APPLICATION OF QUANTITATIVE MICROBIAL RISK ASSESSMENT AND BACTERIAL SOURCE TRACKING TO ASSESS THE ASSOCIATED HUMAN HEALTH RISKS FROM MULTIPLE FECAL SOURCES DURING RECREATIONAL EXPOSURE IN THE LEON RIVER WATERSHED

A THESIS

by

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ABSTRACT

Applying a risk assessment framework, such as quantitative microbial risk assessment (QMRA), can be used to estimate the human health risk associated with recreation in a waterbody impaired for elevated levels of fecal indicator bacteria (FIB). Recent efforts to identify the sources contributing to a waterbody's bacterial impairment have been facilitated by bacterial source tracking (BST) analysis for several watersheds in Texas, including the Leon River Watershed. A QMRA was conducted to calculate the human health risk for a recreational waterbody impacted by both human and non-human sources of fecal contamination. Waterborne reference pathogens were used to represent the different fecal contamination sources and the risk of a GI infection and illness. The GI illness risk for contact exposure to recreational waters within the Walnut Creek tributary of the Leon River Watershed were calculated for site LEO 2, with a geometric mean of 163 cfu 100 mL^{-1}, and the U.S. recreational standard of 126 cfu 100 mL^{-1} for *Escherichia coli* (*E. coli*). Three different scenarios were modeled to estimate the potential risks of a GI illness in recreational waters impacted by different proportions of human and non-human sources of fecal contamination. The analysis found that: a) the dominant fecal source in a waterbody may not be the greatest contributor to the human health risk; b) risks associated with wildlife fecal contamination were significantly lower than that of the cattle/domestic animals and human fecal contamination; and c) while considering norovirus as a representative pathogen for human fecal contamination, the estimated risk was much higher. The results indicate that identifying the sources

contributing to a bacterial impairment and conducting a QMRA for the recreational waterbody can greatly assist in developing site-specific standards, especially if the site is not predominantly impacted by human fecal contamination.

DEDICATION

To my mom, sisters, and friends who supported my passion in studying environmental health and water.

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NOMENCLATURE

BST	Bacterial Source Tracking
CAFO	Concentrated Animal Feeding Operation
E. coli	*Escherichia coli*
FIB	Fecal Indicator Bacteria
GI	Gastrointestinal
HCGI	Highly Credible Gastrointestinal Illness
NEEAR	National Epidemiological and Environmental Assessment of Recreation
NGI	NEEAR-GI illness
OSSF	On-site Sewage Facilities
POTW	Publically Owned Sewage Treatment Works
QMRA	Quantitative Microbial Risk Assessment
RUAA	Recreational Use Attainability Analysis
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TSSWCB	Texas State Soil and Water Conservation Board
U.S. EPA	United States Environmental Protection Agency
WHO	World Health Organization
WPP	Watershed Protection Plan

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Surface water recreation involving a high degree of bodily contact remains a significant epidemiological concern for human health, specifically for the risk of gastrointestinal (GI) illnesses from bacteria, protozoa, and other pathogens (Dorevitch et al., 2011; U.S. EPA, 2012). Initial efforts to assess bacterial water quality began in the 1960s when the United States Environmental Protection Agency (U.S. EPA) opted to use fecal coliforms, a group of Fecal Indicator Bacteria (FIB), to monitor and protect swimmers' and bathers' health (U.S. EPA, 2012). In 1984, the U.S. EPA conducted a study establishing *Escherichia coli (E. coli)* as a useful indicator bacterium to determine the presence of fecal contamination, and fecal pathogens in freshwater sources (Dufour, 1984; Wade et al., 2006).

I.1 Human Health Risk Standards

Dufour's (1984) dose-response data assisted in developing *E. coli* standards to establish the U.S. EPA accepted risk level of eight cases of a Highly Credible Gastrointestinal Illness (HCGI) per 1,000 individuals involved in primary contact recreation (U.S. EPA, 2012). The bacteria standard for *E. coli* was established at a geometric mean of 126 cfu 100 mL^{-1} for continuous sampling (averaging over 30 days) and continues to be used for most freshwater lakes, rivers, and other waterbodies listed to meet primary contact recreational water standards (U.S. EPA, 2012). The 2012 Recreational Water Quality Criteria (RWQC) reviewed the findings of the National Epidemiological and

Environmental Assessment of Recreational (NEEAR) water study conducted between 2003 and 2009 and recorded epidemiological data of GI illnesses and activity in nine different U.S. recreational waters. The findings, as well as review of other published studies, reinforced the use of FIB in detecting the elevated presence of fecal contamination and the potential for other waterborne fecal pathogens.

The epidemiological data gathered from the NEEAR Water study were used to develop a more comprehensive definition for a gastrointestinal illness, termed a NEEAR-GI illness (NGI). A redefinition of a NGI illness was developed to exclude fever from the symptom list and lengthen the incubation time for an illness to occur from 10 to 12 days. The new definition of a NGI illness is defined as an illness occurring within 10 to 12 days after swimming that can include diarrhea, nausea, vomiting, and stomachache or nausea or stomachache which impacts a daily activity (U.S. EPA, 2012). While no longer requiring the incidence of a fever, the number of NGI illnesses within the acceptable risk is considered to be 36 cases per 1,000 recreation events or individuals, a risk to range from 0.03 to 0.04 (Soller et al., 2010b; U.S. EPA, 2012). This risk level of eight HCGI per 1,000 cases of primary contact recreation is considered equivalent to the risk level of 36 NGI per 1,000 individuals engaging in primary contact recreation, as determined by comparing the pre-1986 calculations with the NEEAR-GI illness study (U.S. EPA, 2012).

Several published studies discuss the presence of pathogens in freshwater sources used for contact and non-contact recreation and the epidemiologic potential for disease (Soller et al., 2010a, 2010b; Schoen et al., 2011; Dorevitch et al., 2011). While epidemiologic studies are necessary to identify the bacteria concentrations and number of specific illnesses, such dose-response information tends to reflect local and site-specific environments (Lopez-Pila and Szewzyk, 2000). Dose-response data of bacteria and illness rates from the Great Lakes region have been used in microbial risk assessment studies in other regions. Dufour (1984) developed dose relationships from two inland lakes near Erie, Pennsylvania and Tulsa, Oklahoma to establish bacterial standards inclusive for most freshwater environments. The evidence generated from these studies, however, describes the adverse health risks associated with recreation in waterbodies that are impacted by municipal disinfected wastewater effluent (Schoen et al., 2011). Site-specific standards have begun to be explored for waterbodies that do not meet water quality standards for primary contact recreation, but could meet standards for limited contact or secondary contact recreation in several states in the U.S., including Texas (Winemiller et al., 2010; Dorevitch et al., 2012; Bragg et al., 2015).

I.2 Quantitative Microbial Risk Assessment (QMRA)

The application of Quantitative Microbial Risk Assessment (QMRA) to estimate health risks for individuals recreating in an inland waterbody can assist in establishing site-specific risk levels (Haas et al., 2014). The QMRA framework involves characterizing the hazard, assessing exposure, integrating dose-response data, and estimating health

risks associated with exposure from specific activities. The QMRA framework translates environmental monitoring data, such as FIB concentration levels, to characterize its relationship with human health. Recently, the U.S. EPA has discussed efforts to distinguish different fecal contaminant sources and apply QMRA to quantify the potential human health risk for primary contact recreation in waterbodies impacted by sources other than human waste (U.S. EPA, 2010, 2012).

The U.S. EPA (2010) continues to update and provide a comprehensive understanding of the estimated human GI illnesses that can be expected to occur due to exposure to freshwater recreational waterbodies impacted by fecal contamination. Historically, these epidemiological assessments have focused on the health risk from recreational waters impacted by a combination of treated, disinfected municipal wastewater and less treated or untreated sewage (including bather shedding, poorly operating septic systems, etc.) (Soller et al., 2010a; Schoen et al., 2011). However, many assessed waterbodies are listed as impaired by non-point sources of contamination (U.S. EPA, 2010). Beaches impacted by human sources of pathogens may be contaminated with a different range of pathogens than sites impacted by non-human sources of pathogens, potentially requiring alternative standards to protect human health (Schoen and Ashbolt, 2010). The U.S. EPA (2010) reported that risks associated with agricultural animal waste-impacted waters differ because of the different composition and densities of pathogens in animal waste as compared to human waste.

Few studies discussing the human health risks associated with exposure to non-human sources of bacteria in recreational waterbodies are available (Soller et al., 2010b). The World Health Organization (WHO) considers fecal contamination sources other than human, such as avian or rural agricultural runoff, to be of less risk (Soller et al., 2010b). The mixtures and densities of pathogens found in animal feces are known to be different from the composition of pathogens in municipal wastewater effluent or raw sewage. The U.S. EPA (2010) also mentions that considerations should be made for the different pathogen loading of animal and human excreta, in which animal waste contamination typically is precipitation event-driven, such as by run-off while pathogen contamination from wastewater effluent is a continuous event.

I.3 Bacterial Source Tracking (BST)

BST identifies sources of fecal contamination in a waterbody. Genetic phenotypic tests are applied to identify host-specific bacterial strains and determine the contributing sources of bacterial contamination (Di Giovanni et al., 2013). Several different molecular methods are used; ribotyping, pulse-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD), and repetitive DNA sequences (Rep-PCR) (Meays et al., 2004). Tests used to identify the genomic strains for the Leon River Watershed include automated ribosomal ribonucleic acid genetic fingerprinting (RiboPrinting) and enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR) (Gregory et al., 2013). These two molecular tests are library-dependent, utilizing a reference library of DNA fingerprints of *E. coli* from known

sources (Di Giovanni et al., 2013). The results gathered from BST analysis can help distinguish the predominant sources contributing fecal bacteria to a waterbody.

I.4 QMRA and Different Fecal Waste Sources

Few studies have calculated the exposure risks from both human and non-human sources of fecal pollution (Soller et al., 2010b, 2014, 2015; Schoen and Ashbolt, 2010; McBride et al., 2013). Soller et al. (2010b) constructed hypothetical sites with bacteria concentrations within the regulatory limit of FIB including 126 cfu 100 mL^{-1} of *E. coli* and 35 cfu 100 mL^{-1} of enterococci. The indicator bacteria concentrations were then used to calculate a variety of pathogen concentrations found in different types of fecal waste. Different reference pathogens used included *Giardia* spp., *Cryptosporidium* spp., *Salmonella enterica*, norovirus, *E. coli* O157:H7, and *Campylobacter jejuni.* The calculated risks among fresh, directly deposited, cattle waste and human sewage were not found to be substantially different while the risks associated with bacteria exposure from gull, chicken and pig waste were found to be significantly lower than exposure to human waste. Schoen and Ashbolt (2010) calculated the risk of GI illnesses from different waste sources including gull fecal contamination and publically owned sewage treatment works (POTW), at a geometric mean of 35 cfu 100 mL^{-1} of enterococci. The calculated risks for illnesses due to a variety of reference pathogens including *Campylobacter jejuni* and *Salmonella enterica* for gull waste, and norovirus*, Giardia intestinalis, Cryptosporidium* spp. and *S. enterica* for POTW waste were different for each contamination source. The illness risks for gull fecal contamination were found to

be at least 2 $\log_{10}$ units lower than the illness benchmark of 0.01 and of the risk of illness from POTW fecal contamination (Schoen and Ashbolt, 2010).

Soller et al. (2014) simulated the potential human health risks in recreational waterbodies impacted by a mixture of human and animal waste and found the risk to be primarily influenced by the proportion of the contaminant source with the greatest likelihood for causing a human infection. In waters that are primarily impacted by a combination of animal and non-pathogenic sources with a low contribution from human sources, the numeric criteria can potentially exceed the U.S. EPA standard of 126 cfu 100 mL^{-1} *E. coli* or 35 cfu 100 mL^{-1} enterococci, while continuing to provide equivalent health protection (Soller et al., 2014).

In New Zealand, Till et al. (2008) determined the probability of infection for freshwater recreation using the bacterium *Campylobacter.* After reviewing a variety of contamination sources including avian, dairy, municipal, sheep/pastoral and forestry/undeveloped, revisions to New Zealand's water quality recreational guidelines were undertaken. *E. coli* was measured to have a high degree of correlation with *Campylobacter*. The country's water standards were later revised to reflect the correlated risk between *E. coli* levels and the risk of *Campylobacter* infection (Till et al., 2008). Prior studies have identified differences in GI illness risks for a variety of fecal sources in a waterbody (Soller et al., 2010b, 2014, 2015; Schoen and Ashbolt, 2010). A few different fecal sources have been found to contribute to bacterial impairments in rural

waterbodies, especially for Walnut Creek (within the Leon River watershed) (Gregory et al., 2013). Efforts to distinguish the relative human health risks for the fecal sources can assist in evaluating the associated human health risks with an impaired waterbody and potential management efforts that could be undertaken.

I.5 Study Objectives

The major objective of this study was to evaluate the application of BST analysis and QMRA to assess the associated human health risks for a rural water body. *E. coli* monitoring data and BST results from Walnut Creek within the Leon River Watershed, a rural waterbody located in Central Texas, were used conduct the QMRA. The associated human health risks for a GI infection and illness were calculated using the *E. coli* monitoring data and appropriate reference pathogens for both human and non-human sources of contamination. BST results with a human health risk assessment was integrated to estimate the likelihood of a GI infection and illness associated with exposure to a rural waterbody impacted by fecal contamination sources other than human.

The research findings of this assessment may support re-examination of regulatory guidelines for Texas waterbodies predominantly impaired by non-human sources of bacteria; therefore identifying waterbodies that are of greatest human health concern based upon sources of fecal contamination. Application of QMRA, especially in locations where the investment in BST has already been made, may provide a means for

establishing scientifically defensible, site-specific water quality standards that are protective of human health. To date in Texas, Recreational Use Attainability Analyses (RUAAs) developed from site-specific studies assessing reasonably attainable recreational uses based on stream characteristics and historical recreational use have been utilized to identify and assign more appropriate uses and criteria to individual waterbodies (TCEQ, 2014).

Specific objectives and corresponding hypotheses of this study were to:

1. Conduct a QMRA to estimate the risk of a GI illness for primary contact recreation in the Walnut Creek tributary within the Leon River Watershed.
    a. Determine and calculate the dose of reference pathogens from both human and non-human sources of fecal contamination, as described in the BST data for site LEO 2, located on Walnut Creek.
    b. Estimate the risks of a GI infection and illness from selected reference pathogens for human and non-human bacteria sources.
        a. H_o: The calculated risk of a GI illness will be the same for both human and non-human sources of contamination.
        b. H_a: The calculated risk of a GI illness will not be the same for both human and non-human sources of contamination.
    c. Assess if the risks of a GI infection and illness at site LEO 2, located on Walnut Creek, which has mixture of human and non-human sources of

fecal contamination, are below the human health risk benchmark standard of 0.036.

a. H_o: The calculated risks of a GI infection and illness for the mixture of non-human and human contamination sources at site LEO 2 will be at or above the human health risk benchmark standard.
b. H_a: The calculated risks of a GI infection and illness for the mixture of non-human and human contamination sources at site LEO 2 will be below the benchmark standard.

CHAPTER II

APPLYING BACTERIAL SOURCE TRACKING (BST) AND QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA) TO ESTIMATE THE ASSOCIATED HUMAN HEALTH RISKS WITH A RECREATIONAL WATERBODY

II.1 Synopsis

Quantitative microbial risk assessment (QMRA) was conducted to evaluate the potential risk of a GI illness for swimmers in the Walnut Creek tributary of the Leon River Watershed when accounting for human and non-human fecal contamination. Bacterial source tracking results had identified *Escherichia coli (E. coli)* contributors to the waterbody as human and unidentified (10%), cattle and domestic animals (25%), and wildlife (65%). A modified conservative-risk scenario was simulated with the following proportions; human (7%), cattle (20%), and wildlife/domestic animals/unidentified (73%). The illness risks were calculated through 10,000 Monte Carlo simulations by assigning reference pathogens for each contributing source, assuming ingestion of water occurred during recreational contact, and using pathogen dose-response relationships gathered from the literature. The geometric mean (between 2011 and 2012) for site LEO 2 (163 cfu 100 mL^{-1} *E. coli*) and the contact recreational standard (126 cfu 100 mL^{-1} *E. coli*) were used to calculate the risk of a GI illness. Three scenarios were simulated to better evaluate the potential human health risk associated with this waterbody; 1) the risk of a GI illness when each source contributes 100%, 2) the risk of a GI illness according to the BST results, and 3) the risk of a GI illness when each source contributes equally

(33.3%). While site LEO 2 was predominately affected by non-human sources of fecal contamination, the risk of illness from norovirus, the reference pathogen representing human waste, contributed the greatest amount to human health risk. *Cryptosporidium* (reference pathogen for wildlife feces) and *Campylobacter* (reference fecal pathogen for cattle/domestic animals feces) were found to contribute less to the overall human health risk, even though contributing approximately 90% of the *E. coli* load, according to the BST results. The simulations indicated that identifying the sources contributing to the bacterial impairment, such as with BST, is critical to estimate the human health risk associated with recreation in a waterbody and can assist in developing site-specific standards.

II.2 Introduction

Few studies have been conducted analyzing the human health risk implications from non-human sources of bacterial contamination (Soller et al., 2010b, 2014, 2015; Schoen and Ashbolt, 2011). The use of Bacterial Source Tracking (BST) to identify the sources contributing to bacteria contamination in a waterbody and then employing a microbial risk assessment remains a relatively novel site-specific approach to determine the associated human health risk for a selected waterbody.

Monitoring efforts to improve bacterial water quality gained public health attention in the 1960s when the United States Environmental Protection Agency (U.S. EPA) began using fecal coliforms, a group of Fecal Indicator Bacteria (FIB), to assess the potential

risk for swimmers' and bathers' health in recreational waterbodies (U.S. EPA, 2012). The Dufour (1984) study assisted in determining the presence of waterborne pathogens resulting from fecal contamination in freshwater recreational waterbodies. Data collected by Dufour (1984) aided in establishing the accepted risk level for primary contact recreation of eight cases of a highly credible gastrointestinal illness (HCGI) per 1,000 individuals, which was later accepted by the U.S. EPA (Dufour, 1984; Wade et al., 2006; U.S. EPA, 2012). The national regulatory standard for primary contact recreation in a freshwater surface waterbody used for recreation was established at a geometric mean (over 30 days) of 126 cfu 100 mL^{-1} for *Escherichia coli* (*E. coli*) and continues to be used in most states (U.S. EPA, 2012). The National Epidemiological and Environmental Assessment of Recreational (NEEAR) water study conducted by the U.S. EPA revised the accepted health risk criteria to include a more comprehensive and inclusive definition of a gastrointestinal illness (GI). A NEEAR-GI illness (NGI) was redefined to exclude fever as a symptom and to extend the incubation period of an illness from 10 to 12 days, accounting for viral pathogens. The refined definition is described as an illness occurring 10 to 12 days after primary contact recreation that includes diarrhea, nausea, vomiting and stomachache, or nausea or stomachache which impacts a daily activity (U.S. EPA, 2012). The acceptable risk level has been adjusted to be 36 cases of a NGI illness per 1,000 individuals (U.S. EPA, 2012).

Dose-response information collected from epidemiologic studies tends to represent site-specific environments (Lopez-Pila and Szewzyk, 2000). The work completed by Dufour

(1984) assisted in developing the bacterial standards for freshwater environments, yet the evidence used in determining this standard describe the human health risk for recreation in waterbodies impacted by municipal disinfected wastewater effluent (Schoen et al., 2011).

The application of BST identifies the fecal contamination sources impacting a waterbody. Different laboratory methods, including automated ribosomal ribonucleic acid genetic fingerprinting (RiboPrinting) and enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), are employed to identify the genetic strains that *E. coli* has adapted to, which can include deer, feral hog, livestock or human (Casarez et al., 2007; Gregory et al., 2013; Di Giovanni et al., 2015). The use of microbial source tracking or evaluating both human and non-human sources of bacterial contamination is infrequently used in risk assessments, especially within QMRA.

Using QMRA to predict the probability of risks of infection and illness for a specific waterbody can assist in developing site-specific risk levels. Reference pathogens can be used rather than FIB to evaluate the probable risk of infection and illness (McBride et al., 2013). The U.S. EPA has recently acknowledged the significance and applicability of QMRA to calculate the potential human health risks from non-human sources (U.S. EPA, 2010, 2012). Soller et al. (2010b) assumed hypothetical sites with bacteria concentrations set at the regulatory limit of 126 cfu 100 mL^{-1} of *E. coli* and 35 cfu 100

mL^{-1} of enterococci and extrapolated the risk to human health by assigning reference pathogens for the different bacterial sources, including *Giardia* spp., *Cryptosporidium* spp., *Salmonella enterica*, norovirus, *E. coli* O157:H7, and *Campylobacter jejuni.* Schoen and Ashbolt (2010) assumed the regulatory standard of enterococci (35 cfu 100 mL^{-1}) to calculate the risk for GI illnesses from different fecal contamination sources, including gull fecal waste and publically owned sewage treatment works (POTW). After applying reference pathogens for each contaminant source, *Campylobacter jejuni* and *Salmonella enterica* for gull waste, and norovirus*, Giardia intestinalis, Cryptosporidium* spp. and *S. enterica* for POTW waste, the gull fecal waste was found to be at least 2 log_{10} units lower than the illness benchmark of 0.01 as well as being less than the risk of infection from POTW waste (Schoen and Ashbolt, 2010). Soller et al. (2014) determined the risk of infection and illness in waterbodies impacted by human and non-human fecal waste sources to be predominately influenced by the source that had the greatest risk for human infection.

In this study, BST results from a rural waterbody in the State of Texas were combined with QMRA to calculate the probability of the risks of a GI infection and illness when accounting for both human and non-human sources of fecal contamination. The risk of a GI illness was calculated for a site with *E. coli* levels exceeding the U.S. EPA regulatory standard of 126 cfu 100 mL^{-1}. The total probability of illness, which combined the illness risk from both the human and non-human sources, was calculated to determine if the illness risk was within the acceptable benchmark level of 0.036.

II.3 Methods

II.3.1 Watershed Description

The Leon River watershed, specifically below Proctor Lake and above Belton Lake, is located within the Brazos River Basin. The main stem of the river flows through a predominately rural region encompassing row crops and rangeland (Gregory et al., 2013). Over 74% of the watershed has been classified as rangeland while 18% of the watershed has been classified as forestland (Bragg et al., 2015). The main stem of the river, segment 1221, extends approximately 306 kilometers crossing portions of Comanche, Erath, Hamilton, Mills and Coryell counties until reaching Belton Lake (Figure 2.1). Dairy cattle and concentrated animal feeding operations (CAFOs) exist in the northern part of the watershed (Gregory et al., 2013; Bragg et al., 2015). Due to the rural nature of most of the watershed, many residences and businesses have onsite sewage facilities (Bragg et al., 2015).

The main segment of the Leon River was added to the 303(d) List in 1996 for having bacteria levels exceeding the regulatory standards for its designated use for contact recreation (Gregory et al., 2013). The Leon River underwent extensive monitoring from 2011 to 2012 to conduct BST for 15 sites and to better understand the contributing sources of bacteria pollution in the river. Of the 15 sites, two sites had geometric means that exceeded the primary contact recreational standard of 126 cfu 100 mL^{-1} of *E. coli*

(Gregory et al., 2013). Water quality monitoring of station 11818 located on the Indian Creek tributary and abbreviated as site LEO 3, had the greatest *E. coli* geometric mean of 225 cfu 100 mL^{-1}. Site LEO 2, which included station 17379, had an *E. coli* geometric mean of 163 cfu 100 mL^{-1} and was located on the Walnut Creek tributary. Bacteria data recorded for the Leon River watershed were periodically elevated, most likely due to non-point source pollution flowing into the river during and after runoff events (Gregory et al., 2013). While sampling during 2011, all counties within the watershed experienced extreme to exceptional drought conditions, hindering water sampling due to pooled or dried areas of the river and its tributaries. The drought conditions could have potentially impacted the measured proportions of fecal sources from BST.

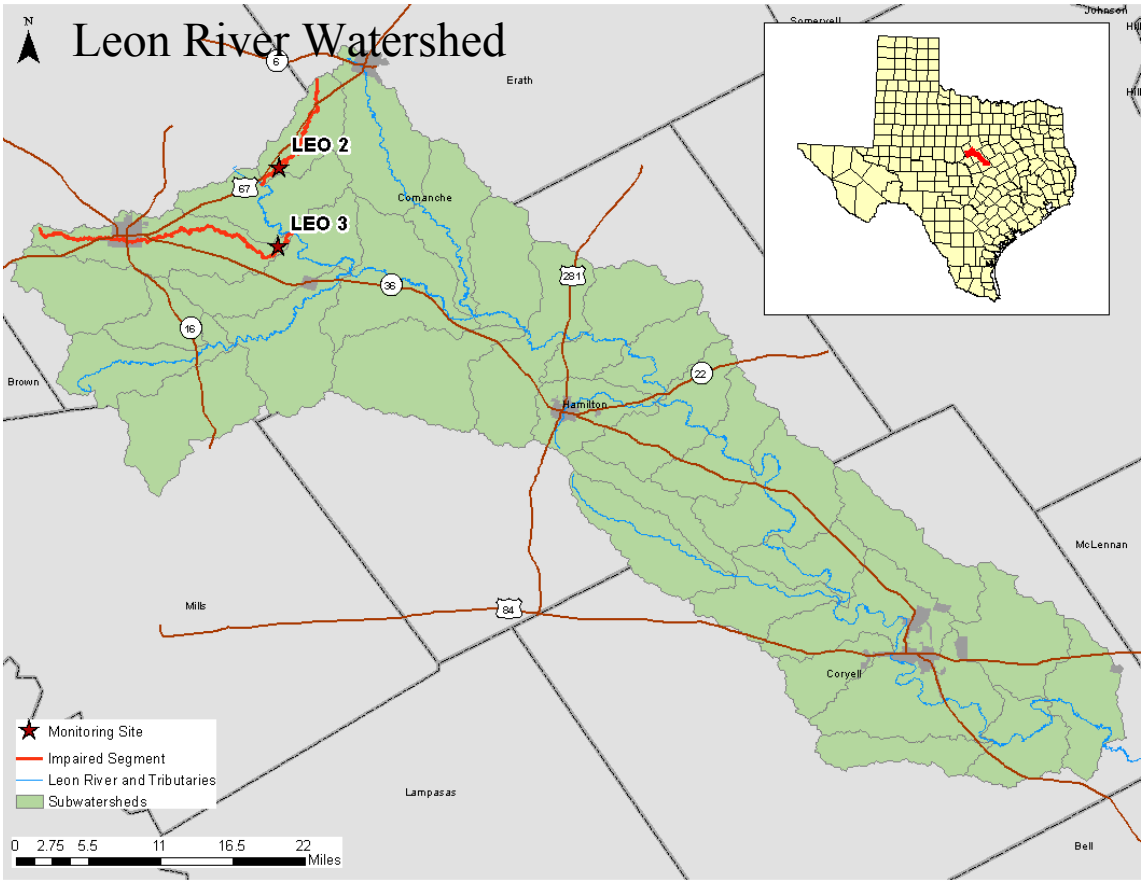

Figure 2.1. Geographical Overview of the Leon River watershed and sites LEO 2 and LEO 3. LEO 2 lies in Walnut Creek while LEO 3 lies in Indian Creek.

II.3.2 *E. coli* Monitoring Data and Reference Pathogens Used

Surface water quality data were obtained from Gregory et al. (2013), which included measured *E. coli* concentrations that were used to calculate the geometric means for sites monitored in the Leon River watershed. The monitoring data were collected between 2011 and 2012 as part of a Texas State Soil and Water Conservation Board (TSSWCB) funded project supplementing the development and implementation of a Watershed Protection Plan (WPP) for the Leon River Watershed. BST percentages were retrieved from the technical report compiled by Gregory et al. (2013). Site LEO 2 of the Leon

River watershed was used as the site of interest to conduct a risk assessment since the available BST data included only 3% unidentified sources. While LEO 3 had *E. coli* levels exceeding the recreational standard, the site was not used in the assessment because more than 25% of the BST results were determined to be from unidentified sources and only four bacteria samples were taken from the site. Site LEO 2 was determined to serve as a better case study site to conduct this risk assessment. The scenarios used to calculate the human health risk applied a range of proportions for each source. When using ribotyping to identify the sources of *E. coli* isolates, not all ribotypes may be matched to a host origin within the specified level of certainty, therefore causing a percentage of results to be labeled as unidentified (Hartel et al., 2003). Therefore, to conduct a worst-case risk assessment scenario, the unidentified source will be considered to be of human origin.

Risk assessments were conducted for two *E. coli* concentrations: the geometric mean of *E. coli* for site LEO 2 and the recreational standard. Reference pathogens were used to assess the fate, transport, and infectivity of microbial groups that cause GI infections and illnesses. The reference pathogen, which has been used in prior studies as the human source of fecal contamination was norovirus (Schoen and Ashbolt, 2011, Soller et al., 2010b, 2014). The human enteric virus, norovirus, has been found to represent the majority of observed swimming-associated gastrointestinal illnesses from contact recreation in human waste-impacted waterbodies (Schoen and Ashbolt, 2011). The reference pathogen used for the non-human sources of fecal contamination from cattle

and domestic animals was *Campylobacter*. The risk agent *Campylobacter* has been found to be a predominant pathogen in water impacted by cattle, pig, and chicken waste (U.S. EPA, 2010).

Prior use of a reference pathogen to determine the risk of infectious human strains transported in wildlife fecal waste remains a novel topic. Schoen and Ashbolt (2010) and Soller et al. (2010b) used *Campylobacter jejuni* and *Salmonella enterica* as reference pathogens for gull fecal waste. *Cryptosporidium* has been a commonly identified pathogen in waterborne outbreaks recorded in the United States. The two species, *C. hominis* and *C. parvum,* have been found to be the dominant species infecting humans and accounting for more than 90% of documented cases (Carmena, 2010). *Cryptosporidium* is also known to infect a variety of vertebrate hosts including humans, rats, dogs, sheep, cattle, birds, fish, mice, and many others. Recent research has indicated that *Cryptosporidium parvum* can be passed easily to water by bird feces, yet the wildlife genotypes of this pathogen can be host-specific and not human adapted (Graczyk et al., 2008; Carmena, 2010). When suspended in natural surface waters, *Cryptosporidium* oocysts can be expected to survive up to six months. Therefore, *Cryptosporidium* spp. appeared to be a useful reference pathogen for addressing the infection and illness risks from wildlife fecal waste present in a surface waterbody used for recreational contact. The selected reference pathogens used to represent each source are described in Table 2.1.

Table 2.1. Sources contributing to the bacteria impairment and corresponding reference pathogens.

Sources	Reference Pathogen	Reference
Human	Norovirus	Soller et al. (2010b); McBride et al. (2013)
Cattle/Domestic Animals	*Campylobacter*	Soller et al. (2010b, 2014)
Wildlife	*Cryptosporidium*	Atwill et al. (2002); Appelbee et al. (2005)

II.3.3 Exposure and Dose Calculations

Several variables and assumptions were made when developing the exposure scenario for the human health risks of primary contact recreation in the Leon River watershed. Adult populations were assessed because of the availability of dose-response data and ingestion rates of water. While other routes of exposure can occur from primary contact recreation in water including inhalation, dermal and conjunctive exposure, they were not included in this risk analysis. Input estimates used for water ingestion for adults have been described as a fitted distribution with an arithmetic mean of 25 mL hr^{-1} and a standard deviation of 5 mL hr^{-1} (Dufour et al., 2006; Sunger and Haas, 2015).

The scenario analyzed was a single recreational event for an adult with the total probability of contracting a NGI illness due to incidental ingestion while swimming or playing in the water. The estimated risk level of the single recreational event was to be compared to the benchmark risk standard for contact recreation (U.S. EPA, 2012). The mixture of *E. coli* found in the Leon River is described in Gregory et al. (2013) as an

estimated portion of the total indicator bacteria load from each contributing source. The BST results that were used in the assessment were gathered from a four-way split, which included cattle, domestic animals, human, and wildlife. Avian and non-avian livestock as well as pets were included with the cattle source. Wildlife included all avian and non-avian animals, such as feral hogs and deer. "Unidentified" was also included in the split for site LEO 2 and was joined with the human source to conduct a worst-case scenario assessment. Wildlife and cattle/domestic animals were each combined into groups and assigned a single reference pathogen due to the limited differentiation between individual species in the BST analysis for site LEO 2 and the lack of available pathogen density, infectivity and illness for each species. When developing the cumulative pathogen dose from the three different fecal sources (human/unidentified, cattle/domestic animals, and wildlife), the contribution of each source to the *E. coli* concentration as described in the BST results was used to calculate the dose of reference pathogens from each source and, therefore, health risk.

The reference pathogen dose was developed from formulas discussed in both Schoen and Ashbolt (2010) and Soller et al. (2010b). The formula to calculate the dose of each reference pathogen used in this study is described below:

$$D_{RP}^{S} = \frac{C_{E.coli} * F^{S}}{R_{E.coli}^{S} \times 100} \times R_{RP}^{S} \times P_{RP}^{S} \times I_{RP}^{S} \times V$$

The input variables are defined as:

D_{RP}^{S}= dose

S= specified source

$C_{E.coli}$= density of the bacterial indicator *E. coli* in the waterbody ($cfu\ 100\ mL^{-1}$)

F^s= fraction of the total amount of indicator bacteria from the specific source

$R^S_{E.coli}$= density of the bacterial indicator, *E. coli*, to the wet mass of the non-human waste or human waste ($cfu\ g^{-1} or\ cfu\ L^{-1}$)

R^S_{RP}= density of the reference pathogen in the fecal waste (wet mass) or in sewage ($cfu\ g^{-1} or\ cfu\ L^{-1}$)

P^S_{RP}= prevalence of infection for the reference pathogen and source

I^S_{RP}= infectious potential of the reference pathogen in humans

V= water volume ingested (mL)

Cattle and other domestic animals were grouped together and represented by the reference pathogen, *Campylobacter*. The human source, including poorly treated and primary sewage as well as municipal effluent, was represented by norovirus. The reference pathogen, *Cryptosporidium*, was used to represent the fecal contamination input by wildlife (Table 2.1). The range of *E. coli* concentration in wildlife fecal waste was calculated by taking the $\log_{10}$ of the lowest and highest *E. coli* concentration measured in a variety of Texas wildlife waste, as described in Padia et al. (2012) and in Telesford-Checkley (2014). Table 2.2 lists the input parameters for calculating the ingested dose of each pathogen.

Table 2.2. Parameters applied for calculating the dose ingested, the risk of infection, and the risk of illness.

Parameters	*Input Data*	*Comments*	*Distribution*	*Source*
Volume of Water Ingested	Arithmetic mean: 25 mL hr^{-1}		Normal	Dufour et al. (2006); Sunger and Haas (2015)
	Standard deviation: 5 mL hr^{-1}			
Density of *E. coli* in Fecal Waste (Log_{10} range)	Human: 0.5-8.0	Log_{10} range	Log-Uniform	Soller et al. (2010b)
	Cattle/Domestic Animal: 5.0-6.7			Soller et al. (2010b)
	Wildlife: 2.0-9.5			Padia et al. (2012); Telesford-Checkley (2014)
Density of Reference Pathogen in Fecal Waste (Log_{10} range)	Human (Norovirus): 3.0-7.5	Log_{10} range	Log-Uniform	Soller et al. (2010b); Schoen and Ashbolt (2010); assumptions based on pathogenic strains found in Soller et al. (2014) and Atwill et al. (2002)
	Cattle/Domestic Animal (*Campylobacter*): 1.2-7.3			
	Wildlife (*Cryptosporidium*): 2.3-3.8			
Prevalence of Infection	Human: 100%	Percent ranges	Uniform	Appelbee et al. (2005); Soller et al. (2010b), (2014), (2015)
	Cattle/Domestic Animal: 5-38%			
	Wildlife: 5-50%			
Infectious Potential	Human: 100%	Percent ranges	Uniform	Soller et al. (2010b), (2015)
	Cattle/Domestic Animal: 67-100%			
	Wildlife: 0-33%			
Dose-Response Values[1]	Norovirus: α=0.04, β=0.055	Beta-Binomial (ID50: 26 viral particles, 60% morbidity)[2]	Point Estimate	Teunis et al. (2005); U.S. EPA (2006); McBride et al. (2013)
	Campylobacter: α=0.145, N_{50}=7.59	Beta-Poisson (ID50: 800 cfu, morbidity 28%)[2]		
	Cryptosporidium: r=0.09	Exponential (ID50: 8 oocysts, morbidity 50%)[2]		
E. coli Data	LEO 2 (geometric mean: 163)	point estimates of the geometric mean (cfu 100 mL^{-1})	Point Estimate	Gregory et al. (2013)
	Recreational Standard (geometric mean: 126)			

1. The dose-response parameters are numerical values used in a statistical distribution to describe the host-pathogen interaction. The parameters listed above are for infection risk.
2. The ID50 is the median infective dose of pathogens contributing to infection.

Input parameters for *E. coli* density, reference pathogen density, and prevalence of infection for human and cattle/domestic animal sources were obtained from Soller et al. (2010b) and Soller et al. (2014). *Cryptosporidium* pathogen density in wildlife fecal waste was retrieved from a California study (Atwill et al., 2002). The prevalence of infection describes the percentage of animals likely shedding the selected reference pathogen at any given time. Similar to Soller et al. (2015), herd level prevalence was not assessed and conservatively assumed to be 100%. Herd level prevalence, unlike the prevalence of infection, describes the fraction of herds that would have at least one individual shedding the pathogen at any time (Soller et al., 2015). The range for the prevalence of infection of *Cryptosporidium* in wildlife was gathered from Appelbee et al. (2005), who synthesized studies reviewing the concentrations of *Cryptosporidium* oocysts in wildlife fecal waste. The range for the prevalence of infection was selected for wildlife species in the United States and for values that were measured to be statistically significant.

The human infectious potential or relative fraction of human infectious strains of each reference pathogen for the respective sources remains uncertain. Ranges for the infectivity potential were not found in the literature, restricting assignment of quantitative values. Following the methods by Soller et al. (2015), qualitative values can be assigned for pathogenicity. Since not all pathogens shed by animals can infect humans, a range of infectivity is necessary. For cattle/domestic animals, infectivity is considered high, with the infectious potential ranging from 67 to 100% (Soller et al.,

2010b; Soller et al., 2015). Most *Cryptosporidium* genotypes have been determined to have a narrow host range and while humans can be included in the host range, most wildlife mammals are considered to not contribute to a significant public health concern (Appelbee et al., 2005). Infectivity for wildlife is therefore considered low, ranging from 0 to 33% (according to the low infectivity ranges in Soller et al., 2010b, 2015). Human infectivity is considered 100% for norovirus. The infectivity for the enteric virus was 100% since the human waste source is assumed to not be individual fecal samples but sewage composite.

The dose-response model parameters described in Table 2.2 were used to determine the probability of infection for each source and its reference pathogen in the risk characterization phase of the risk assessment. Both dose-response models for *Campylobacter jejuni* and *Cryptosporidium* spp. have previously been used in other QMRA studies, including Soller et al. (2010b) and Soller et al. (2014), and have established distribution models. Few dose-response studies have been conducted for norovirus. One model distribution developed by Teunis et al. (2008) selected a confluent hypergeometric function to model norovirus when assuming viral aggregation of particles and basing the model upon an average dose in a clinical setting. McBride et al. (2013) presented a beta-binomial function model, based upon the beta-Poisson model that was developed by Teunis et al. (2008). Assumptions made in McBride et al.'s model included modeling the infection based on individual exposure, which is therefore the "average dose for an individual." It is necessary to assume there is no Poisson

distribution associated with the dose ingested to develop smooth cumulative frequencies, which permit the dose-response curve for norovirus to be simplified to a beta-binomial distribution (McBride et al., 2013). The beta-binomial distribution was used to calculate the probability for the risk of infection from norovirus as opposed to the confluent hypergeometric function developed by Teunis et al. (2008). The McBride et al. model also made the assumption to ignore aggregation, which helped prevent overestimating the risk of infection at low doses (McBride et al., 2013).

The total probability of illness was calculated to account for the overall human health risk from a mixture of sources. The total probability for the risk of illness (P_{ill}) is the product of multiplying the complement risk for each source. The parameter P_{ill}^{S} describes the probability for a GI illness from each reference pathogen (representing a fecal source). The formula computes the overall risk in the case that the individual risks are low or high, ensuring that a total probability risk value is below one (provided by J. Soller via personal communication).

$$P_{ill} = 1 - \prod (1 - P_{ill}^{S})$$

II.3.4 Calculating and Characterizing the Human Health Risk

The risk assessment evaluated the probabilities of a gastrointestinal illness (as defined previously as NGI) for waterbodies exceeding the bacteria standards for primary contact recreation when a mixture of fecal sources is present. A probabilistic analysis was employed for several of the input parameters used in the dose formula. Certain input

parameters were given distributions to account for the uncertainty and variability associated with the parameter (see Table 2.2). Water ingestion was calculated under the probabilistic approach of a lognormal distribution with an arithmetic mean of 25 mL hr^{-1} of water ingested and a standard deviation of 5 mL hr^{-1} (Dufour et al., 2006; Sunger and Haas, 2015). The percent of each source contributing to the bacteria concentration was calculated as a point estimate. A log-uniform distribution was used to describe the density of *E. coli* and reference pathogens in human, cattle/domestic animals and wildlife waste.

Applying the probabilistic approach to develop probability distributions for several of the parameters will assist in evaluating the uncertainty in the risk model and in identifying which parameters contribute to the greatest amount of uncertainty (Eisenberg et al., 1996). Similar distribution assumptions were made by Schoen and Ashbolt (2010) when the input parameters gathered from the literature ranged several orders of magnitude. Eisenberg et al. (1996) used a log-uniform distribution to prevent any bias toward a specific value that was gathered from the literature. A uniform distribution was used to describe the prevalence of infection for the pathogen as well as the human infectious fraction. Values used for the prevalence of infection were gathered from the literature. Rather than using the point estimate of the midpoint of the ranges of the human infectious fraction for each non-human source as done in Soller et al. (2010b), a uniform distribution was used to account for the natural variability of human infectious

fractions that could potentially exist.

Monte Carlo simulations were conducted to develop a distribution of the pathogen dose from each source. The estimates of the probable pathogen dose were then applied to calculate the probability of infection risk. Each probability for the risk of infection was then multiplied by the best estimate of the conditional probability (of an infection occurring) of illness. The Monte Carlo assessment was conducted with 10,000 simulations to calculate the probability for the risk of illness (Schoen and Ashbolt, 2010). The dose-response parameters and conditional probability of illness rates used in the simulations were point estimates.

The simulations estimated the probability of a GI illness risk based upon the reference pathogen input from the three different sources. Crystal Ball Pro®, distributed by Oracle, was used to conduct the Monte Carlo simulations. Risk estimates were calculated with a 95% confidence level, and the median, 5^{th}, 25^{th}, 75^{th}, and 95^{th} percentile values of each risk estimate were used in the analyses. The simulated scenarios are described as follows:

Scenario 1: Each source contributes 100% of the FIB load when calculating the risk of a GI illness.

Scenario 2: The total probability for the risk of illness is calculated based upon the BST

results (four-way split); 10% human (combined with unidentified), 25% cattle/domestic animals, and 65% wildlife.

Scenario 2 Modified: The BST results are modified, separating cattle and domestic animals, and are applied to calculate the total probability of illness risk (four-way split); 7% human, 20% cattle, and 73% wildlife/domestic animals/unidentified.

Scenario 3: Each source contributes equally to the bacteria load (33.3%) when calculating the total probability for the risk of illness.

II.4 Results

The total probability for the risk of a NGI illness for site LEO 2 and the recreational standard was calculated under each scenario. The regulatory standard for *E. coli*, 126 cfu 100 mL^{-1}, was incorporated to compare the predicted infection and illness risk for site LEO 2, which has been listed as impaired with a geometric mean of *E. coli* of 163 cfu 100 mL^{-1}. The risk estimates were calculated with a 95% confidence level.

II.4.1 Human Health Risks under Different Scenarios

Scenario 1: Each Source Contributing 100%

Under Scenario 1, the predicted risks for a GI illness were calculated for the *E. coli* concentration at site LEO 2 and for the contact recreational standard (Figure 2.2). The human source, as estimated by norovirus infectivity and illness, resulted in the greatest

median risk (0.31) for GI infection and illness (Figure 2.2). Wildlife source, as measured through *Cryptosporidium* infectivity and illness, had the lowest median risk for a GI illness of all three sources. However, the variance was much greater for the wildlife source than either the human or cattle/domestic animal sources. Cattle/domestic animals, as measured through *Campylobacter* infectivity and illness, had a risk for a GI illness that was slightly greater than for wildlife, but considerably less than human. The median calculated risk for a GI illness at LEO 2 for each source was as follows: human: 0.31, cattle/domestic animals: 0.13, and wildlife: 0.03. The median calculated risk for a GI illness when using the recreational standard was similar to LEO 2, except cattle/domestic animals had a slightly lower risk of 0.12 (Figure 2.2).

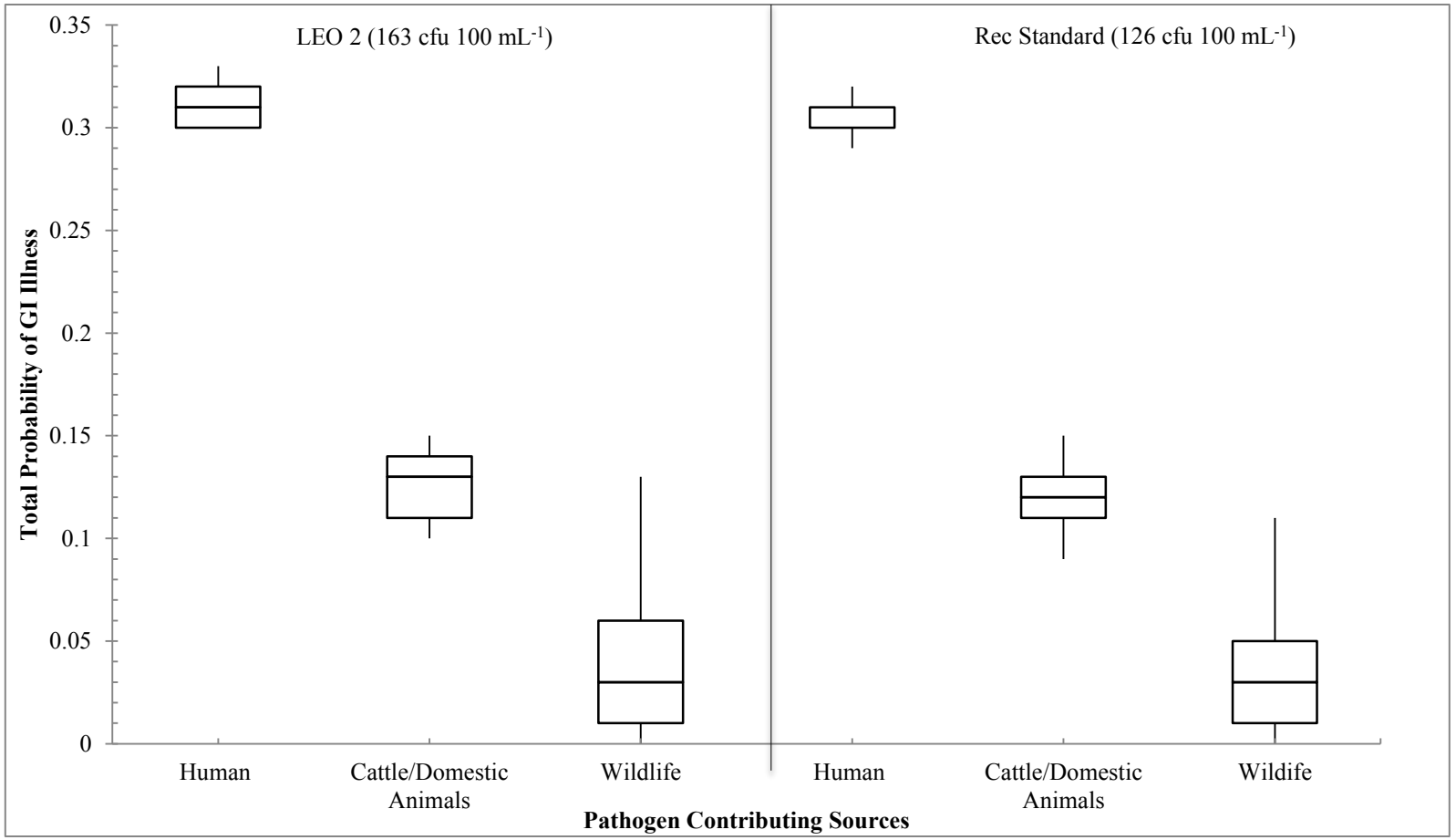

Figure 2.2. The calculated probabilities for a GI illness under Scenario 1 (each source contributes 100%). The 5th, 25th, 75th and 95th percentiles as well as the median are presented for the actual *E. coli* concentration (163 cfu 100 mL^{-1}) and current contact recreational standard (126 cfu 100 mL^{-1}).

Scenario 2: Contributing Sources Based upon Actual BST Results

Under Scenario 2, the human health risk was calculated for when each source contributed a specific proportion of the *E. coli* load, as measured by BST (Figure 2.3). Scenario 2 was also modified to combine domestic animals and unidentified sources with wildlife to produce a less conservative calculation. As expected from the previous scenario results, the human source contributed the greatest health risk while having the least contribution to the bacterial impairment for both site LEO 2 and the recreational standard. Wildlife, the largest contributing source, resulted in the least human health risk. There was not a large difference between the risks for site LEO 2 and the recreational standard, with the cattle/domestic animal source contributing a slightly increased risk of 0.09 (Figure 2.3).

While Scenario 2 (modified) served as the least conservative estimate for the risk to human health, the calculated human health risk was similar to that of Scenario 2. The human source was again found to contribute the greatest to overall risk followed by cattle, and wildlife/domestic animals (Figure 2.4). The overall human health risk for LEO 2 and the recreational standard was similar with only cattle/domestic animals contributing a slightly greater risk of 0.08.

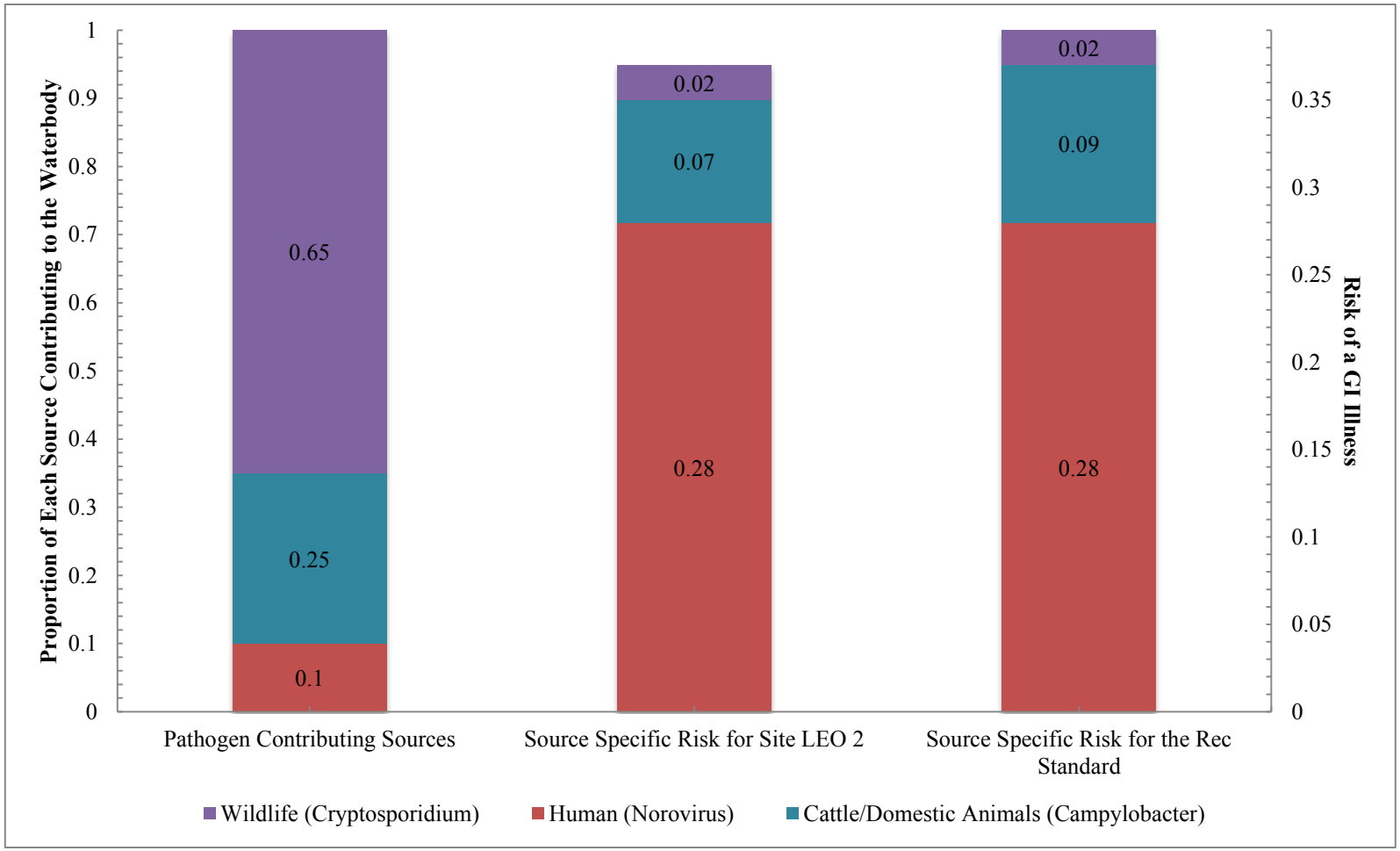

Figure 2.3. Contribution of each source to the health risk under Scenario 2 (LEO 2 had an *E. coli* concentration of 163 cfu 100 mL^{-1} and the recreational standard had an *E. coli* concentration of 126 cfu 100 mL^{-1}).

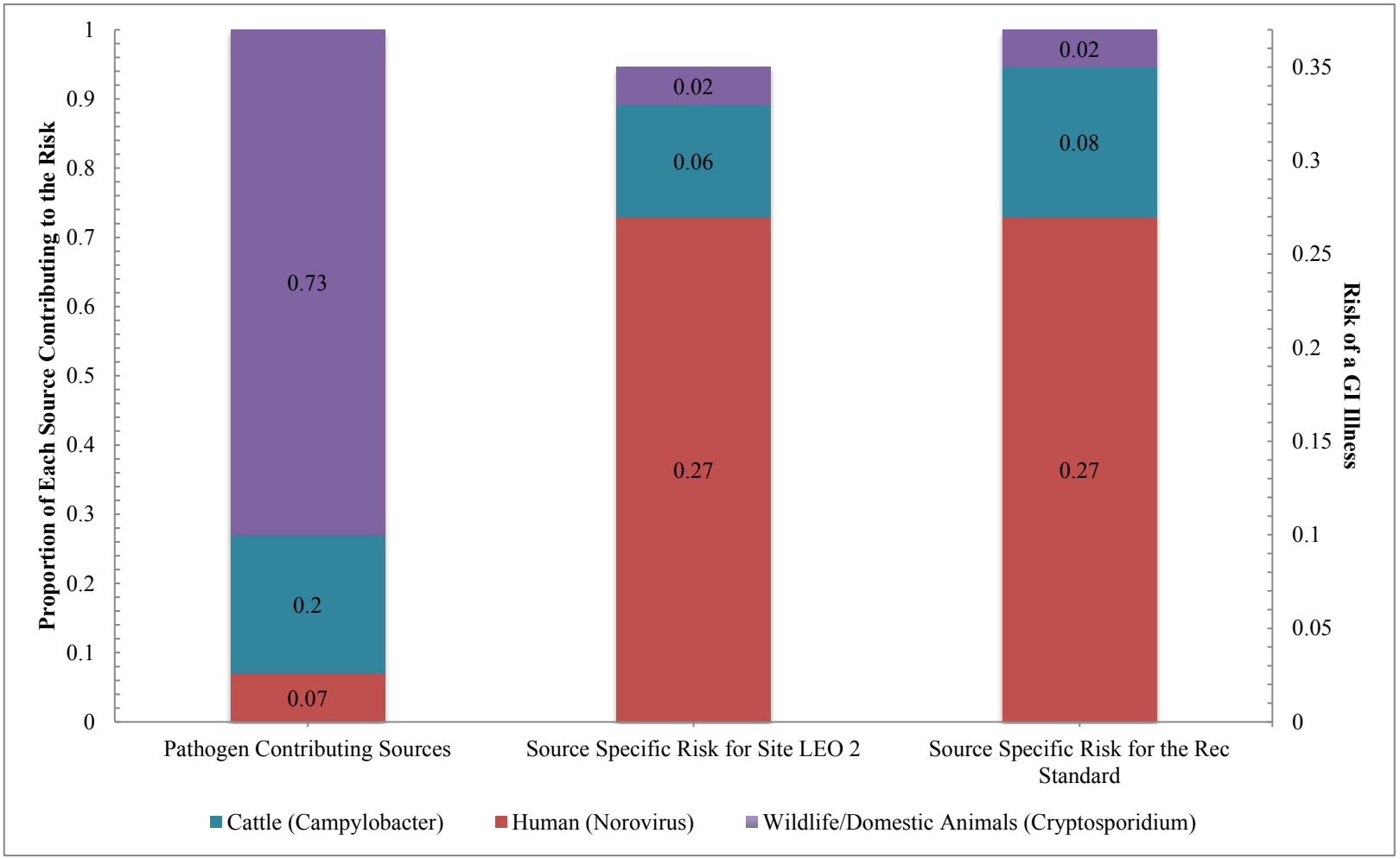

Figure 2.4. Contribution of each source to the health risk under Scenario 2 modified (LEO 2 had an *E. coli* concentration of 163 cfu 100 mL^{-1} and the recreational standard had an *E. coli* concentration of 126 cfu 100 mL^{-1}).

Scenario 3: Each Source Contributing Equally (33.3%)

When each source contributed equally to the bacteria load, the greatest contribution to the total probability of illness was from norovirus (0.3), followed by *Campylobacter* (0.07) and *Cryptosporidium* (0.01) for site LEO 2 (Figure 2.5). Under the recreational standard, norovirus had a slightly lower risk (0.29) and *Campylobacter* had a slightly greater risk (0.1). The estimated risk for a GI illness was similar to the other scenarios conducted (Figures 2.3, 2.4, and 2.5). Scenario 3 was conducted to evaluate the estimated risk when each source was found to contribute equally in a waterbody.

Comparison of the Total Probability of Illness Risks

Every scenario, a mixture of fecal sources, had an elevated human health risk of at least 0.34 with the greatest risk proportion from the human source (Figure 2.6). The difference in health risk when the human source contributed 33.3% as opposed to 7% was negligible (Figure 2.4 and 2.5). Due to the high host infectivity of norovirus, the risk remained relatively similar across each scenario, indicating that the proportion of cattle/domestic animals and wildlife fecal loading had a minimal impact on the overall risk for a GI illness (Figure 2.6).

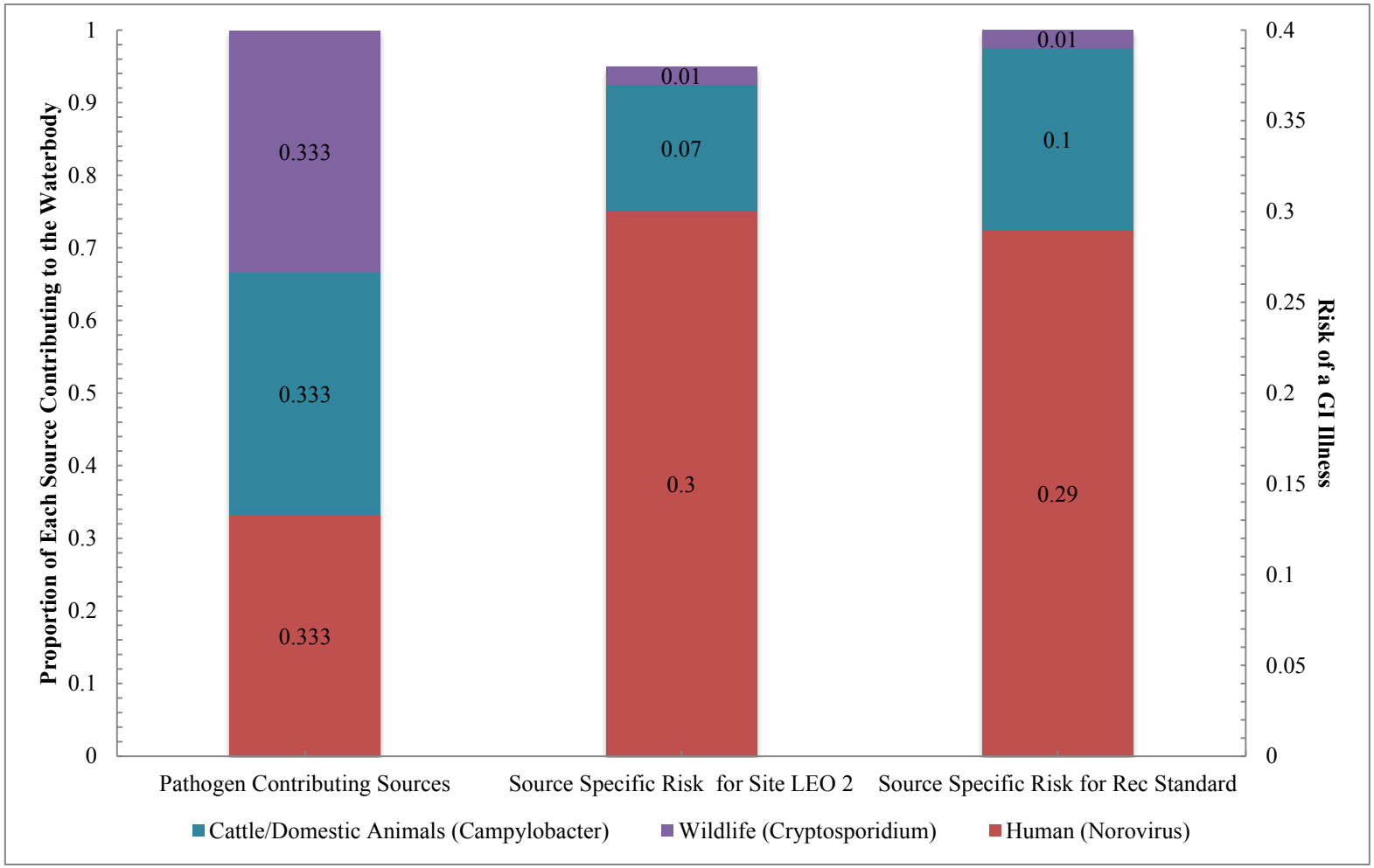

Figure 2.5. Contribution of each source to the human health risk under Scenario 3 (LEO 2 had an *E. coli* concentration of 163 cfu 100 mL^{-1} and the recreational standard had an *E. coli* concentration of 126 cfu 100 mL^{-1}).

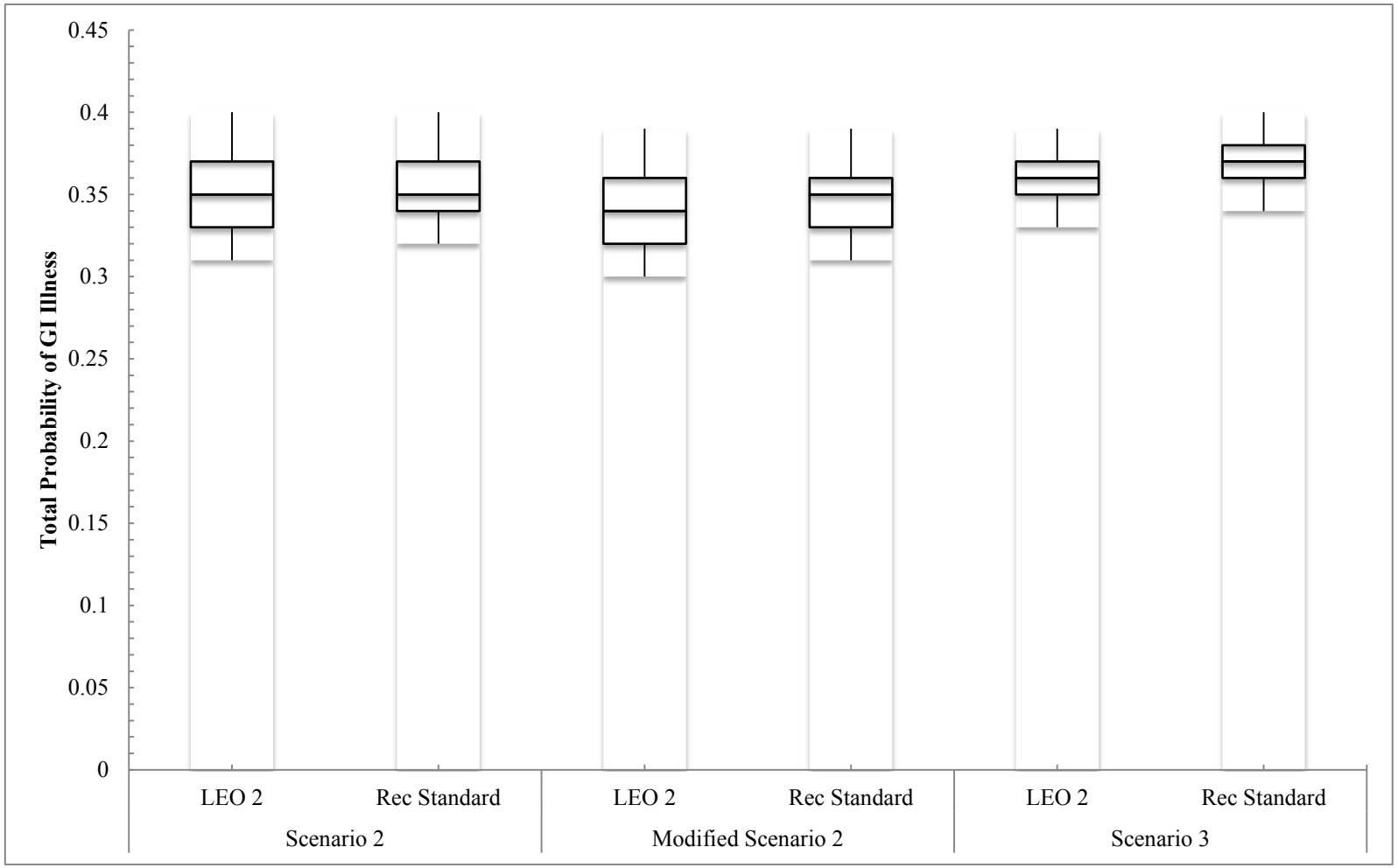

Figure 2.6. The calculated probabilities for a GI illness under Scenario 2, Modified Scenario 2, and Scenario 3. The 5th, 25th, 75th and 95th percentiles as well as the median are presented. LEO 2 had an *E. coli* concentration of 163 cfu 100 mL^{-1} and the recreational standard had an *E. coli* concentration of 126 cfu 100 mL^{-1}.

II.4.2 Sensitivity Analysis of the Uncertainty in the Simulations

A sensitivity analysis of the risk of illness was conducted for each scenario to identify the assumptions that contributed the greatest uncertainty. The assumptions, prevalence of infection and infectious potential of *Campylobacter* and *Cryptosporidium,* repeatedly contributed the greatest amount of uncertainty when calculating the risk of illness for each pathogen/source in each scenario (Appendix A). The assumption contributing the greatest amount of uncertainty when calculating the risk of illness from the human source (represented by norovirus) was attributed to the density of *E. coli* in human waste; the assumptions for the infectious potential and prevalence of infection for norovirus were point estimates of 100%. The proportion of each pathogenic source changed in each scenario, but those three assumptions were found to be the most important variables contributing uncertainty in the assessment. The assumptions for the volume of water ingested and density of *E. coli* in cattle/domestic animal and wildlife waste were found to be less important.

II.5 Discussion

II.5.1 Scenario Assessment and Risks of a GI Illness

The risk assessment quantified the probable risk of a GI infection and consequently illness from both human and non-human sources of fecal contamination. Three scenarios were evaluated to identify the influence of each source in different proportions and its selected reference pathogen on the total probable risk of a GI illness.

The findings indicated that the greatest risk for human health (in terms of a GI illness) was from the human source, as measured by the reference pathogen norovirus. None of the assessments met the recreational risk standard of 0.036, due to the elevated risk of a GI infection and illness from the potential exposure to norovirus in recreational waters. The risk for a GI illness ranged from 0.34 to 0.37. The risk of illness from *Campylobacter*, representing cattle/domestic animals, consistently had a greater median calculated risk for a GI illness than the wildlife source, which was measured by *Cryptosporidium* exposure. The proportion of each contributing source was not found to directly relate the overall human health risk. Norovirus, due to its host specificity, was considered to have 100% infectious prevalence and infectious potential and was found to have the greatest risk for human health when representing the human source.

II.5.2 Using Norovirus as a Reference Pathogen

As anticipated, there was a difference in risk levels for each source and the respective reference pathogen. Norovirus was selected as the human reference pathogen since recent research has indicated that enteric viruses, specifically norovirus, have caused the majority of swimming-associated GI illnesses (Soller et al., 2010a). The NEEAR study supported this and indicated the importance of considering enteric viruses as significant contributors for GI illnesses (Soller et al., 2010a, Schoen et al., 2011). While prior studies have found using norovirus as a reference pathogen to yield high infection and illness risks, the pathogen's human dose-response relationship still requires further research (Soller et al., 2010a, 2014; Schoen and Ashbolt, 2010; Schoen et al., 2011;

McBride et al., 2013). Dose-response models available for norovirus are based on young, healthy adults and the infection risk from a small dose of the virus is overestimated by the models when extrapolating from clinical doses to smaller environmental doses (Schoen et al., 2011). Generally, limited knowledge exists for the infectivity of noroviruses and previous models for most enteric viruses had been based on rotavirus (Teunis et al., 2008; McBride et al., 2013). Several limitations in developing a dose-response model for norovirus stems from various factors, including the difficulty of culturing the viruses as well as the limited published human studies that discuss the dose of the virus (Teunis et al., 2008). While using norovirus was an appropriate pathogen for this assessment, a more refined dose-response model would aid in understanding the risks of infection and illness in recreational waters from this pathogen.

II.5.3 Source Contributing the Greatest Human Health Risk

The human health risks associated with Scenarios 2 and 3 (when calculating the illness risk from a mixture of sources) were greatly driven by the human fecal source even when it was only contributing 7% of the total bacteria load (Figure 2.4). In Schoen and Ashbolt (2010), the human health risk was estimated for different ratios of fresh gull waste and POTW. The percentage of human waste, as represented by norovirus, was found to dominate the human health risk until gull waste contributed 98% of the fecal contamination load. The proportion of a single source contributing to the overall fecal indicator concentration is not an indicator of the overall human health risk.

Schoen et al. (2011) found similar results when 90% of the fecal bacteria came from non-pathogenic sources, and a large portion of the total risk was driven by recently discharged disinfected municipal wastewater. The risks of a GI illness from a recreational waterbody impacted by a mixture of fecal contamination sources was identified to be influenced by the infectious pathogen for humans (Schoen and Ashbolt, 2010; Schoen et al., 2011; Soller et al., 2014). The findings from this study indicate that the human health risk, in regards to recreation, is predominately driven by the most infectious source instead of the largest contributing source to a waterbody (Figures 2.3, 2.4, and 2.5). Similar to these findings, Soller et al. (2014) identified that when the proportion of human contamination was elevated, the risk was dominated by the human sources regardless of the other fecal sources present. Determining which source represents the most dominant human health risk can assist in targeting management efforts regarding sources contributing the greatest risk. The human source, while in the context of norovirus infectivity and illness, was identified as contributing the majority of the overall human health risk. Reducing point source and non-point source contributions of human waste to recreational waterbodies can mitigate this risk. Ideally, identifying the maximum proportion at which a human source can be present in a waterbody while continuing to meet the benchmark standard risk would facilitate those management efforts. Quantifying the maximum proportion of the human source to meet the recreational risk standard will require further evaluation of norovirus dose-response.

Studies have evaluated the human health risk associated with elevated concentrations of non-human fecal sources in recreational waters (Schoen and Ashbolt, 2010; Soller et al., 2010b, 2014). Schoen and Ashbolt (2010) determined that an enterococci concentration of 20,000 cfu 100 mL^{-1} from gull waste would be sufficient to elevate the human health risk to 0.01. While *Cryptosporidium* was selected as a representative reference pathogen for wildlife feces after an extensive literature review indicating its predominance in wildlife fecal samples, the specific wildlife species, location, and time of the year could influence which pathogens are present or in high concentrations (Soller et al., 2014).

II.5.4 Study Limitations

As with most risk assessments, some limitations and caveats exist for this study. For simplicity, unidentified and human sources from the BST analysis were combined while cattle and other domestic animals (including dogs, cats, and avian and non-avian livestock) were combined. Combining these sources aided in simplifying the calculations and was necessary due to limited literature values for reference pathogens for different contributing fecal sources in a waterbody. The cattle/domestic animal source was found to have a significantly lower human health risk than the human source. Previous studies have found fresh, directly deposited cattle manure to have a similar risk for a GI illness as human fecal waste (Soller et al., 2010b). Combining cattle with other domesticated animals (included in the density of *E. coli* and ranges in infectious risk and prevalence) may have underestimated the potential human health risk resulting from this source.

The FIB levels used in the calculations were assumed to be directly derived from fresh fecal contamination and not from other sources. Indicator bacteria, such as *E. coli*, can be re-suspended from sediment or have originated from other natural sources (Wheeler Alm et al., 2003; Pandey and Soupir, 2013). Sediment resuspension has been found to dramatically increase *E. coli* concentrations when compared to only measuring waterborne *E. coli* concentrations (Pandey and Soupir, 2013). Other sources that may have been potentially contributing to the measured FIB levels were not included in this assessment, but could potentially influence the proportion of identified fecal sources. BST can illustrate which sources are potentially contributing to the human health risk and therefore direct management efforts toward mitigating human health risks (Schoen et al., 2011).

The dose-response models used did not account for immunocompromised individuals, pregnant women, or children. These sensitive subpopulations may be more susceptible to pathogen infection or at a greater risk for a GI illness (Gerba et al., 1996). Few studies have identified children having stronger infection responses to the pathogen *Campylobacter* (Teunis et al., 2005; cited in McBride et al., 2013). The potential uncertainties in dose-response model parameters were not considered in the assessment since point estimates were used. While the dose-response model can assist in calculating the probability of infection, the probability of illness can be more difficult to determine and require mathematical distributions or point estimates, whichever is available (McBride et al., 2013).

II.5.5 QMRA Applicability for Site-Specific Standards

The negligible difference in the human health risk across scenarios raises the question as to the appropriateness of the current recreational standard for protecting human health (Figure 2.6). The elevated human health risk under the recreational standard when the human source contributed 7% indicated that even if the waterbody met the FIB standard of 126 cfu 100 mL^{-1}, the risk of infection and illness from norovirus would exceed 0.036 (Figures 2.4 and 2.6). Current efforts to assign site-specific recreational water quality standards, at least in Texas, typically require conducting Recreational Use Attainability Analyses (RUAAs), monitoring efforts, BST analyses, and Total Maximum Daily Loads (TMDL) and/or WPP development. Incorporating QMRA into this "toolbox" could assist in assessing the human health risks for a site, particularly if the site exceeds the FIB recreational standard and none of the contributing sources are human. Emphasizing the human health risk associated with a site, based upon BST, may be a more accurate, effective, and cost-efficient method for determining which waterbodies are of greatest risk for human health. While previous efforts have been directed at reducing FIB concentrations to the recreational standard, efforts to minimize the human health risk by targeting the sources representing the greatest risk may be more protective of human health, especially when funding is limited. However, additional analyses investigating pathogens especially transmissible from particular wildlife populations to humans should be reviewed and conducted.

Routine monitoring data can indicate if there is reason for concern, but management efforts to mitigate water quality issues must be selected based upon funding availability, time limitations, and effectiveness. Not only identifying potential causes and sources of impairment, but also understanding the routes by which pathogens reach the waterbody and how individuals may be exposed, can assist in developing a comprehensive narrative of the human health risk for different activities in the waterbody (Ashbolt et al., 2010). Incorporating QMRA into the water management "toolbox" can answer questions related to which activities are safe in a waterbody and which economical remediation efforts will lower the risk.

The integration of BST and QMRA has the potential to facilitate site-specific standards and to guide science-based management efforts (Ashbolt et al., 2010). The Leon River WPP included information derived using several tools including TMDL, RUAA results, BST analyses, and monitoring data to develop a comprehensive understanding of the attainability of contact recreation throughout the watershed (Bragg et al., 2015). As indicated in this risk assessment, the predominant human health risk stemmed from human sources. Waterbodies with BST data could benefit from QMRA to determine which measured fecal source is contributing the greatest risk and therefore, which funded management efforts would potentially mitigate that source. Management strategies for pollutant sources listed in the Leon River WPP included efforts to mitigate human sources of pollution, including improving and maintaining wastewater treatment facilities, replacing sewers, addressing failing OSSFs, planning and managing sanitary

sewer overflows, as well as management efforts for addressing direct deposition and non-point source pollution from wildlife and domesticated animals (including livestock) (Bragg et al., 2015). Based upon the risk assessment, mitigation of human pollution sources would assist in reducing the human health risk much more so than reduction from other non-human sources.

Stakeholder discussions could include QMRA findings on the risks associated with the different fecal sources. Incorporating these findings could facilitate which management strategies are chosen to be practiced or implemented in a waterbody. Informing stakeholders of the differences in risks between sources is necessary, especially since management efforts recommended by WPPs are voluntary and selected based upon stakeholder input (Bragg et al. 2015).

II.5.6 Future Research Considerations

This risk assessment relied greatly upon previous QMRAs of human and non-human fecal sources. The reference pathogens selected, except when assessing the risk of illness from wildlife, have been previously used in the published literature (Soller et al., 2010a, 2010b, 2014, 2015; McBride et al., 2013, Schoen and Ashbolt, 2010). Additional data, especially for the variables infectious prevalence and potential for infection, may assist in refining the potential human health risks calculated. The risk analysis was conducted assuming that pathogen loads reaching the water were fresh and no pathogen decay occurred. There is differential persistence of pathogens and FIB, which could potentially

yield differing results since not all pathogenic organisms or FIB decay at similar rates (Soller et al., 2010b, Cheng et al., 2013). Pathogen and FIB persistence could potentially be another variable incorporated into future risk assessment work, especially when calculating the human health risk associated with rural and urban run-off (McBride et al., 2013; Soller et al., 2014, 2015). The recreational risk standard discussed in the 2012 RWQC was developed based upon an aged human contamination mixture; therefore, the risk results reported in this study, which are based upon fresh fecal contamination, are expected to be higher than the standard (RWQC, 2012; Soller et al., 2014). Further consideration of FIB and pathogen die-off and inactivation could impact the human health risk, but remains poorly understood in real-world context (Soller et al., 2014).

Regulatory and management considerations can be developed from these results, especially for waterbodies that are not impacted by human sources. Documented and future BST results integrated with a QMRA for impaired waterbodies can be used to better understand the human health risk associated with the contributing bacterial sources and whether developing site-specific water quality standards is appropriate. The infectivity of norovirus requires further review to identify an acceptable percentage of human fecal waste in a waterbody that would meet the regulatory risk standard. Improvements to the norovirus model and more data to support the assumptions in a risk model such as inactivation and decay rate, infectious prevalence and human infectious fraction would assist in developing a risk model that may better reflect site-specific environmental conditions. The risk assessment conducted served as a worst-case

scenario. The estimated risk results however do question whether the current primary contact recreational standard for FIB is appropriate since researchers now have a better understanding of which pathogens are causing GI illnesses and their infectivity.

II.6. Conclusions

Specifying the sources contributing to a bacterial impairment in a waterbody can assist in identifying the potential human health risk, especially when differentiating human and non-human sources. Depending on the source, pathogens will have different infectivity and prevalence and, therefore, a different human health risk. Identifying the sources contributing to the FIB impairments and applying QMRA may be a useful practice to develop site-specific standards, especially when FIB may exceed the recreational *E.coli* standard. The estimated human health risk for a GI illness did not differ when LEO 2 was listed as impaired for FIB, even if the site did meet the recreational standard. Assessing the risk for a waterbody based upon the sources contributing to the FIB concentrations and estimated human health risk can be used to supplement management decisions. Human fecal waste sources tend to have a greater health risk contribution than non-human waste sources, and differentiating risk sources would improve human health risk estimations.

CHAPTER III

CONCLUSIONS

Risk assessments, specifically QMRA, have been used to establish food safety standards and develop safe drinking water and air quality regulations. QMRA can be used to characterize the human health risks associated with impaired recreational waterbodies, especially when mixtures of fecal sources are present (Soller et al., 2015). The risk assessment conducted in this study provides a quantitative comparison of human health risks associated with different fecal sources in a rural waterbody. The hypotheses tested attempted to clarify the difference in risks associated with human and non-human fecal sources.

- The first hypothesis calculated whether the GI infection and illness differed among the three sources and their representative reference pathogens. When calculating the human health risk for each source, the calculated risk for a GI illness was found not to be the same for the human and non-human sources. The human source, norovirus, had a calculated risk that was at least one log order greater than the calculated risk for the wildlife source, therefore causing rejection of the null hypothesis.
- Under the second hypothesis, the risk for a GI illness at site LEO 2 and the recreational standard were calculated to determine if identifying the sources affected the human health risk and if the calculated risk was below the human health benchmark of 0.036. In all simulations, the human health risk exceeded

the benchmark standard, therefore the null hypothesis was not rejected. Norovirus was found to dominate the risk calculations, causing the probabilities for a GI illness to exceed the benchmark standard by one log order.

Based upon the assumptions made in this assessment, the human fecal source, represented by norovirus infection and illness, was found to be the predominant source and pathogen affecting human health. Identifying the maximum percentage the human fecal source can be present in a waterbody (based upon the selected reference pathogen such as norovirus) and the recreational risk standard can be met can facilitate the use of BST and QMRA in identifying waterbodies that may potentially pose a risk for human health. Modeling pathogen transport and decay in a waterbody would provide a more accurate representation of the potential concentration ingested. A risk assessment that modeled pathogen transport and decay would also assist in developing a representation of discharge and run-off and the potential contribution of pathogens from precipitation-driven events. Applying two or three reference pathogens could help develop an overall infection and illness risk for a GI illness for that source. The highly infectious nature of norovirus indicates that sites that are impaired at least by a measurable percentage of a human source would benefit from efforts targeted towards the management of human fecal source.

The relative proportion of the contributing fecal source in a waterbody was not found to represent the risk of illness, but rather the presence of human fecal waste was found to

elevate the human health risk to at least one log order greater than the recreational risk standard of 0.036 for a GI illness. The study provided evidence that while a non-human fecal source may be predominate in a waterbody, the presence of human fecal waste may cause an elevated human health risk. Identifying the fecal sources contributing to a waterbody's bacterial impairment is necessary to direct management efforts to improve recreational water quality and protect human health.

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APPENDIX A

Scenario 1

Forecast Results from LEO 2 Simulations

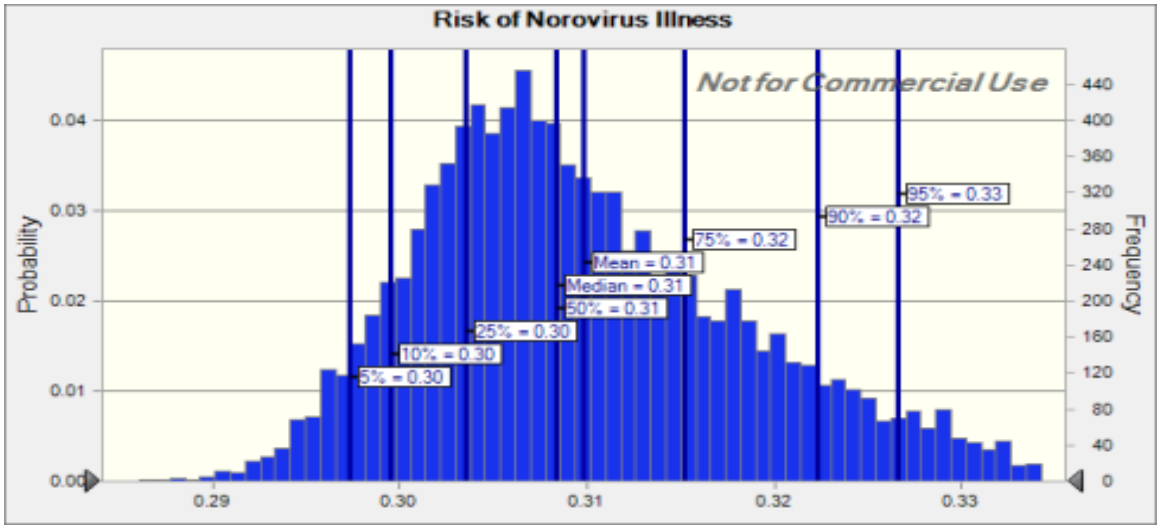

Figure A-1. Risk of a GI illness from norovirus infections (human source).

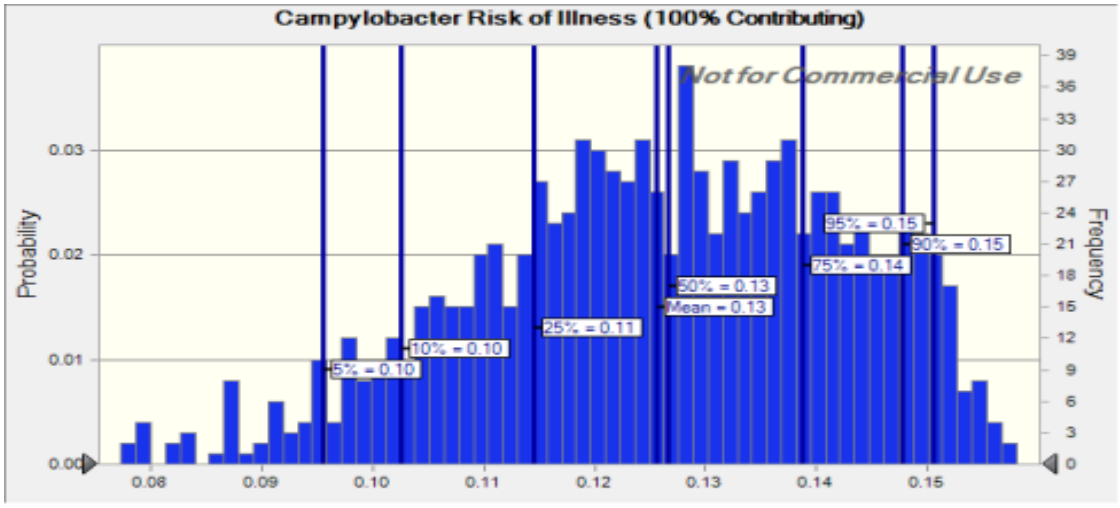

Figure A-2. Risk of a GI illness from *Campylobacter* infections (cattle/domestic animal source).

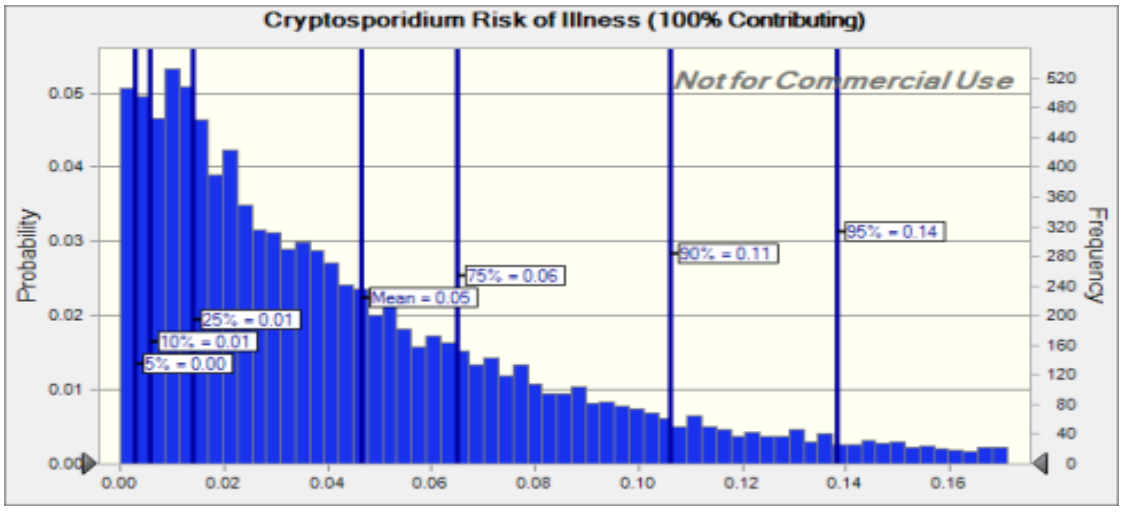

Figure A-3. Risk of a GI illness from *Cryptosporidium* infections (wildlife source).

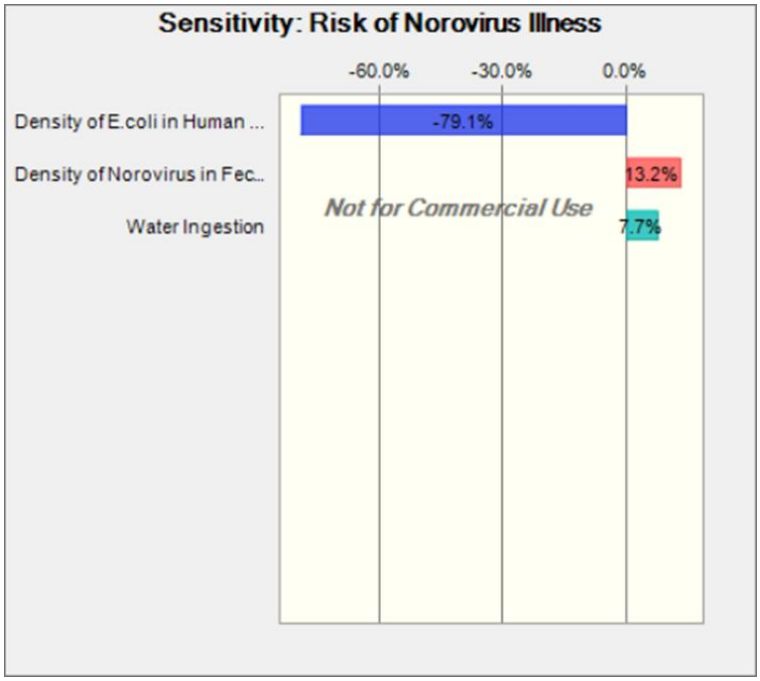

Figure A-4. Sensitivity analysis results for calculating the risk of a GI illness from norovirus (human source).

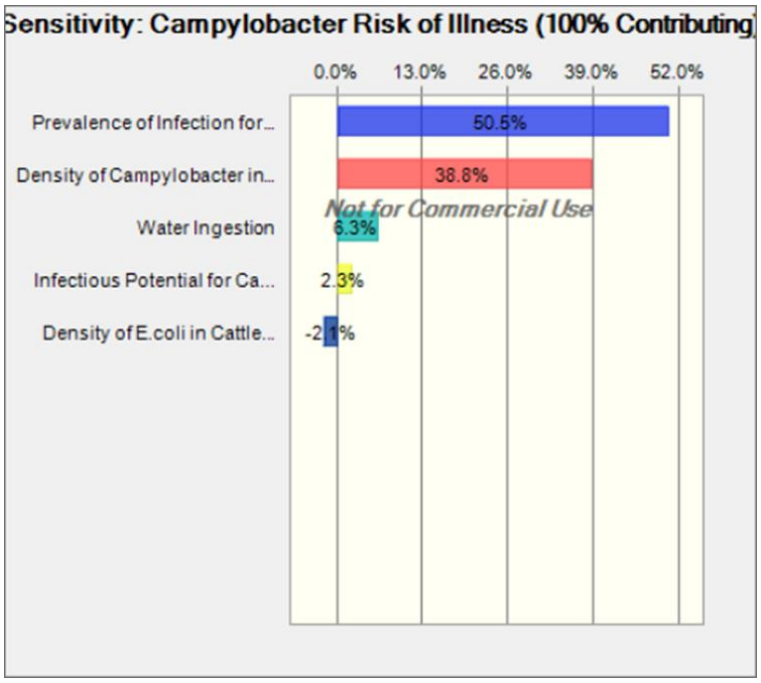

Figure A-5. Sensitivity analysis results for calculating the risk of a GI illness from *Campylobacter* (cattle/domestic animal source).

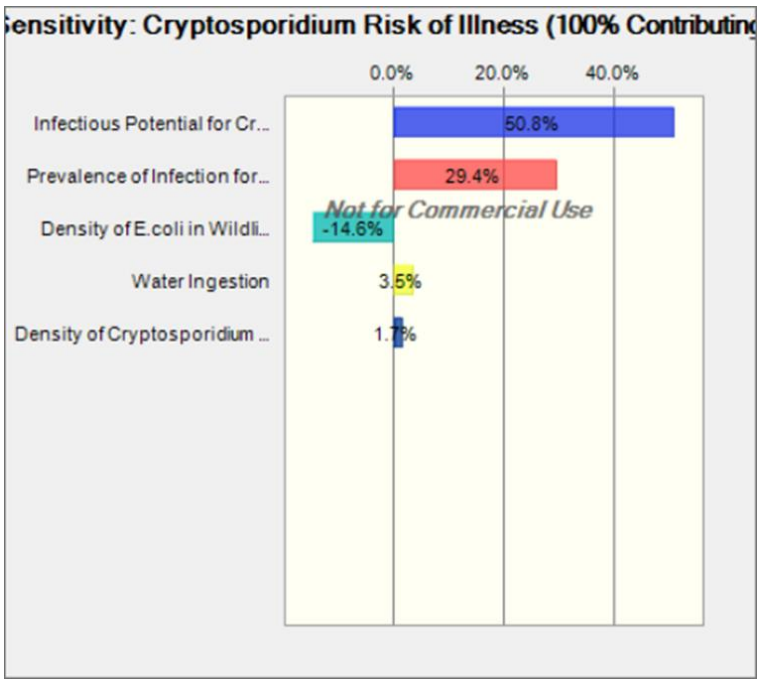

Figure A-6. Sensitivity analysis results for calculating the risk of a GI illness from *Cryptosporidium* (wildlife source).

Forecast Results from the Recreational Standard Simulations

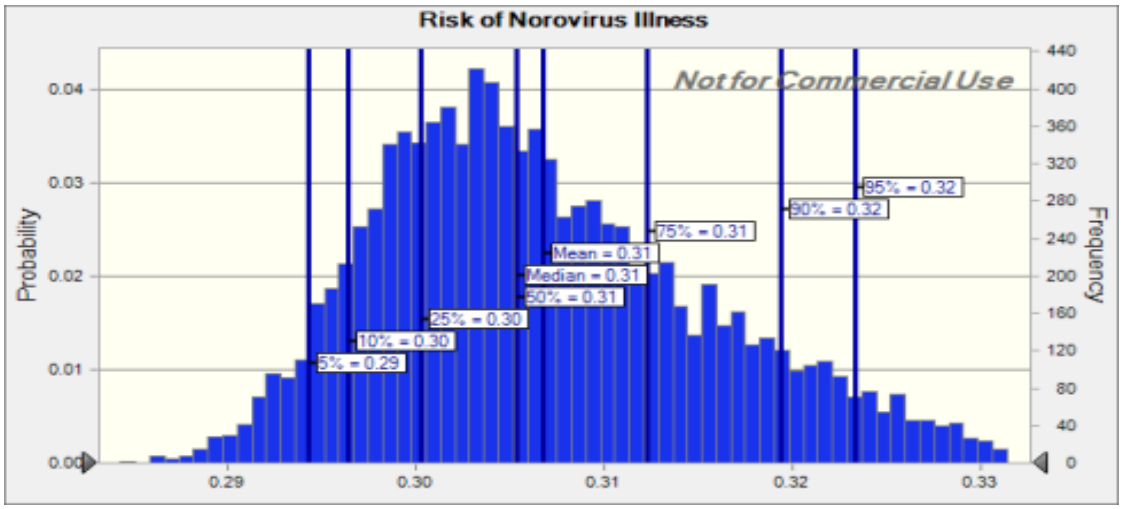

Figure A-7. Risk of a GI illness from norovirus infection (representing human source).

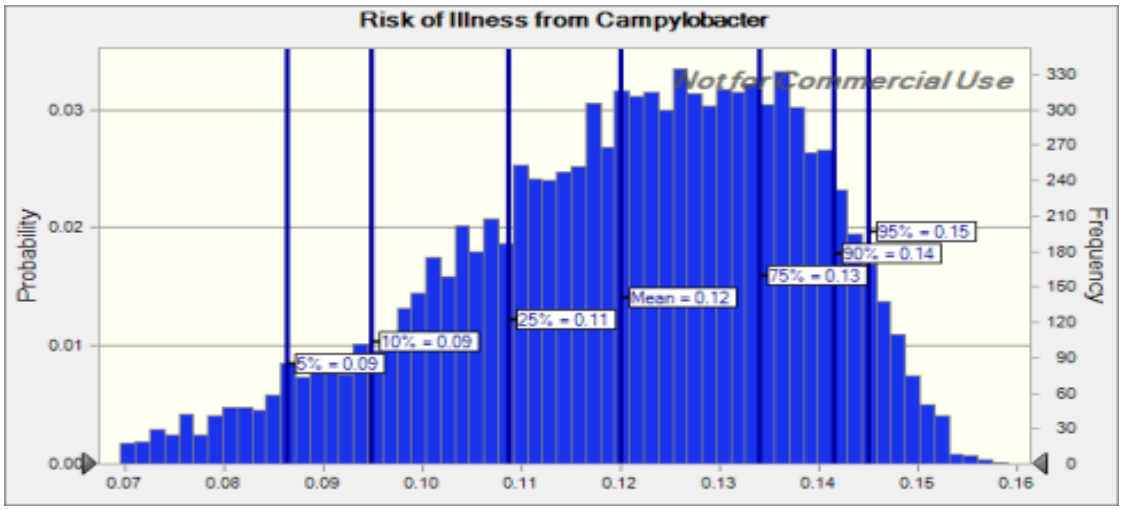

Figure A-8. Risk of a GI illness from *Campylobacter* infection (representing cattle/domestic animal source).

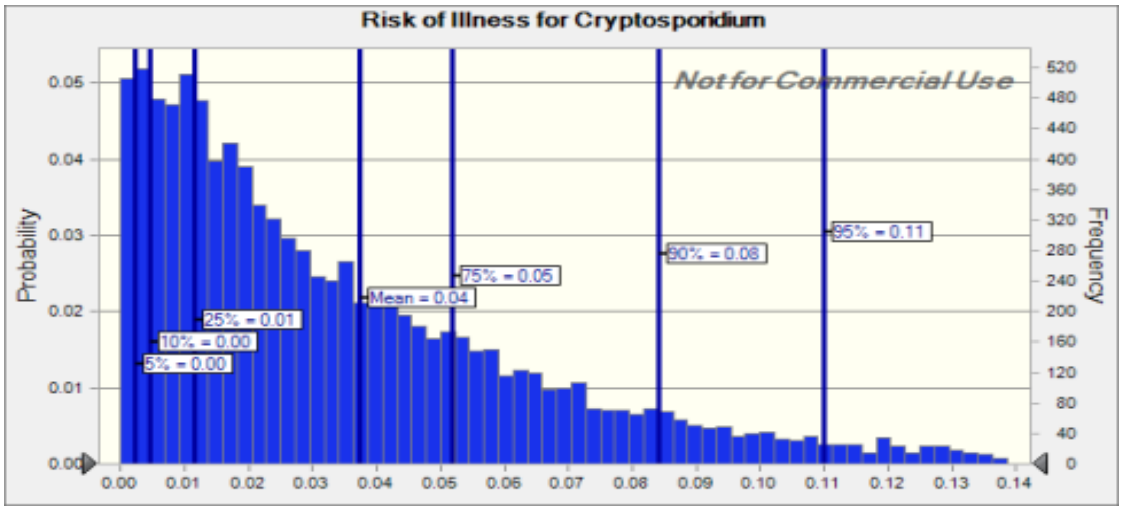

Figure A-9. Risk of a GI illness from *Cryptosporidium* infection (representing wildlife source).

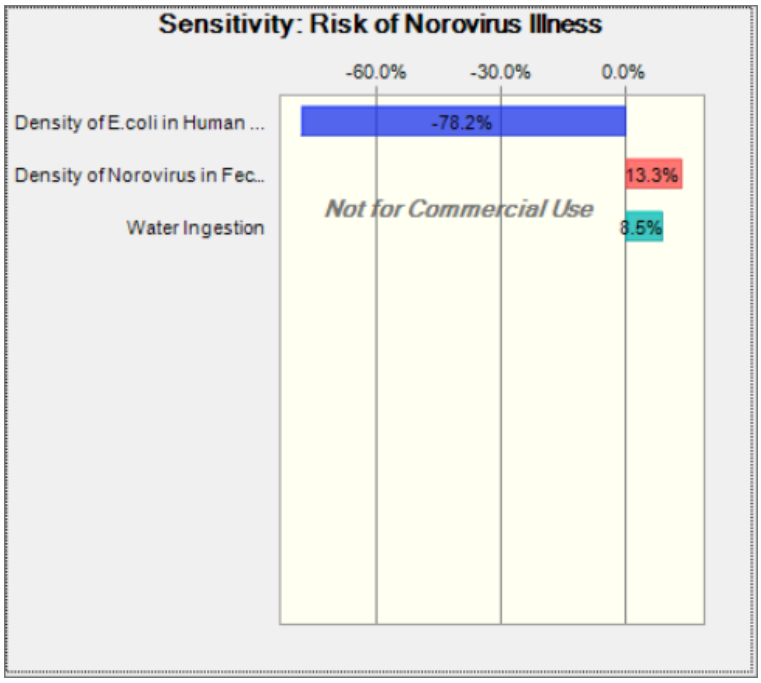

Figure A-10. Sensitivity analysis results for calculating the risk of a GI illness from norovirus (human source).

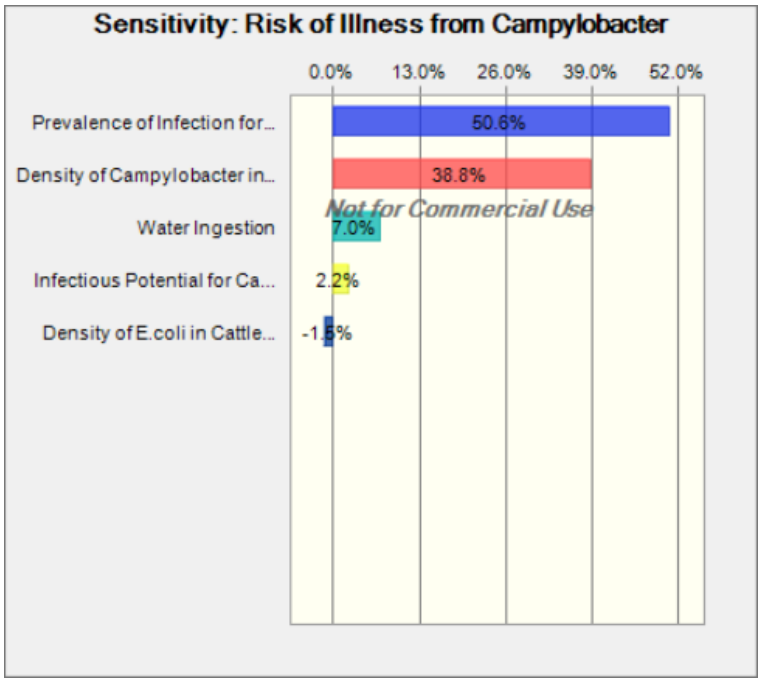

Figure A-11. Sensitivity analysis results for calculating the risk of a GI illness from *Campylobacter* (cattle/domestic animal source).

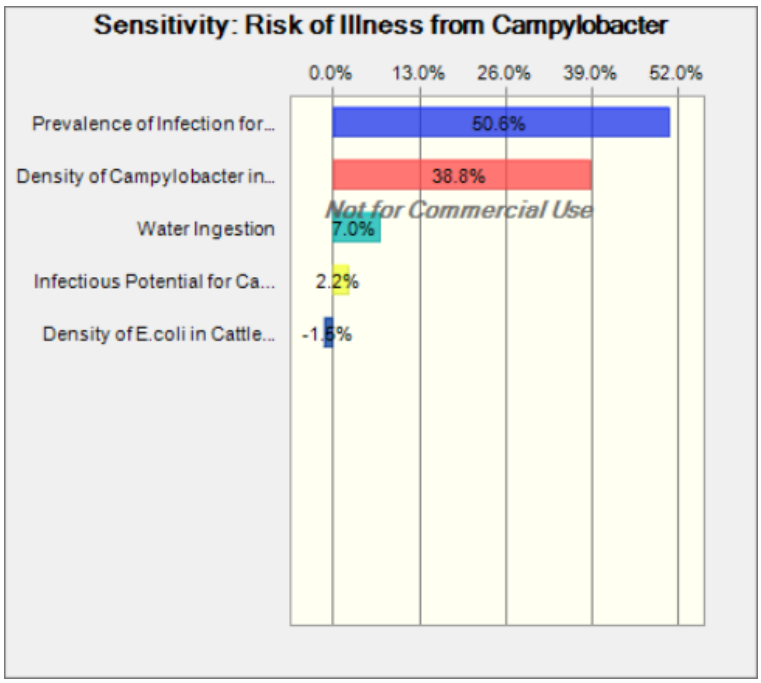

Figure A-12. Sensitivity analysis results for calculating the risk of a GI illness from *Cryptosporidium* (wildlife source).

Scenario 2

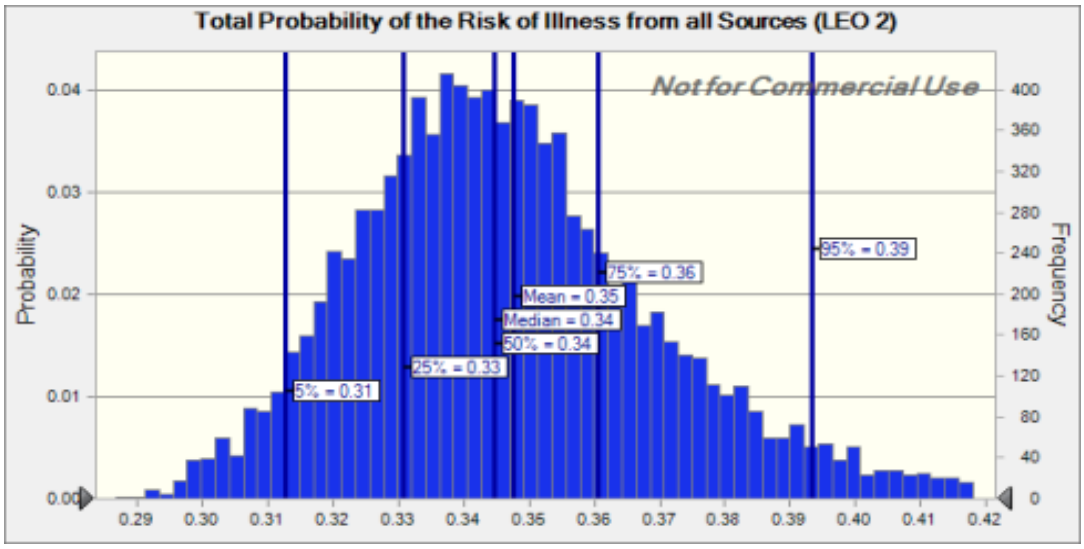

Figure A-13. Total Probability for the risk of illness for all sources at site LEO 2.

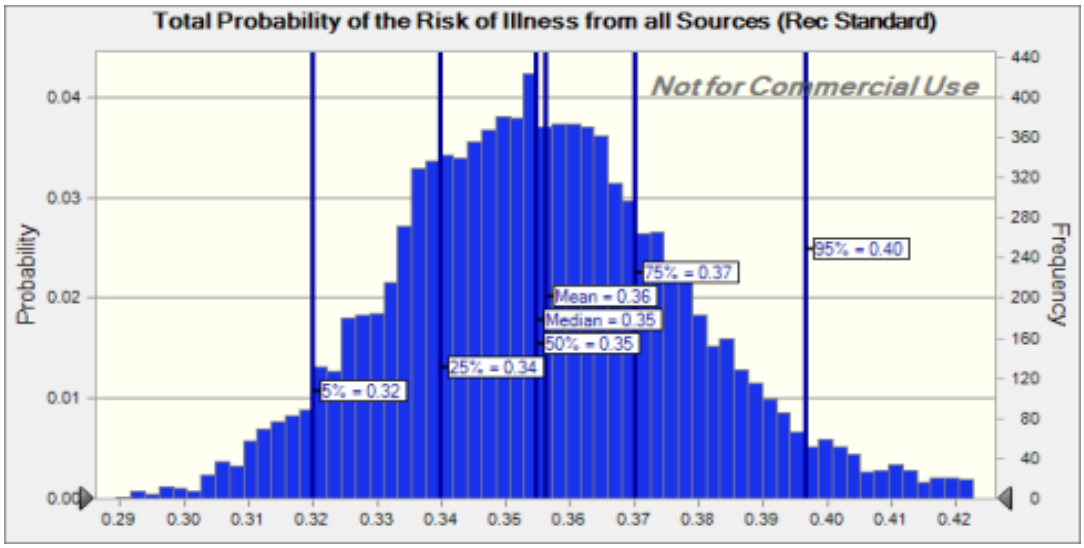

Figure A-14. Total probability for the risk of a GI illness for all sources under the recreational standard.

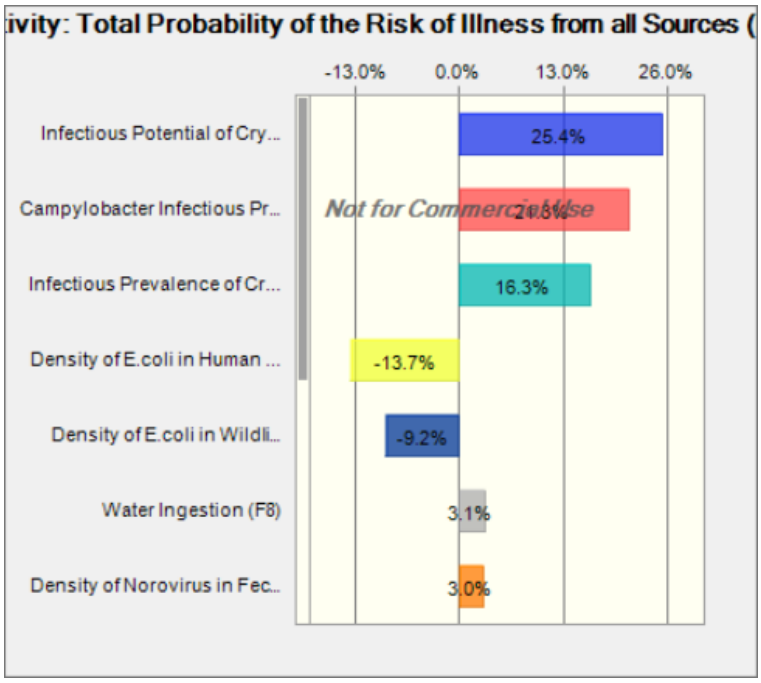

Figure A-15. Sensitivity analysis results when calculating the total probability of the risk of a GI illness for site LEO 2 under Scenario 2.

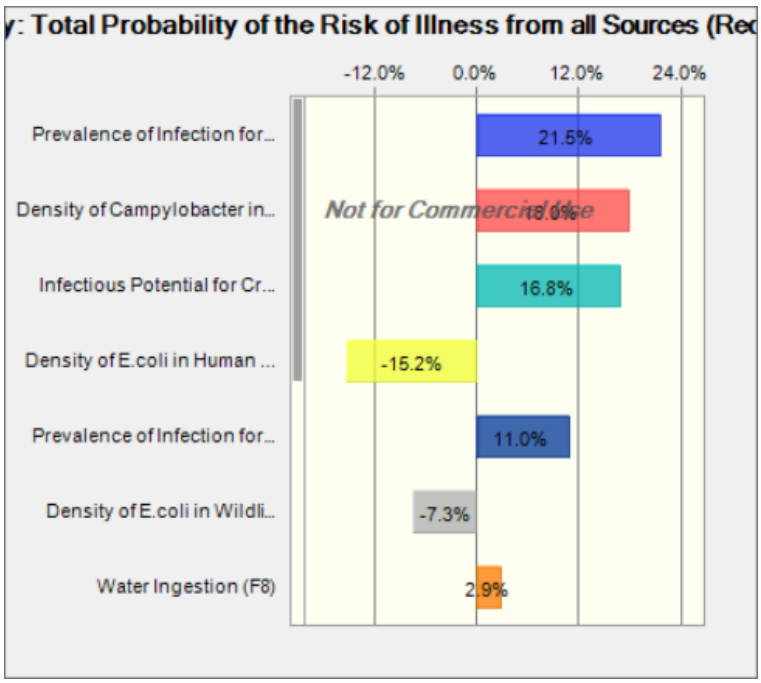

Figure A-16. Sensitivity analysis results when calculating the total probability of the risk of a GI illness for the recreational standard under Scenario 2.

Modified Scenario 2 Results

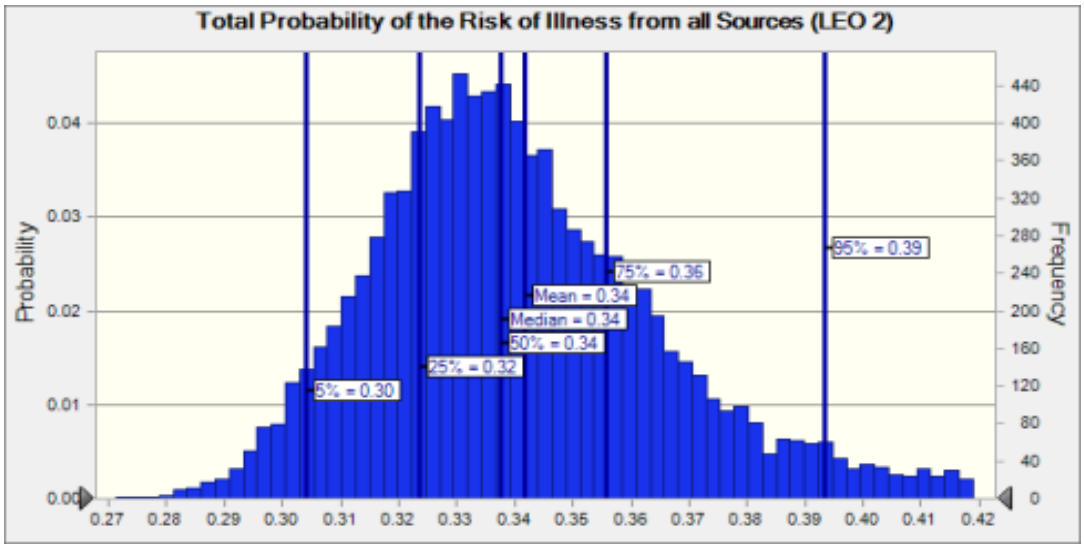

Figure A-17. Total probability for the risk of a GI illness of all sources at site LEO 2.

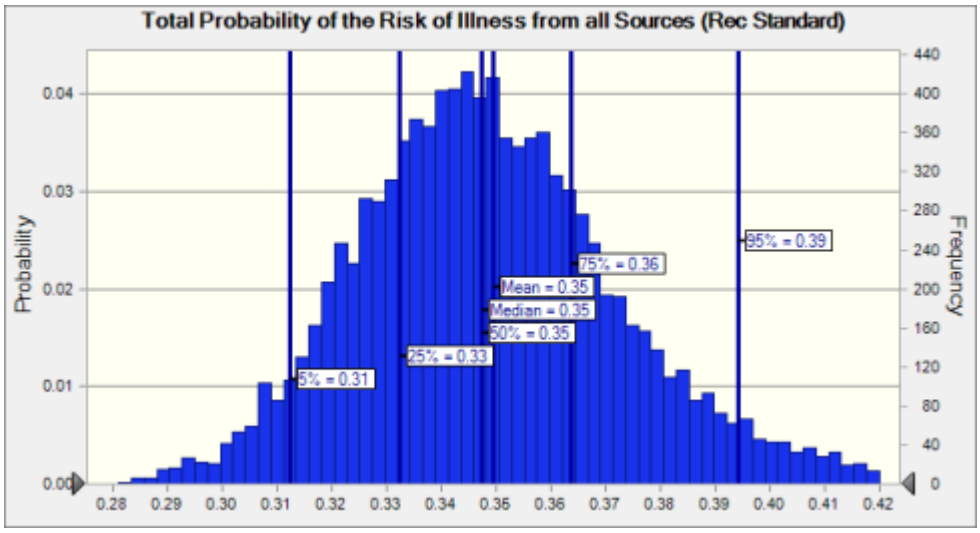

Figure A-18. Total probability for the risk of a GI illness of all sources under the recreational standard.

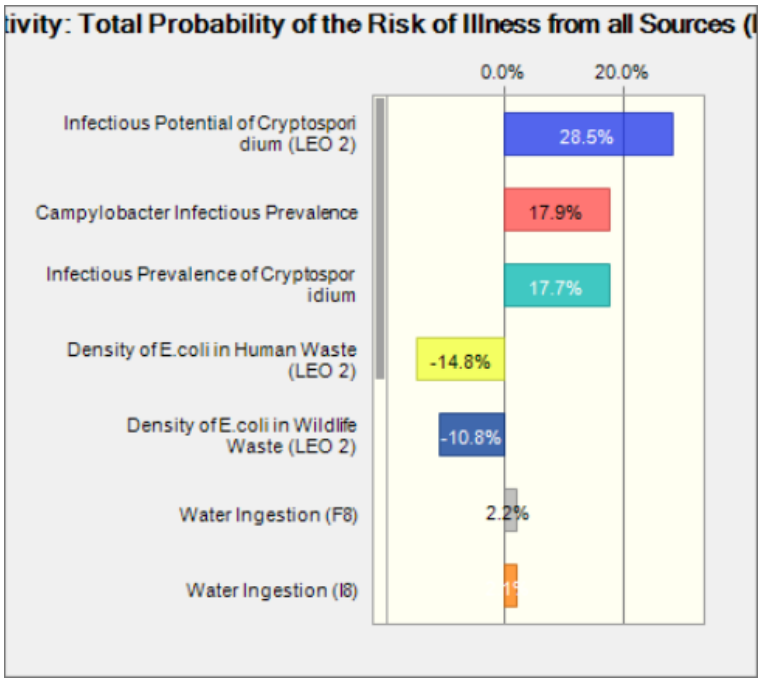

Figure A-19. Sensitivity analysis for the total probability of the risk of a GI illness from all sources at site LEO 2.

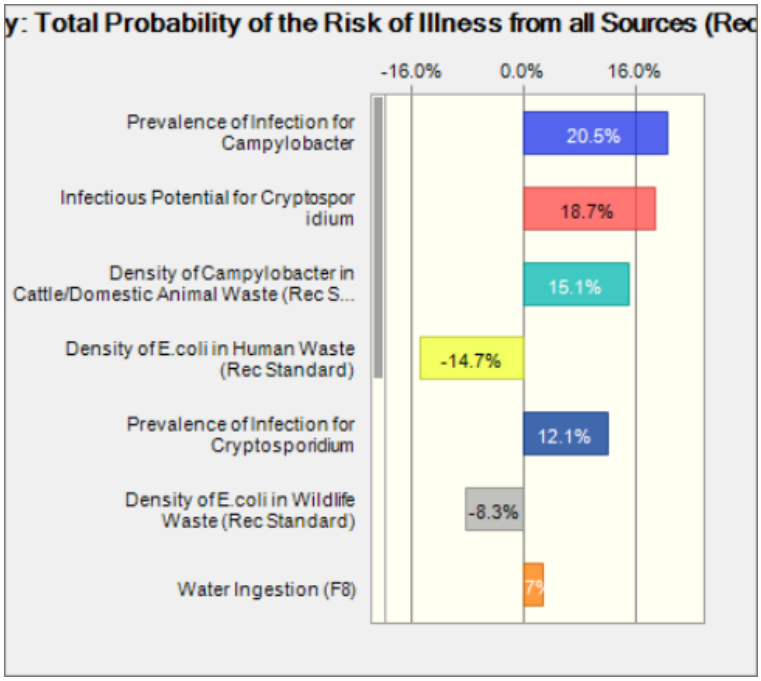

Figure A-20. Sensitivity analysis of the total probability of the risk of a GI illness from all sources under the recreational standard.

Scenario 3

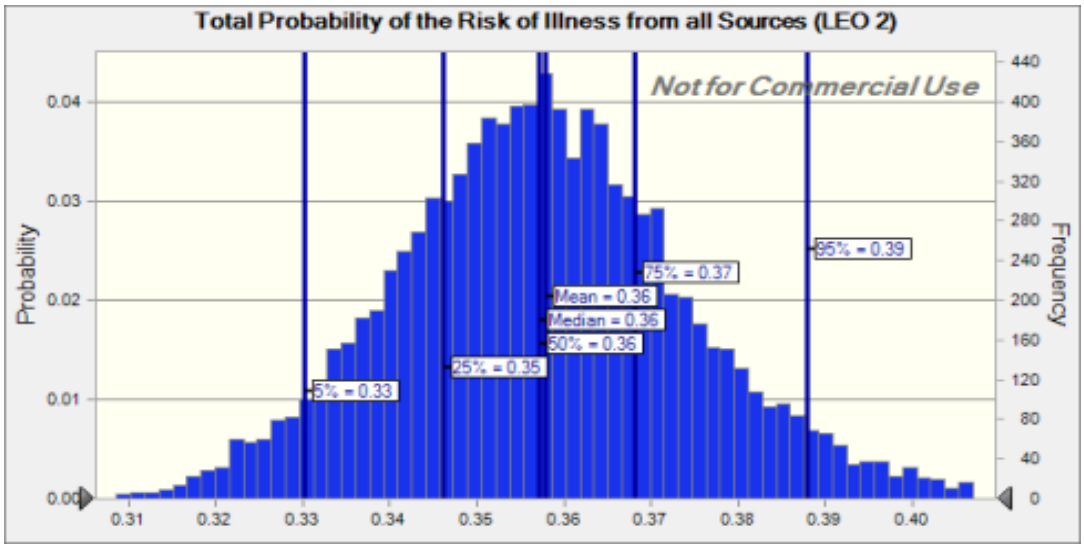

Figure A-21. Total probability for the risk of a GI illness from all sources at site LEO 2.

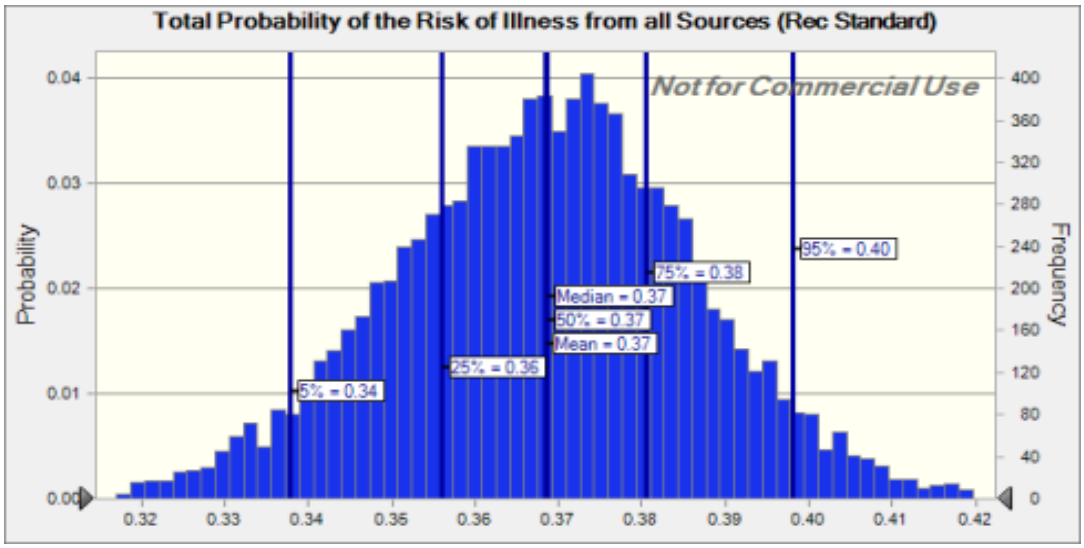

Figure A-22. Total probability for the risk of a GI illness from all sources under the recreational standard.

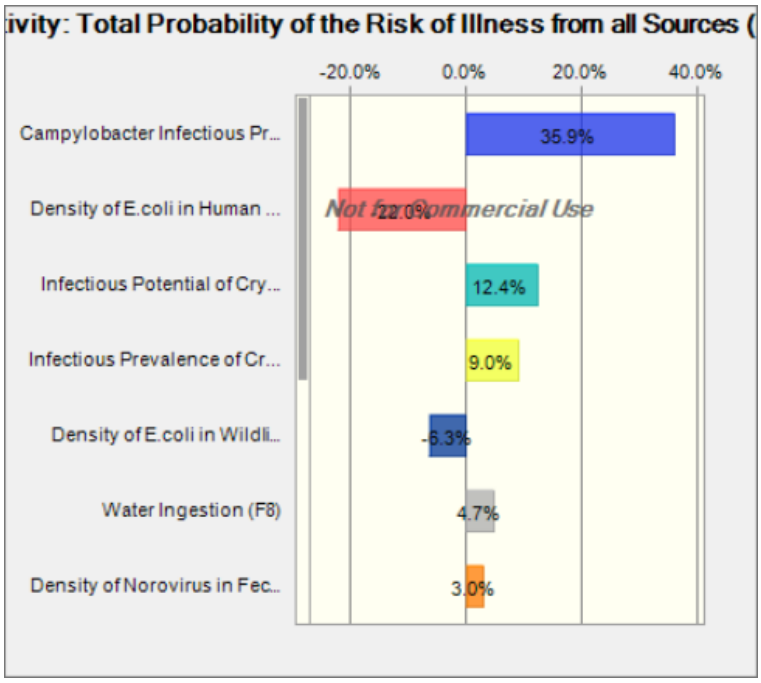

Figure A-23. Sensitivity analysis of the total probability for the risk of a GI illness from all sources at site LEO 2.

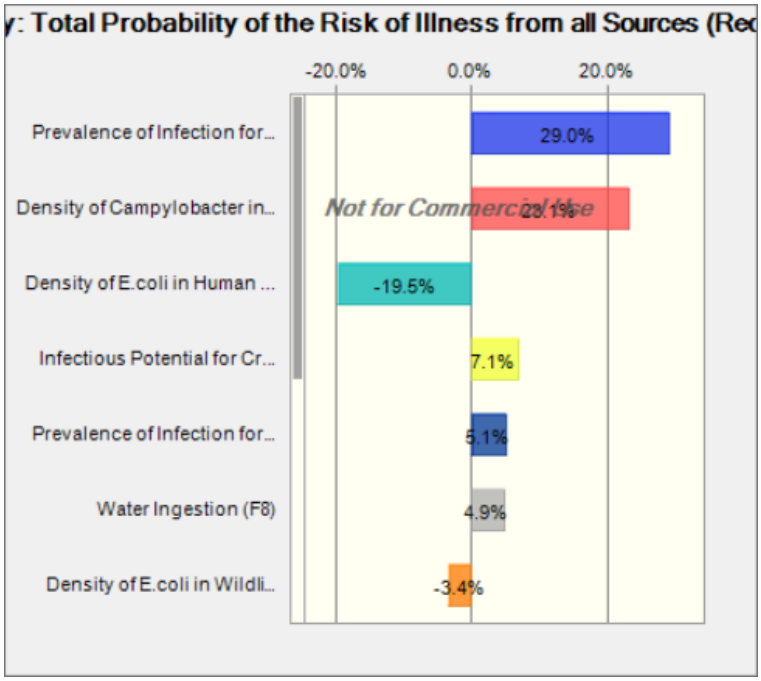

Figure A-24. Sensitivity analysis of the total probability for the risk of a GI illness from all sources under the recreational standard.