source specific in-stream *E. coli* concentrations from the fecal loads estimated by SELECT. SWAT-simulated in-stream *E. coli* concentrations were used to estimate reference pathogen doses which were input into a QMRA to estimate human health risk associated with exposure to fecal contamination from contact recreation in impaired waters. Across all flow conditions, the WWTP had the most significant impact on human health risk

even though it was not the largest contributor to fecal contamination. The probability of GI illness associated with the WWTP exceeded the acceptable GI illness rate but the WWTP was meeting water quality regulations.

Water bodies are regulated by developing a total maximum daily load (TMDL) to determine the largest contributor of fecal contamination and make appropriate load reductions to that contributor. For regulation and remediation to have significant impacts, it should be focused on sources that are the riskiest to human health.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Smith, and my committee members, Dr. Lacey, Dr. Mena, and Dr. Srinivasan, for their guidance and support throughout the course of this research.

I would also like to thank my fellow graduate students, Cherish Vance, Anna Gitter, Meaghan Owens, and Maryam Habibi, for their friendship and help while completing this dissertation.

Thanks also need to be given to my fellow military spouses, especially my friend Nikki Opbroek. Even though we no longer live near each other, you have been an immense source of support during the multiple deployments and moves that occurred over the course of completing this dissertation.

I would like to thank my husband, Matthew Borel, whose encouragement and support enabled me to pursue and persist in my studies to complete this dissertation. Finally, I would like to thank my parents, brother, and sister-in-law who helped me with many moves throughout the course of my studies and encouraged me to finish this dissertation.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Patricia Smith serving as chair from the Department of Biological and Agricultural Engineering. Additional committee members include Professor Ronald Lacey from the Department of Biological and Agricultural Engineering, Professor Raghavan Srinivasan of the Department of Ecosystem Science & Management Department, and Professor Kristina Mena of the Department of Epidemiology, Human Genetics, and Environmental Sciences at UTHealth School of Public Health.

All other work conducted for the dissertation was completed by the student independently.

Funding Sources

Graduate study was partially supported by a grant from North Texas Municipal Water District.

NOMENCLATURE

BST	Bacterial Source Tracking
CCN	Certificate of Convenience and Necessity
CFU	Colony Forming Units
CSO	Combined Sewer Overflow
DNA	Deoxyribonucleic Acid
E. coli	Escherichia coli
FDC	Flow Duration Curve
FIB	Fecal Indicator Bacteria
GI	Gastrointestinal
GIS	Geographic Information System
HRU	Hydrologic Response Unit
HSPF	Hydrologic Simulation Program Fortran
LDC	Load Duration Curve
MST	Microbial Source Tracking
NASS	National Agricultural Statistics Service
NEEAR	National Epidemiological and Environmental Assessment of Recreational Water
NLCD	National Land Cover Database
NSE	Nash-Sutcliffe Efficiency
OWTS	Onsite Wastewater Treatment Systems
QMRA	Quantitative Microbial Risk Assessment

qPCR	Quantitative Polymerase Chain Reaction
R^2	Coefficient of Determination
RCC	Rate of Correct Classification
Rep-PCR	Repetitive Sequence Polymerase Chain Reaction
RMU	Resource Management Unit
rRNA	Ribosomal Ribonucleic Acid
RWQC	Recreational Water Quality Criteria
SELECT	Spatially Explicit Load Enrichment Calculation Tool
SUFI-2	Sequential Uncertainty Fitting
SWAT	Soil and Water Assessment Tool
SWAT-CUP	SWAT Calibration Uncertainty Programs
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TPWD	Texas Parks and Wildlife Department
USDA NASS	United States Department of Agriculture National Agricultural Statistic Service
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization
WPP	Watershed Protection Plan
WWTP	Wastewater Treatment Plant

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1. INTRODUCTION AND LITERATURE REVIEW

Microbial contamination in recreational waters was the cause of 44% more waterborne disease outbreaks when compared to contaminated drinking water in the United States between 1995 and 2005 (Craun et al., 2005). In the United States, 510 outbreaks associated with microbial contamination in recreational water occurred over the course of only 12 years from 2001 to 2012 (Dziuban et al., 2006; Hlavsa et al., 2011; Hlavsa et al., 2014; Hlavsa et al., 2015; Yoder et al., 2004; Yoder et al., 2008). The United States Environmental Protection Agency (USEPA) microbial water quality guidelines for recreational waters were established to prevent these outbreaks from occurring. However, in 93% of outbreaks where the information was available, the water quality of a recreational water body was meeting the local water quality standards at the time of the outbreak (Craun et al., 2005).

The purpose of the recreational water quality standard is to provide fecal indicator bacteria (FIB) criteria concentrations that correspond to acceptable gastrointestinal (GI) illness rates from recreational exposure (Dufour and Ballentine, 1986; USEPA, 2012). Higher concentrations of FIB have a direct relationship to higher fecal microbial contamination. Epidemiology studies have shown that GI illness can be linked with FIB concentrations in recreational water impacted by human sources (Cabelli, 1983; Dufour, 1984; Wade et al., 2003; Wu et al., 2011). The link between GI illness and non-human fecal sources such as livestock and wildlife is not well established (Field and Samadpour, 2007). Multiple epidemiology studies conducted at locations impacted by non-human fecal sources did not show a link between GI illness and FIB concentrations (Calderon et al., 1991; Colford et al., 2007; Colford et al., 2012; Mcbride et al., 1998). This suggests that there is less risk of GI illness from recreation in waters

contaminated by non-human sources compared to waters contaminated by human sources. Currently, the USEPA's FIB recreational water quality criteria do not differ based on the source of contamination. (USEPA, 2012).

The USEPA recreational water quality standard for marine and fresh waters was developed in 1986 and updated in 2012 (Dufour and Ballentine, 1986; USEPA, 2012). The USEPA (1986) recreational water quality standard for marine and fresh waters was developed based on waters contaminated with human sources, such as treated wastewater treatment plant (WWTP) effluents (Cabelli, 1983; Dufour, 1984; Dufour and Ballentine, 1986). The FIB chosen for fresh waters are *Escherichia coli* (*E. coli*) with a geometric mean not to exceeded 126 colony-forming units (CFU) per 100 milliliters (Dufour and Ballentine, 1986). Enterococcus was chosen as the FIB for salt waters with a geometric mean not to exceed 35 CFU per 100 milliliters (Cabelli, 1983). Even though the USEPA updated the recreational water quality criteria, the FIB concentration criteria remained the same.

Differences between the 1986 USEPA standard and the updated 2012 standard are the acceptable rate of swimming associated illness and what symptoms constitute a GI illness. The recreational standard for fresh waters was developed using the Dufour (1984) study with an acceptable swimming associated gastroenteritis rate of 8 per 1000 swimmers (Dufour and Ballentine, 1986). Study participants were considered ill if they showed highly credible gastrointestinal symptoms including: vomiting, diarrhea with fever, and stomach ache or nausea accompanied by fever (Dufour and Ballentine, 1986).The USEPA updated the recreational water quality criteria in 2012. The illness rates upon which the water quality criteria were developed were based on the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) definition of GI illness, which does not require fever to be exhibited. The

USEPA standard for acceptable illness rate was increased to 36 per 1000 people (0.036) participating in primary contact recreation (USEPA , 2012).

The main purpose of water quality regulations is to protect human health with every other consideration being secondary, such as protection of the environment (Field and Samadpour, 2007). A shortcoming of FIB are that they do not identify the source of the contamination. A correlation between FIB and human health risks due to pathogens in human fecal sources has been established (Cabelli, 1983; Dufour, 1984; Wade et al., 2003; Wu et al., 2011). However, a correlation between FIB and health risks from water impacted by sources other than human has not been shown in epidemiology studies (Calderon et al., 1991; Colford et al., 2007; Colford et al., 2012; Mcbride et al., 1998). Even though animal feces contains pathogens, the human health risk from animal feces is assumed to be less than human fecal sources because many pathogen strains are host specific to the infected animal species and therefore not pathogenic to humans (Field and Samadpour, 2007). Accurately estimating human health risks from fecal contamination requires knowledge of the contribution from human and non-human sources.

Total Maximum Daily Loads (TMDLs) are used to remediate contamination in water bodies in the United States. The TMDL is the maximum amount of pollutant load a water body can receive and still meet the Recreational Water Quality Criteria (RWQC) (USEPA, 2008). In a TMDL, point and non-point sources contributing microbial contamination in a watershed are identified and wasteload or load allocations are determined for each fecal source. All pollutant sources are treated equally when developing a TMDL (USEPA, 2008). However, fecal sources are not equal in their likelihood to cause human illness and this should be taken into account when remediating a water body for pathogens. The TMDL approach solely focuses on remediation to meet the numerical FIB water quality standard regardless of the source.

Outbreaks are still occuring in recreational waters even when the water quality is meeting the local water quality standards at the time of the outbreak. Generally, epidemology studies need to be performed to determine the cause of oubreaks or assess the health impacts from exposure to potential sources of pathogens. Human enteric viruses, specifically norovirus, were suggested as the cause of a majority of GI illnesses from swimming in recreational waters impacted by human sources during an epidemiology study in the Great Lakes in 2003 and 2004 (Soller et al., 2010a). Epidemiology studies are costly and difficult to implement for multiple exposure scenarios that can cause negative health impacts. Quantitative microbial risk assessment (QMRA) is a tool that can be applied for multiple exposure scenarios to estimate associated health risks.

QMRA is an approach to estimate the health risk from exposure to infectious microorganisms by applying the principles of risk assessment (Haas et al., 2014). QMRA studies estimated human health risks from exposure to water contaminated with human and non-human sources using FIB concentrations to calculate a pathogen dose (Schoen and Ashbolt, 2010; Schoen et al., 2011; Soller et al., 2010a; Soller et al., 2010b; Soller et al., 2014; Soller et al., 2015). These studies only consider hypothetical FIB concentrations, in particular the recreational water quality standards and not the actual FIB concentrations occurring in a watershed.

QMRA has been used in conjunction with watershed modeling to estimate potential human health risks from exposure to simulated FIB concentrations from human and non-human sources during rainfall events (Eregno et al., 2016; Liao et al., 2016). These studies were not able to include all sources of potential contamination particularly non-avian wildlife. Other QMRA studies have modeled the dispersion of pathogens to estimate a potential pathogen dose from exposure but there was a single source of contamination (Andersen et al., 2013; Sokolova et al.,

2012; Sokolova et al., 2015). Additional QMRA studies have also estimated human health risks from exposure to water contaminated with a single source by measuring pathogen concentrations in the water body to calculate a pathogen dose (Betancourt et al., 2014; McBride et al., 2013; Rijal et al., 2011; Wilkes et al., 2013). Routinely monitoring water bodies for waterborne pathogens is not feasible because it is technically difficult and costly to culture and identify pathogens in water samples (Harwood et al., 2014).

The distribution of pathogens in environmental waters typically are variable over the area of the water body. Pathogens also occur at low concentrations which makes them not readily detectable. However, pathogens are highly infective at low doses having the potential to cause negative health impacts from exposure even when they are not able to be detected in environmental waters (Field and Samadpour, 2007; Harwood et al., 2014; Scott et al., 2002). Because monitoring for all pathogens is not possible in water bodies, FIB are monitored to protect human health.

Microbial source tracking (MST) also known as bacterial source tracking (BST) is a method that identifies the sources of fecal bacteria from environmental samples (Field and Samadpour, 2007; Meays et al., 2004). The premise behind MST is that certain characteristics in the fecal microorganisms from a source are strongly associated with particular hosts. This is an identifying trait that can be used as a marker for fecal contamination from the source that can be detected in water (Field and Samadpour, 2007; Harwood et al., 2014). An assumption used to quantify MST is that identifying markers within the fecal material of a specific species remain the same over time and after the feces enters the water. If the markers are quantitatively detected, then the proportion of the contribution from each particular source can be estimated (Field and Samadpour, 2007). The estimated proportion of each source is applied to identify the sources of

FIB, which would aid in identifying the likely pathogens in water bodies. Ultimately, this would enable the estimation of human health risk associated with exposure to the water contaminated with fecal material from multiple sources (Field and Samadpour, 2007).

There are multiple methods to identify fecal sources using MST but these methods are typically categorized as library-dependent or library-independent methods. A library is a collected database which includes a set of bacterial isolates from multiple known fecal sources that are tested using a method where patterns or fingerprints unique to the source are identified (Field and Samadpour, 2007). The fingerprints of bacterial isolates taken from environmental samples are then compared to the library to identify the contributing fecal sources (Field and Samadpour, 2007; Harwood et al., 2014). Library-independent methods identify a specific bacterial species or type that are host-specific. An environmental sample is analyzed for each host specific marker, when applying library-independent methods (Harwood et al., 2014). This study will focus on two library-dependent methods; ribotyping and repetitive sequence polymerase chain reaction (rep-PCR).

Ribotyping and rep-PCR are both culture-based, library-dependent deoxyribonucleic acid (DNA) fingerprinting techniques (Field and Samadpour, 2007). Ribotyping involves the identification of microorganisms through analysis of their highly conserved 16S ribosomal ribonucleic acids (rRNA) genes after they have been enzymatically restricted (Meays et al., 2004; Scott et al., 2002). Unique strains of *E. coli* for specific host species are identified and the 16S rRNA genes for those strains are then compared to the genetic fingerprints of bacterial isolates from water samples to identify the animal sources of the bacteria occurring in the water sample (Meays et al., 2004). Ribotyping is labor intensive and geographically specific (Field and

Samadpour, 2007). Advantages of ribotyping are that it is highly reproducible and able to classify isolates from multiple sources (Meays et al., 2004).

Rep-PCR uses primers corresponding to repetitive intergenic DNA sequences to differentiate among sources of fecal pollution to generate specific genomic fingerprints for each source (Meays et al., 2004; Scott et al., 2002). The DNA fingerprint patterns in water samples are then analyzed using pattern recognition software to identify the fecal sources within that water sample (Meays et al., 2004). Similar to ribotyping, a library is required; with rep-PCR, the variability increases as the size of the library increases (Field and Samadpour, 2007; Meays et al. , 2004). Rep-PCR is a simple and rapid method to identify sources within water samples once a library has been established (Meays et al., 2004).

Fecal pathogens entering surface water are dependent not only on the source of fecal matter but also on the transport of those pathogens from the source into water bodies. Curriero et al. (2001) found that 51% of waterborne disease outbreaks in the United States were preceded by extreme precipitation events. Outbreaks occurring from surface water contamination had the strongest correlation with extreme precipitation events during the month of the outbreak (Curriero et al., 2001). A study in Canada also showed that extreme rainfall events increase the likelihood of a waterborne disease outbreak by a factor of 2.3 (Thomas et al., 2006). Contamination of the water source through runoff or inundation following an extreme rain event was the most common cause of outbreaks in both developing and developed countries (Cann et al., 2013). Surface water bodies are more vulnerable than groundwater to microbial contamination caused by runoff from precipitation events. Precipitation events need to be included in the analysis when estimating potential health risks from exposure to microbial contaminants in surface water bodies.

Collecting monitoring data for a water body is time intensive and expensive, especially for a long consistent record. The cost to collect additional monitoring data to support total maximum daily loads (TMDL) is $17.3 million annually (USEPA, 2001a). Often, water quantity and quality monitoring records for a water body are not consistently measured over the period of record. Water quantity and quality modeling can be used to fill monitoring data gaps and provide a consistent data record for a water body. A model also can be applied to an ungauged watershed to provide water quantity and water quality data. Watershed models are a vital tool to supplement water quality and quantity monitoring.

The ideal microbial model should be able to simulate four factors: land use, climate, topography, and hydrology (Coffey et al., 2010; Jamieson et al., 2004). Climate, topographical, and hydrological factors all play a role in the fate and transport of pathogens over the land surface through runoff and infiltration from rainfall events, whereas, land use affects runoff and infiltration, but also, the sources and amounts of potential pathogen input (Coffey et al., 2010; Jamieson et al., 2004). Many models that simulate in-stream bacteria concentrations utilize runoff models to simulate the fate and transport of fecal microorganisms over the land surface (Baffaut and Sadeghi, 2010; Coffey et al., 2010; Iudicello and Chin, 2014; Pachepsky et al., 2006; Srivastava et al., 2007). Wet and dry periods should be differentiated when using these models to increase the accuracy of the fecal source inputs. Two popular watershed-scale models have incorporated modules to simulate the fate and transport of FIB. These models are Hydrologic Simulation Program Fortran (HSPF) (Bicknell et al., 2001) and Soil and Water Assessment Tool (SWAT) (Neitsch et al., 2011).

HSPF is a watershed-scale model that simulates in-stream bacteria concentrations by modeling bacteria fate and transport from runoff events (Pachepsky et al., 2006; Srivastava et al.,

2007). Bacteria fate and transport in HSPF are modeled for an hourly time step through overland flow and interflow of bacteria inputs that are deposited directly to land surfaces (Iudicello and Chin, 2014). The bacteria module in HSPF was developed for fecal coliforms therefore, the inputs and outputs need to be converted into and from fecal coliforms into other FIB or pathogens (Ferguson et al., 2003; Liao et al., 2015). HSPF has had limited success modeling in-stream bacteria concentrations at the watershed-scale due to high variability associated with observed concentrations. A difference of one order of magnitude between simulated and observed bacteria concentrations is considered acceptable (Liao et al., 2015). Iudicello and Chin (2015) modeled fecal coliform concentrations using HSPF separating modeling results into corresponding dry and wet conditions. This resulted in a majority of model predicted concentrations to be within one order of magnitude of the observed fecal coliform concentrations for both dry and wet conditions. Liao et al. (2015) was also able to achieve HSPF simulated bacteria concentrations to be within one order of magnitude of observed concentrations for both in-stream water and sediment bacteria concentrations. However, HSPF is considered more difficult to apply compared to other watershed-scale models due to large amounts of data and input parameters that are required in order to calibrate HSPF (Ferguson et al., 2007; Saleh and Du, 2004; Srivastava et al., 2007).

Similar to HSPF, SWAT is a watershed-scale model that simulates in-stream bacteria concentrations using runoff events to drive the fate and transport of bacteria. SWAT is considered an easier model to run compared to HSPF (Saleh and Du, 2004). Bacteria concentrations are modeled in SWAT on a daily time step and the fate and transport of bacteria are modeled in runoff from land surfaces (Iudicello and Chin, 2014; Pachepsky et al., 2006). Modeling FIB with SWAT has resulted in variable success (Baffaut and Sadeghi, 2010; Coffey

et al., 2010; Coffey et al., 2013; Frey et al., 2013; Iudicello and Chin, 2014). It is difficult to model the actual spatial and temporal loading patterns of bacteria, additionally, observed bacteria measurements are highly variable and this contributes to the difficulty in calibrating and validating a model (Frey et al., 2013; Pachepsky et al., 2006). Both HSPF and SWAT only have modules that predict FIB and they are not able to predict pathogen concentrations (Coffey et al., 2010; Ferguson et al., 2003). Additionally, both models have difficultly accurately modeling bacteria concentrations at extremely low and high flows (Benham et al., 2006; Chin et al., 2009).

The concentrations of pathogens occurring in a water body have been modeled by applying hydrologic and dispersion models (Ferguson et al., 2007; Medema and Schijven, 2001). Ferguson et al. (2007) applied a hydrologic model to estimate the mobilization of pathogens from land deposited feces due to rainfall (Ferguson et al., 2007). Medema and Schijven (2001) applied emission (PROMISE) and dispersion (WATNAT) models to estimate the concentrations of pathogens downstream from sewage discharges. Both of these studies only considered one source contributing to the microbial contamination in the water body. For the results to be accurate, all potential sources need to be considered when estimating the pathogen concentrations in water bodies.

Spatially Explicit Load Enrichment Calculation Tool (SELECT) is a geographic information system (GIS) tool that uses spatial factors such as land use, fecal source population densities, and soil to assess potential *E. coli* loads within a watershed (Teague et al., 2009). SELECT is able to estimate *E. coli* loads for multiple non-point and point sources including: livestock, wildlife, on-site wastewater treatment systems (OWTS), dogs, and wastewater treatment plants (WWTPs) (Teague et al., 2009; Riebschleager et al., 2012; Borel et al., 2012a). SELECT outputs were combined with a simple curve number based runoff model to predict *E.*

coli concentrations occurring in a water body from the transport of fecal material from rain events (Borel et al., 2012b). The SELECT model can be used as an input for other rainfall-runoff models such as HSPF or SWAT to predict FIB concentrations. The purpose of modeling FIB or pathogen concentrations in a water body is to determine if the modeled concentrations are above a water quality standard and if exposure to the fecal contamination will impact human health when measured data are not available.

A limitation to applying QMRA to recreational water bodies is the current lack of specific water quality data for pathogens. Also, FIB are typically the only measure of microbial water quality and the prevalance of specific pathogens vary considerably depending on the source population and seasonality (WHO, 2003). The main advantage of QMRA is the health risk for multiple exposure scenarios can be estimated and used for risk management decisions without performing costly epidemiology studies that may be infeasible.

QMRA has been applied extensively in recreational water bodies to determine health risk (Andersen et al., 2013; Eregno et al., 2016; Liao et al., 2016; Rijal et al., 2011; Schoen and Ashbolt, 2010; Schoen et al., 2011; Soller et al., 2010a; Soller et al., 2010b; Soller et al., 2014; Soller et al., 2015; Wilkes et al., 2013). In recreational waters, QMRA has been applied to multiple sources but only avian wildlife has been considered in previous studies. There is a gap when it comes to wildlife sources because non-avian wildlife, such as deer, need to be considered as a source of fecal contamination. In multiple MST studies performed in Texas, wildlife sources contributed a majority of the fecal contamination to the water body (Di Giovanni and Casarez, 2006; Gregory et al., 2013; Martin and Gentry, 2014). Estimates of health risk from exposure to fecal contamination in a water body cannot be accurately determined if major source of fecal contamination is not included in those calculations. Therefore, non-avian wildlife should

be included as a source of fecal contamination when applying QMRA to develop a more accurate estimate of health risk from exposure to fecal contamination in water bodies.

One purpose of this dissertation was to address current gaps in QMRA research when estimating health risk from exposure to fecal contamination in recreational water bodies. MST was incorporated with the QMRA analysis to determine the bacteria contribution from specific fecal sources in water bodies to estimate health risk from exposure to fecal contamination in the water bodies. Non-avian wildlife was included in QMRA analysis as a source of fecal contamination in water bodies to determine health risks from exposure to recreational water bodies contaminated with fecal material. SELECT and SWAT were used to estimate *E. coli* concentrations from specific sources to predict health risks related to flow regimes.

1.1. Objectives and Hypotheses

The objective of this dissertation is to determine the health risk, likelihood of illness, from exposure to fecal contamination in recreational water bodies using QMRA, MST, SELECT, and SWAT. Each objective will focus on a different parameter impacting health risk.

1.1.1. Apply QMRA and MST to calculate the likelihood of GI illness from exposure in two recreational water bodies in Texas to fecal contamination from human, cattle, and non-avian wildlife sources

a. Hypothesis: Applying uncertainty ranges to the point inputs of *E. coli* concentration in the water body and fecal source contribution will significantly change the resulting health risk.
b. Hypothesis: Exposure to the reference pathogens norovirus, *Cryptosporidium parvum, Giardia lamblia, Campylobacter*, *Salmonella*, and *E. coli* O157:H7 in human sources of fecal contamination of recreational waters will result in a higher

health risk than exposure to those same reference pathogens in fecal contamination of recreational water by wildlife and cattle.

c. Hypothesis: At the recreational bacterial standard of 126 CFU/100mL *E. coli*, the health risk from all sources will equal the USEPA acceptable illness rate, 0.036.

1.1.2. Apply SWAT in conjunction with SELECT to simulate source-specific *E. coli* concentrations for input into a QMRA to determine likelihood of GI illness

Hypothesis: SWAT, SELECT, and QMRA methodologies can be applied together for future predictive capabilities to determine health risk in watersheds under specific hydrologic conditions.

2. ESTIMATING HUMAN HEALTH RISK IN RECREATIONAL WATER BODIES IMPACTED BY MULTIPLE FECAL SOURCES

2.1. Introduction

In 1986 the United States Environmental Protection Agency (USEPA) recommended recreational water quality criteria (RWQC) to prevent outbreaks from occurring. However, Craun et al. (2005) found that where such information was available, in 93% of outbreaks in recreational water bodies the water body was meeting the local water quality standards at the time of the outbreak. The purpose of the RWQC is to provide standards for fecal indicator bacteria (FIB) concentrations that correspond to acceptable gastrointestinal (GI) illness rates from recreational exposure (Dufour and Ballentine, 1986; USEPA, 2012). Epidemiology studies have linked GI illness to FIB concentrations in recreational water impacted by human sources, most frequently wastewater treatment plants (WWTP) (Cabelli, 1983; Dufour, 1984; Wade et al., 2003; Wu et al., 2011). Multiple epidemiology studies conducted at locations impacted by non-human fecal sources were inconclusive in the link between GI illness and FIB concentrations (Calderon et al., 1991; Colford et al., 2007; Colford et al., 2012). However, the non-human fecal source of cattle may be as risky as human sources at impacting human health (Mcbride et al., 1998; USEPA, 2010; USEPA, 2012). As a conservative measure, the USEPA (1986) RWQC for marine and fresh waters do not differ based on the source of contamination. (USEPA, 2012).

The USEPA updated the RWQC in 2012. The FIB determined for fresh waters, remained the same, *Escherichia coli* (*E. coli*) with a geometric mean not to exceeded 126 colony-forming units (CFU) per 100 milliliters (Dufour and Ballentine, 1986). The USEPA standard for acceptable illness rate was increased from 8 to 36 per 1000 people (0.036) participating in

primary contact recreation (USEPA, 2012). The USEPA (2010) has established eight waterborne reference pathogens; norovirus, rotavirus, adenovirus, *Cryptosporidium spp*., *Giardia lamblia*, *Campylobacter spp*., *Salmonella spp.*, and *E. coli O157:H7*. These reference pathogens can be present in human and animal fecal waste as well as in recreational water. The eight reference pathogens are representative of other waterborne pathogens, have the ability to survive in the environment, and have dose-response relationships

Currently, water bodies are regulated using Total Maximum Daily Loads (TMDLs), the maximum amount of pollutant load a water body can receive while still meeting water quality standards (USEPA, 2008). During the process to develop a TMDL, every pollutant source is treated equally (USEPA, 2008). While this approach helps to facilitate diverse stakeholder involvement, fecal sources vary significantly in their likelihood to cause human illness. As part of a TMDL, wasteload allocations and load allocations are determined for each point and non-point source. If load reductions are necessary in a water body, typically the largest contributing source will be targeted for remediation to reduce the pollutant loading in the water body. This approach solely focuses on remediation to meet the numerical FIB water quality standard and does not take into account the differing health risks associated with the contributing fecal sources.

Quantitative Microbial Risk Assessment (QMRA) is an approach to estimate the health risk from exposure to infectious microorganisms by applying the principles of risk assessment (Haas et al. , 2014). QMRA studies estimated human health risks from exposure to water contaminated with human and non-human sources using FIB concentrations to calculate a pathogen dose (Schoen and Ashbolt, 2010; Schoen et al., 2011; Soller et al., 2010b; Soller et al., 2014; Soller et al., 2015). These studies only consider hypothetical FIB concentrations, in

particular the recreational water quality standards and not the actual FIB concentrations occurring in a watershed. Additionally, the non-human sources used in these studies are agricultural animals or avian wildlife (seagulls). This study aims to address these gaps by applying the actual FIB concentrations occurring in a water body and to take non-avian wildlife into consideration to determine human health risks.

2.1.1. Objectives and Hypotheses

The hypotheses of this study are threefold: (1) applying uncertainty ranges to the point inputs of *E. coli* concentration in the water body and fecal source contribution will significantly change the resulting health risk. (2) exposure to the reference pathogens norovirus, *Cryptosporidium parvum*, *Giardia lamblia*, *Campylobacter*, *Salmonella*, and *E. coli* O157:H7 in human sources of fecal contamination of recreational waters will result in a higher health risk than exposure to those same reference pathogens in fecal contamination of recreational water by wildlife and cattle, and (3) at the recreational bacterial standard of 126 CFU/100mL *E. coli* the health risk from all sources will equal the USEPA acceptable GI illness rate, 0.036.

The objective of this study is to use QMRA and MST to calculate the likelihood of GI illness from exposure in two recreational water bodies in Texas to fecal contamination from human, cattle, and non-avian wildlife sources.

2.1.2. Study Areas

Two watersheds in Texas were selected for this study: one rural, the Lampasas River Watershed, and one urban, the Salado Creek Watershed. Watershed selection was based on impairment and previous MST studies (Di Giovanni and Casarez, 2006; Gregory et al., 2013). The Lampasas River Watershed is 3,231 square kilometers (1,247 square miles) primarily situated in rural and agricultural areas (Figure 2-1) (Prcin et al., 2013). The Salado Creek

Watershed encompasses an area of 565 square kilometers (218 square miles) with the middle and lower parts of the watershed located in dense urban areas (Figure 2-2) (Atkins, 2011).

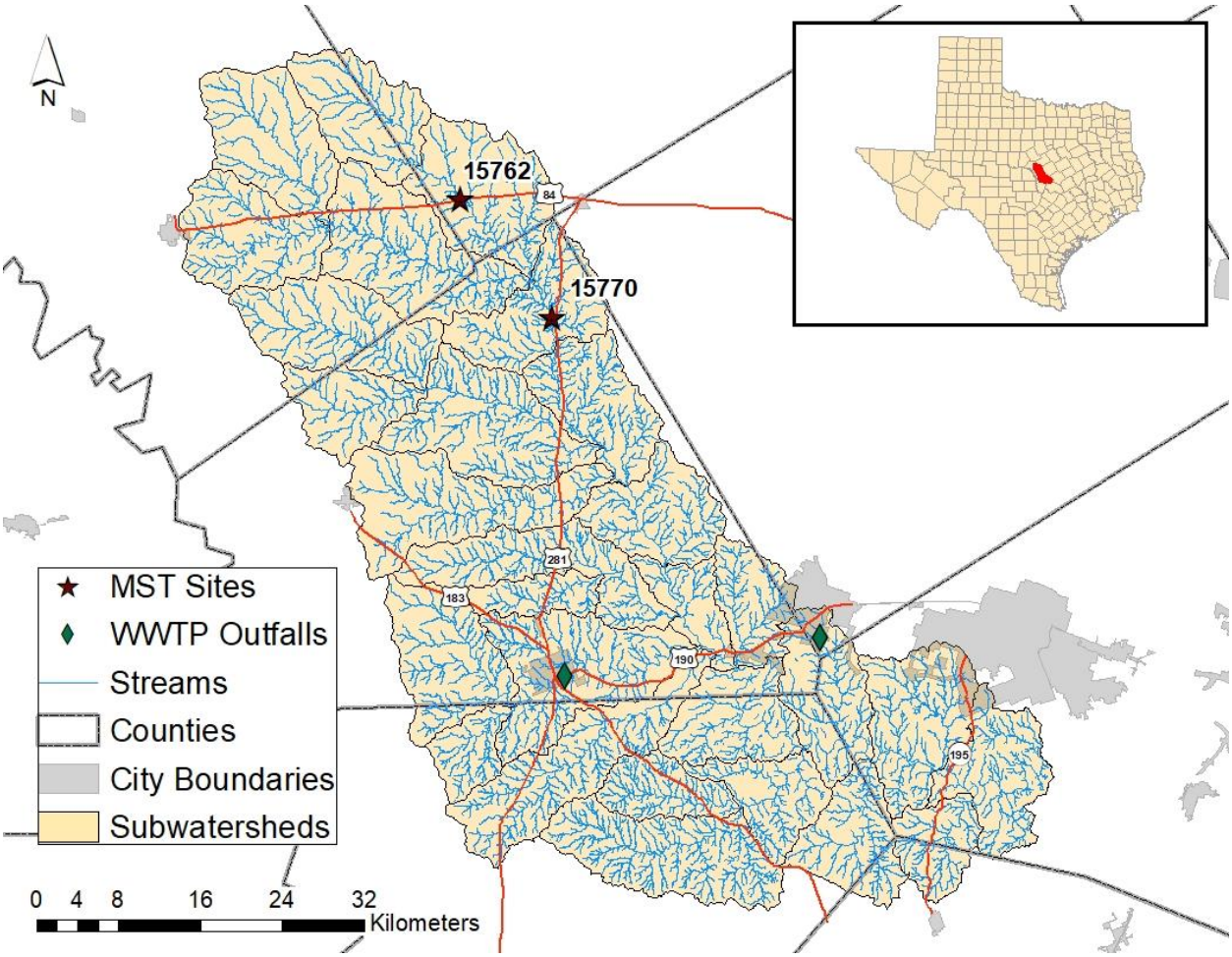

Figure 2-1. Location of the Lampasas River Watershed with MST sites and WWTP outfalls.

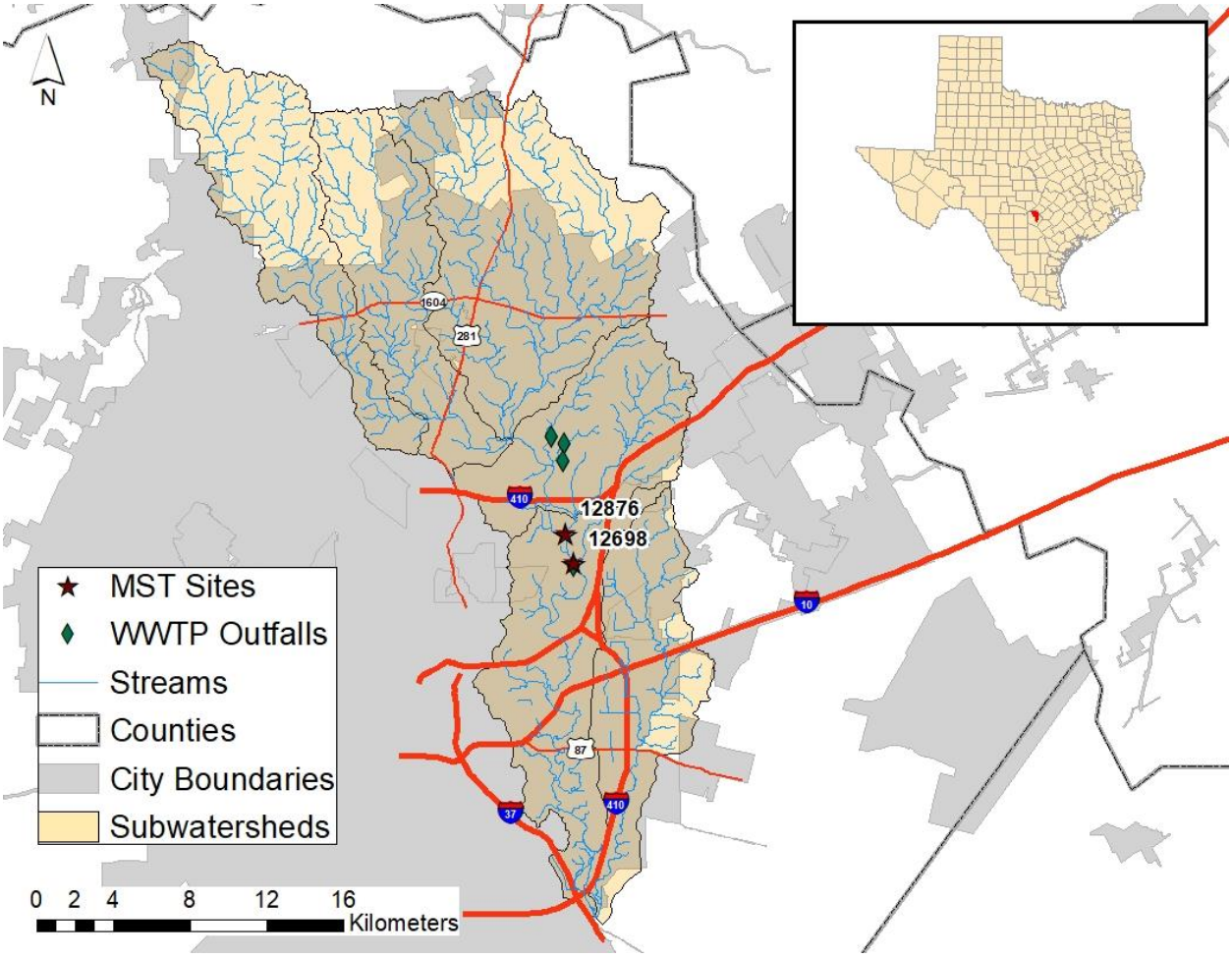

Figure 2-2. Location of the Salado Creek Watershed with MST sites and WWTP outfalls.

2.2. Methodology

QMRA was used to estimate the human health risk from recreational exposure, specifically accidental ingestion from swimming in impaired water bodies, associated with microbial contamination from human, cattle, and non-avian wildlife sources.

Of the eight waterborne reference pathogens established by USEPA (2010) only six were used in the QMRA as described below. Adenovirus was not used because there is currently no published ingestion dose- response relationship (USEPA, 2010). Rotavirus can be shed by calves and piglets but the strains are host specific and not likely to infect humans (Martella et al.,

2010; USEPA, 2010). Therefore, rotavirus was also excluded as a reference pathogen in this study.

Measured *E. coli* concentrations (C_{FIB}) in the water bodies were used to estimate reference pathogen (rp) doses, μ_{rp}^{S}, from each of three fecal source (s): human, cattle, and non-avian wildlife for each of the six reference pathogens: norovirus, *Cryptosporidium spp*., *Giardia lamblia*, *Campylobacter spp*., *Salmonella*, and *E. coli* O157:H7 using equation 2-1 which Gitter (2016) modified from Schoen and Ashbolt (2010). The human sources were assumed to include both secondary disinfected effluent and raw sewage. Cattle sources were assumed to be fresh manure. The wildlife sources were limited to deer and feral hogs also known as wild boar or feral swine.

The reference pathogen dose, μ_{rp}^{S}, (number of pathogens or genomes) was calculated as:

$$\mu_{rp}^{S} = \frac{C_{FIB} \times F^{S}}{R_{FIB}^{S} \times 100} \times R_{rp}^{S} \times p_{rp}^{S} \times I_{rp}^{S} \times V \qquad (2\text{-}1)$$

C_{FIB} is the *E. coli* concentration in the water body measured by a culture-based method (CFU/100mL)

F^{S} is the fraction of *E. coli* from source S

R_{FIB}^{S} is the *E. coli* concentration in feces (wet mass) (CFU/g) or in sewage from source S (CFU/L)

R_{rp}^{S} is the concentration of pathogen species in feces (wet mass) (number of pathogens or genomes/g) or in sewage from source S (number of pathogens or genomes/L)

p_{rp}^{S} is the fraction of human-infectious pathogenic strains from source S

I_{rp}^{S} is the prevalence of infection in the non-human source, i.e. cattle and non-avian wildlife, S (proportion of animals shedding the pathogen)

V is the volume of water ingested (mL)

Two values of C_{FIB} were used in the analysis of each watershed; the geometric mean of the measured *E. coli* concentrations sampled during the MST sampling period (Table 2-1) and the regulatory standard for recreational water bodies of 126 CFU/100mL. The fraction of the *E. coli* concentration (F^S) from each source was determined using the MST data from two sites for each watershed in the study (Table 2-1).

Table 2-1. C_{FIB} and F^S from MST results for 2 study watersheds.

Location	C_{FIB}	F^S (%)			Reference
	cfu mL^{-1}	Human[a]	Cattle[b]	Wildlife[c]	
Lampasas River					*Gregory et al., 2013*
Site 15762	162	27	6	60	
Site 15770	158	12	0	77	
Salado Creek					*Di Giovanni and Casarez, 2006*
Site 12876	299	28	28	44	
Site 12698	498	34	37	29	

a Unidentified sources have been combined with human to represent a worst-case scenario
b Other domesticated animals were added to cattle in the Salado Creek Watershed, but not the Lampasas River Watershed. Also includes pets for the Salado Creek Watershed.
c Avian wildlife also included in the Salado Creek Watershed wildlife F^s.

The MST results for two sites (15762 and 15770) in the Lampasas River Watershed consisted of human, cattle, other domesticated animals, wildlife, and unidentified (Gregory et al., 2013). Unidentified was combined with human sources to represent a worst-case scenario. Domesticated animals were kept separate from cattle because one of the sites had 0% cattle contribution.

The Salado Creek watershed contained two MST sites, 12876 located on the main stem and Walzem Creek site 12698 located on a tributary. The MST results for the Salado Creek

watershed comprised of sewage, cattle, pet, other livestock non-avian, other livestock avian, wildlife non-avian, wildlife avian, and unidentified (Di Giovanni and Casarez, 2006). Pet and other livestock were included with cattle for the cattle MST source inputs. In order to be consistent with the Lampasas River watershed MST results, both non-avian and avian wildlife percentages were included as non-avian wildlife for the Salado Creek watershed.

Concentrations of *E. coli* (Table 2-2) and the concentrations of reference pathogens in human waste (Table 2-3), cattle (Table 2-4) and wildlife (Table 2-5) were taken from the literature. The range of values of *E. coli* concentrations (R^S_{FIB}) and pathogen concentrations (R^S_{rp}) in the source waste represent the lowest value and highest values reported for each source. The R^S_{FIB} values for human waste takes its low from reported values of secondary chlorinated effluent and its high from primary sewage (Table 2-2 and 2-3).

Table 2-2. Concentration of *E. coli* in source waste (R^S_{FIB}).

Fecal Source			Low	High	Reference
Human		CFU/L	3.16	100,000,000	(Soller et al., 2010b)
Cattle		CFU/g	335	17,400,000	(Padia et al., 2012; USEPA, 2010)
Wildlife	Deer	CFU/g	46,000	26,900,000	(Gallagher, 2012)
	Feral Hog	CFU/g	79,500	41,600,000	(Gallagher, 2012)

Table 2-3. Concentration of reference pathogens in human waste (R^S_{rp}).

Organism	Low	High	Reference
Norovirus (genomes/L)	158	1,000,000	(Soller et al., 2010b)
Giardia (cysts/L)	0.1	10,000	(Soller et al., 2010b)
Cryptosporidium (oocysts/L)	0.1	398	(Soller et al., 2010b)
Salmonella (CFU/L)	0	1,000	(Soller et al., 2010b)
Campylobacter (CFU/L)	0	199.5	(Soller et al., 2010b)
E. coli O157:H7 (CFU/L)	0	1995	(Soller et al., 2010b)

The concentration and prevalence values of reference pathogens in cattle feces (Table 2-4) were assumed to be from fresh manure similar to the USEPA (2010) and Soller et al. (2010) recreational QMRA studies.

Table 2-4. Concentration (R^S_{rp}) and prevalence (I^S_{rp}) of reference pathogens in cattle feces.

Organism	Low(mean)*	High(st dev)*	Prevalence (%)	References
Giardia (cysts/g)	1.58	3162	0.2 - 37	(USEPA, 2010)
Cryptosporidium (oocysts/g)	0.5	1585	0.6 - 23	(USEPA, 2010)
Campylobacter (cfu/g)	63	31,623	5 - 38	(USEPA, 2010)
Salmonella (cfu/g)	398	39,811	5 - 18	(USEPA, 2010)
E. coli O157:H7* (cfu/g)	(1202)	(30.9)	9.7 - 28	(USEPA, 2010)

*lognormal distribution

Only *Giardia* and *Cryptosporidium* R^S_{rp} were available for deer fecal matter and only *Campylobacter* and *Salmonella* R^S_{rp} were available for feral hog fecal matter. Therefore, the pathogen doses from the wildlife source, μ^W_{rp}, for *Giardia* and *Cryptosporidium* were based on deer only and the pathogen doses from wildlife source for *Salmonella* and *Campylobacter* were based on feral hogs only. Insufficient data were available relating pathogen concentration of *E.*

coli O157:H7 in either deer or feral hog fecal matter, so *E. coli* O157:H7 was not included in the wildlife calculations.

There are a limited number of studies quantifying the concentration of *Giardia* and *Cryptosporidium* in deer fecal matter, none of which were located in the United States (Castro-Hermida et al., 2011; Cox et al., 2005; Garcia-Presedo et al., 2013; Heitman et al., 2002a; Paziewska et al., 2007). Heitman et al. (2002) conducted a study in Alberta, Canada which measured a mean number of 1168 of *Giardia* cysts per gram and 12 *Cryptosporidium* oocysts per gram of deer feces. In Spain, two studies provided ranges of *Giardia* and *Cryptosporidium* pathogen concentrations in roe deer fecal matter with ranges of 5-320 and 5-47 of *Giardia* cysts per gram and 5-225 and 5-200 of *Cryptosporidium* oocysts per gram (Castro-Hermida et al., 2011; Garcia-Presedo et al., 2013). The geometric mean concentrations of *Giardia* and *Cryptosporidium* in roe deer fecal matter were measured as 1.1 and 1.3 *Giardia* cysts per milliliter and 1.8 and 3 *Cryptosporidium* oocysts per milliliter in Poland (Paziewska et al., 2007). The lowest and highest concentrations of each reference pathogen from these studies, R_{rp}^{S},were used as the range for these pathogens in Table 2-5 (Garcia-Presedo et al., 2013; Heitman et al., 2002a; Paziewska et al., 2007). The lowest and highest concentrations for *Salmonella* and *Campylobacter* came from an unpublished study by Brooks (2017), where concentrations were measured in the fecal matter of three feral hogs using two approaches, enrichment combined with quantitative polymerase chain reaction (qPCR) and traditional cultivation (Table 2-5).

Giardia and *Cryptosporidium* prevalence in deer fecal matter were measured in multiple studies (Castro-Hermida et al., 2011; Garcia-Presedo et al., 2013; Hamnes et al., 2006; Heitman et al., 2002a; Lalle et al., 2007; Ng et al., 2011; Paziewska et al., 2007; Rickard et al., 1999; Santin and Fayer, 2015; Trout et al., 2003). Amongst all of the studies the prevalence of *Giardia*

ranged from 0.15% to 21.2% (Heitman et al., 2002a; Ng et al., 2011). *Cryptosporidium* prevalence in deer fecal matter ranged from 0.15% to 14.4% (Heitman et al., 2002a; Paziewska et al., 2007).

Multiple studies in the United States and Europe measured the prevalence (I^S_{rp}) of *Salmonella* and *Campylobacter* in feral hog fecal matter using an isolate test (Diaz-Sanchez et al., 2013; Jay-Russell et al., 2012; Magnino et al., 2011; Thakur et al., 2011; Vieira-Pinto et al., 2011; Wacheck et al., 2010; Wahlstrom et al., 2003; Zottola et al., 2013). The prevalence of *Salmonella* ranged from a low of 0% and a high of 22% (Vieira-Pinto et al., 2011; Wahlstrom et al., 2003). The prevalence of *Campylobacter* was measured in four studies as 0%, 12%, 40% and 66%, resulting in a range of 0% to 66% (Diaz-Sanchez et al., 2013; Jay-Russell et al., 2012; Wacheck et al., 2010; Wahlstrom et al., 2003).

Table 2-5. Concentration (R^S_{rp}) and prevalance (I^S_{rp}) of reference pathogens in wildlife feces.

Organism	Low	High	Prevalence (%)	References
Giardia (cysts/g)	1.1	1168	0.15-21.2	(Heitman et al., 2002a; Paziewska et al., 2007)
Cryptosporidium (oocysts/g)	1.8	225	0.15-14.4	(Garcia-Presedo et al., 2013; Paziewska et al., 2007)
Salmonella (MPN/dry g enrichment)	0	11	0-22	(J. Brooks, personal communication, February 2, 2017)
Campylobacter (MPN/dry g enrichment)	0	420	0-66	(J. Brooks, personal communication, February 2, 2017)

For human sources, the fraction of human-infectious pathogenic strains from each source (p^S_{rp}) and prevalence of infection (I^S_{rp}) was both assumed to be 1.0. The fraction of human-infectious pathogenic strains (p^S_{rp}) in sources other than human were estimated qualitatively as

low (0-33%), medium (34-66%), and high (67%-100) (Table 2-6). These percentages were applied as a fraction and uniform distribution. USEPA (2010) and Soller et al. (2010) assigned low, medium, and high values to describe the ability of zoonotic-derived reference pathogens to infect humans in livestock. These values were chosen as low for all reference pathogens in wildlife because deer and feral hogs are not considered major hosts of human-pathogenic strains of *Cryptosporidium*, *Giardia*, *Salmonella*, and *Campylobacter* (USEPA, 2009).

Table 2-6. Human infectious potential of reference pathogens (p^S_{rp}) from cattle and wildlife.

Organism	Cattle	Wildlife
Giardia	H	L
Cryptosporidium	H	L
Campylobacter	H	L
Salmonella	M	L
E. coli O157:H7	H	NA

The parameters used to calculate pathogen dose contain a large amount of uncertainty therefore a Monte Carlo process using 10,000 simulations was used to produce distributions for the pathogen dose from each source. The Monte Carlo simulations were generated using Crystal Ball Pro® software (Oracle Corp., Redwood Shores, CA).

Harmel et al. (2016) examined measurement uncertainty for in stream *E. coli* measurements considering sample collection, storage, and laboratory analysis. They determined under a "good" scenario *E. coli* concentrations had ±34% uncertainty associated with the measurements (Harmel et al., 2016). To describe the measured *E. coli* concentrations (C_{FIB}) probabilistically, ±34% of the geometric mean of the measured *E. coli* concentrations for each

sampling site and of the regulatory standard were used as the upper and lower bounds of the uniform distribution.

The average rate of correct classification (RCC) was used to estimate the uncertainty of the fecal source contributions from the MST results. It was suggested that an average RCC of 67% is more accurate and conservative than the published RCCs associated with the Lampasas River Watershed MST report (T. Gentry, personal communication, February 26, 2018). Therefore, to describe the MST source percentage probabilistically, each MST source percentage was multiplied by ±33% to create the upper and lower bounds of a uniform distribution.

Variables provided as a range of numbers (R_{FIB}^{S}, R_{rp}^{S}, I_{rp}^{S}, p_{rp}^{S}) were approximated as uniform distributions with the minimum and maximum values used as the α and β bounds. The volume of water ingested (V) was approximated as a lognormal distribution for an adult during an hour swimming event with a mean of 25 milliliters and a standard deviation of 5 milliliters (Dufour et al., 2006; Sunger and Haas, 2015).

Four scenarios were run varying the in-stream *E. coli* concentration and the MST results: 1) the regulatory standard as the in-stream *E. coli* concentration input with the measured MST source percentages as point number inputs, 2) the regulatory standard and the measured MST source percentages with uncertainty applied to the point numbers, *E. coli* concentration and fecal source contribution, to create a range of numbers, 3) measured geometric mean in-stream *E. coli* concentration input with the measured MST source percentages as point number inputs, and 4) measured geometric mean in-stream *E. coli* concentration input with the measured MST source percentages with uncertainty applied to the point numbers, *E. coli* concentration and fecal source contribution, to create a range of numbers.

2.2.1. Estimating health risk

The risk of illness was estimated using the pathogen dose and the corresponding dose response model for the reference pathogen in Table 2-7. The norovirus dose-response model used was simplified from a hypergeometric function to a beta-binomial function because Crystal Ball Pro® software (Oracle Corp., Redwood Shores, CA) was unable to calculate a hypergeometric function.

The risk of illness from exposure to all of the reference pathogens associated with each fecal source was estimated using equation 2-2 (Soller et al., 2015).

$$P_{ill_S} = 1 - \prod_{rp}(1 - P_{ill_{rp}}) \quad (2\text{-}2)$$

where:

P_{ill_S} is the total probability of illness from the fecal source, S

$P_{ill_{rp}}$ is the probability of illness from the reference pathogen, rp, associated with the fecal source, S

The probability of illness associated from exposure to all of the sources for each individual reference pathogen and for all of the reference pathogens was estimated using equation 2-3 (Gitter, 2016).

$$P_{ill} = 1 - \prod_{S}(1 - P_{ill_S}) \quad (2\text{-}3)$$

where:

P_{ill} is the total probability of illness associated with all fecal sources

P_{ill_S} is the probability of illness associated with the fecal source, S

Table 2-7. Reference pathogen dose response models.

Reference Pathogen	Dose-Response Model	Dose-Response Equations	Model Parameters	Parameter Values	Morbidity	Reference
Norovirus	Hypergeometric as Beta binomial	$P_{inf} = 1 - \frac{B(\alpha, \beta + dose)}{B(\alpha, \beta)}$	alpha beta	0.04 0.055	60%	(McBride et al., 2013; Teunis et al., 2008a)
Cryptosporidium	Exponential	$P_{inf} = 1 - \exp(-r \times dose)$	r	0.09	50%	(USEPA, 2006)
Giardia lamblia	Exponential	$P_{inf} = 1 - \exp(-r \times dose)$	r	0.0199	45%	(Rose and Gerba, 1991)
Campylobacter	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.145 7.59	28%	(Medema et al., 1996; USEPA, 2010)
Salmonella	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.3126 2884	20%	(Haas et al., 1999)
E. coli O157:H7	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.4 45.9	28%	(Teunis et al., 2008b)

2.2.2. Sensitivity Analysis

The sensitivity of each input parameter was calculated to determine if changes to any one input would have a significant impact on the results. The sensitivity analysis was performed using Crystal Ball Pro® (Oracle Corp., Redwood Shores, CA) while applying 10,000 Monte Carlo simulations. Spearman rank correlation coefficients, Spearman's ρ, were computed between all inputs and predictions in Crystal Ball while the simulations were running (Oracle Corporation, 2008). Spearman's ρ is a nonparametric measure of correlation ranging from -1 to 1 (USEPA, 2001b). A value of ±1 indicates a perfect monotonic linear relationship between two variables, with a value of -1 indicating a perfect negative relationship and 1 indicating a perfect positive relationship (Hamby, 1994; Pirie, 1988; USEPA, 2001b). Zero indicates the variables are independent (Hamby, 1994; Pirie, 1988).

The sensitivity analysis performed by Crystal Ball Pro® (Oracle Corp., Redwood Shores, CA) of the measured + uncertainty scenario for each sampling site was examined to determine the significance of each input parameter to impact the total probability of illness and the total probability of illness excluding norovirus. The measured + uncertainty scenario was analyzed to account for the largest variation associated with all of the input parameters. Spearman's ρ was calculated for 84 input parameters, comprising of each input variable in Equation 2-1 for each source and reference pathogen.

2.3. Results

The probability of illness (P_{ill}) for four scenarios was estimated for both watersheds each containing two sites where MST and water quality measurements were taken (Figures 2-3-2-6). The addition of uncertainty to the *E. coli* concentration in the water body (C_{FIB}) and the MST

source fractions (F^S) did not impact the health risk estimates for any sites at the two study areas (Figure 2-3-2-6).

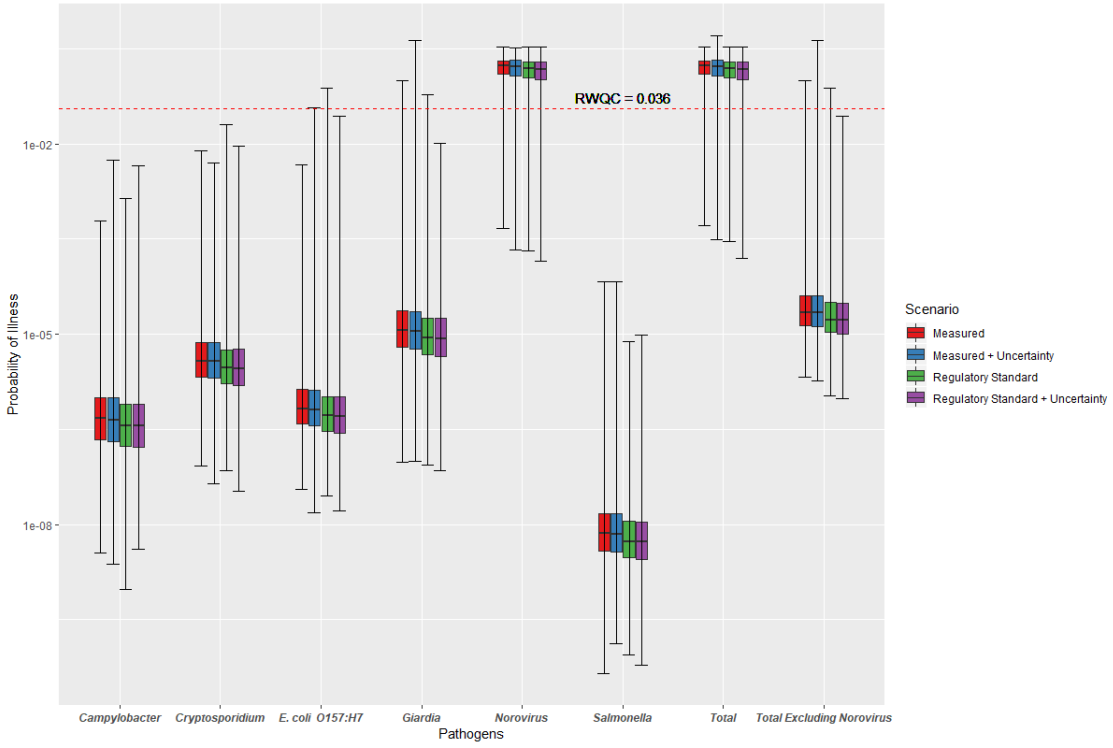

Figure 2-3. Lampasas River Watershed site 15762 total probability of illness associated with all fecal sources per reference pathogen for all run scenarios.

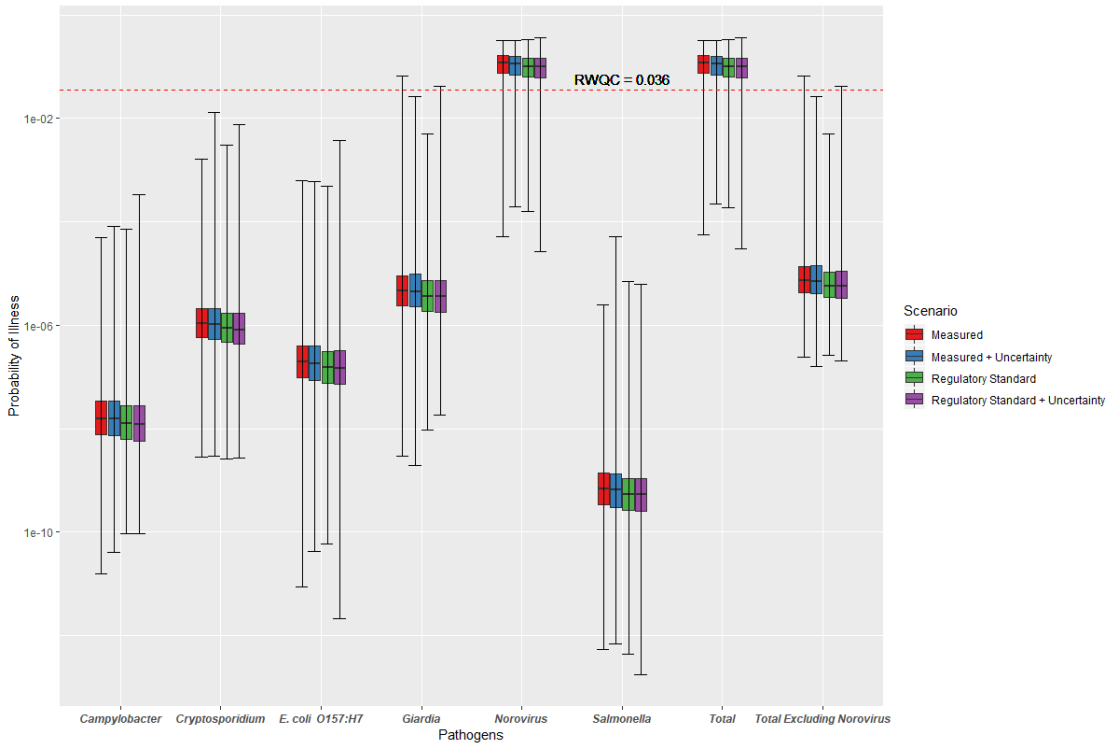

Figure 2-4. Lampasas River Watershed site 15770 total probability of illness associated with all fecal sources per reference pathogen for all run scenarios.

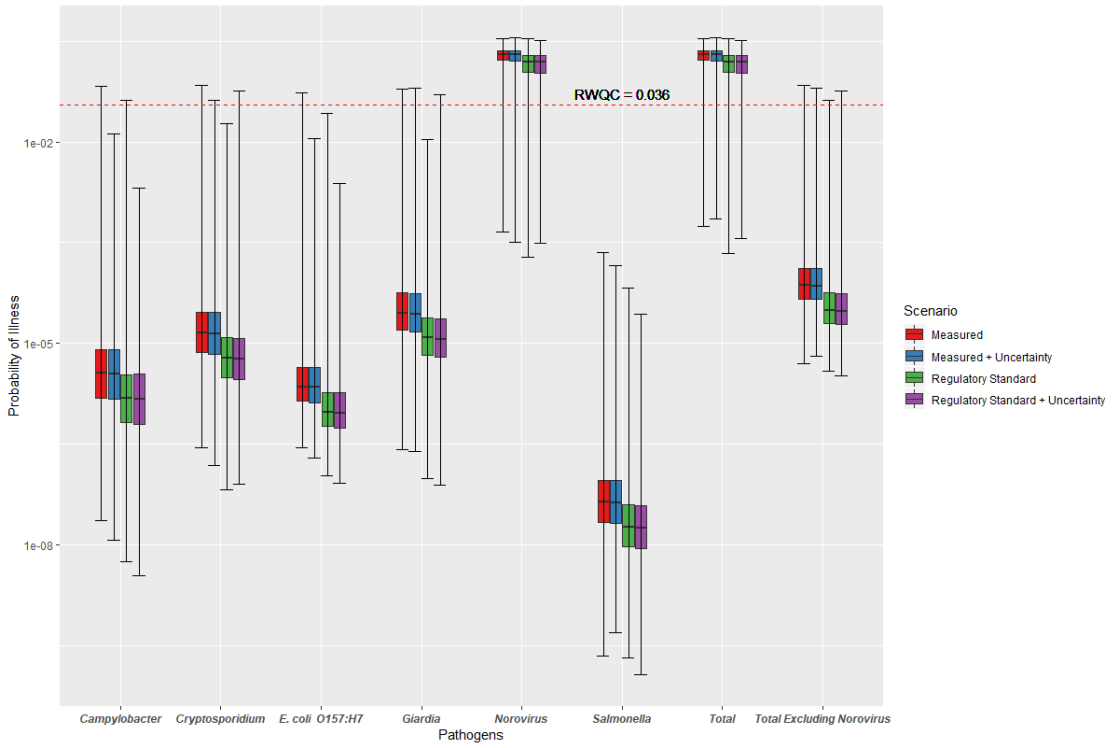

Figure 2-5. Salado Creek Watershed site 12876 total probability of illness associated with all fecal sources per reference pathogen for all run scenarios.

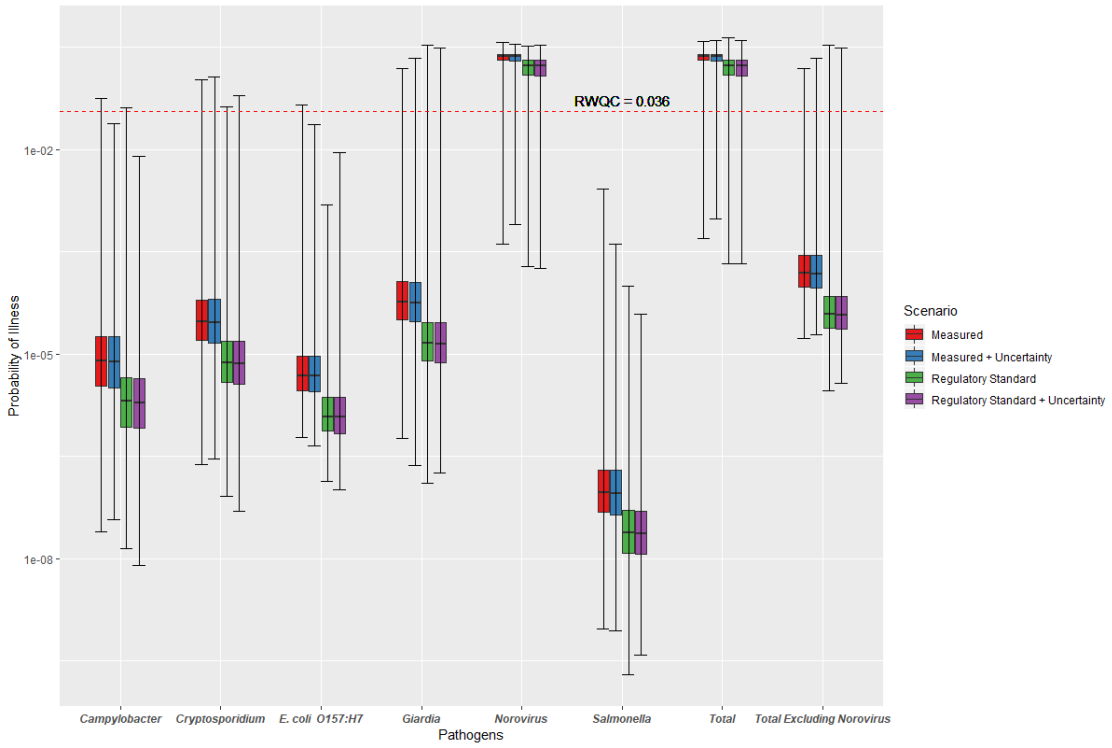

Figure 2-6. Walzem Creek Watershed site 12698 total probability of illness associated with all fecal sources per reference pathogen for all run scenarios.

Among all sites and run scenarios, the total probability of illness associated with all fecal source and reference pathogens exceeded the acceptable GI illness rate of 0.036 (Figures 2-3-2-6). Norovirus was the overwhelming driver of risk amongst all sites and run scenarios. The acceptable GI illness rate of 0.036 was exceeded even at the regulatory standard at all sampling sites due to the contribution of norovirus. The probability of illness between the 25 and 75 percentiles associated with all of the reference pathogens other than norovirus was well below 0.036.

To better examine the impacts on risk between all of the sources, the total probability of illness associated with all sources and reference pathogens and the total probability of illness associated with only the human source were both calculated omitting norovirus at all sampling

sites (Figures 2-3-2-6). The total probability of illness associated with all reference pathogens did not exceed the acceptable GI illness rate once norovirus was omitted (Figures 2-3-2-6). *Giardia* was the driver of health risk once norovirus was omitted. The health risk for the other reference pathogens, with the exception of *Salmonella,* were within one order of magnitude of the health risk associated with *Giardia*.

The total probability of illness from the human source and the cumulative total probability of illness associated from all of the fecal sources was calculated excluding norovirus (Figures 2-3-2-6). Because norovirus was the overwhelming driver of risk and only associated with the human fecal source, it was excluded from the total probability of illness to enable a better comparison between the fecal sources impacting health risk through reference pathogens associated with all of the fecal sources. Once norovirus was excluded from the total probability of illness associated with both only human and all fecal sources, none of the sampling sites exceeded the acceptable GI illness rate of 0.036. The human source remained the driver of risk for both sampling sites located in the Lampasas River Watershed. However, once norovirus was excluded from the probability of illness associated with the human source, cattle and human were equally the drivers of risk for the sampling sites located in the Salado Creek Watershed (Figures 2-5 and 2-6). The majority of the MST measured fecal contribution was from wildlife for the Lampasas River watershed, but the wildlife source contributed the least to the probability of GI illness across all of the study areas and sampling sites (Figures 2-3 and 2-4).

The total probability of GI illness associated with all fecal sources per reference pathogen for the measured (Figure 2-7) and regulatory standard (Figure 2-8) scenarios were compared among the sampling sites.

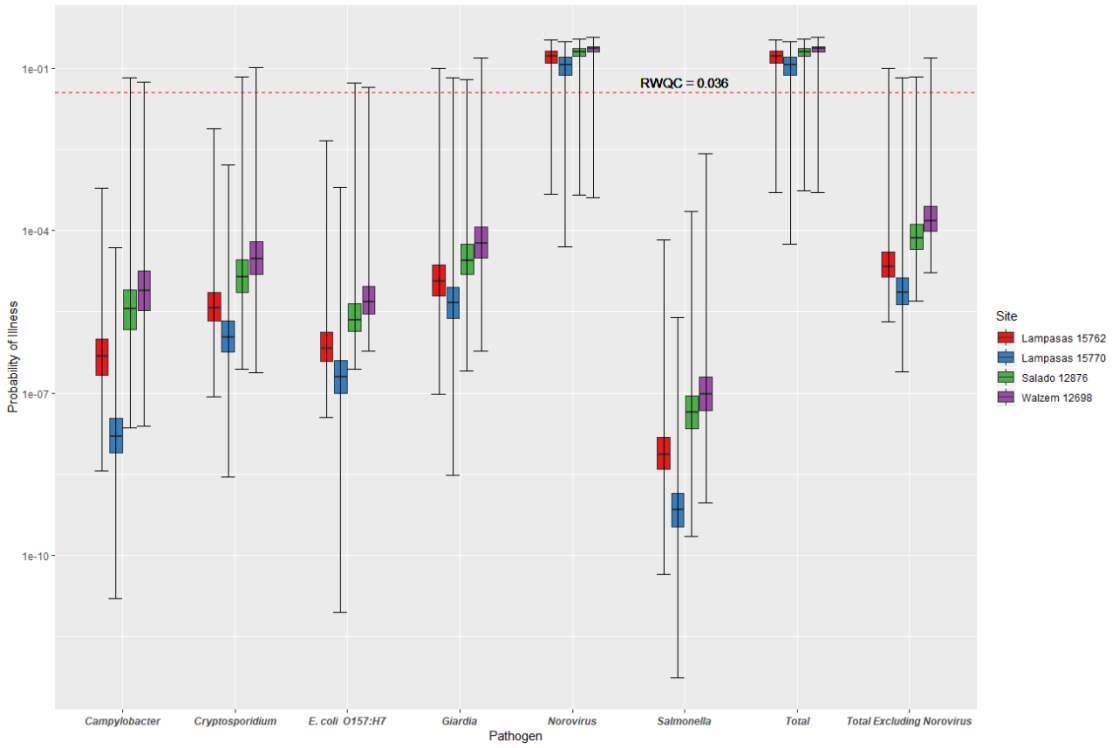

Figure 2-7. The total probability of GI illness from all associated fecal sources per reference pathogen at each sampling site for the measured scenario.

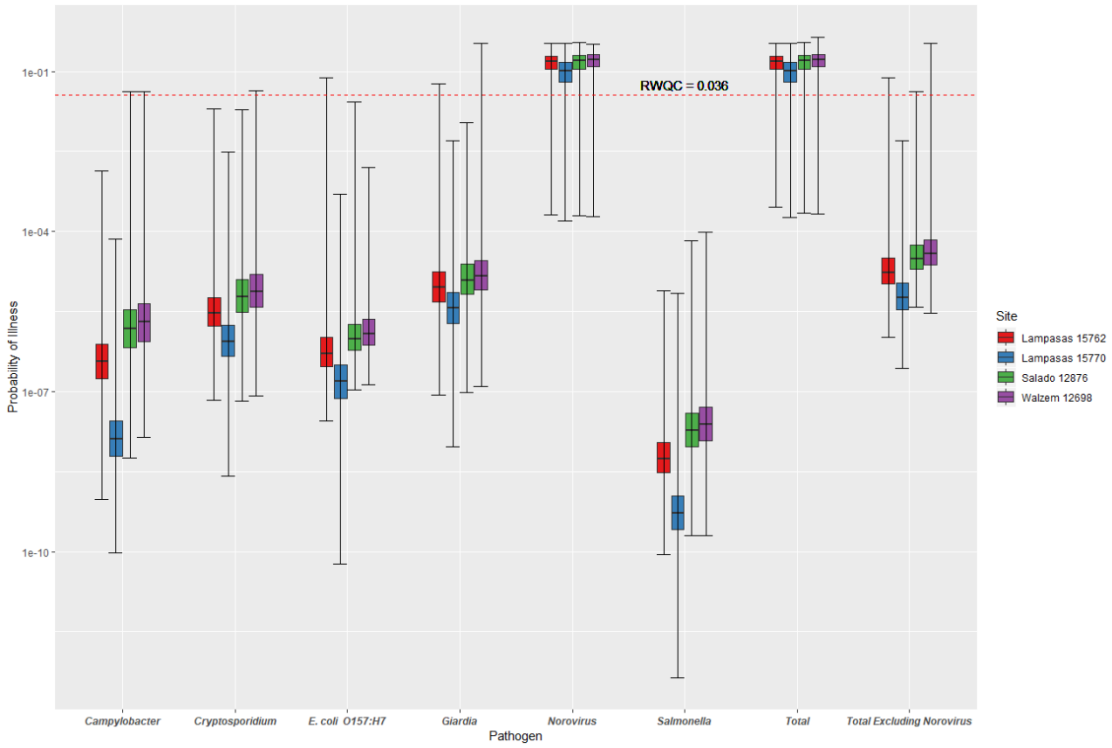

Figure 2-8. The total probability of GI illness from all associated fecal sources per reference pathogen at each sampling site for the regulatory standard scenario.

For all four sampling sites, norovirus was the driver of total health risk and the only reference pathogen to exceed the acceptable GI illness rate. The probability of illness associated with norovirus and the total of all reference pathogens was within one order of magnitude across all four sampling sites. However, the probability of illness for both Lampasas River watershed sites for the measured scenario was below one order of magnitude compared to the other sampling sites associated with five reference pathogens: *Campylobacter*, *Cryptosporidium*, *E. coli* O157:H7, *Giardia*, and *Salmonella* (Figure 2-7). For the regulatory standard scenario, only the probability of illness associated with the Lampasas River site 15770 was below one order of magnitude compared to the other sampling sites associated with the same five reference pathogens (Figure 2-8).

The total probability of GI illness associated with all reference pathogens per fecal sources for the measured (Figure 2-9) and regulatory standard (Figure 2-10) scenarios were compared among the sampling sites.

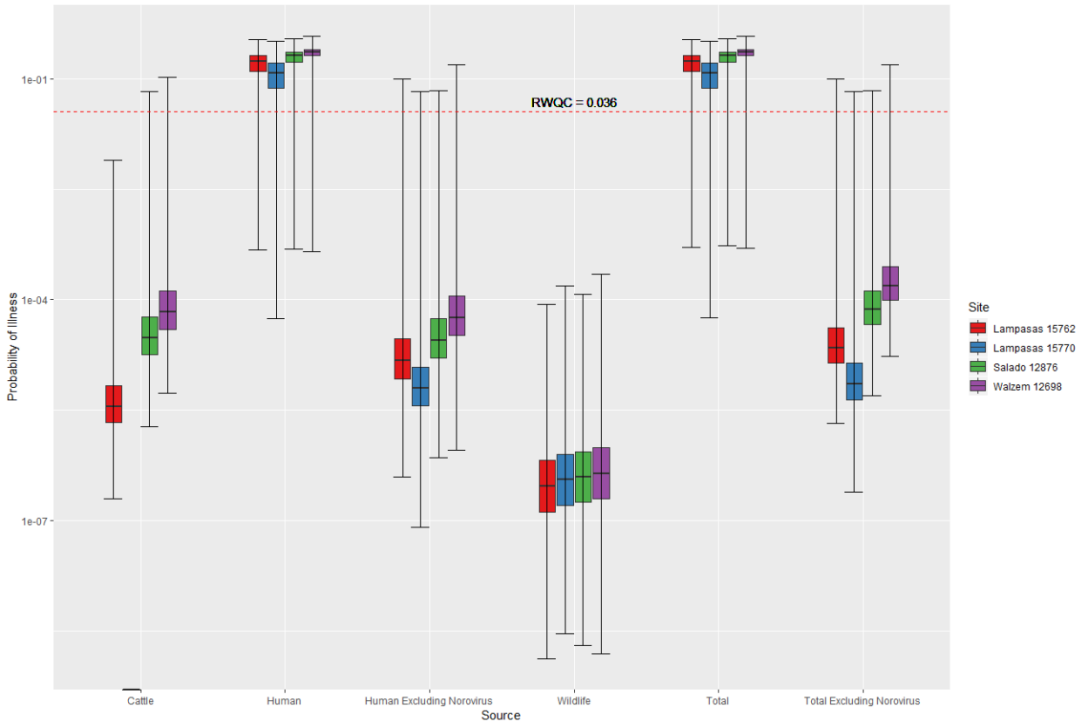

Figure 2-9. The total probability of GI illness from all associated reference pathogens per fecal source at each sampling site for the measured scenario.

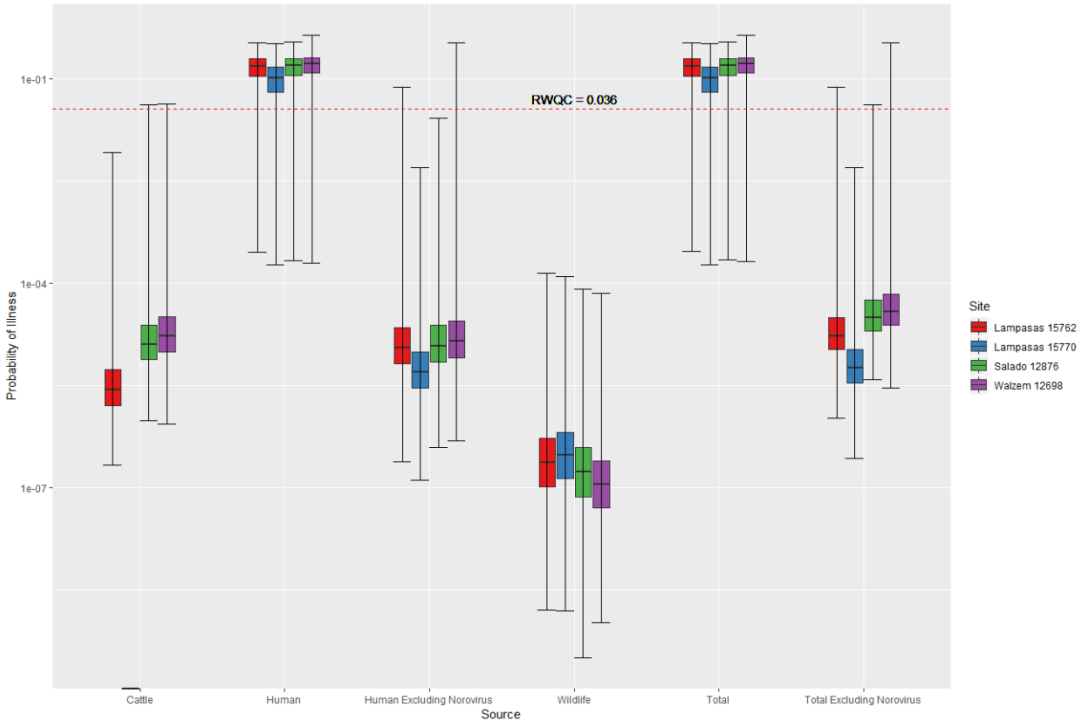

Figure 2-10. The total probability of GI illness from all associated reference pathogens per fecal source at each sampling site for the regulatory standard scenario.

The probability of illness for human sources and the total of all sources excluding norovirus did not exceed the acceptable GI illness rate for the 0 to 75 percentiles across all sampling sites for both measured and regulatory standard scenarios (Figures 2-9 and 2-10). When norovirus was excluded from the probability of illness calculations, the probability of illness for cattle, human, and the total of all sources were within one order of magnitude of each other across all four sampling sites with the exception of the Lampasas 15770 site for the cattle fecal source. The probability of illness associated with the wildlife fecal source differed from the other fecal sources by more than one order of magnitude lower across all four sampling sites for both the measured and regulatory standard scenarios, suggesting that wildlife fecal sources have the least impact on health risk (Figure 2-9 and 2-10).

2.3.1. Sensitivity Analysis

Across all of the sampling sites, the five input parameters associated with calculating the dose of norovirus had the most significant impact on the total probability of illness associated with all sources and reference pathogens (Figure 2-11).

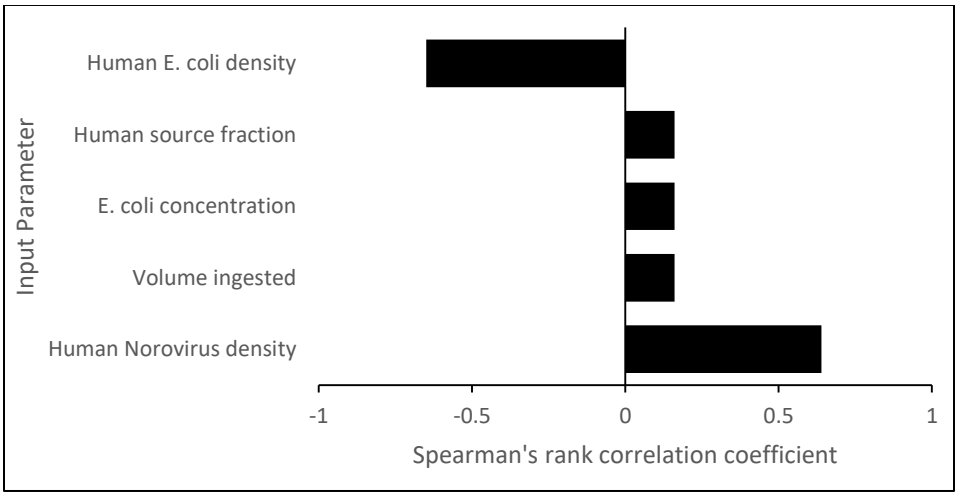

Figure 2-11. Maximum Spearman rank correlation coefficients for dose input parameters to calculate the total probability of illness across all sampling sites.

The five input parameters used to calculate the norovirus dose were: concentration of norovirus in human sewage (R^{H}_{noro}), *E. coli* concentration in human sewage ($R^{H}_{E.coli}$), fraction of *E. coli* from human source (F^{H}), *E. coli* concentration in the water body (C_{FIB}), and volume of water ingested (V). These input parameters were the only parameters that had a Spearman's ρ greater than ± 0.03 for all sampling sites to the total probability of illness associated with all sources and reference pathogens (Figure 2-11). The input parameters R^{H}_{noro} and $R^{H}_{E.\ coli}$ had maximum Spearman's ρ over ±0.5 across all sampling sites. The three remaining parameters (F^{H}, C_{FIB}, and V) were less significant with a maximum Spearman's ρ less than ±0.2.

Once norovirus was excluded from the total probability of illness calculations at all of the sampling sites, fourteen input parameters had Spearman's ρ equal to or greater than ±0.1 for at least one out of the four sampling sites (Figures 2-12 and 2-13).

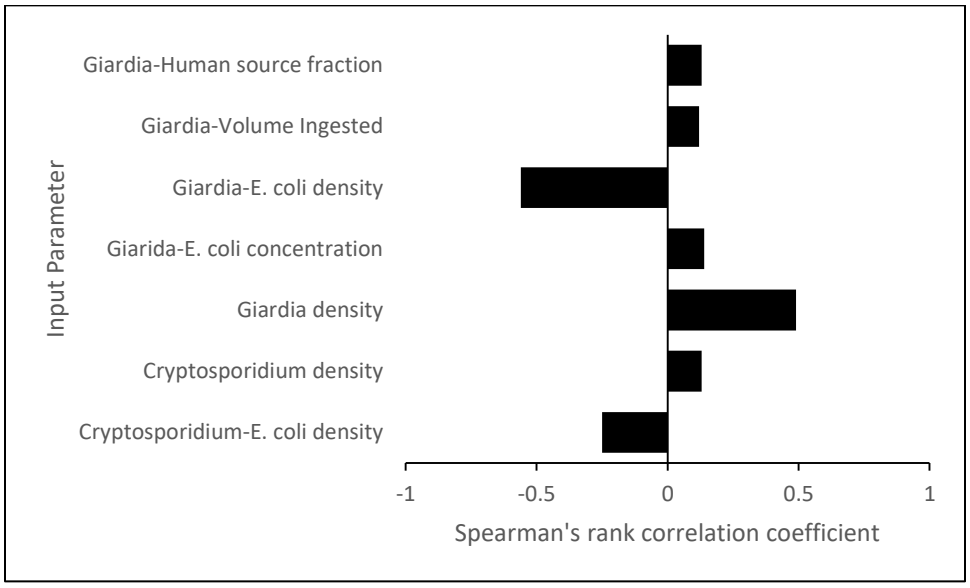

Figure 2-12. Maximum Spearman's rank correlation coefficient for dose input parameters associated with the human fecal source to calculate the total probability of illness excluding norovirus across all sampling sites.

Out of the fourteen input parameters with Spearman's ρ equal or greater than ±0.1, only four input parameters had Spearman's ρ equal to or greater than ±0.1 across all four sampling sites (Figure 2-12). The input parameters, $R^{H}_{E.coli}$ and $R^{H}_{Giarida,}$ for the human source associated with calculating the dose for *Giardia* were the most significant parameters contributing to variance with Spearman's ρ greater than or equal to ±0.45 (Figure 2-12). The four input parameters with Spearman's ρ greater than ±0.1 across all four sampling sites were only associated with the human fecal source. The input parameters associated with cattle had a

Spearman's ρ of 0 for the Lampasas 15770 sampling site because there was a 0 percent contribution of cattle at that site.

Only input parameters associated with human and cattle fecal sources received Spearman's ρ values greater than ±0.1. These parameters were mostly associated with the pathogens, *Cryptosporidium*, *Giardia*, and *Campylobacter*, while only one parameter was associated with *E. coli* O157:H7. The results of the sensitivity analysis indicated that wildlife and *Salmonella* were not significant drivers of risk.

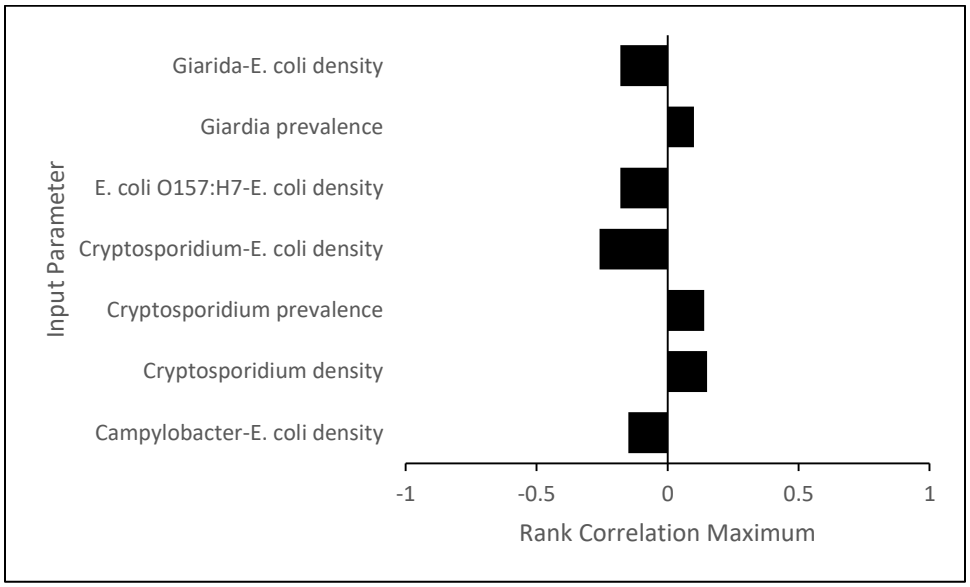

Figure 2-13. Maximum Spearman's rank correlation coefficient for dose input parameters associated with the cattle fecal source to calculate the total probability of illness excluding norovirus across all sampling sites.

2.4. Discussion

Adding measurement uncertainty to the *E. coli* concentration in the water body (C_{FIB}) and the fraction of source in the water body (F^S) did not appear to impact the risk results therefore, the hypothesis that adding measurement uncertainty would significantly impact the health risk

results was not supported. The sensitivity analysis showed that these parameters were not significant to the results across all four of the sampling sites compared to the *E. coli* concentration in feces or in sewage from the source (R_{FIB}^{S}) and the concentration of reference pathogen in feces or in sewage from the source (R_{rp}^{S}). This is likely why the risk results were not impacted when these input variables were adjusted. Currently, fresh water bodies are regulated solely on the *E. coli* concentration in the water body and remediated based on *E. coli* loads of the highest contributing fecal sources. Therefore, it was extremely surprising that the *E. coli* concentration in the water body and the fraction of source in the water body did not significantly impact health risk.

Norovirus was the overwhelming driver of risk even though it was the only pathogen that was applied to a singular source, human, that contributed less than half of the fecal contamination to the water bodies. Human fecal matter was the most significant source impacting the total probability of illness associated with all of the fecal sources and reference pathogens because norovirus was only associated with human fecal sources, therefore the second hypothesis of this work was supported. MST results across all of the sampling sites showed human fecal sources contributed as little as 12% to as much as 34% of the total *E. coli* contamination. Yet the probability of GI illness associated with norovirus exceeded the USPEA's acceptable level of risk (0.036) for all of the run scenarios at all four sampling sites. The acceptable level of risk was exceeded for norovirus even when the *E. coli* concentration in the water body met the regulatory standard. This did not support the third hypothesis that the health risk associated with all of the fecal sources would equal the USEPA's acceptable level of risk at the regulatory standard.

Norovirus was excluded from the total probability of illness calculations associated with all other reference pathogens and all fecal sources to further compare the impact of the individual fecal sources on health risk. *Giardia* was the main driver of risk once norovirus was excluded, but other reference pathogens, with the exception of *Salmonella*, were within one order of magnitude of *Giardia*. *Salmonella* did not significantly impact the total probability of illness even though it was included for all of the fecal sources, unlike norovirus and *E. coli* O157:H7.

Human fecal sources were still the main driver of risk once norovirus was excluded from the total probability of illness calculations. However, cattle fecal sources were also a significant driver of risk in addition to human fecal sources. Even though wildlife fecal sources were the largest contributor to *E. coli* contamination in the water bodies, with a contribution of 77% at one sampling site, wildlife did not significantly impact the total probability of illness for any pathogens associated with wildlife fecal sources.

For remediation of water bodies contaminated with microbial pollutants, pathogen TMDLs are a flawed approach. Pathogens are regulated in water bodies through FIB water quality criteria. Therefore, TMDLs are solely focused on lowering the FIB load within a watershed through the largest contributing source. This approach diverges from the purpose of the water quality criteria which is to protect human health. The results of this study showed that the largest contributing source (wildlife) can have no impact on human health. Meanwhile, the lowest contributing source (human) can still have a significant impact on human health, particularly if present due to the infectivity of the pathogens associated with that source. For remediation to have significant impacts, it should be focused on sources that are the riskiest to human health instead of those contributing the largest FIB load. Remediation of water bodies

contaminated with microbial pollutants should move away from focusing on meeting a numerical FIB standard and instead focus on meeting the acceptable illness rate.

2.5. Conclusions

The probability of GI illness from exposure to fecal matter originating from human, cattle, and wildlife sources was calculated in three water bodies. The probability of GI illness was calculated by applying QMRA and MST using four different run scenarios. The source contributing the largest percentage of fecal matter into the water body (wildlife) did not have a significant impact on human health. Meanwhile, human fecal sources contributed as little as 12% to the microbial contamination in a water body, but were the overwhelming driver of risk. Remediation efforts aimed at improving human health should focus on the riskiest sources impacting human health.

3. COUPLING THE SOIL AND WATER ASSESSMENT TOOL WITH QUANTITATIVE MICROBIAL RISK ASSESSMENT TO ESTIMATE HEALTH RISK FROM EXPOSURE TO FECAL CONTAMINATION IN RECREATIONAL WATER BODIES FROM POINT AND NON-POINT SOURCES

3.1. Introduction

Pathogens are the primary cause of impairment for rivers and streams in the United States (USEPA , 2019). A water body is considered impaired by pathogens if it is exceeding the Recreational Water Quality Criteria (RWQC) of the Fecal Indicator Bacteria (FIB) concentration. For fresh waters, the RWQC FIB is *Escherichia coli* (*E. coli*) with a geometric mean of 126 colony-forming units (CFU) per 100 milliliters (mL) (Dufour and Ballentine, 1986). The purpose of the RWQC is to protect human health with the assumption that the FIB concentration corresponds to an acceptable gastrointestinal (GI) illness rate of 36 per 1000 people (0.036) from exposure through contact recreation to microbial contamination (Dufour and Ballentine, 1986; USEPA, 2012). The USEPA standard FIB concentrations are strongly linked to GI illness from exposure to water bodies contaminated with human sources (Cabelli, 1983; Dufour, 1984; Wade et al., 2003; Wu et al., 2011). The connection between FIB concentrations and GI illness was inconclusive for epidemiology studies in recreational water contaminated with non-human sources, but pathogens from cattle may result in similar health risks as human sources (Calderon et al., 1991; Colford et al., 2007; Mcbride et al., 1998; USEPA, 2010). Additionally, FIB are not the cause of waterborne disease but an indication fecal contamination which may contain pathogens that directly cause waterborne disease. The RWQC do not differ based on the source of contamination (USEPA, 2012).

Total Maximum Daily Loads (TMDLs) are used to remediate contamination in water bodies in the United States. The TMDL is the maximum amount of pollutant load a water body can receive and still meet the RWQC (USEPA, 2008). In a TMDL, point and non-point sources contributing microbial contamination in a watershed are identified and wasteload or load allocations are determined for each fecal source. All pollutant sources are treated equally when developing a TMDL (USEPA, 2008). However, fecal sources are not equal in their likelihood to cause human illness and this should be taken into account when remediating a water body for pathogens. The TMDL approach solely focuses on remediation to meet the numerical FIB water quality standard regardless of the source.

Quantitative Microbial Risk Assessment (QMRA) is an approach to estimate the health risk from exposure to infectious microorganisms by applying the principles of risk assessment (Haas et al. , 2014). QMRA has been used in conjunction with watershed modeling to estimate potential human health risks from exposure to simulated FIB concentrations from human and non-human sources during rainfall events (Eregno et al., 2016; Liao et al., 2016). These studies applied the watershed-scale model Hydrological Simulation Program-FORTRAN (HSPF) and were not able to include all sources of potential contamination particularly non-avian wildlife. Soil and Water Assessment Tool (SWAT) is a watershed-scale model that simulates in-stream bacteria concentrations using runoff events to drive the fate and transport of bacteria (Saleh and Du, 2004). Spatially Explicit Load Enrichment Calculation Tool (SELECT) is a geographic information system (GIS) tool that uses spatial factors such as land use, fecal source population densities, and soil properties to predict potential *E. coli* loads within a watershed (Teague et al., 2009). SELECT can be used as the bacterial source input for rainfall-runoff models such as HSPF or SWAT to predict in stream FIB concentrations (Borel et al., 2012b). Other QMRA

studies have modeled the dispersion of pathogens to estimate a potential pathogen dose from exposure but only included a single source of contamination (Andersen et al., 2013; Sokolova et al., 2012; Sokolova et al., 2015). The aim of this study is to address these gaps by using model simulated *E. coli* concentrations from model predicted human and non-human source loads including non-avian wildlife to predict QMRA human health risks.

The objective of this study is to test the hypothesis that SWAT in conjunction with SELECT can be used to simulate source-specific *E. coli* concentrations for input into a QMRA to determine the likelihood of GI illness from exposure by contact recreation to fecal contamination in a water body. If successful the methodology proposed by this study can be applied to predict health risk under a range of hydrologic conditions from contact with pathogens associated with fecal contamination in watersheds.

3.1.1. Study Area

The Lampasas River (Figure 3-1) flows southeast for 121 kilometers (75 miles) into the Stillhouse Hollow Reservoir (Gregory et al., 2013). The river was listed on the 2008 Texas 303(d) list for elevated levels of bacteria exceeding the water quality criteria for contact recreation use (TCEQ, 2015). The Lampasas River Watershed is 3,231 square kilometers (1,247 square miles) with the majority of land used for rangeland and grasslands (Gregory et al., 2013; Prcin et al., 2013).

That Lampasas River Watershed contains three United States Geological Survey (USGS) gauging stations, two Texas Commission on Environmental Quality (TCEQ) water quality monitoring stations, and two WWTP outfalls which are all located in the southern portion of the watershed (Figure 3-1). Both TCEQ water quality monitoring stations are located on the main stem of the Lampasas River and at the same location as a USGS gauging station. Fecal coliform

and *E. coli* monitoring was conducted at both TCEQ stations. At TCEQ station 11897 fecal coliform and *E. coli* data were collected from 1988 to the present, resulting in 133 data points of fecal coliform and *E. coli* concentrations with 117 unique days tested. USGS station 8103800 is co-located with TCEQ station 11897 and has a continuous record of daily discharge ranging from 1962 to the present. TCEQ station 11896 has a corresponding location with USGS station 8103940, however, there were not enough continuous matching data to use this station.

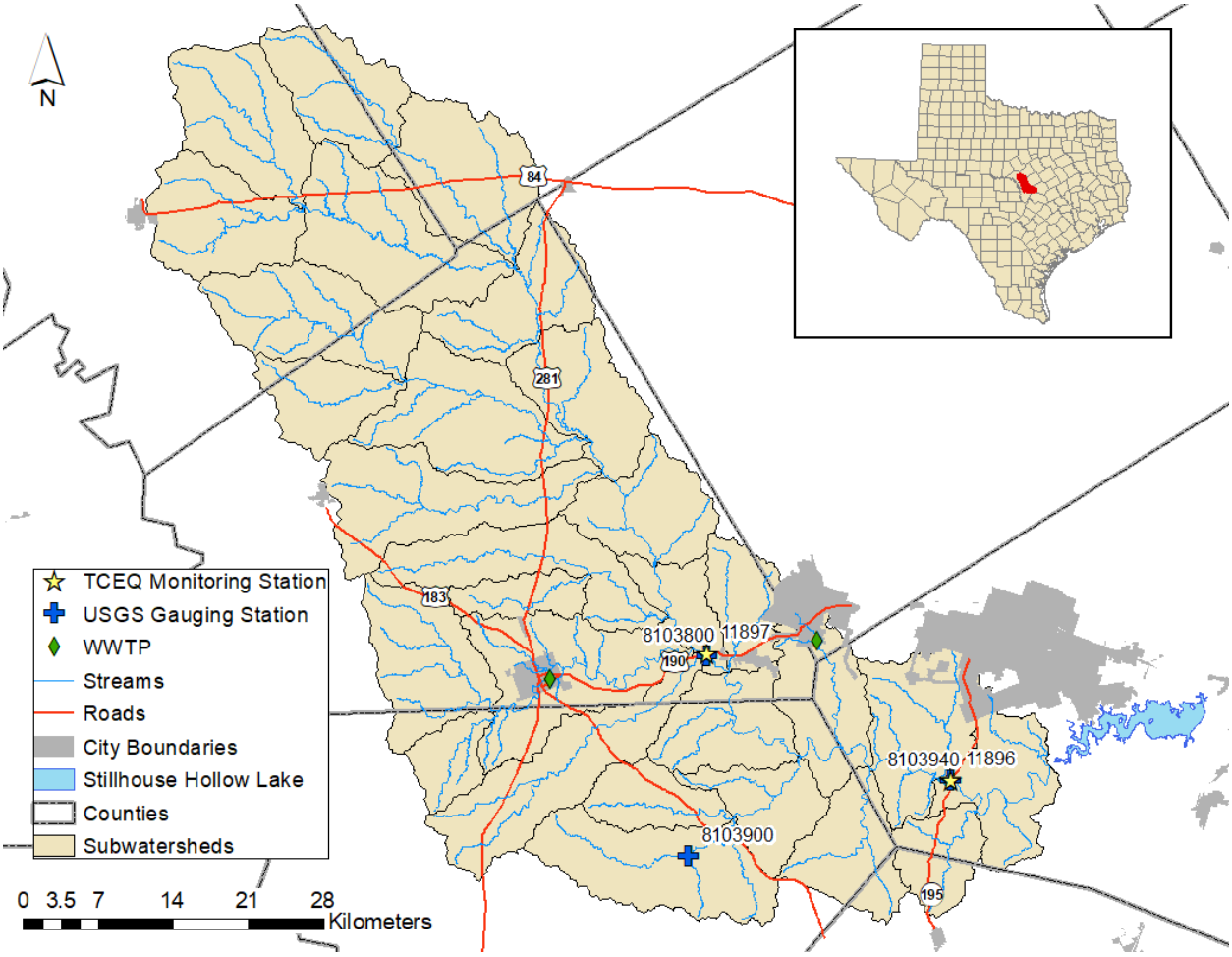

Figure 3-1. Location of the Lampasas River Watershed with USGS gauging stations, TCEQ water quality stations, and WWTP outfalls.

3.2. Methodology

Non-point source fecal loads were spatially estimated within the Lampasas River Watershed using SELECT from three general sources: cattle, human, and non-avian wildlife. SWAT was used to simulate source specific in-stream *E. coli* concentrations from the fecal loads estimated by SELECT. SWAT-simulated in-stream *E. coli* concentrations were used to estimate reference pathogen doses which were input into a QMRA to estimate human health risk associated with exposure to fecal contamination from contact recreation in impaired waters (Figure 3-2).

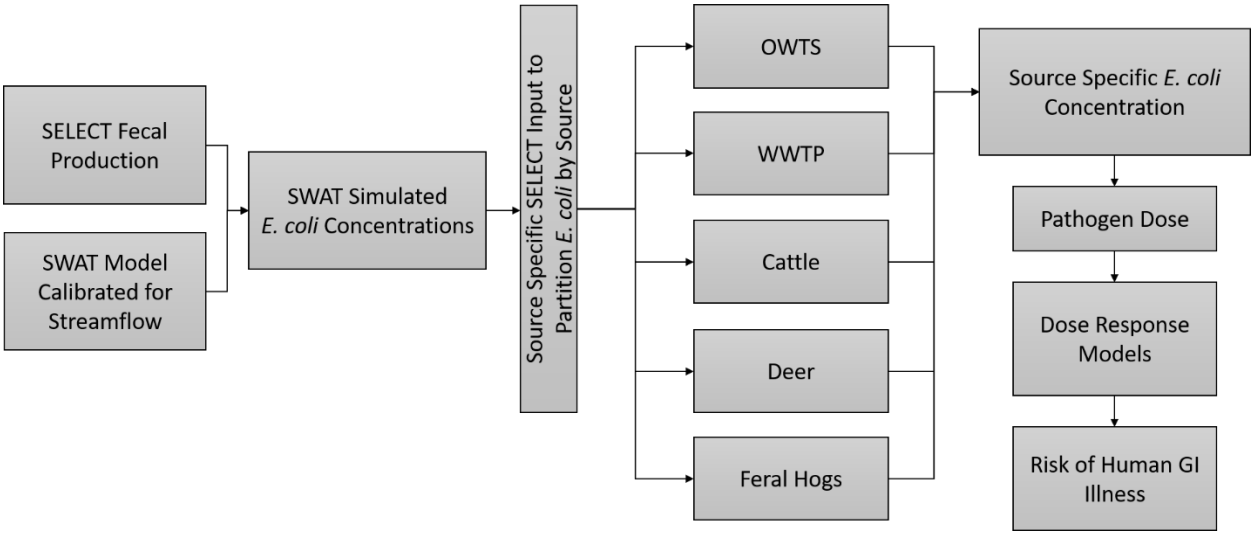

Figure 3-2. Flowchart to estimate health risk using SELECT and SWAT.

3.2.1. Estimating *E. coli* surface loads with SELECT

Daily fecal production densities (kg/ha/day) for non-point sources such as livestock, non-avian wildlife, and failing Onsite Wastewater Treatment Systems (OWTS) were estimated using SELECT. SELECT uses GIS to spatially distribute potential sources within a watershed.

SELECT was used to spatially distribute the daily fecal production densities for each non-point source across unique subwatershed and land use areas. The livestock sources located in the watershed and the animal counts were determined using the U.S. Department of Agriculture (USDA) National Agricultural Statistic Service (NASS) Census of Agriculture (USDA NASS, 2012). The Lampasas River Watershed contained cattle, goats, sheep, and horses as likely livestock sources. Census of Agriculture livestock animal counts are provided on a county basis, therefore animal counts within the Lampasas River watershed were estimated using the proportion of the watershed that was within each county and summed to estimate the proportion of livestock within the watershed. A livestock density within each county area in the watershed was calculated by distributing the livestock on suitable areas of land cover they are found on within the watershed using the National Land Cover Database (NLCD) (Homer et al., 2015).

The non-avian wildlife sources of deer and feral hogs were chosen based on the availability of animal density estimations and the likelihood of being significant wildlife sources of fecal contamination. Deer densities within the Lampasas River watershed were estimated from deer densities associated with two resource management units (RMUs) located in the Lampasas River Watershed (Lockwood, 2006). The deer population for the two RMUs within the Lampasas River Watershed was distributed across corresponding suitable areas in the watershed. The feral hog density determined by the Lampasas River Watershed stakeholders, local land owners and governmental agency employees, was distributed on suitable riparian areas within 100 meters of a stream (Borel et al., 2012a; Prcin et al., 2013). Fecal production densities per source were calculated per subwatershed by dividing the animal count and the animal daily fecal production rate by the suitable habitat area (Borel et al., 2015; Wagner and Moench, 2009).

The distribution of failing OWTS across the watershed was estimated using the Python SELECT (pySELECT) potential *E. coli* load GIS module (Borel et al., 2017). The number of people using OWTS was estimated from census blocks (USCB, 2012) and by removing the areas serviced by sanitary sewer systems determined from the certificate of convenience and necessity (CCN) (Public Utility Commission of Texas, 2017). The number of failing OWTS was estimated using the drain field limitation class for soils (USDA-NRCS , 2016) by applying a failure rate to the classes of soils (Borel et al., 2017). Daily fecal production rate (L/day) from failing OWTS was calculated per subwatershed by dividing the number of people on failing OWTS and the volume of wastewater produced per person (265 L/person/day) (Riebschleager et al., 2012) by the areas within each subwatershed not serviced by sewer (Borel et al., 2017). The City of Lampasas Wastewater Treatment Plant (WWTP) was the only WWTP in the Lampasas Watershed located upstream from the sampling station. As a conservative measure, it was assumed the City of Lampasas WWTP was meeting the regulatory discharge standard for WWTPs of 126 CFU per 100 mL of *E. coli* (TCEQ, 2009). Therefore, the regulatory standard was the bacteria estimation input into SWAT at the subwatershed location for the City of Lampasas WWTP.

The fecal coliform density for each source was multiplied by the fecal coliform to *E. coli* conversion factor of 0.63 to calculate the *E. coli* density per source (Borel et al., 2017; Borel et al., 2015; USEPA, 2001c; Wagner and Moench, 2009). The fecal coliform to *E. coli* conversion factor was determined dividing the primary contact recreation regulatory standard for surface waters for fecal coliform, 200 colony forming units (CFU) per 100 milliliters, into the primary contact recreation regulatory standard for *E. coli*, 126 CFU per 100 mL (Dufour and Ballentine, 1986).

3.2.2. SWAT Setup and Hydrology Calibration

The SWAT model inputs for the Lampasas River Watershed were setup in Hydrologic and Water Quality System (HAWQS) to ease input data collection and formatting (Spatial Sciences Laboratory Texas A&M Agrilife Research, 2017). The SWAT model was calibrated using Sequential Uncertainty Fitting (SUFI-2) with SWAT Calibration Uncertainty Programs (SWAT-CUP) (Abbaspour, 2015; Arnold et al., 2012). The sixteen SWAT parameters: ALPHA_BF, GW DELAY, CN2, SOL_AWC, ESCO, RCHRG_DP, REVAPMN, GW_REVAP, ALPHA_BF_D, CH_K(2), CH_K(1), SLSOIL, LAT_TTIME, CNCOEF, ICN, and GWQMN were adjusted to calibrate the model on a monthly time step with monthly average USGS streamflow measurements at the 8103800 USGS gauge located in the Lampasas River Watershed (Appendix A). SWAT was calibrated for streamflow from 2008-2014 on a monthly time step resulting with a Nash-Sutcliffe Efficiency (NSE) coefficient of 0.86 and a coefficient of determination (R^2) of 0.87.

3.2.3. SWAT Bacteria Calibration

Measured *E. coli* concentrations were collected as grab samples, or single sample measurements taken at a specific time, on a monthly or quarterly time step. Therefore, to account for the variability in the *E. coli* samples, calibration of the measured *E. coli* concentrations was performed in three ways, on the exact day of measurement, and after the days prior to and following the day the measurement was taken, and the best calibration scenario was chosen. Calibrating against the day prior to the day on which the measurement was taken resulted in the best values of the objective functions, an NSE of 0.84 and R^2 of 0.87. SWAT was calibrated from 2008 to 2014 with 44 observed *E. coli* concentrations on a daily time step using fecal source inputs from cattle, goats, sheep, horses, deer, feral hogs, and OWTSs and WWTPs

estimated by SELECT. SELECT calculated fecal production rates associated with all sources per land use type and subwatershed were input into the SWAT model using SWAT Editor. The fecal production rates were entered into SWAT as daily grazing operations as part of the management parameters to distribute the fecal matter over the land surface. SWAT was calibrated using SUFI-2 in SWAT-CUP with the nine parameters: BACTKDQ, BACTKDDB, THBACT, BACT_SWF, WDPRCH, WDPF, WOF_P, BACTMX, and BACTMINP (Appendix A) (Thilakarathne et al., 2018). The values of the nine parameters input into SWAT-CUP associated with the best simulation out of 1000 simulations were input into the SWAT model using SWAT Editor as the calibrated model. After the SWAT simulated *E. coli* concentrations were calibrated using all fecal source inputs, they were partitioned into source-specific *E. coli* concentrations for the individual sources: OWTSs, WWTPs, cattle, feral hogs, and deer. The previous SELECT calculation fecal production rates associated with all sources were cleared from the model using SWAT Editor. Then the SELECT calculated fecal production rates associated with each particular source were input individually per land use type and subwatershed per source and the model was run individually for each source. This resulted in source-specific *E. coli* concentrations for each individual source.

3.2.4. Quantitative Microbial Risk Assessment (QMRA)

Human health risk was estimated from the SWAT simulated source-specific *E. coli* concentrations by applying QMRA. Reference pathogen doses associated with exposure from fecal contamination three fecal sources (s): human, cattle, and non-avian wildlife in recreational water bodies were estimated using Equation 3-1 modified from Schoen and Ashbolt (2010). The reference pathogens used to represent pathogens in fecal matter that cause waterborne illness in humans were: norovirus, *Cryptosporidium spp*., *Giardia lamblia*, *Campylobacter spp*.,

Salmonella, and *E. coli* O157:H7. Eight waterborne reference pathogens were established by USEPA (2010) however, only six were used in the QMRA as described below. There is currently no published ingestion dose- response relationship for adenovirus, therefore adenovirus was not included (USEPA, 2010). Rotavirus can be shed by calves and piglets but the strains are host specific and not likely to infect humans, so rotavirus was excluded from this study (Martella et al., 2010; USEPA, 2010).

Human fecal sources included OWTSs and WWTPs as raw sewage and secondary disinfected effluent, respectively. For livestock fecal sources, only cattle were considered in the health risk calculations due to a lack of data available related to pathogen concentrations, prevalence, and infectivity in the other livestock fecal sources. Non-avian fecal sources included both deer and feral hogs.

The reference pathogen (rp) dose (μ_{rp}^{S}) (number of pathogens or genomes) was calculated as:

$$\mu_{rp}^{S} = \frac{C_{FIB}^{S}}{R_{FIB}^{S} \times 100} \times R_{rp}^{S} \times p_{rp}^{S} \times I_{rp}^{S} \times V \quad (3\text{-}1)$$

C_{FIB}^{S} is the source-specific(S) SWAT simulated *E. coli* concentration in the water body (CFU/100mL) for a particular FIB.

R_{FIB}^{S} is the *E. coli* concentration in feces (wet mass) (CFU/g) or in sewage from source S (CFU/L)

R_{rp}^{S} is the concentration of pathogen species in feces (wet mass) (number of pathogens or genomes/g) or in sewage from source S (number of pathogens or genomes/L)

p_{rp}^{S} is the fraction of human-infectious pathogenic strains from source S

I_{rp}^{S} is the prevalence of infection in the non-human source, i.e. cattle and non-avian wildlife, S (proportion of animals shedding the pathogen)

V is the volume of water ingested (mL)

The input parameters into the dose equation (Equation 3-1) contain a large degree of uncertainty, therefore 10,000 Monte Carlo simulations were generated with Crystal Ball Pro® software (Oracle Corp., Redwood Shores, CA) to produce distributions for the pathogen dose with each source. Load Duration Curves (LDCs) were developed using SWAT simulated daily streamflow and source-specific *E. coli* concentrations at the calibration site from 2008-2014. The LDCs were divided into five flow regimes: high flows (0-10%), moist conditions (10-40%), mid-range flows (40-60%), dry conditions (60-90%), and low flows (90-100%) (USEPA, 2007). C_{FIB}^{S} are the source-specific daily SWAT simulated *E. coli* concentrations within each flow regime and were input as a left skewed triangular distribution. The triangular distribution within each flow regime was calculated by determining the minimum, maximum, and likeliest values. The minimum and likeliest values were zero across all flow regimes and fecal sources, and therefore they were not included in Table 3-1, whereas the maximum and mean values differed across all flow regimes and fecal sources (Table 3-1).

Table 3-1. Mean and maximum values of SWAT simulated *E. coli* concentrations (C^S_{FIB}) (CFU/100 mL).

Source		High Flows	Moist Conditions	Mid-Range Flows	Dry Conditions	Low Flows
WWTP	Maximum	5.64	4.24	0.74	0.45	0.46
	Mean	0.19	0.06	0.09	0.11	0.22
OWTS	Maximum	20.11	11.02	1.12	0.40	0.02
	Mean	0.43	0.03	0.01	0.0009	0.0001
Cattle	Maximum	511.9	308.9	74.27	8.09	0.32
	Mean	13.31	0.76	0.47	0.03	0.002
Wildlife	Maximum	5.62	4.13	0.56	0.05	0.004
	Mean	0.13	0.01	0.0023	0.0001	0.00002
All Sources	Maximum	47340	30390	7689	836	31
	Mean	1261	81	48	2.99	0.71

The variables: $R^S_{FIB}, R^S_{rp}, p^S_{rp}, I^S_{rp}$ were estimated as uniform distributions with the minimum and maximum values used as the α and β bounds (Table 3-2). The volume of water ingested (V) was an adult during a one hour swimming event approximated as a lognormal distribution with a mean of 25 mL and a standard deviation of 5 mL (Dufour et al., 2006; Sunger and Haas, 2015).

Table 3-2. Reference pathogen dose input parameters

Fecal Source		R_{FIB}^{S} (cfu/g or L)	Reference Pathogen	R_{rp}^{S} (pathogens/g or L)	p_{rp}^{S}	I_{rp}^{S}	References
Human	WWTP	3.16-10,000	norovirus	47-7,499	100%	100%	(Katayama et al., 2008; Lodder and de Roda Husman, 2005; Soller et al., 2010b; USEPA, 2010)
			Giardia	0.1-126	100%	100%	
			Cryptosporidium	0.1-32	100%	100%	
	OWTS	5,010,000-100,000,000	norovirus	1,000-1,000,000	100%	100%	(Soller et al., 2010b; USEPA, 2010)
			Giardia	6-10,000	100%	100%	
			Cryptosporidium	1-398	100%	100%	
			Salmonella	3-1,000	100%	100%	
			Campylobacter	1-200	100%	100%	
			E. coli O157:H7	1-1,995.0	100%	100%	
Livestock	Cattle	335-17,400,000	*Giardia*	1.58-3162	67-100%	0.2-37%	(Padia et al., 2012; Soller et al., 2010b; USEPA, 2010)
			Cryptosporidium	0.5-1,585	67-100%	0.6-23%	
			Salmonella	398-39,811	34-66%	5-18%	
			Campylobacter	63-31,623	67-100%	5-38%	
			E. coli O157:H7[*]	1202,30.9[*]	67-100%	9.7-28%	
Wildlife	Deer	46,000-26,900,000	*Giardia*	1.1-1,168	0-33%	0.15-21.2%	(Gallagher, 2012; Garcia-Presedo et al., 2013; Heitman et al., 2002b; Ng et al., 2011; Paziewska et al., 2007)
			Cryptosporidium	1.8-225	0-33%	0.15-14.4%	
	Feral Hogs	79,500-41,600,000	*Salmonella*	0-11	0-33%	0-22%	(Brooks, 2017; Diaz-Sanchez et al., 2013; Gallagher, 2012; Vieira-Pinto et al., 2011; Wacheck et al., 2010; Wahlstrom et al., 2003)
			Campylobacter	0-420	0-33%	0-66%	

[*]lognormal distribution mean and standard deviation

The risk of GI illness stated as the probability of GI illness, was estimated using the reference pathogen dose and the corresponding dose response model in Table 3-3. The hypergeometric function used for the norovirus dose-response model was simplified to a beta-binomial function because Crystal Ball Pro® software (Oracle Corp., Redwood Shores, CA) was unable to calculate a hypergeometric function (McBride et al., 2013; Van Abel et al., 2017).

The source specific risk of illness from exposure to all reference pathogens associated with a source was estimated using Equation 3-2 (Soller et al., 2010b).

$$P_{ill_S} = 1 - \prod_{rp}(1 - P_{ill_{rp}}) \quad (3\text{-}2)$$

where:

P_{ill_S} is the total probability of illness from the fecal source, S

$P_{ill_{rp}}$ is the probability of illness from the reference pathogen, rp, associated with the fecal source, S

The total probability of illness from exposure to all of the sources was calculated using Equation 3-3 (Gitter, 2016; Soller et al., 2014).

$$P_{ill} = 1 - \prod_{S}(1 - P_{ill_S}) \quad (3\text{-}3)$$

where:

P_{ill} is the total probability of illness associated with all fecal sources

P_{ill_S} is the probability of illness associated with the fecal source, S

Table 3-3 Reference pathogen dose response models.

Reference Pathogen	Dose-Response Model	Dose-Response Equations	Model Parameters	Parameter Values	Morbidity	Reference
Norovirus	Hypergeometric as Beta binomial	$P_{inf} = 1 - \frac{B(\alpha, \beta + dose)}{B(\alpha, \beta)}$	alpha beta	0.04 0.055	60%	(McBride et al., 2013; Teunis et al., 2008a)
Cryptosporidium	Exponential	$P_{inf} = 1 - \exp(-r \times dose)$	r	0.09	50%	(USEPA, 2006)
Giardia lamblia	Exponential	$P_{inf} = 1 - \exp(-r \times dose)$	r	0.0199	45%	(Rose and Gerba, 1991)
Campylobacter	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.145 7.59	28%	(Medema et al., 1996; USEPA, 2010)
Salmonella	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.3126 2884	20%	(Haas et al., 1999)
E. coli O157:H7	Beta-Poisson	$P_{inf} = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$	alpha beta	0.4 45.9	28%	(Teunis et al., 2008b)

3.2.5. Sensitivity Analysis

The sensitivity of each input parameter was calculated to determine the ability of each input parameter to impact the total probability of illness and the total probability of illness excluding norovirus. The sensitivity analysis was performed using Crystal Ball Pro® (Oracle Corp., Redwood Shores, CA) while applying 10,000 Monte Carlo simulations. Spearman rank correlation coefficients, Spearman's ρ, were computed between all inputs and predictions in Crystal Ball (Oracle Corporation, 2008). Spearman's ρ is a nonparametric measure of correlation ranging from -1 to 1 (USEPA, 2001b). A value of ±1 indicates a perfect monotonic linear relationship between two variables and zero indicates the variables are independent (Hamby, 1994; Pirie, 1988; USEPA, 2001b). Spearman's ρ was calculated for 75 input parameters (i.e. each input variable in Equation 3-1 for each source and each reference pathogen).

3.3. Results

The probability of GI illness (P_{ill}), or health risk, associated with all QMRA fecal sources (WWTP, OWTS, cattle, deer, and feral hogs) was estimated for each flow regime. The total probability of illness associated with all fecal sources and reference pathogens exceeded the RWQC of 36 per 1000 people (0.036) across all flow regimes (Figures 3-2 and 3-3). For all flow regimes, norovirus was the overwhelming driver of health risk. The RWQC was exceeded across all flow regimes for norovirus. The probability of GI illness between the 25^{th} and 75^{th} percentiles for all reference pathogens other than norovirus was well below the RWQC (Figure 3-3).

The total probability of GI illness associated with all fecal sources was calculated excluding norovirus to better compare the health risk amongst all sources because norovirus is only associated with human sources. Additionally, the source specific health risk associated with all reference pathogens for total human sources, OWTS, and WWTP was calculated excluding

norovirus (Figure 3-4). The total probability of GI illness excluding norovirus did not exceed RWQC. Once norovirus was excluded, *Cryptosporidium* and *Giardia* were the reference pathogens most significantly contributing to the health risk. *Cryptosporidium* and *Giardia* had health risk results within the same order of magnitude of each other but were four orders of magnitude less than norovirus. The health risks associated with *Campylobacter* and *E. coli* O157:H7 were within the same order of magnitude and one order of magnitude less than *Cryptosporidium* and *Giardia*. *Salmonella* was the reference pathogen that had the least impact on health risk and was seven orders of magnitude less than norovirus (Figure 3-3).

The probability of illness between the flow conditions differs considerably between high flows and low flows. The total health risk and the total excluding norovirus health risk differ from high flows to low flows by an entire order of magnitude. The difference between the probability of illness during high flows and low flows associated with norovirus, *Giardia,* and *Cryptosporidium* was one order of magnitude. The bacteria reference pathogens (*Campylobacter*, *E. coli* O57:H7, and *Salmonella*) had health risk results that differed by three orders of magnitude from high flows to low flows (Figure 3-3).

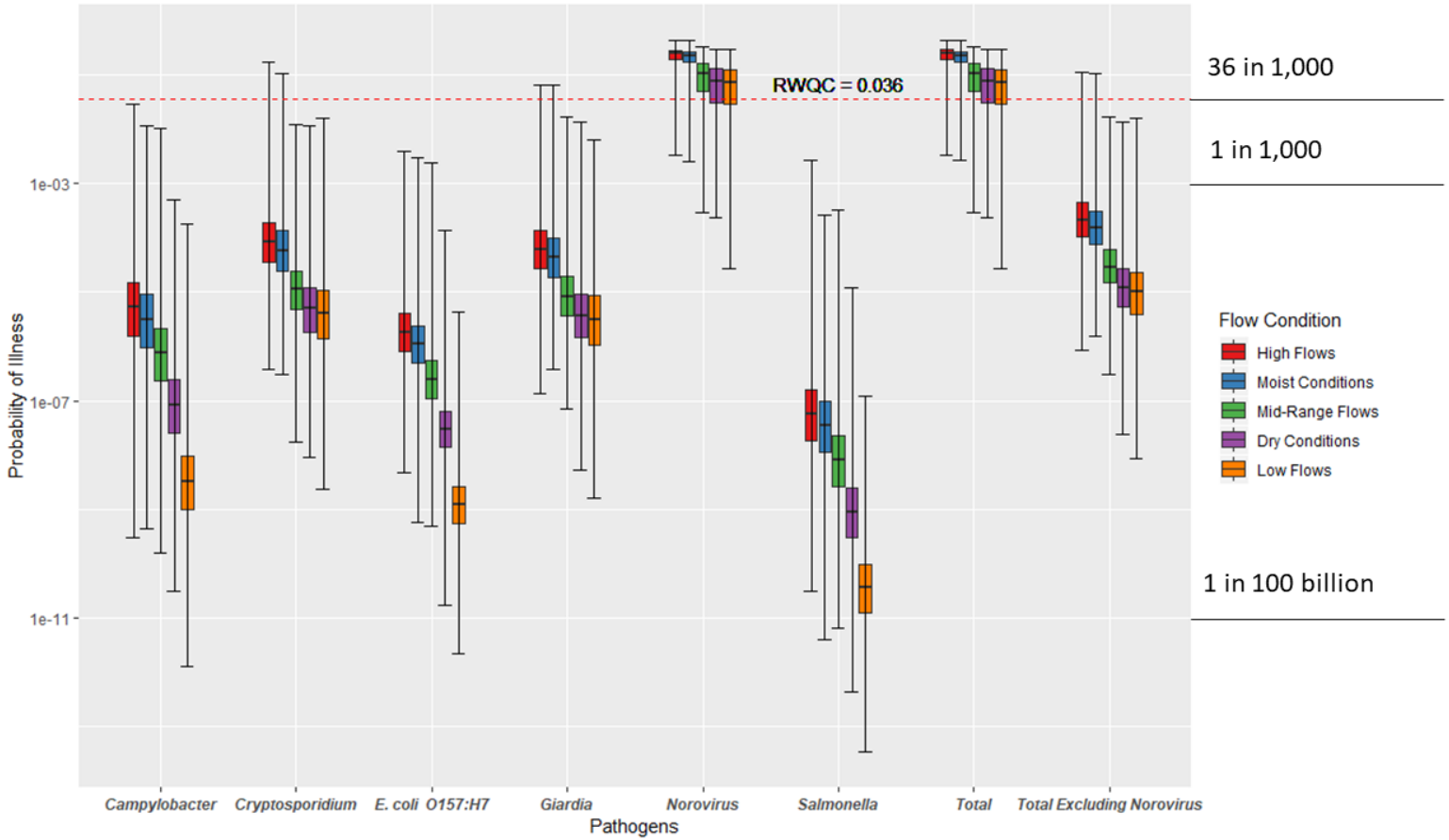

Figure 3-3. The total probability of GI illness from all associated fecal sources per reference pathogen for each flow condition.

The probability of illness associated with the WWTP is within the same order of magnitude across all flow regimes. However, once norovirus was excluded the probability of illness associated with the WWTP differed by one order of magnitude from high flows to low flows. All other fecal sources (OWTS, OWTS excluding norovirus, cattle, deer, and feral hogs) had a difference of three orders of magnitude for health risk from high flows to low flows (Figure 3-4).

The probability of GI illness associated with the WWTP for all reference pathogens exceeded the RWQC across all flow regimes (Figure 3-4). The health risk associated with OWTS for all reference pathogens only exceeded the RWQC between the 25th and 75th percentiles for high flows and moist conditions. All sources other than human (cattle, feral hogs, and deer) had a probability of GI illness below the RWQC across all flow conditions (Figure 3-4). Once norovirus was excluded, the WWTP and OWTS fecal sources did not exceed the RWQC between the 25th and 75th percentiles.

The probability of GI illness associated with WWTP was within the same order of magnitude as the total probability of GI illness associated with all fecal sources across all flow regimes, suggesting that the WWTP fecal source is the main driver of health risk. The health risk associated with OWTS varied from one order of magnitude to three orders of magnitude less than the total health risk associated with all sources from high flows to low flows. The probability of illness associated with cattle during high flows and low flows was four to six orders of magnitude lower than the total probability of illness associated with all sources. Both wildlife sources contributed the least to health risk. Total probability of illness for feral hogs was ten to twelve orders of magnitude and deer ranged between eight to ten orders of magnitude lower than the total health risk associated with all sources (Figure 3-4).

Once norovirus was excluded from the probability of GI illness estimations, the health risk associated with the WWTP fecal source was also within the same order of magnitude as the total health risk associated with all sources across all flow regimes. This suggests that the WWTP source was the main contributor to health risk once norovirus was excluded. From high flows to low flows, the probability of GI illness associated with cattle was one to three orders of magnitude lower than the total probability of illness associated with all sources. The health risk associated with OWTS was two to four orders of magnitude lower than the total health risk associated with all sources from high flows to low flows. Wildlife remained the least contributing source when norovirus was excluded, ranging from five to seven orders of magnitude less than the total health risk (Figure 3-4).

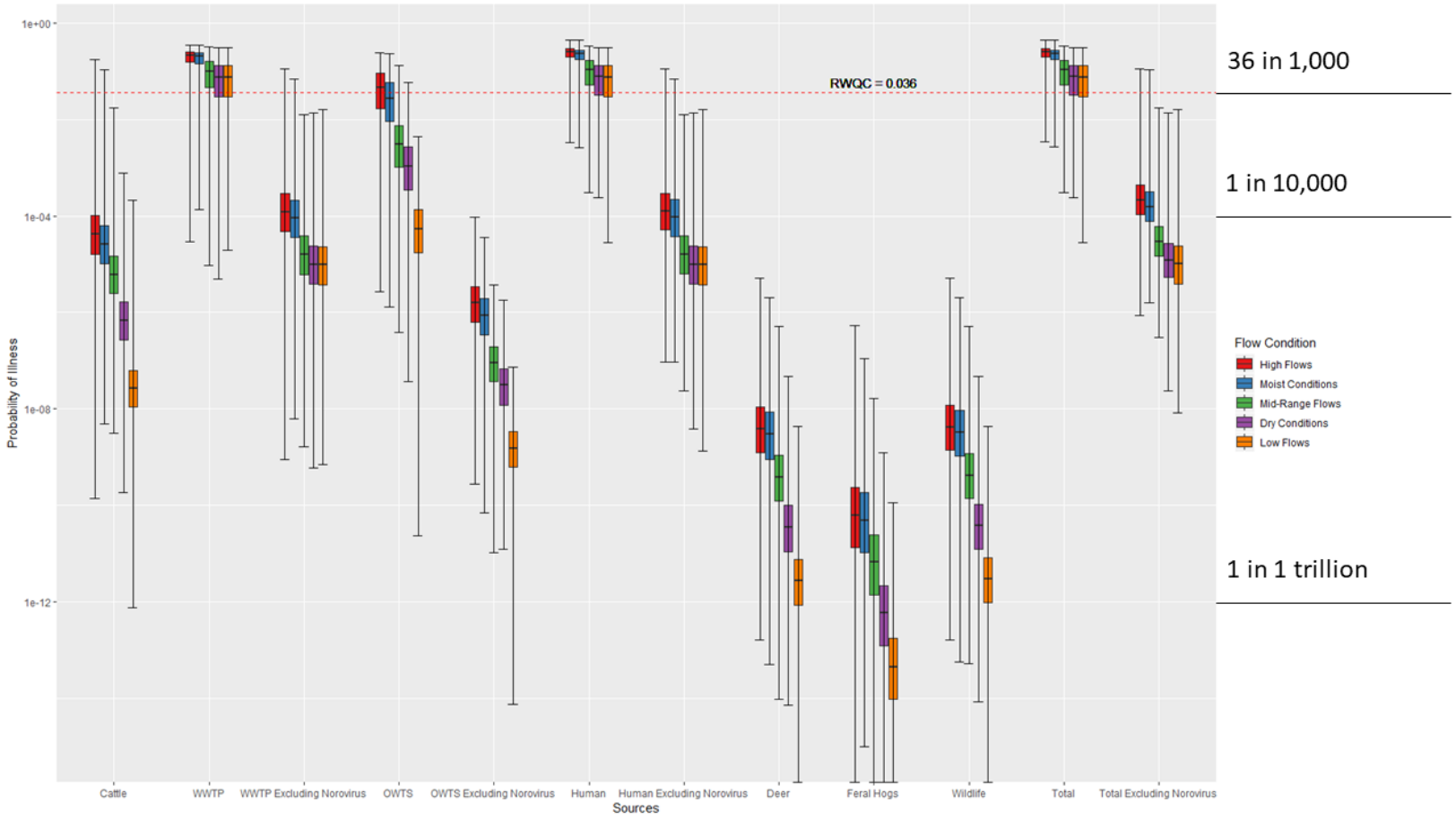

Figure 3-4. The total probability of GI illness from all associated reference pathogens per fecal source for each flow condition.

3.3.1. Sensitivity Analysis

Across all flow regimes, the input parameters associated with calculating the dose of norovirus had the greatest impact on the total probability of GI illness associated with all fecal sources and reference pathogens (Figure 3-5). The seven input parameters used to calculate the dose of norovirus were: concentration of norovirus in raw sewage (R^{O}_{noro}), *E. coli* concentration in raw sewage ($R^{O}_{E.\ coli}$), OWTS *E. coli* concentration in the water body (C^{O}_{FIB}), WWTP *E. coli* concentration in the water body (C^{W}_{FIB}), concentration of norovirus in secondary disinfected effluent (R^{W}_{noro}), *E. coli* concentration in secondary disinfected effluent ($R^{W}_{E.\ coli}$), and the volume of water ingested (V). These seven input parameters were the only input parameters with Spearman's ρ greater than or equal to ±0.1 for at least one flow regime. The input parameters R^{W}_{noro}, $R^{W}_{E.\ coli}$, and C^{W}_{FIB} had Spearman's ρ greater than ±0.3 across all flow regimes. Three input parameters, C^{O}_{FIB}, R^{O}_{noro}, and $R^{O}_{E.\ coli}$, had Spearman's ρ greater than ±0.2 only for high flows and moist conditions, while the other flow conditions for these parameters had Spearman's ρ less than or equal to ±0.05. The input parameter V had Spearman's ρ greater than or equal to ±0.1 for all flow regimes except high flows.

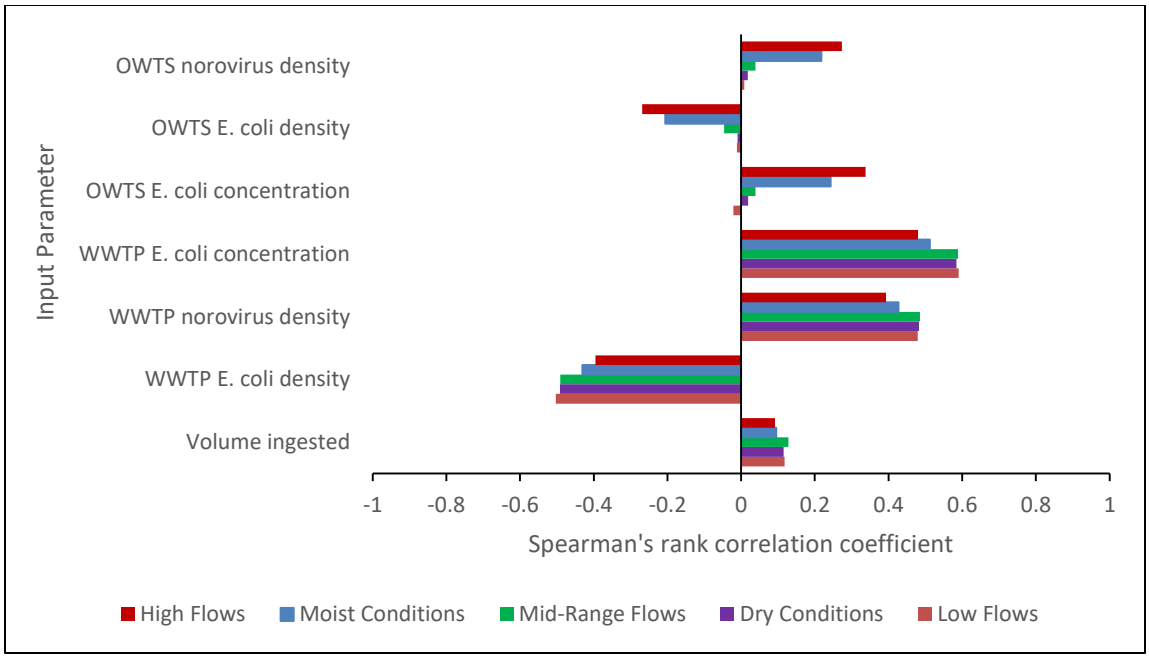

Figure 3-5. Spearman rank correlation coefficients for dose input parameters to calculate the total probability of illness.

Once norovirus was excluded from the total probability of GI illness associated with all reference pathogens and fecal sources, eight input parameters had Spearman's ρ values greater than ±0.1 for at least one flow regime (Figure 3-6). The input parameter of the WWTP *E. coli* concentration in the water body (C_{FIB}^{W}) had the most significant impact on the total probability of GI illness, with Spearman's ρ values greater than or equal to ±0.5 across all flow regimes. Four input parameters associated with the WWTP had Spearman's ρ values greater than ±0.1 across all flow regimes. These four input parameters were *E. coli* concentrations in secondary disinfected effluent ($R_{E.\ coli}^{W}$) associated with calculating the dose of *Cryptosporidium* and *Giardia*, the concentration of *Cryptosporidium* in secondary disinfected effluent (R_{Crypto}^{W}), and the concentration of *Giardia* in secondary disinfected effluent ($R_{Giardia}^{W}$). The two input parameters of the cattle *E. coli* concentration in the water body (C_{FIB}^{C}) and the *E. coli*

concentration in cattle feces ($R^{C}_{E.\ coli}$) associated with calculating the dose of *Cryptosporidium* had Spearman's ρ values greater than ±0.1 for only high flows, moist conditions, and mid-range flows. The input parameter of the *E. coli* concentration in cattle feces ($R^{C}_{E.\ coli}$) associated with calculating the dose of *Giardia* had Spearman's ρ values greater than ±0.1 for only high flows and mid-range flows.

The input parameters associated with WWTP and cattle fecal sources were the only parameters that had Spearman's greater than ±0.1. Additionally, these input parameters were only associated with the reference pathogens of *Cryptosporidium* and *Giardia*. This sensitivity analysis suggests that the wildlife fecal source and the bacteria reference pathogens (*Campylobacter*, *E. coli* O157:H7, and *Salmonella*) were not significant contributors to health risk.

Flow condition does not appear to greatly impact the sensitivity related with the input parameters associated with the WWTP because the Spearman's ρ do not vary considerably between flow conditions (Figure 3-5 and 3-6). However, the Spearman's ρ associated with the OWTS (Figure 3-5) and cattle (Figure 3-6) input parameters decreased considerably from high flows to low flows which suggests that flow condition had a large impact on the sensitivity of the input parameters associated with OWTS and cattle. This suggests that changes in flow conditions have a larger impact on non-point sources (OWTS and cattle) than point sources (WWTP).

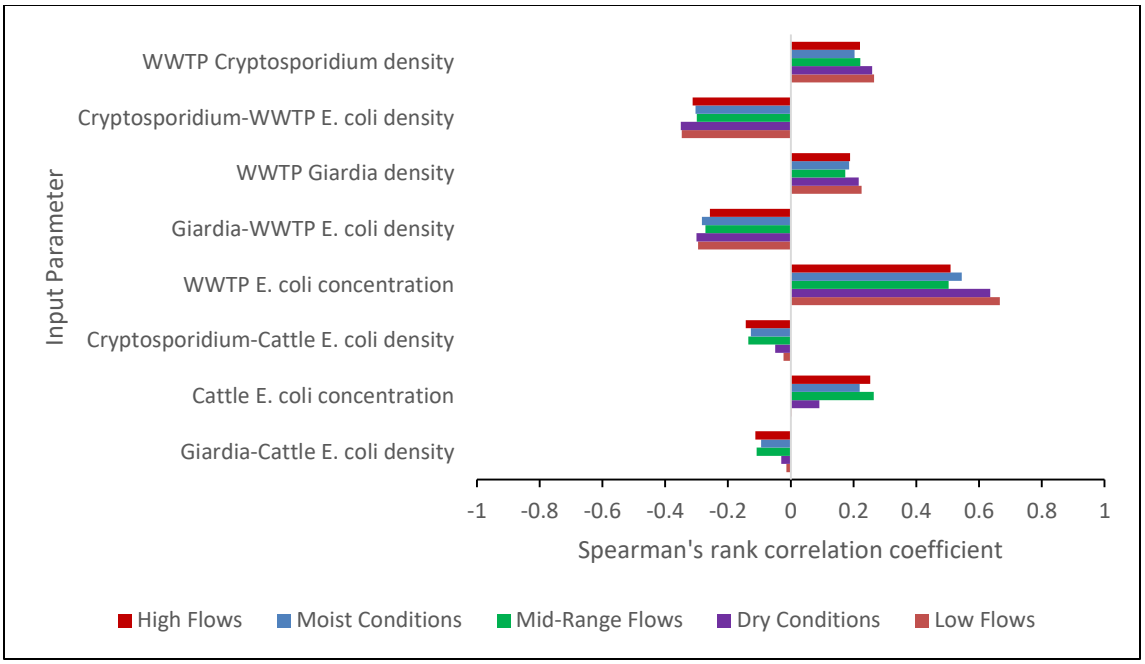

Figure 3-6. Spearman rank correlation coefficients for dose input parameters to calculate the total probability of illness excluding norovirus.

3.4. Discussion

SWAT, SELECT, and QMRA models were successfully applied together to estimate health risk associated with different hydrologic conditions. Previous studies integrating hydrodynamic models with QMRA focused solely on the impact of heavy rainfall events on health risk associated with contact with sewage which concluded an increased health risk after heavy rainfall events (Andersen et al., 2013; Eregno et al., 2016). This study found similar conclusions showing an increased health risk associated with *E. coli* concentrations during high flows compared to low flows particularly for non-point sources (OWTS, cattle, feral hogs, and deer). However, the riskiest source (WWTP) was not impacted by flow conditions due to it being a point source, suggesting that flow conditions only impact the associated health risk of non-point sources.

Norovirus was the reference pathogen with the largest contribution to health risk even though it was only contained in two out of five sources. Norovirus was also the only reference pathogen that exceeded the RWQC. The human source (WWTP and OWTS) input parameters associated with calculating the norovirus dose were the only significant input parameters that impacted the total probability of GI illness associated with all fecal sources and reference pathogens.

The norovirus dose-response model used was simplified from a hypergeometric function to a beta-binomial function because Crystal Ball Pro® software (Oracle Corp., Redwood Shores, CA) was unable to calculate a hypergeometric function. The use of this simplified norovirus dose-response model may have impacted the risk estimates for the probability of illness due to exposure to norovirus. The beta-binomial dose-response model predicts a higher risk at low doses compared to the hypergeometric dose-response model (Van Abel et al., 2017). Typically, the overestimation of risk at low doses would have a greater impact on a study examining drinking water instead of recreational water due to the increased level of contamination in recreational water. There is not a universally accepted best application of the norovirus dose-response model, so whichever application is chosen will impact the final results.

In addition to the dose-response model used, the reference pathogens chosen can significantly impact the results, especially for human enteric viruses, such as norovirus. Human enteric viruses were suggested as the cause of a majority of GI illnesses from swimming in recreational waters impacted by human sources during an epidemiology study in the Great Lakes in 2003 and 2004 (Soller et al., 2010a). Adenovirus was included as a USEPA (2010) reference pathogen to represent the fate and transport of waterborne diseases in recreational water bodies. However, adenovirus was not included in this study because there is not a corresponding

ingestion dose-response model for adenovirus. The inclusion of adenovirus and other enteric viruses would show if the driver of risk is specifically norovirus or human enteric viruses in general.

Once norovirus was excluded, *Giardia* and *Cryptosporidium* were the main drivers of health risk. However, the other reference pathogens were within one order of magnitude of *Giardia* and *Cryptosporidium* with the exception of *Salmonella*. The WWTP remained the main contributor to health risk once norovirus was excluded. Cattle were a larger driver of health risk than OWTS, the other human source. Wildlife sources (deer and feral hogs) contributed the least to health risk for both the total health risk and the total health risk excluding norovirus.

The means of the SWAT-simulated source-specific *E. coli* concentrations were below the *E. coli* regulatory standard of 126 CFU per 100 mL across all flow regimes (Table 3-1). However, the probability of GI illness associated with all fecal sources and reference pathogens exceeded the RWQC across all flow regimes. Additionally, the means of the SWAT-simulated *E. coli* concentrations associated with all of the SELECT input sources exceeded the *E. coli* regulatory standard only during high flows. The *E. coli* concentration in the water body is the sole factor considered for the regulation of fresh water bodies. Therefore, based on the SWAT-simulated *E. coli* concentrations, this water body would not be regulated even though the RWQC is exceeded.

Furthermore, cattle were the only fecal source that had SWAT-simulated source-specific maximum *E. coli* concentrations that exceeded the *E. coli* regulatory standard, but the health risks associated with cattle did not exceed the RWQC for any flow regime. The WWTP was the main contributor to health risk, but the SWAT-simulated source-specific maximum *E. coli* concentrations for WWTP were well below the *E. coli* regulatory standard across all flow

regimes. Moreover, the *E. coli* concentration input into SWAT at the WWTP location upstream from the sampling site was the *E. coli* regulatory standard. Therefore, this WWTP effluent was meeting the regulations for bacteria (TCEQ, 2009). Currently, the remediation of water bodies focuses on decreasing the source that has the highest *E. coli* load contribution, which in this case would be cattle. However, this approach would not effectively impact health risk, which is the ultimate goal of regulations and remediation efforts.

3.5. Conclusions

TMDLs and current water quality regulations are solely focused on meeting a numerical FIB concentration by lowering the FIB load of the largest contributing source. The results of this study show that this approach does not protect human health, which is the main purpose of water quality regulations. TMDLs are not an effective approach to remediate water bodies contaminated with microbial pollutants. Instead remediation efforts should be focused on sources that are the riskiest to human health instead of those contributing to the largest FIB load. Remediation efforts for water bodies contaminated with microbial pollutants should account for the health risk impacts of specific sources instead of meeting a numerical FIB standard regardless of the source of microbial contamination.

Previous studies have shown that avian wildlife are large contributors to fecal contamination and a significant contributor to human health risk (Liao et al., 2016; Schoen and Ashbolt, 2010). Additional research should include other sources of fecal contamination such as avian wildlife and pet waste for a better representation of the fecal source contribution into a water body. This study assumed a direct relationship between *E. coli* concentration and infective pathogen dose in a water body. Future research should take into account the survival time of pathogens in a water body compared to the survival time of FIB, such as *E. coli*, when estimating

health risks. A better understanding of the relationship between FIB and pathogens would aid in the ability to more accurately predict health risk. There is no way to validate the health risk estimates from this study, therefore, further research can be performed applying this methodology along with an epidemiology study to assess the accuracy of the health risk results.

4. CONCLUSIONS

The purpose of this dissertation was to address the flaws of the microbial recreational water quality criteria (RWQC) and remediation by applying Total Maximum Daily Loads (TMDLs) at protecting human health and to provide an alternative method. The current remediation approach of TMDLs only focuses on meeting a numerical FIB RWQC regardless of the source of contamination. Outbreaks associated with microbial contamination are still occurring in water bodies where the water quality is meeting the regulatory standards (Craun et al., 2005). This research focused on addressing this by taking into account health impacts of the particular sources of contamination (Chapter 2) and the fate and transport of microbial contamination during different hydrologic conditions (Chapter 3).

Fecal Indicator Bacteria (FIB) criteria concentrations are used as RWQC to regulate water bodies for microbial contamination. The RWQC does not differ based on the source of contamination, however, studies have not been able to establish a correlation between FIB from sources other than human and health risk (Calderon et al., 1991; Colford et al., 2007; Colford et al., 2012; Mcbride et al., 1998). Additionally, FIB only show that fecal contamination is present but not the source of the contamination. However, FIB must be used in place of pathogens because measuring pathogens is technically difficult and costly and therefore infeasible to routinely monitor (Harwood et al., 2014). In order to address the contribution from human and non-human sources to estimate health risk, this research (Chapter 2) applied Microbial Source Tracking (MST) in conjunction with Quantitative Microbial Risk Assessment (QMRA). Previous QMRA studies were not able to include all sources potential fecal contamination particularly,

non-avian wildlife, or they were only able to include one source of contamination. This study included multiple sources of fecal contamination, including non-avian wildlife.

The probability of GI illness from exposure to fecal matter originating from human, cattle, and wildlife sources was calculated in three water bodies. The probability of GI illness was calculated by applying QMRA and MST using four different run scenarios. The source contributing the largest percentage of fecal matter into the water body (wildlife) did not have a significant impact on human health. Meanwhile, human fecal sources contributed as little as 12% to the microbial contamination in a water body, but were the overwhelming driver of risk.

Waterborne disease outbreaks are not only related to the source of contamination but also the transport of fecal matter by rainfall events. Curriero et al. (2001) found that outbreaks occurring from surface water contamination had a strong correlation with extreme precipitation events. Therefore, the fate and transport of fecal contamination during rainfall events should be taken into account when estimating health risk. Load Duration Curves (LDCs) are often a used as a tool when developing TMDLs to assess pollutant loads at different hydrologic conditions. The fate and transport of microbial contamination associated with multiple fecal sources was modeled using Spatially Explicit Load Enrichment Calculation Tool (SELECT) to spatially distribute the fecal matter of sources overland and the Soil and Water Assessment Tool (SWAT) to model the fate and transport into the water body.

SWAT, SELECT, and QMRA were applied together to estimate the probability of gastrointestinal (GI) illness from exposure to fecal contamination due to a Wastewater Treatment Plant (WWTP), Onsite Wastewater Treatment Systems (OWTS), cattle, and non-avian wildlife during different flow conditions. The WWTP had the most significant impact on health risk across all five flow conditions but was not the largest contributor (cattle) to fecal contamination.

The hydrologic flow conditions considerably impacted the health risk associated with the non-point sources (OWTS, wildlife, and cattle) but did not impact the point source (WWTP).

The source of fecal contribution had a more significant impact on health risk compared to the hydrologic conditions. However, the hydrologic conditions are more significant for non-point sources at determining health risk. Fecal source contributions in a watershed can be estimated by using MST or SELECT and SWAT to determine the riskiest sources impacting health risk. Water quality regulations and remediation efforts should take into account the impacts of a fecal contamination source on health risk. Current water quality regulations are not effective at protecting human health because the acceptable rate of GI illness was exceeded for both studies even when the *E. coli* concentrations in the water body were meeting the regulatory standard.

The fecal source contributions resulting from MST and SELECT and SWAT differed considerably within the same watershed (Lampasas River Watershed). Additional research could compare the results between the MST and SWAT source contributions. This research was not able to take into account all fecal sources contributing fecal contamination, particularly avian wildlife and domestic pets. Future research can apply these fecal sources in addition to the other sources applied in this study.

Across both studies (Chapter 2 and Chapter 3), norovirus and human sources were the drivers of risk despite having low fecal contributions. This dissertation assumed a prevalence and infectivity of norovirus to be 100% in both wastewater effluent and raw sewage. Additional research relating FIB densities and enteric viruses, such as norovirus, density concentrations in wastewater effluent and sewage is needed to improve the pathogen dose estimation. Overall, a better understanding of the relationship between FIB and pathogens would improve our ability to determine the impact of both FIB and pathogens on human health.

The only enteric virus included in this study was norovirus because there was no published ingestion dose-response model for adenovirus. Further research to develop an ingestion dose-response model for adenovirus would enable another enteric virus to be included in this research. The choice of reference pathogens used to estimate the probability of illness significantly impacts the results. If an enteric virus other than norovirus were included in this study, then the impact of norovirus as the main contributor to risk could examined to determine if other enteric viruses are similar drivers of risk.

Lastly, the health risk results of these studies (Chapter 2 and Chapter 3) are not able to be confirmed. Future research could include an epidemiology study to be conducted in addition to the MST measurements or SELECT and SWAT modeling. The watersheds used for this research (Chapter 2 and Chapter 3) were chosen because preexisting MST studies were conducted in the water bodies. An assessment of the how people are using a water body should be performed to determine if the assumed exposure scenario used in the QMRA is accurate. These studies (Chapter 2 and Chapter 3) assumed that swimming was the exposure scenario however, if people are not swimming in these watersheds then the risk estimates would not apply to them. To perform a successful epidemiology study, large numbers of people should be actively recreating within the water body. A water body where large numbers of people are known to recreate within should be chosen to perform an epidemiology study. Ideally, the collection of MST data and FIB and pathogen concentration data would occur concurrently with an epidemiology study and watershed modeling.

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APPENDIX A SWAT CALIBRATION PARAMETERS

ALPHA_BF	Baseflow alpha factor (1/days)
GW_DELAY	Groundwater delay time (days)
CN2	Initial SCS runoff curve number for moisture condition II
SOL_AWC	Available water capacity of the soil layer (mm H_2O/mm Soil)
ESCO	Soil evaporation compensation factor
RCHRG_DP	Deep aquifer percolation fraction
REVAPMN	Threshold depth of water in the shallow aquifer for "revap" or percolation to the deep aquifer to occur (mm H_2O)
GW_REVAP	Groundwater "revap" coefficient
ALPHA_BF_D	Alpha factor for groundwater recession curve of the deep aquifer (1/days)
CH_K(2)	Effective hydraulic conductivity in mail channel alluvium (mm/hr)
CH_K(1)	Effective hydraulic conductivity in tributary channel alluvium (mm/hr)
SLSOIL	Slope length for lateral subsurface flow (m)
LAT_TTIME	Lateral flow travel time (days)
CNCOEF	Plant ET curve number coefficient
ICN	Daily curve number calculation method
GWQMN	Threshold depth of water in the shallow aquifer required for return flow to occur (mm H_2O)
BACTKDQ	Bacteria soil partitioning coefficient
BACTKDDB	Bacteria partition coefficient
THBACT	Temperature adjustment factor for bacteria die-off/growth

BACT_SWF	Fraction of manure applied to land areas that has active colony forming units
WDPRCH	Die-off factor for persistent bacteria in water bodies at 20°C (1/day)
WDPF	Die-off factor for persistent bacteria on foliage at 20°C (1/day)
WOF_P	Wash-off fraction for persistent bacteria
BACTMIX	Bacteria percolation coefficient (10 m^3/Mg)
BACTMINP	Minimum daily bacteria loss for persistent bacteria (# cfu/m^2)