Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination

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FOREWARD

Managing and minimizing the public health threat associated with fecal pollution in recreational water are important aspects of policy development and regulation for the U.S. Environmental Protection Agency (EPA) Office of Water. The Beaches Environmental Assessment and Coastal Health Act of 2000 (BEACH Act) amended the Clean Water Act Sections 104 (v) and 304(a)(9) to require EPA to conduct studies associated with pathogens and human health, and to publish new or revised Recreational Water Quality Criteria (RWQC). To meet these requirements, EPA is conducting a series of studies that will inform the development of new or revised RWQC.

This document describes a quantitative microbial risk assessment (QMRA) that was conducted to to estimate illness in freshwater impacted by agricultural animal sources of fecal contamination. This assessment was based on the EPA/International Life Sciences Institute Framework for Microbial Risk Assessment (ILSI, 1996), and the structure follows EPA's peer-reviewed Microbiological Risk Assessment (MRA) Tools, Methods, and Approaches for Water Media (MRA Tools document), which has been peer-reviewed by renowned microbial risk assessors and the EPA Science Advisory Board.

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ACRONYMS AND ABBREVIATIONS

ARS	Agricultural Research Service
AWQC	ambient water quality criteria
BEACH Act	Beaches Environmental Assessment and Coastal Health Act of 2000
BMP	best management practice
BPW	buffered peptone water
CD	Consent Decree
CFU	colony forming units
CWA	Clean Water Act
DNA	deoxyribonucleic acid
EHEC	enterohemorrhagic *E. coli*
EPA	U.S. Environmental Protection Agency
EtOH	ethyl alcohol
FIB	fecal indicator bacteria
FSIS	Food Safety and Inspection Service
GI	gastrointestinal
GM	geometric mean
HCGI	Highly Credible Gastrointestinal Illness
ILSI	International Life Sciences Institute
IMS	immunomagnetic separation
IU	infectious units
mEI	membrane-enterococus indoxyl-ß-D-glucoside (agar)
mL	milliliters
MPN	Most Probable Number
MRAIT	Microbial Risk Assessment Interface Tool
ND	non-detect
NEEAR	National Epidemiological and Environmental Assessment of Recreational (Water Study)
NM	not measured
NRC	National Research Council
NRCS	Natural Resources Conservation Service
PBS	phosphate buffered saline
PBW	phosphate buffered water
PCR	polymerase chain reaction
PFU	plaque forming units
POTW	publicly owned (sewage) treatment works
QMRA	quantitative microbial risk assessment
qPCR	quantitative polymerase chain reaction

RT-PCR	reverse transcriptase polymerase chain reaction
RWQC	recreational water quality criteria
SA	Settlement Agreement
SD	standard deviation
STEC	Shiga toxin (producing) *E. coli*
TSC	tryptose sulfite cycloserine (agar)
U.K.	United Kingdom
U.S.	United States
USDA	U.S. Department of Agriculture
UV	ultraviolet (light)
VBNC	viable but non-culturable
WHO	World Health Organization (United Nations)
WWTP	wastewater treatment plant

1. Executive Summary

Under the Beaches Environmental Assessment and Coastal Health Act of 2000 (BEACH Act), EPA committed to "conduct quantitative microbial risk assessment (QMRA) (based on measurement of pathogenic organisms and indicators[1]) to estimate illness at a freshwater beach impacted by agricultural animal sources of fecal contamination."[2] This report documents EPA's activities to meet this commitment and addresses the following two questions:

1. What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, and chicken) sources of fecal contamination? and
2. How do those risks compare to risks associated with freshwater beaches impacted by human sources of fecal contamination?

The QMRA characterizes risks on a single recreation event basis for the general population and is defined by the following assumptions: (1) fresh cattle manure, pig slurry, and poultry litter (fecal materials) are land-applied at standard agronomic (maximum U.S. allowable) rates adjacent to a freshwater beach; (2) the fresh fecal materials contain fecal indicator bacteria (FIB) and reference pathogens consistent with levels reported in the peer-reviewed literature; (3) FIB and reference pathogens from the fresh land-applied fecal materials reach the freshwater beach via runoff from an intense rainfall event; (4) FIB and reference pathogens are mobilized during the rainfall event at levels consistent with those observed during the EPA environmental monitoring studies; (5) primary contact recreation (e.g., swimming) occurs in the undiluted runoff; and (6) exposure to reference pathogens occurs through water ingestion during recreation. This scenario is intentionally formulated to result in health-protective estimates of risk[3] (conservative).

The QMRA indicates that the median risk of illness from recreational exposure to the cattle-impacted waterbody is equivalent to the risk associated with the 1986 (current) recreational water quality criteria (RWQC)[4] (USEPA, 1986). The median risk of illness from exposure to the pig-impacted waterbody is approximately four-times lower than the risk associated with the current RWQC, and the median risk of illness from exposure to the chicken-impacted waterbody is approximately 300-times lower than the risk associated with the current RWQC.

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[1] Fecal indicator bacteria provide an estimation of the amount of feces, and indirectly, the presence and quantity of fecal pathogens in the water (NRC, 2004).

[2] Case 2;06-cv-04843-PSG-JTL Document 159-3 Files 08/08/2008 Page 3 of 15, http://www.epa.gov/waterscience/criteria/recreation/pdf/sa.pdf

[3] "Conservative" is used here to note that risk estimates will err on the side of a higher value and thus be more protective of human health.

[4] The 1986 RWQC were based on the results of a series of epidemiology studies conducted in human fecal matter-impacted (human impacted) water and establish a level of health protection in recreational freshwaters at 8 cases of Highly Credible Gastrointestinal Illness (HCGI) per 1000 recreation events.

In comparing risks in waterbodies that contain FIB at the current RWQC from land-applied agricultural animal fecal material, the predicted median risks of illness are at least 20- to 30-times lower than the risk associated with human-impacted water (risks for cattle and chicken impacted waters are lower depending on the FIB used). If FIB are present at the current RWQC from fecal material deposited directly into a waterbody, pig- and chicken-impacted water risks are similar to the land-applied risks, whereas cattle-impacted water risks are similar to the current RWQC.

1.1. QMRA Methods

This QMRA follows the EPA and International Life Sciences Institute Framework for Microbial Risk Assessment (ILSI, 1996) and employs peer-reviewed tools and approaches (USEPA, 2010). A traditional forward QMRA characterizes the risk of illness associated with recreation at a freshwater beach (first question above). A relative QMRA provides a comparison of the estimated risks at the current RWQC from recreation in water impacted by agricultural sources of fecal contamination to those associated with human-impacted water (second question above).

In this QMRA, we use a probabilistic framework and characterize each model parameter using a statistical distribution where the parameters of those distributions account for variability and/or uncertainty.

1.1.1. Forward QMRA methods

For each of the animal sources (fresh cattle manure, swine slurry, and poultry litter), the density of reference pathogens[5] in the runoff (USEPA, 2009b) is calculated based on data (see Appendix B) describing the reference pathogen density in land-applied fecal material, the prevalence of infection (percent of infected animals), the human infectious potential of the reference pathogens from the agricultural animals, and the proportion of the applied reference pathogens that run-off following a rain event (based on data collected specifically for this risk assessment; see Appendix D for further information). These data are referred to hereafter as the EPA environmental monitoring program).

That density is multiplied by the volume of water ingested during recreational activities to estimate the "dose" of pathogens for this exposure scenario. That dose is input to the appropriate dose-response relationship resulting in a probability of infection. The probability of infection is multiplied by a morbidity factor to estimate a probability of illness. The risk associated with

[5] In this report, a set of reference pathogens for the EPA recreational water QMRA work was established and is described herein that represents a large proportion of illnesses in the United States, are representative of the fate and transport of waterborne pathogens of concern, are present in human and animal waste and recreational waters, can survive in the environment, and have corresponding dose-response relationships in the peer-reviewed literature. For animal-impacted waters, the reference pathogens are *Cryptosporidium*, *Giardia*, *Salmonella enterica*, *Campylobacter jejuni*, and *E. coli* O157:H7. Other pathogens were also considered for inclusion as reference pathogens (e.g., Hepatitis E virus, *Listeria monocytogenes*, or *Leptospira)*; however, by comparison, these pathogens are thought to cause few illnesses from recreational water exposure and/or do not have available dose-response relationships based on human data.

each fecal contamination source is characterized as the total probability of gastrointestinal (GI) illness from each source-specific reference pathogen.

1.1.2. Relative QMRA methods

For the relative QMRA, previously developed methods for direct fecal contamination (fecal material deposited directly into a waterbody) (Schoen and Ashbolt, 2010; Soller et al., 2010b) are extended by including land application of fecal material, and FIB and reference pathogen mobilization (proportion of FIB and reference pathogens that run-off) during rainfall events. The estimated risks are calculated for a hypothetical waterbody that contains geometric mean FIB densities at the U.S.-recommended RWQC for recreational freshwaters (33 colony forming units [CFU] 100 mL^{-1} enterococci and 126 CFU 100 mL^{-1} *E. coli*, respectively). We provide separate calculations for each fecal source/FIB combination.

Pathogen dose is calculated based on observed and literature-based ranges of pathogen and FIB densities in fecal waste, the prevalence of infection, the fraction of human-infectious strains, and the proportion of the FIB and pathogens that mobilize during a rain event. Similar to the forward QMRA, doses are input to the appropriate dose-response relationship resulting in a probability of infection. The probability of infection is multiplied by a morbidity factor to produce a probability of illness. The risk associated with each fecal contamination source is characterized as the total probability of GI illness from each source-specific reference pathogen. The resulting risk distributions are then compared to benchmark risks for human-impacted waters.

1.2. Results

1.2.1. Risk of illness associated with recreation at a freshwater beach impacted by agricultural animal sources of fecal contamination

The forward QMRA predicts risk of illness from recreational exposure to the animal-impacted waterbodies during and immediately after an intense rain event. The forward QMRA simulation results for the cattle manure, pig slurry, and chicken litter-impacted recreational water are presented in boxplot format in Figure 1.[6,7]

[6] In Figure 1 and subsequent boxplots, the edges of the box represent the 25th and 75th percentiles of the simulation results (probability of infection or illness), the line in the center of the box is the median value, the whiskers represent the 10th and 90th percentiles, and the diamonds below and above the whiskers represent the 5th and 95th percentiles, respectively.

[7] In Figure 1 and several subsequent figures, a reference line labeled "Current geometric mean RWQC" is provided. This line represents an estimate of the GI illness risk associated with the FIB densites that are specified by the geometric mean RWQC (USEPA, 1986). Simulation median values can be compared to this line to evaluate how the simulation results compare to the level of risk associated with the current RWQC.

These results can be summarized as follows:

- The predicted median cumulative risk of illness from recreational exposure to the cattle-impacted waterbody is effectively equivalent to the risk of illness that is associated with the current RWQC.
- The predicted median cumulative risk of illness from recreational exposure to the pig-impacted waterbody is approximately 4-times lower than the risk of illness that is associated with the current RWQC.
- The predicted median cumulative risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 300-times lower than the risk of illness that is associated with the current RWQC.
- *E. coli* O157 is the predicted dominant risk agent in cattle-impacted water, followed by *Campylobacter* and *Cryptosporidium*. For pig-impacted water, *Campylobacter* and *Cryptosporidium* are the predicted dominant risk agents, followed by *Giardia*. For chicken-impacted water, *Campylobacter* is the predicted dominant risk agent.
- The predicted variability is greatest for chicken-impacted water and least for pig-impacted water.

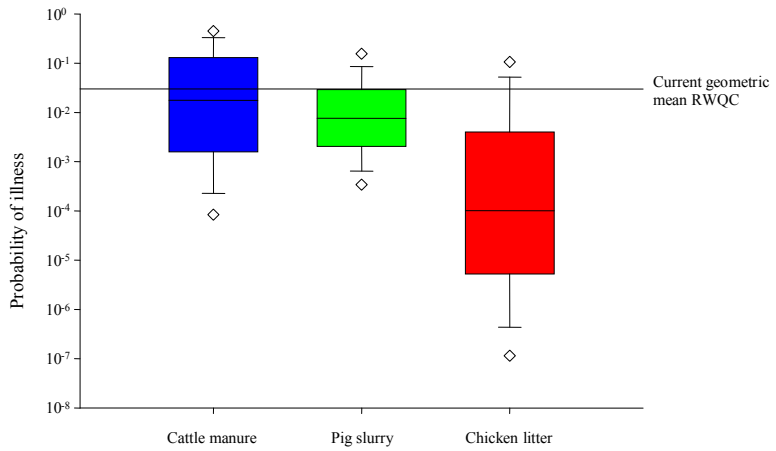

Figure 1. Summary of forward QMRA results

1.2.2. Comparison of risks for freshwater beaches impacted by agricultural animal and human sources of fecal contamination

The relative QMRA simulation results for the cattle manure, pig slurry, and chicken litter-impacted recreational water are presented in Figure 2.

These results can be summarized as follows:

- At the current geometric mean RWQC, the predicted median risk of illness from recreational exposure to the cattle-impacted waterbody is approximately 25- to 150-times lower than risk of illness associated with human sources of contamination.
- At the current geometric mean RWQC, the predicted median risk of illness from recreational exposure to the pig-impacted waterbody is approximately 30-times lower than the risk of illness that is associated with human sources of contamination.
- At the current geometric mean RWQC, the predicted median risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 20- to 5000-times lower than risk of illness that is associated with human sources of contamination.

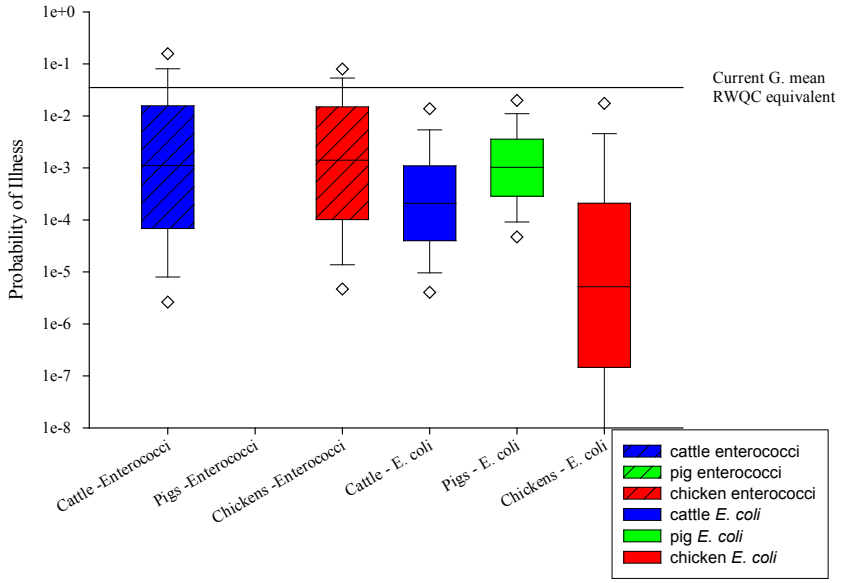

Figure 2. Summary of relative QMRA results

1.3. Considerations

Like any scientific study, this work has a number of important conceptual constraints. In this risk assessment, we consolidated a vast range of disparate data and information to support an improved understanding about risks to human health that would have been difficult or impossible to characterize through an observational (e.g., epidemiology) study.

To facilitate this risk assessment, we necessarily made several simplifying, health-protective assumptions to limit the scope of the assessment to ensure it could be completed defensibly and in a timely manner. The most important conceptual constraints and caveats are that (1) the analyses only considered one intentionally limited exposure scenario; (2) FIB and pathogen mobilization was modeled on a simulated intense rain event in a single location—we used a modest set of reference pathogens that represent a large proportion of illnesses in the United States, however, it is possible that animal-impacted water could contain pathogens of potential public health concern that we did not evaluate; and (3) we summarized our results to facilitate comparison to the existing (1986) RWQC and, as such, do not describe the risks associated with extreme or rare events.

Risk assessment is widely used by governmental and regulatory agencies worldwide to protect public health from exposure to a myriad of contaminants through numerous routes of exposure. Air pollution regulations, protection of the food supply chain, and drinking water regulations are large-scale examples that illustrate the effective use of risk assessment methodologies within a environmental regulatory context. To date, epidemiology studies have been the primary tool used to characterize human health risks from exposure to recreational water. Those epidemiology studies have generally focused on waters impacted by wastewater (human sewage) effluent. Substantial progress has been made in improving the quality of wastewater effluent in the United States. However, greater attention is being paid to other contamination sources. In fact, non-point fecal contamination is one of the most common reasons that U.S. waterbodies are classified as impaired with respect to their recreational use. Epidemiology studies are not likely to be effective in characterizing risks in many waters of this type due to technical, logistical, and/or financial constraints. As illustrated in this report, QMRA is a viable and valuable complement to epidemiology for waters where epidemiology data are not available, do not apply, or are impractical to collect. Finally, the data, results, and caveats of this study provide context for understanding recreational risks in diverse waterbodies, and could help to facilitate implementation of upcoming new or revised RWQC.

2. Problem Formulation

2.1. Statement of Concern

Managing and minimizing the public health threat associated with fecal pollution in recreational water are important aspects of policy development and regulation for the U.S. Environmental Protection Agency (EPA) Office of Water. Human exposure to recreational water impacted by fecal contamination is known to cause a variety of adverse health effects including gastrointestinal (GI) and respiratory illness (Craun et al., 2005; NRC, 2004; Parkhurst et al., 2007). Microbial hazards in recreational water contaminated by feces include pathogenic bacteria, viruses, and parasitic protozoa of human and animal origin. Risks to swimmers may differ depending on the source (human or animal) of the excreta because (1) the pathogens in animal manure differ in type, occurrence, and abundance from those in human sewage (WHO, 2004b); and (2) the routes by which human-infectious pathogens of animal origin (zoonoses) reach swimmers can differ from human enteric pathogens (e.g., intermittent rainfall transport as compared to wastewater treatment plant effluent with relatively constant flow).

2.2. Purpose and Context

2.2.1. Purpose

This quantitative microbial risk assessment (QMRA) estimates human GI illness associated with recreation at a freshwater beach contaminated by fecal material from agricultural animal sources (livestock). It compares those risks to those associated with recreation in water impacted by human sewage sources. The assessment follows the EPA/International Life Sciences Institute peer-reviewed microbial risk assessment framework (ILSI, 1996) and employs peer-reviewed microbial risk assessment tools and approaches (USEPA, 2010).

2.2.2. Context for using QMRA to estimate recreational water risks

The Beaches Environmental Assessment and Coastal Health Act of 2000 (BEACH Act) and the associated Consent Decree (CD) and Settlement Agreement (SA) require EPA to publish new or revised recreational water quality criteria (RWQC) by October 2012. To meet these requirements, EPA is conducting a series of studies as part the *Critical Path Science Plan for Development of New or Revised Recreational Water Quality Criteria* (science plan) to form the technical basis of new or revised RWQC (USEPA, 2007). This QMRA was conducted to meet the SA requirement to "conduct QMRA (based on measurement of pathogenic organisms and indicators) to estimate illness at a freshwater beach impacted by agricultural animal sources of fecal contamination."[8]

[8] Case 2;06-cv-04843-PSG-JTL Document 159-3 Files 08/08/2008 Page 3 of 15, http://www.epa.gov/waterscience/criteria/recreation/pdf/sa.pdf

Epidemiology studies have linked swimming-associated illnesses with FIB densities in point source human-impacted recreational water (see reviews by Prüss, 1998; Wade et al., 2003; Zmirou et al., 2003). For a more recent review, see WERF (2009). In these epidemiology studies, FIB were used to detect the possible presence of microbial contamination from human waste (NRC, 2004).

Although several epidemiology studies have considered non-point sources of contamination, these studies do not specifically link FIB densities to risks from agricultural animals. At a given level of FIB, risks for animal-impacted water may differ from human-impacted water because the mix and densities of pathogens in animal manure are different from those in human excreta. Another important distinction is that pathogen loading to recreational water from animal manure typically differs (event-driven) from wastewater outfall loading (continuous). Because of these issues, it would be technically and logistically difficult to conduct epidemiology studies on predominately agricultural animal-impacted waters. QMRA provides a scientifically defensible mechanism to characterize risks for agricultural animal-impacted water.

QMRA applies risk assessment principles (NRC, 1983) to approximate the consequences from exposure to selected infectious pathogens (Haas et al., 1999). For recreational water contact, QMRA can be used to

- estimate the risk of GI illness for recreational water where no epidemiological data are available (forward);
- understand which pathogens caused GI illnesses in epidemiological studies (reverse);
- compare the relative levels of risk to human health associated with fecal contamination from various sources (relative); and
- harmonize QMRA models with epidemiology studies (anchoring).

The QMRA presented in this report uses both forward (traditional) and relative approaches. The forward QMRA quantifies risks associated with specific animal waste (cattle, swine, and chicken) runoff scenarios. The relative QMRA compares risks associated with cattle, swine, and chicken-impacted water to risks associated with recreation in human-impacted water with FIB densities at the current RWQC levels (USEPA, 1986).

2.2.2.1. Forward QMRA

The pathogen densities to which swimmers are exposed depend upon myriad factors, the most important of which is the primary source of fecal pollution at the site (Dorevitch et al., 2010; Schoen and Ashbolt, 2010; Soller et al., 2010a; 2010b; WERF, 2009). In a forward QMRA, knowledge of prevalence of infection and abundance of pathogens in sources is used to predict risks of infection or illness associated with recreational activities. In this traditional QMRA approach, an exposure assessment (statistical analyses of pathogen occurrence, ingestion volumes, and abundance in sources and fate and transport modeling) is used to estimate the

pathogen density in water and the volume of water ingested. An estimate of human health risk is then computed based on pathogen specific dose-response relationships (Figure 3). Thus, forward QMRA assesses risks based on particular site or exposure features (e.g., water primarily affected by runoff events). The QMRA described in this report includes a forward QMRA.

Forward QMRAs provide a mechanism to link animal manure exposure with human health risks. The few epidemiology studies based on inland water affected by animal waste have not produced risk estimates associated with recreation or data linking FIB with risk. Epidemiology studies may be limited in this regard due to (1) the temporally sporadic pathogen loading to recreational water from animal manure, and (2) a decoupling between the FIB that are traditionally used in epidemiology studies and the mix of pathogens present in animal manure.

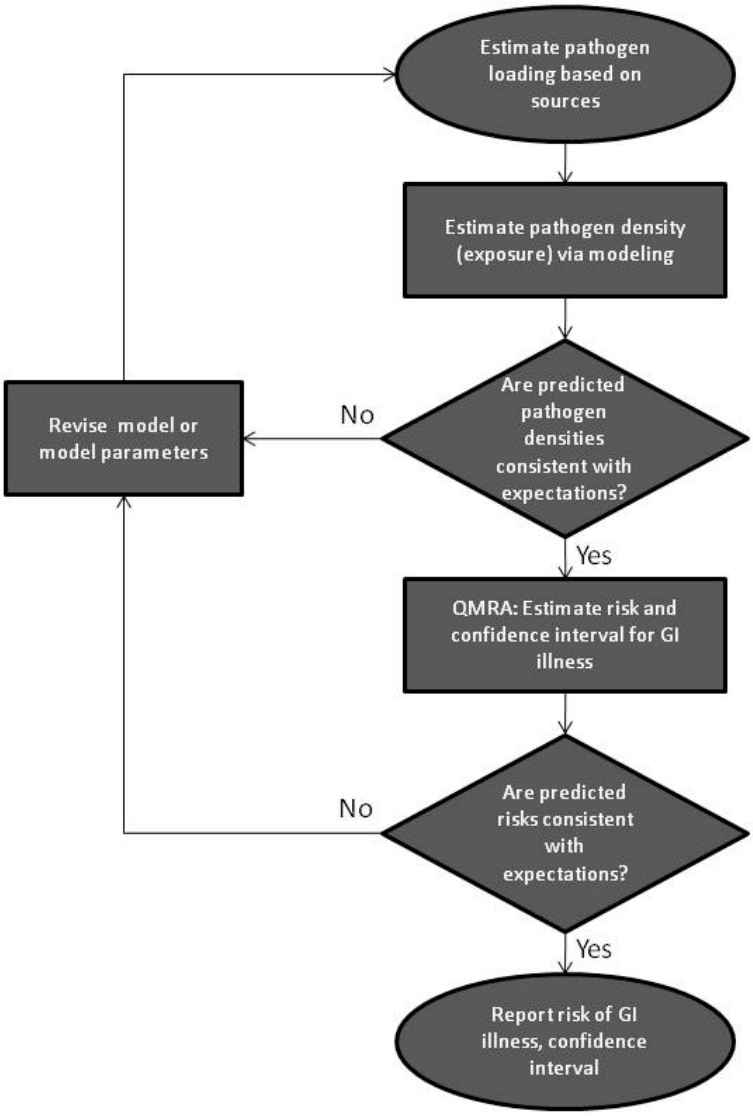

Figure 3. Flowchart for forward QMRA

2.2.2.2. Reverse QMRA

In a reverse QMRA, infection or illness rates associated with recreational activities are used in conjunction with knowledge about contamination source to make inferences about likely pathogen presence in a waterbody. The same components are used as with the forward QMRA approach (the volume of water ingested, pathogen specific dose-response relationships). The reverse QMRA output (i.e., pathogen densities) is the starting point for forward QMRA (and vice versa) (Figure 4).

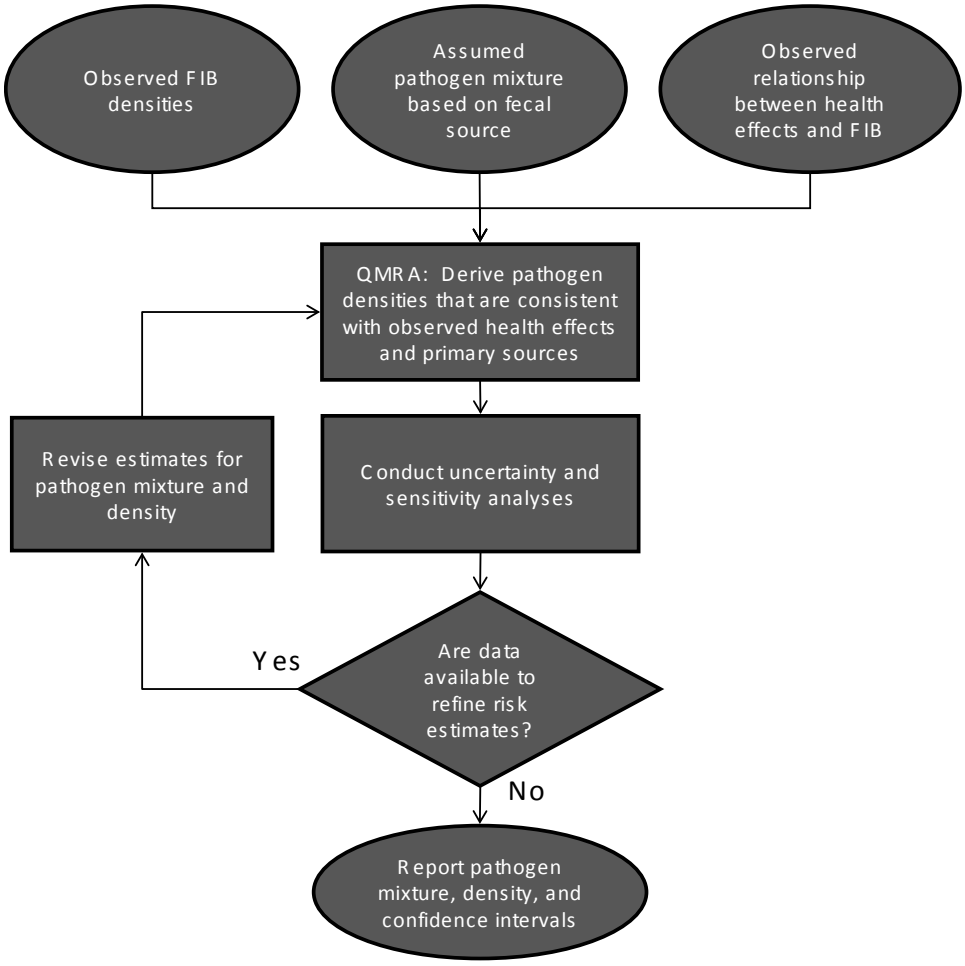

Figure 4. Flow chart for a reverse QMRA

In the absence of direct pathogen monitoring, reverse QMRA provides a mechanism to infer pathogen densities in a recreational waterbody from specific sources of fecal contamination and can clarify epidemiology study results (Soller et al., 2010a). For example, Soller et al. (2010a) used reverse QMRA to identify human enteric viruses, particularly norovirus, as the likely causes of the observed illnesses from the epidemiology studies conducted in 2003 to 2004 on the Great Lakes in the United States as part of EPA's National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study.

Because of the lack of epidemiological data associating illness caused by swimming in livestock-impacted water with water quality measures, a reverse QMRA was not conducted as part of this report.

2.2.2.3. Relative QMRA

The relative QMRA approach compares risks associated with recreation in water affected by human and non-human sources of fecal pollution. The relative QMRA approach allows direct comparison of the risks for various sources because the approach assumes that each source contributes a given level of FIB (e.g., 33 CFU enterococci/100 mL). Estimated distributions of FIB and reference pathogens in each source are used to calculate the relative levels of risk (Schoen and Ashbolt, 2010; Soller et al., 2010b). This report includes a relative QMRA.

2.2.2.4. QMRA anchoring

QMRA anchoring harmonizes QMRA models with epidemiology studies. These assessments require both water quality information (as measured by FIB) and epidemiological data for a given site. The anchoring process compares health impacts predicted using QMRA based on water quality data with observed health effects from epidemiology studies. Next, QMRA model parameters are adjusted to improve agreement between observations and predictions. QMRA anchoring can be used to extend QMRA models to sites where epidemiological studies are impractical or unavailable (Figure 5). This QMRA approach has only recently been proposed (WERF, 2009) and has not yet appeared in the literature.

2.2.3. Prior use of QMRA to estimate risks associated with waterborne pathogens

EPA conducted a detailed literature review to document the use of QMRA to estimate the risks associated with recreational water impacted by cattle, swine, or poultry waste in (Annex 1). That review established the QMRA state-of-the-science for waterborne contaminants and provides insight into the techniques available for use in a QMRA of animal-impacted water. The literature search yielded approximately 300 QMRA studies and was used to

- identify the pathogens that QMRAs most commonly address;
- identify how QMRA studies address variability and uncertainty;
- assess how often QMRAs include secondary transmission;
- identify which QMRA elements support RWQC; and
- compare the methods used for sensitivity analyses and risk characterization.

Sixteen of those studies estimated risks associated with waterborne recreation and all but one was a forward QMRA. Appendix A provides a synopsis of the 16 studies. The literature review indicates that QMRA has been used in a variety of scenarios and is useful when other techniques such as epidemiology studies are impossible or cost-prohibitive.

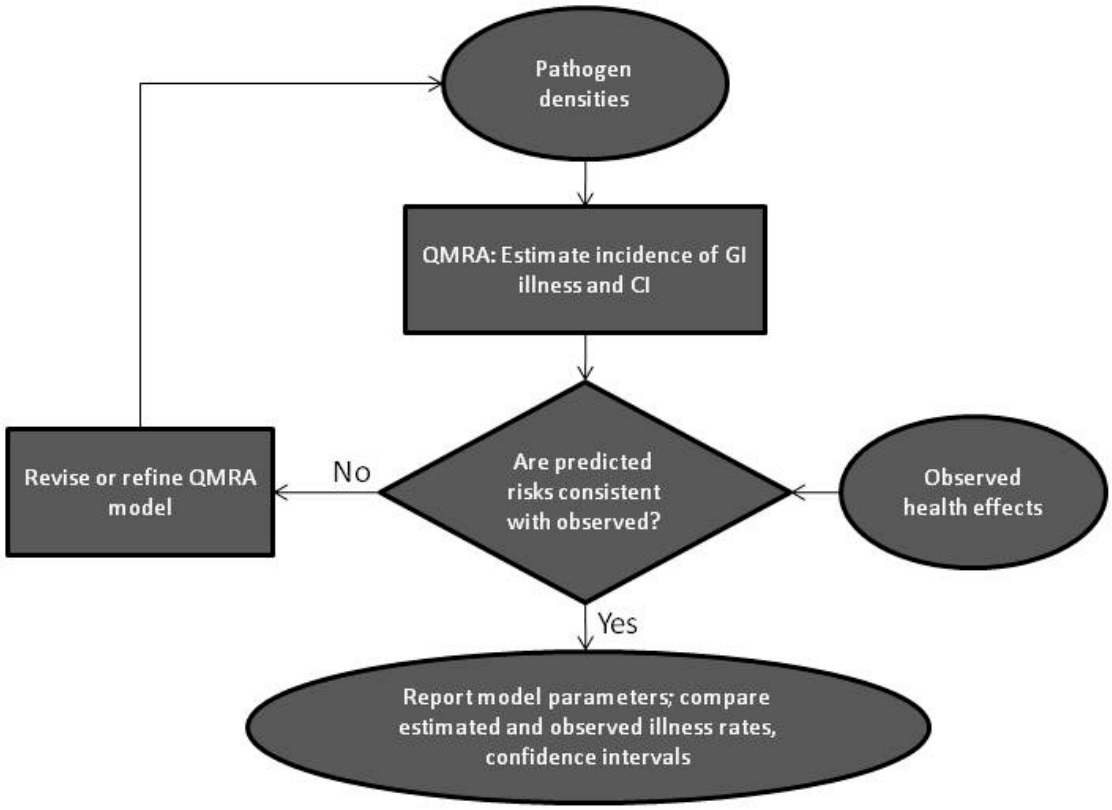

Figure 5. Anchoring a QMRA using observed pathogen densities and health effects

Several observations may be drawn from these studies. First, the studies focused on a small subset of pathogens that may be important in waterborne exposure during recreational activities. The two pathogens analyzed most frequently—rotavirus and *Cryptosporidium*—are important contributors to risk of GI illness, primarily due to their high infectivity, frequent occurrence in sewage, and relatively high persistence in environmental matrices. Other human enteric viruses, particularly noroviruses, have been implicated in numerous outbreaks since the 1950s (Sinclair et al., 2009), making their absence in QMRA studies notable. However, a recently published dose-response relationship for norovirus (Teunis et al., 2008a) has helped address this gap (Schoen and Ashbolt, 2010; Soller et al., 2010a).

Second, a lack of comprehensive data on pathogen occurrence in sources and pathogen fate and transport characteristics limits the ability to model variability in pathogen sources. In the literature review, the two most common methods to account for source variability were (1) using empirical distributions for pathogen density based on relatively limited data, and (2) assuming log-normally distributed pathogen densities. The QMRA effort described in this document explicitly models variability and uncertainty based on FIB and pathogen density data drawn from the peer-reviewed literature, and from EPA field studies conducted specifically for this risk assessment.

Third, these studies used limited dose-response modeling, and most did not account for variability and uncertainty in dose-response model parameters because high quality and diverse dose-response model data are limited. Our QMRA analyses rely on dose-response models from peer-reviewed studies. While those models represent the state-of-the-art, they may not adequately describe risks associated with susceptible sub-populations. Understanding this limitation, we used a probabilistic QMRA framework[9] to address susceptibility to the extent possible.

Finally, most risk estimations do not account for secondary transmission and immunity. Several QMRA studies show that infectious disease transmission attributes can influence risk in unintuitive ways (Eisenberg et al., 2004, 2008; Riley et al., 2003; Soller et al., 2006, 2009); however, our QMRA analyses do not explicitly address these parameters, this is because previous work indicates that they are unlikely to substantially affect the estimated risks, given the pathogens present in livestock manure, and the relatively infrequent exposure to recreational water (via incidental ingestion of water during recreation) (Soller and Eisenberg, 2008).

2.3. Scope and Risk Range

2.3.1. Hazards

Although human and animal waste can contain numerous pathogenic microbes, recreational water monitoring data, public health reporting, epidemiology studies, outbreak reports, and dose-response studies suggest that a modest subset of these pathogens are representative of the majority of hazards in human and livestock-impacted recreational water (reference pathogens). The use of reference pathogens to represent the infectivity and the likely environmental fate and transport of each microbial group (WHO, 2004a) is a widely accepted practice in the field of QMRA (Roser et al., 2007; Soller et al., 2010b).

Reviews of waterborne transmission of zoonotic pathogens identified pathogens of primary concern based on their occurrence in water, abundance in animal feces, and persistence and ability to multiply in the environment (Bicudo and Goyal, 2003; Goss and Richards, 2008; Rosen, 2000; USEPA, 2009a, 2009b). Based on those criteria, the protozoans *Cryptosporidium* and *Giardia* and the bacterial pathogens *E. coli* O157:H7, *Salmonella*, and *Campylobacter* are the primary pathogens of concern in livestock waste (Bicudo and Goyal, 2003; Goss and Richards, 2008; Rosen, 2000). Pathogens and diseases of secondary concern include *Yersinia enterolitica* (Bicudo and Goyal, 2003), brucellosis, and leptospirosis (Rosen, 2000). Transmission of fecally-associated viruses of animal origin to humans is considered rare (Rosen, 2000; Sobsey et al., 2006), but is an emerging issue.

[9] In this QMRA, all parameters, including the dose-response relationship, are characterized by statistical distributions to the extent that data were available to support the use of a distribution.

Data published in 1999 indicated that known pathogens accounted for an estimated 38.6 million illnesses each year in the United States, including 5.2 million due to bacteria, 2.5 million due to parasites, and 30.9 million due to viruses (Table 1) (Mead et al., 1999). Of those illnesses, 13.8 million were thought to be foodborne, leaving 24.8 million illnesses of which some portion was due to waterborne exposures (including, but not limited to, recreational water contact).

Several researchers have developed illustrative lists of waterborne pathogens to consider as reference pathogens for QMRAs of recreational water (Olivieri and Soller, 2002; Rosen, 2000; Soller et al., 2010b). For example, Rosen (2000) compiled a list of pathogens in human and animal waste and ranked them in terms of their risk to human health (Table 2).

2.3.2. Reference pathogens

For the EPA recreational water QMRA described in this report, we established a set of eight reference pathogens that (1) cause a large proportion of non-foodborne illnesses in the United States from Mead et al. (1999) (Figure 6 and Table 1); (2) are representative of the fate and transport of other waterborne pathogens of concern (Ferguson et al., 2009); (3) are present in human and animal waste and recreational water (USEPA, 2009b); (4) can survive in the environment; and (5) have corresponding peer-reviewed dose-response relationships (USEPA, 2010). The reference pathogens are

- Norovirus
- Rotavirus
- Adenovirus
- *Cryptosporidium* spp.
- *Giardia lamblia*
- *Campylobacter* spp.
- *Salmonella*[10]
- *E. coli* O57:H7.

[10] In keeping with the usual convention, in this report, *Salmonella* refers to *Salmonella enterica* spp. *enteric*, except in specific reference to a different *Salmonella* species.

Table 1. Estimated annual illnesses in the United States from known pathogens (SOURCE: adapted from Mead et al., 1999)

Pathogen Class	Pathogen	Total Estimated Annual Cases	% Foodborne	# Foodborne	# Non-foodborne
Bacteria	*Bacillus cereus*	27,300	100	27,360	0
	Botulism, foodborne	58	100	58	0
	Brucella spp.	1554	50	77	777
	Campylobacter spp.	2,453,926	80	1,963,141	490,785
	Clostridium perfringens	248,520	100	248,520	0
	Escherichia coli O157:H7	73,480	85	62,458	11,022
	E. coli, non-O157 (Shiga toxin-producing *E. coli* [STEC])	36,740	85	31,229	5,511
	E. coli, enterotoxigenic	79,420	70	55,594	23,826
	E. coli, other diarrheogenic	79,420	30	23,826	55,594
	Listeria monocytogenes	2518	99	2493	25
	Salmonella Typhi	824	80	659	165
	Salmonella, non-typhoidal	1,412,498	95	1,341,873	70,625
	Shigella spp.	448,240	20	89,648	358,592
	Staphylococcus food poisoning	185,060	100	185,060	0
	Streptococcus, foodborne	50,920	100	50,920	0
	Vibrio cholerae, toxigenic	54	90	49	5
	V. vulnificus	94	50	47	47
	Vibrio, other	7880	65	5122	2758
	Yersinia enterolitica	96,368	90	86,731	9637
	Subtotal	**5,204,934**		**4,175,565**	**1,029,369**
Parasitic	*Cryptosporidium parvum*	300,000	10	30,000	270,000
	Cyclospora cayetanesis	16,264	90	14,638	1626
	Giardia lamblia	2,000,000	10	200,000	1,800,000
	Toxoplasma gondii	225,000	50	112,500	112,500
	Trichinella spiralis	52	100	52	0
	Subtotal	**2,541,316**		**357,190**	**2,184,126**
Viral	Norwalk-like virus (norovirus)	23,000,000	40	9,200,000	13,800,000
	Rotavirus	3,900,000	1	39,000	3,861,000
	Astrovirus	3,900,000	1	39,000	3,861,000
	Hepatitis A	83,391	5	4170	79,221
	Subtotal	**30,883,391**		**9,287,170**	**21,601,221**
	Total	**38,629,641**		**13,814,924**	**24,814,717**

Table 2. Pathogenic organisms in animal waste of concern to human health (SOURCE: adapted from Rosen, 2000)

Type of Oganism	Pathogens of Primary Concern	Pathogens of Secondary Concern
Protozoa	*Cryptosporidium* spp. (*C. parvum*, *C. hominis*) *Giardia* spp.	*Cryptosporidium* spp. (others) *Toxoplasma gondii*† *Balantidium coli*
Bacteria	*Campylobacter* spp. (*C. jejuni*, *C. coli*) *E. coli* O157:H7 *E. coli*, non-O157 STEC *E. coli*, enterotoxigenic *E. coli*, other diarrheogenic *Listeria monocytogenes* *Salmonella enterica* (particularly serotypes associated human infection, including enteritidis, newport, typhimurium) *Shigella* spp. *Vibrio cholerae*, toxigenic	*Yersinia enterocolitica*‡ *Brucella* spp. *Leptospirosis interrogans*
Viruses	Adenovirus† Astrovirus† Calciviruses† Coxsackievirus† Echovirus† Hepatitis A† Hepatitis E Norovirus† Rotavirus	Bovine rotavirus

† Not known to originate from livestock sources
‡ Not considered a major source of waterborne infection

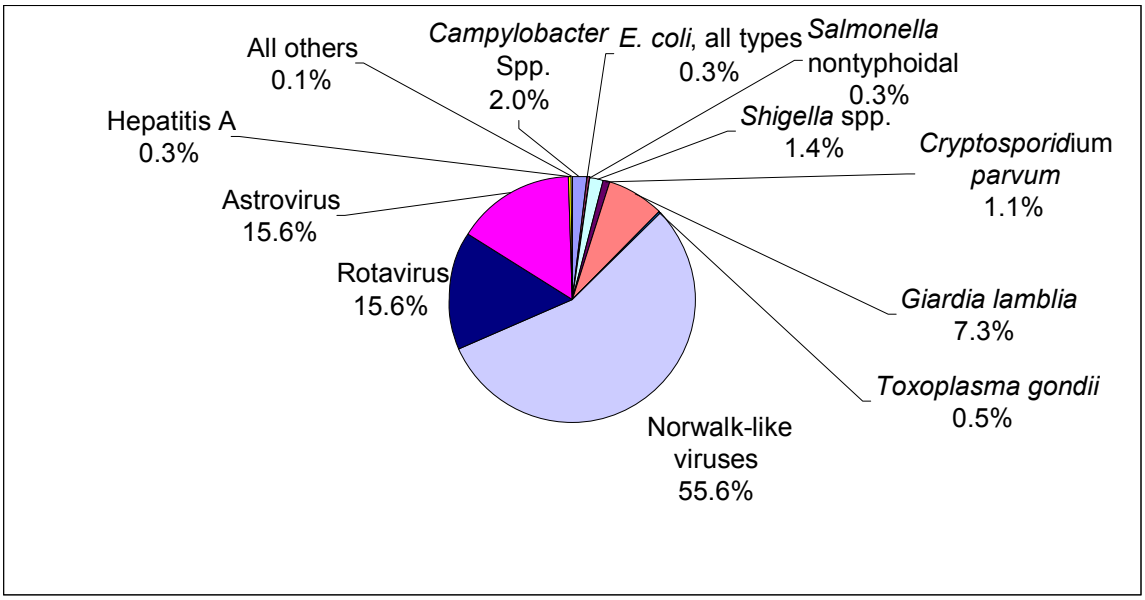

Figure 6. Non-foodborne illnesses in the United States (SOURCE: adapted from Mead et al., 1999)

These eight reference pathogens adequately represent the risk from pathogens potentially present in fecal matter and in the diverse range of U.S. recreational waters. In addition, their selection is consistent with previous EPA work that suggested prioritizing standard methods and recreational and drinking water guidelines for *Salmonella* spp., *Campylobacter jejuni, E. coli* O157:H7, *Cryptosporidium*, *Giardia*, and selected viral contaminants (USEPA, 2005b).

In this QMRA, we use the bacterial and parasitic protozoan reference pathogens to characterize the risk associated with animal-impacted waters (since the viruses are primarily species-specific, we do not need them to estimate the risk of human GI illness from animal-based water contamination). In previous related work, we used viral, bacterial, and parasitic protozoan reference pathogens to evaluate risks associated with human-impacted waters (Schoen and Ashbolt, 2010; Soller et al., 2010a, 2010b).

Appendix B presents data on the occurrence of the reference pathogens in the fecal pollution sources of interest, and the analysis chapter (3) describes dose-response models for each of the reference pathogens. Justification follows for inclusion of each of the eight microorganisms as a reference pathogen for the EPA recreational water QMRA work.

2.3.2.1. Norovirus

Noroviruses are an important cause of human enteric infection and illness. They are estimated to cause approximately 23,000,000 illnesses in the United States annually (Mead et al., 1999) and are associated with up to 90% of the epidemic nonbacterial gastroenteritis (GI illness) worldwide (Lindesmith et al., 2003). Norovirus illness is not limited to young children (Dolin, 2007); however, a portion of the general population appears to be immune to infection from specific norovirus genotypes, perhaps due to memory immune response (Lindesmith et al., 2003). Teunis et al. (2008a) recently published a norovirus dose-response study that expresses dose in terms of quantitative polymerase chain reaction (qPCR) genome equivalents. Noroviruses are resistant to water treatment (Haramoto et al., 2006; Laverick et al., 2004; Lodder and de Roda Husman, 2005; Pusch et al., 2005; van den Berg et al., 2005), and remain infective for prolonged periods of time in the environment (Allwood et al., 2005; Lee et al., 2008). Strains of norovirus also exist that are uniquely associated with animals (Mattison et al., 2007). Direct zoonotic transmission appears to be rare, but genetic mixing of animal and human viruses seems plausible with the finding that common human strains replicate in pigs and cattle (Koopmans, 2008).

2.3.2.2. Adenovirus

Adenoviruses are primarily of human origin, although some animals are known to be infected by and shed host-specific variants. Adenovirus types vary widely in their pathology, with strains 40 and 41 causing enteric infections in young children, and with secondary contributions by strains 2 and 31 (Heirholzer, 1992; Jiang, 2006). A significant limitation in the use of adenovirus as a reference pathogen is that no dose-response relationship has been published for the ingestion route of exposure. Experimental studies of adenovirus 4 and 7 with healthy adult volunteers

indicate that inhalational exposure results in a higher rate of infection at the same dose than intranasal and oral exposure (Couch et al., 1969). Thus, the inhalation dose-response model appears to be a conservative estimator for ingestion. Adenoviruses are detected frequently in sewage, surface water (e.g., Xagoraraki et al., 2007), and surface water affected by stormwater (e.g., Jiang et al., 2001).

2.3.2.3. Rotavirus

Rotavirus is the leading cause of GI morbidity and mortality among young children and is of greater public health concern to young children and immunocompromised persons and populations than the general population. Reinfection of adults is common, but is usually asymptomatic (Molyneaux, 1995). Dose-response studies indicate that a low dose of rotavirus (<10 focus forming units) is sufficient to infect a significant proportion of the exposed population (Haas et al., 1993; Ward et al., 1986). Large numbers of rotavirus (on the order of 10^{10} organisms/g) can be shed in stool (McNulty, 1978), and rotavirus can survive for weeks on fomites and in environmental waters (Boone and Gerba, 2007). Although pigs also shed rotavirus, those strains appear to be host-adapted and not likely to pose a significant risk to humans (Martella et al., 2010).

2.3.2.4. Cryptosporidium and Giardia spp.

Cryptosporidium and *Giardia* spp. have been implicated in many U.S. and international waterborne disease outbreaks. Dose-response models are available for both protozoa, and both parasites can infect a significant proportion of the exposed population at low doses. The dose-response characteristics of *Cryptosporidium*, however, may vary among isolates—*C. parvum* and *C. hominis* are the two species of primary importance in human infections (Messner et al., 2001; Teunis et al., 2002; USEPA, 2005a). *Cryptosporidium* and *Giardia* spp. are frequently isolated from publicly owned treatment works (POTW) effluent, stormwater, and livestock manure, and their respective oocysts and cysts can survive for extended periods of time in the environment. The high environmental loading of potentially human infectious *Cryptosporidium* in calves makes *Cryptosporidium* of particular interest in estimating risk related to livestock sources of fecal pollution.

2.3.2.5. Campylobacter spp.

Two species of *Campylobacter*—*C. jejuni* and *C. coli*—cause most *Campylobacter* infections in humans, with the majority caused by *C. jejuni*. Several dose-response relationships for *C. jejuni* have been published (Medema et al., 1996; Teunis et al., 2005). *Campylobacter* spp. is prevalent in livestock, particularly poultry and sheep, has been implicated in outbreaks associated with consumption of milk, and is present in levels as high as 79,000 Most Probable Number (MPN)/100 mL in wastewater treatment plant (WWTP) effluent.

2.3.2.6. E. coli O157:H7

E. coli O157:H7 is representative of Shiga toxin producing *E. coli* (STEC), possesses the potential for serious adverse health outcomes, and has been implicated in waterborne outbreaks. A peer-reviewed dose-response model is available (Teunis et al., 2008b). *E. coli* O157:H7 is frequently isolated from cattle manure, often in very high densities, but less often from swine manure and seldom from poultry manure (Appendix B). *E. coli* O157:H7 can potentially grow in soil, sediment, water, and possibly other environmental matrices—all of which emphasizes its potential to be found in POTW- and livestock-impacted waters.

2.3.2.7. Salmonella

The most heterogeneous of the reference pathogens is *Salmonella*, whose serotypes have adapted to a wide variety of host-specific environments. Because *Salmonella* serotypes also vary widely in their ability to infect humans, dose-response modeling of *Salmonella* can be somewhat complex. *Salmonella* are of particular interest as reference pathogens because they have many sources; have been associated with outbreaks (primarily foodborne); occur in abundance in chicken, cattle, and swine manure; and because some serotypes pose serious human health hazards (Berg, 2008; O'Reilly et al., 2007). *Salmonella* can persist in soils for 180 days or longer (Holley et al., 2006), depending on several factors including soil moisture, presence of manure, and clay content. *Salmonella* densities may increase in manures and manure-soil mixtures (You et al., 2006). In surface waters, *Salmonella* can be detected throughout the year, with densities and serotype diversity typically higher during summer months than winter months (Haley et al., 2009).

2.3.3. Livestock-impacted sites

The reference pathogens in livestock manure are primarily bacterial and protozoan (Appendix B). Among human viruses of potential concern, only hepatitis E is associated with livestock operations (Banks et al., 2004; Legrand-Abravanel et al., 2009; Rutjes et al., 2009; Sinclair et al., 2009; Takahashi et al., 2009). Although the presence of Hepatitis E antibodies in pigs is notable (Meng et al., 1999; Smith, 2001), including Hepatitis E in QMRA is limited by the lack of dose-response relationships available to estimate risks to humans. In this regard, experiments with monkeys indicate that oral inoculation with hepatitis E is inefficient in producing disease. In addition, in countries with well-developed sewage treatment facilities and practices, the prevalence of Hepatitis E in environmental waters is relatively low (Smith, 2001). Therefore, using bacterial and protozoan reference pathogens to evaluate livestock-impacted water with QMRAs is more appropriate at this stage of our understanding.

Livestock-derived pathogens reach surface water primarily through runoff from land with fresh or treated manure during and immediately after rainfall events. This mechanism requires pathogens to be in fecal material when the manure is applied to land (occurrence), present in sufficient numbers to contaminate runoff, and carried in runoff to receiving water (mobilization).

Such processes vary between different livestock handling practices and within a particular livestock manure type. For example, the proportion of animals that are infected by a specific pathogen (shedding pathogens in their feces) is variable in time and space; the level of storage/land treatment varies among farms and even between applications on a given farm; and mobilization of pathogens depends on the rain event (and the antecedent rainfall), the slope of land where manure is applied, the groundcover and soil characterization of the application site, among other factors (Ferguson et al., 2007).

Because the pathogens in livestock manure are not necessarily the same species or serotypes that cause human illness, estimating the proportion of human-infectious strains of each reference pathogen in each animal source is important. For example, the overlap between *Salmonella* serotypes prevalent in humans and livestock can be used to develop a lower bound on the potential loading of human-infectious *Salmonella* from livestock (see Chapter 3 for further information).

Appendix B summarizes the prevalence and abundance of pathogen shedding from animal sources, including cattle, pigs, chickens, and gulls.

2.3.4. Human-impacted sites

At sites affected by humans, pathogen sources include treated sewage and other human-based sources such as on-site septic systems and swimmers (Elmir et al., 2007; Loge et al., 2009). A literature review identified representative concentrations of the reference pathogens in disinfected secondary sewage effluent (Appendix B). Table 3 provides an overview of that review.

Although all of the reference pathogens are found at substantial levels in human wastewater, research indicates that relatively few reference pathogens accounted for the vast majority of swimming-associated GI illnesses observed in EPA's 2003 to 2004 NEEAR epidemiology studies (Wade et al., 2006, 2008) conducted at POTW-impacted recreational sites on the Great Lakes (Soller et al., 2010a). The scenario evaluated in this QMRA does not cover human-impacted water specifically; however, EPA's literature review and preliminary QMRA actitivies included human-impacted sites (Schoen and Ashbolt, 2010; Soller et al., 2010a, 2010b).

Table 3. Estimated densities of reference pathogens in disinfected secondary effluent

Reference Pathogen	Estimated Density in Chlorinated Secondary Effluent	Summary Justification (Citation[s])
Rotavirus	10 plaue forming units (PFU)/L	Rao et al. (1987)
Norovirus	1000 qPCR genomes/L	Lodder and de Roda Husman (2005); Katayama et al. (2008)
Adenovirus	10 virions/L	Irving and Smith (1981); He and Jiang (2005); MWRDGC (2008)
Cryptosporidium	40 oocysts/L	McCuin and Clancy (2006)
Giardia	13 cysts/L	Rose et al. (2004); Soller et al. (2007b)
Campylobacter	100 MPN/L	Stampi et al. (1993)
Salmonella	100 MPN/L	Koivunen et al. (2001); Lemarchand and Lebaron (2003); Jiménez-Cisneros et al. (2001)
E. coli O157:H7	2.5 stx_2 gene carrying bacteria/L	García-Aljaro et al. (2004)

2.3.5. Shorebird-impacted sites

Pathogens in shorebird feces are primarily bacterial and to a lesser degree protozoan, including the reference pathogens. Thus, using bacterial and protozoan reference pathogens is appropriate for QMRAs evaluating shorebird-impacted recreational waters. Although the scenario evaluated in this QMRA does not cover shorebird-impacted water, EPA's literature review and preliminary work included shorebirds (Schoen and Ashbolt, 2010; Soller et al., 2010b). Appendix B summarizes the prevalence of reference pathogens in shorebird feces.

2.4. Populations Included in the Risk Assessment Model

The QMRA analyses characterize risks of illness from a single recreation event for the general population. In this case, the recreational "event" is defined to be as consistent as possible with exposures that occurred during EPA's water epidemiology studies (USEPA, 1986; Wade et al., 2006, 2008). Those studies reported statistically relevant relationships between FIB and GI illness in subjects engaging in self-reported body contact recreation. Here, we assume that water ingestion (consistent with the ingestion rates reported by Dufour et al. [2006]) is conservative and representative of the body-contact recreation activities that occurred during the EPA's water epidemiology studies (USEPA, 1986; Wade et al., 2006, 2008).[11]

Sub-populations can have variable risks because of differences in water contact times, water ingestion rates, and susceptibility to infection for some pathogens (Gerba et al., 1996). However, conducting QMRA for specific sub-populations is not currently feasible given the uncertainty in the differences between susceptible populations and the general population (Parkin et al., 2003),

[11] New or revised RWQC will provide a specified level of public health protection to the population, as defined by the tolerable or acceptable level of risk. In the 1986 RWQC, this level of protection was specified not to exceeed 8 cases of HCGI per 1000 recreation events. Thus, RWQC are not designed to provide a specific level of public health protection to an individual during any specific recreation event.

and a lack of data on dose-response relationships for specific subpopulations (USEPA, 2010). However, because this risk assessment uses a stochastic framework, susceptible sub-populations are accounted for to the extent that reported variations in the values of dose-response model parameters reflect response variations among different sub-populations.

2.5. Reference Health Outcomes

Water recreation can cause adverse health outcomes including GI illness, respiratory infection and illness, skin infection and disease, conjunctiva infection and disease, and ear infections and disease (e.g., "swimmer's ear"). Although swimmers might suffer from any of these outcomes, epidemiology studies indicate that water quality as measured by FIB is generally predictive of GI illness[12] (Prüss, 1998; Wade et al., 2003; Zmirou et al., 2003) and less frequently respiratory illness (Fleisher et al., 1996, 2010). Moreover, QMRA-compatible exposure data are strongest for the ingestion route of exposure and most dose-response relationships are consistent with a GI infection endpoint. Therefore, to ensure that the QMRA analyses described in this report are as compatible as possible with the water epidemiology studies, the reference health outcomes in the QMRA include (1) infection via exposure to reference pathogens through ingesting surface water during recreation, and (2) GI illness conditional on infection.

Evaluating infection differs by pathogen, based on how the dose-response models defined infection. Available models mostly defined infection as seroconversion or shedding pathogens in feces. Similarly, GI illness definitions varied among studies, but were generally related to diarrhea, and/or vomiting (Colford et al., 2002; Payment et al., 1991, 1997).

2.6. Units of Exposure and Route of Concern

The units of exposure are the number of pathogens ingested per recreation event. The number of pathogens is estimated based on the volume of water ingested during recreation and the estimated pathogen densities in the ingested water. Bacteria measurement units are usually MPN or CFU; for most viruses, PFU, although for norovirus, units are qPCR genome copies. For protozoa, the units are oocysts or cysts.

The route of concern is ingestion of water during recreational activities. This QMRA does not include aerosol exposure, ingestion of sediment or soil, or skin, eye, or ear exposures. It is important to note that EPA's water epidemiology results (USEPA, 1986; Wade et al., 2006, 2008) are based on self-reported body contact recreation, which does not necessarily require

[12] Several different definitions of GI illness have been used in water epidemiology studies. For example, the 1986 EPA AWQC are based on HCGI that was defined as a symptom category including any one of the following unmistakable or combinations of symptoms: (1) vomiting; (2) diarrhea with fever or a disabling condition (remained home, remained in bed or sought medical advice because of the symptoms); and (3) stomachache or nausea accompanied by a fever. In the 2003/2004 NEEAR Great Lakes epidemiology studies, GI illness was defined as any of the following: diarrhea (3 or more loose stools in a 24-hour period), vomiting, nausea and stomachache, and nausea or stomachache that affects regular activity (inability to perform regular daily activities). This recent definition of GI illness occurs more frequently as it excludes the requirement of fever. It is also consistent with GI illness definitions used in other recent epidemiology studies (e.g., Colford et al., 2002; Payment et al., 1991, 1997).

water ingestion. For this QMRA, we assume that the observed water contact recreation-associated illnesses observed during the water epidemiology studies were a result of water ingestion. Based on the results of a recent reverse QMRA reported by Soller et al. (2010a), water ingestion during recreation is a reasonable approximation of the epidemiology study exposure metric.

2.7. Target Risk Level

The target risk level defined by the 1986 AWQC for fresh water was 8 cases of HCGI per 1000 exposures (swimming events), which was established based on epidemiology and water quality studies conducted by EPA (USEPA, 1986). However, the more recent water epidemiology studies (Wade et al., 2006, 2008) use GI illness rather than HCGI as the target health outcome (see Footnote 12 above). For this QMRA, we use an estimated equivalent risk target based on GI illness to the 8 cases of HCGI per 1000 exposures that provides a similar overall level of public health protection. Based a preliminary review of the available epidemiology information, a target risk level of 30 GI illnesses per 1000 exposures is used as a preliminary equivalent benchmark. This estimate takes into account the more frequent occurrence of GI illness compared to HCGI.

2.8. Scenarios Modeled

We selected the QMRA scenario to evaluate illnesses resulting from recreation at a freshwater beach impacted by agricultural animal sources of fecal contamination. As part of this effort, several assumptions were made to limit the scope of the scenario and to ensure that the QMRA results would be conservative, including the following:

- Exposure is via primary contact recreation (e.g., swimming) at a freshwater beach.
- Water ingestion is the predominant route of exposure during primary contact recreation. The scenario does not evaluate the risk of ingesting water from activities such as wading, boating, or fishing; however, we expect those risks to be less than those associated with primary contact recreation.
- Fresh cattle manure, pig slurry, and poultry litter are applied at agronomic rates (the highest rate at which manure should be applied in the United States) to land adjacent to the freshwater beach to minimize the uncertainty and variability associated with environmental fate and transport of FIB and reference pathogens.
- The cattle manure, pig slurry, and poultry litter contain FIB and reference pathogens consistent with levels reported in the peer-reviewed literature.
- The fresh, solid, untreated fecal contamination from cattle, pigs, and chickens reaches the freshwater beach via runoff from an intense rainfall event and undergoes minimal dilution in receiving water. An intense rainfall event produces a higher load of pathogens

and FIB than a less intense precipitation event. This results in a conservative risk estimate via forward QMRA.

- The FIB and reference pathogens are present in cattle manure, pig slurry, and poultry litter runoff at levels consistent with the observed mobilization of FIB and reference pathogens from the EPA environmental monitoring studies (see Section 2.12 and Appendix D for further information).
- Assuming that the mobilized pathogens and FIB are applied adjacent to receiving waters presents the highest exposure and produces a more conservative risk estimate relative to conditions with best management practices.

2.9. Questions to be Addressed

The QMRA is designed to address the following primary and secondary questions:

1. What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, or chicken) sources of fecal contamination during or immediately after a rain event?
2. How do those risks compare to risks associated with freshwater beaches impacted by human sources of fecal contamination (effluent from a POTW)?

The forward QMRA estimates the risk of illness associated with recreation (swimming) at a beach impacted by agricultural animal sources of fecal contamination. Numerical simulations are used in which the pathogens in land-applied manure are selected from ranges derived from the literature; pathogen mobilization (from the manure) proportions are based on observed mobilization rates for each pathogen and manure type; and the swimmers are assumed to be exposed to untreated and undiluted runoff.

The relative QMRA compares risks from recreation in the animal-impacted water to those associated with human-impacted water. To achieve this, we extended previous related work that evaluated the estimated human health risks from exposure to recreational water impacted directly by fecal contamination from human and non-human sources (Schoen and Ashbolt, 2010; Soller et al., 2010b). This relative QMRA extends that evaluation to include land application of manure that contains FIB and reference pathogens and their mobilization (proportion of FIB and reference pathogens that run-off) during and immediately after rainfall events based on the results of EPA environmental monitoring studies.

2.10. Conceptual Models

2.10.1. Top-tier models

Figure 7 and Figure 8 illustrate the top-tier conceptual models for the QMRA. Forward QMRA (Figure 7) is used to answer question 1 (above) while the relative QMRA approach (Figure 8) is used to address question 2. A previously conducted reverse QMRA (Figure 9) provides context

about human-impacted water and justification for the use of specific reference pathogens for human-impacted waters (Soller et al., 2010a).

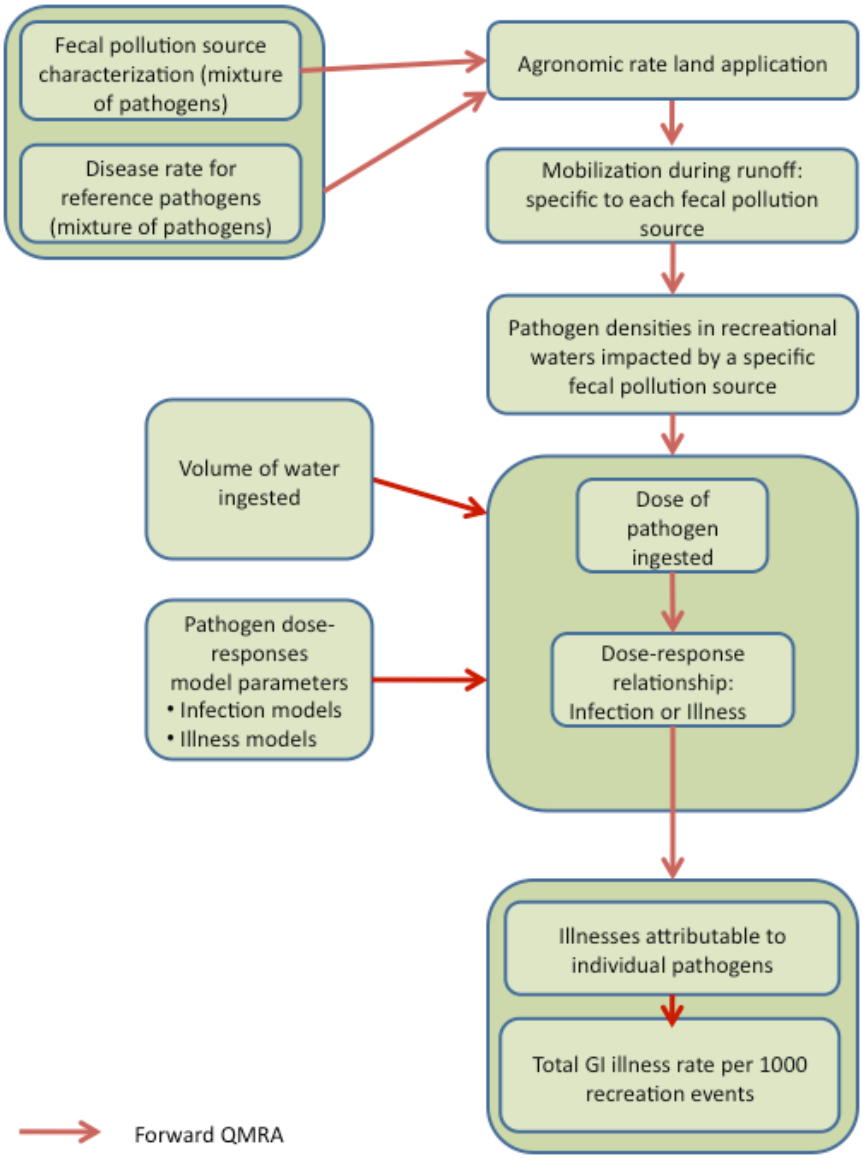

Figure 7. Forward QMRA conceptual model

In Figure 7, the input data characterize pathogens present in fecal pollution source(s), the fraction of human-infectious pathogenic strains in each fecal source of interest, the prevalence of infection in the non-human source (proportion of animals shedding the pathogen), ingested volumes, dose-response models and parameters, and pathogen mobilization. Output of the forward QMRA model is the probability of infection and illness associated with exposure to water during recreation.

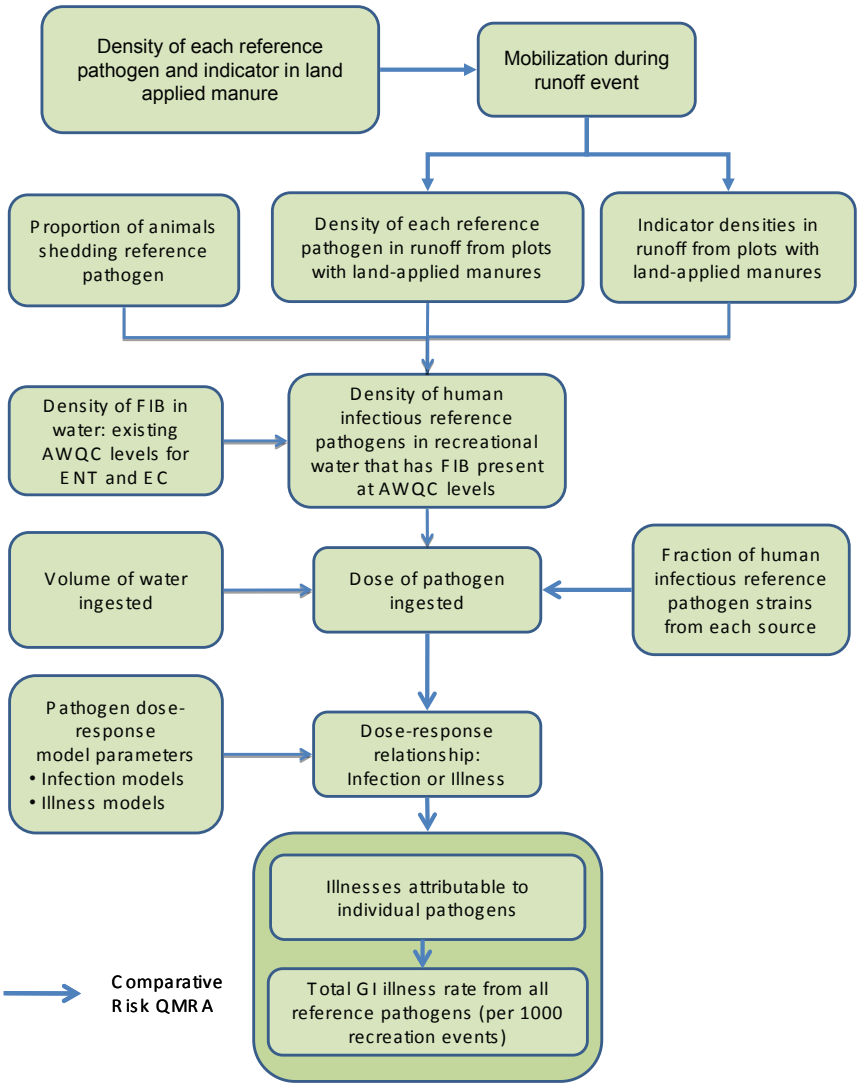

Figure 8. Relative QMRA conceptual model

In Figure 8, input data are somewhat different than those used in the forward QMRA. For the relative QMRA, we assume a specific level of FIB is in the waterbody—in this case, the current RWQC levels for enterococci or *E. coli*. These FIB levels are used in conjunction with the FIB and reference pathogen levels in the land-applied material, the fraction of human-infectious pathogenic strains in each fecal source of interest, the prevalence of infection in the non-human source (proportion of animals shedding the pathogen), the proportions of FIB and reference pathogens that mobilize during a rain event, and the volume of water ingested (Schoen and Ashbolt, 2010; Soller et al., 2010b). The relative QMRA model output is the probability of infection and illness associated with exposure to water during recreation for each source of interest referenced to the chosen level of FIB.

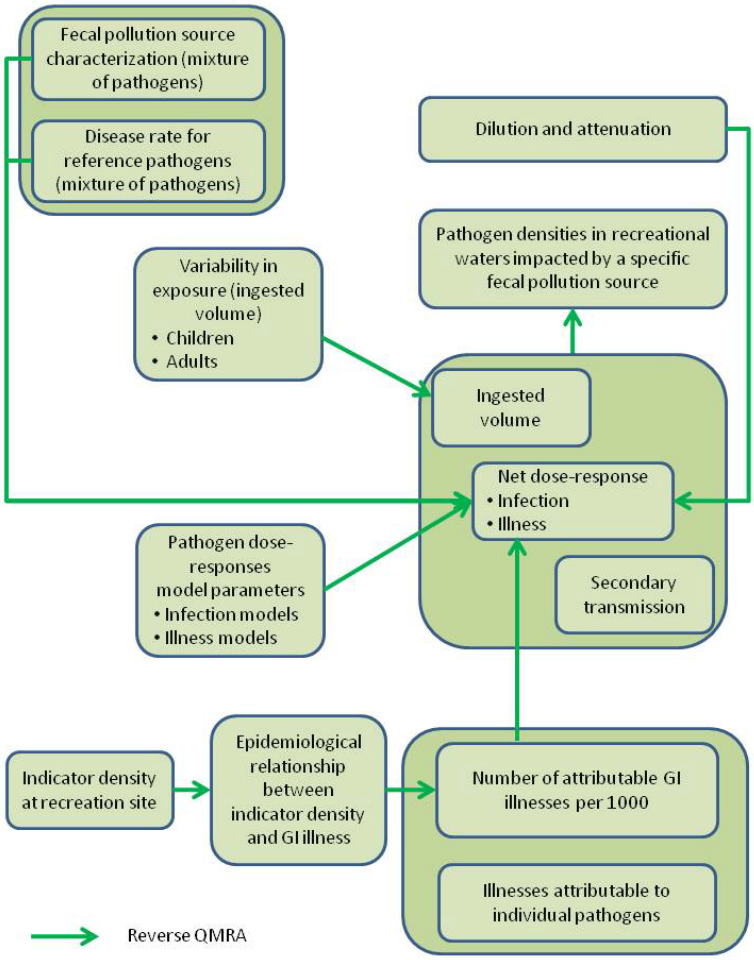

Figure 9. Reverse QMRA conceptual model

In Figure 9,[13] input data characterize the pathogens present in fecal pollution source(s) (either based on the relative abundance of pathogens in the fecal pollution source or on the observed health effects for each reference pathogen); ingested volumes; dose-response models and parameters; and observed illness rate (i.e., number of illnesses per day per 1000 swimmers). The output of the reverse QMRA model is an estimate of pathogen densities at a recreation site with a known fecal pollution source (Soller et al., 2010a).

2.10.2. Sub-tier model: model parameter form and estimation

In this report, we modeled parameter uncertainty and variability explicitly by treating each parameter as a random variable. In cases with insufficient data to justify a specific statistical

[13] Again, no reverse QMRA was conducted as part of this study. The discussion of reverse QMRA and Figure 9 are provided because EPA previously conducted reverse QMRA, and in this study it provides context about the relative importance of the reference pathogens in human-impacted recreational waters.

distribution, we used point estimates. This approach is consistent with previous QMRAs (Eisenberg et al., 1996; 1998; Soller et al., 2010b). The stochastic parameters in the model include the following:

- reference pathogen density (abundance) in animal manure;
- FIB density in animal manure;
- prevalence of reference pathogen shedding in the animal-source;
- proportion of FIB and reference pathogens that mobilize during a rainfall event;
- volume of water ingested during recreation;
- dose-response parameters (to the extent that peer-reviewed literature supports it); and
- morbidity fraction (proportion of infections that result in illness).

Abundance, prevalence, and mobilization of all pathogens differ with manure type. Different distributions are used for each pathogen-manure type combination.

2.10.3. Sub-tier model: animal-impacted water pathogen-loading model

FIB and reference pathogen loading to a recreational waterbody can occur through direct or indirect contamination (Figure 10). Direct contamination occurs when fresh undiluted fecal material is deposited into a waterbody. Indirect contamination occurs during transport from adjacent land into a waterbody via rainfall runoff. Soller and colleagues (2010b) reported the risks from direct fecal contamination from agricultural animals into a recreational waterbody. Those results indicated that the GI illness risks associated with exposure to recreational waterbody directly impacted by fresh cattle feces might not differ substantially from water impacted by human sources; however, the risks associated with exposure to recreational water directly impacted by gull, chicken, and pig wastes appear to be lower than those impacted by human sources (Soller et al., 2010b). The QMRA described in this report extends that work by considering indirect contamination (described below). These two routes represent reasonable conservative stream loading scenarios for livestock fecal pollution.

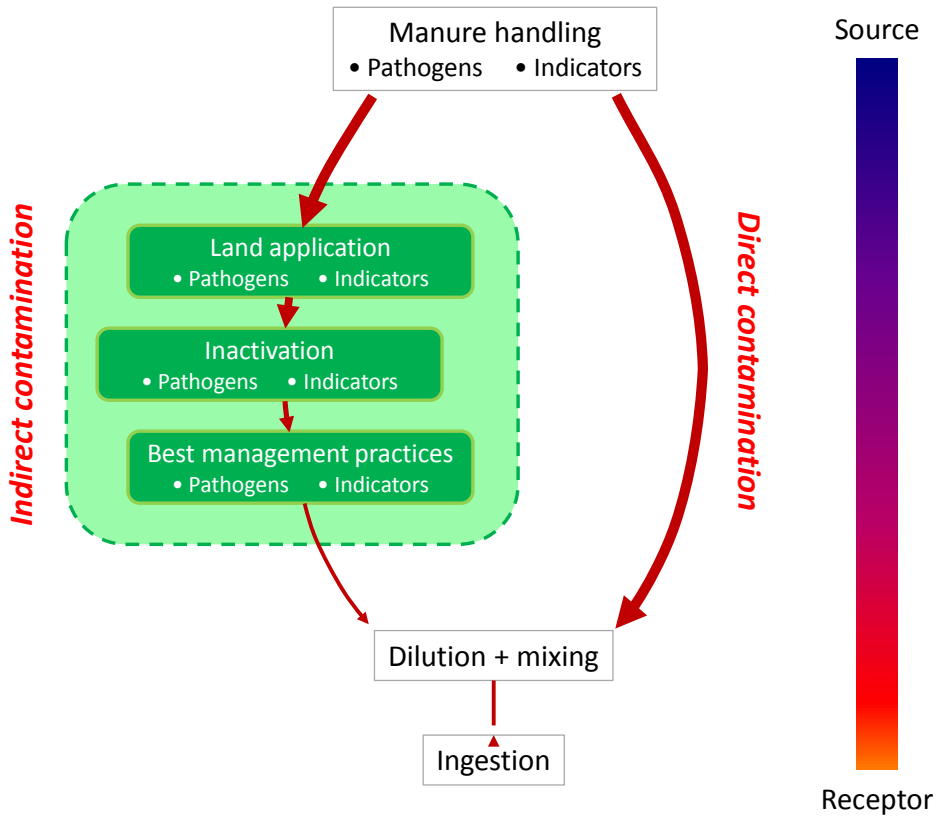

Figure 10. Transport of pathogens and indicators to swimmers from livestock manure

An important distinction between direct and indirect contamination is that the source material of concern for direct contamination is feces from an individual or individuals; for indirect contamination the source material of interest is effectively a composite sample of fecal material. The data used to characterize the abundance of FIB and reference pathogens for direct (Schoen and Ashbolt, 2010; Soller et al., 2010b) and indirect (present QMRA) contamination reflect this distinction. Using data from individual fecal samples to characterize abundance in the previous work corresponded to an exposure that was assumed to be in close proximity to manure deposited directly into recreational water. In this QMRA, data from an intensive environmental sampling program conducted by EPA (see Section 2.12) were used to characterize the mobilization of FIB and reference pathogens due to rainfall and subsequent runoff.

Pathogen loading from agricultural animal sources depends on the prevalence of animals infected by reference pathogens, the abundance of reference pathogens in fresh manure, manure handling practices (particularly storage time and timing of application), time between application and rainfall, and the path by which pathogens reach receiving water. During transport to receiving water, pathogens may be inactivated or removed in buffer strips or other physical barriers.

Figure 11 provides a conceptual model showing how indirect FIB and reference pathogen loading can occur for agricultural animal sources: (1) they may be deposited directly on fields during grazing and be mobilized during rainfall and transported, (2) they may be in treated or untreated manure that is spread on fields and mobilized or transported during a rain event, (3) they may run-off from feedlot pens, or (4) they may escape storage due to an extreme rainfall event or mishap.

EPA conducted a preliminary literature review to evaluate the important factors associated with animal-impacted waterbodies (USEPA, 2009a), developed preliminary exposure models for the pathways described above, and conducted exploratory analyses to determine which model parameters most strongly affected QMRA output (Annex 2). Salient findings from the exposure modeling and preliminary QMRA work included the following:

- Collecting and storing fecal material on site can be an effective barrier to pathogen mobilization. Depending on storage time, land application may cause short-term pathogen risk spikes immediately following application. These spikes can be roughly equivalent to the risk associated with open grazing operations.
- Provided sufficient time is provided, storage can effectively reduce pathogens.
- Managing land application to avoid periods of high rainfall reduces risk.
- Prevalence of a herd's infection changes over time.
- Understanding the prevalence (and dynamics) of human infectious pathogenic strains in animal-impacted water is important to estimate its risk.
- Pathogen densities in manure are highly uncertain.
- Pathogen super-shedders have the potential to drive the risk during a rainfall event.
- Environmental inactivation rates of pathogens are highly uncertain. Therefore, reducing pathogens through uncontrolled environmental processes is not feasible unless extended residence times are guaranteed.

Based on the goals of the QMRA and the available data, the microbial loading model used in this QMRA addresses land application of manure and subsequent mobilization of FIB and reference pathogens at the beach that is adjacent to the animal-impacted runoff.

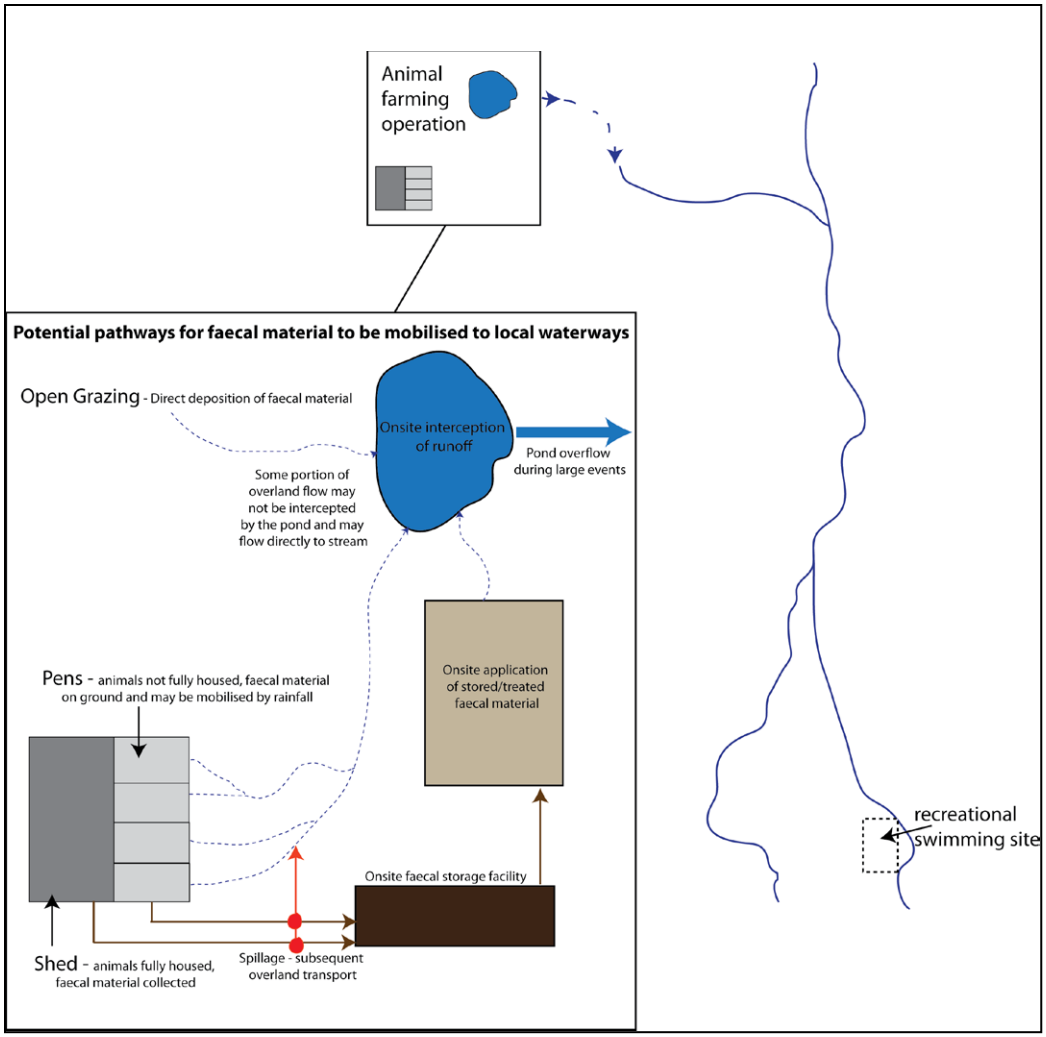

Figure 11. Conceptual model of paths for livestock pathogens reaching recreation sites

2.10.4. Sub-tier model: reference pathogen dose-response models

The QMRA dose-response models for reference pathogens come from peer-reviewed studies (see Section 3.2.2 for further information). A brief overview of dose-response modeling is presented below, while the EPA MRA Tools document (USEPA, 2010) provides a more comprehensive review of this topic.

The infection process requires that a person ingests pathogens, at least one pathogen initiates an infection, and a proportion of infections proceed to illness. All three of these processes can be described with probability distributions.

When the probability of ingesting a dose of pathogens is Poisson-distributed and all of the ingested pathogens have an equal probability of initiating infection, the exponential dose-response model is appropriate:

$$P_{\text{infect}}(d;r) = 1 - e^{-rd} \qquad [1]$$

where P_{infect} is the individual probability of infection, d is dose (number of pathogens), and r is a parameter of the distribution equal to the probability that an individual pathogen initiates infection.

When the probability of ingesting pathogens is Poisson-distributed and the probability that individual pathogens initiate infection is beta-distributed, the beta-Poisson model is appropriate:

$$P_{\text{infect}}(d;\alpha,\beta) = 1 - {}_1F_1(\alpha, \alpha+\beta, -d) \qquad [2]$$

where α and β are parameters of the Beta distribution and ${}_1F_1$ denotes a confluent hypergeometric function. A commonly used approximation to the beta-Poisson may be used when $\beta >> 1$ and $\beta >> \alpha$. This approximation is:

$$P_{\text{infect}}(d;\alpha,\beta) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \qquad [3]$$

When pathogens are aggregated (are no longer Poisson-distributed), a Poisson-stopped log-normal distribution can describe the distribution of doses in an inoculum. When dose is assumed to follow a Poisson-stopped log-normal distribution, and the ability of individual ingested pathogens to initiate infection is beta-distributed, the resulting dose-response model is:

$$P_{\text{infect}}(d;\alpha,\beta,a) = 1 - {}_2F_1\left(\alpha, \left(\tfrac{1-a}{a}\right)d, \alpha+\beta, -\left(\tfrac{a}{1-a}\right)\right) \qquad [4]$$

where the parameter a is related to the degree of aggregation in the pathogen dose, α and β are parameters of the Beta distribution, and ${}_2F_1$ denotes a hypergeometric function. Note that equation 4 reduces to the exact beta-Poisson dose-response model as $a \to 0$.

Published studies have used empirical dose-response models (which cannot be derived using assumed distributions for exposure and infection initiation) based on fitting those models to data or that are based on those models mimicking observed patterns of infections among humans. Among these empirical models, the Gompertz-log model (equation 5) describes response (illness) of humans to doses of *Salmonella* of numerous serotypes:

$$P_{\text{illness}}(d;a,b) = 1 - e^{-e^{-\ln a + b \ln d}} \qquad [5]$$

where a and b are parameters of the distribution that take on different values for different *Salmonella* serotypes, and P_{illness} denotes the individual probability of illness.

Two types of models describe the progression of illness to infection—a constant rate model and a dose-dependent model. The constant rate model, which is the most common in published QMRA studies, assumes a fixed proportion of individuals infected by a given pathogen

progresses to illness. The proportion progressing to illness may be estimated based on results of feeding studies or epidemiology studies. The dose-dependent model assumes the conditional probability of illness given infection is given by:

$$P(\text{illness} \mid \text{infection}; d, \eta, \kappa) = 1 - (1 + \eta f(d))^{-\kappa} \qquad [6]$$

where η and κ are parameters of the distribution and $f(d)$ is a function of dose. Studies have explored functions of dose for progression of infection to illness and have identified data sets in which progression was independent of dose, was dependent on the inverse of dose, and was dependent on dose.

2.10.5. Sub-tier model: volume of water ingested during recreational activities

Results reported by Dufour et al. (2006) characterize the volume of water ingested during recreational activities for swimming episodes of 45 minutes duration. The data can be fit to log-normal distributions for children and adults combined (Figure 12) or individually (Figure 13) (Soller et al., 2007b). In this QMRA, we use the distribution for children and adults combined to represent water ingestion during recreational activities for the general population.

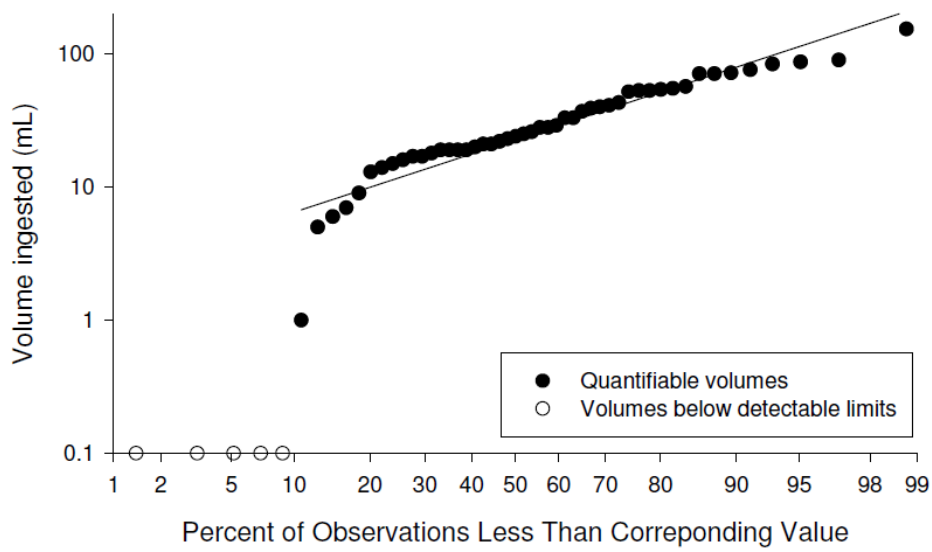

Figure 12. Ingested volumes for the combined data (children and adults)

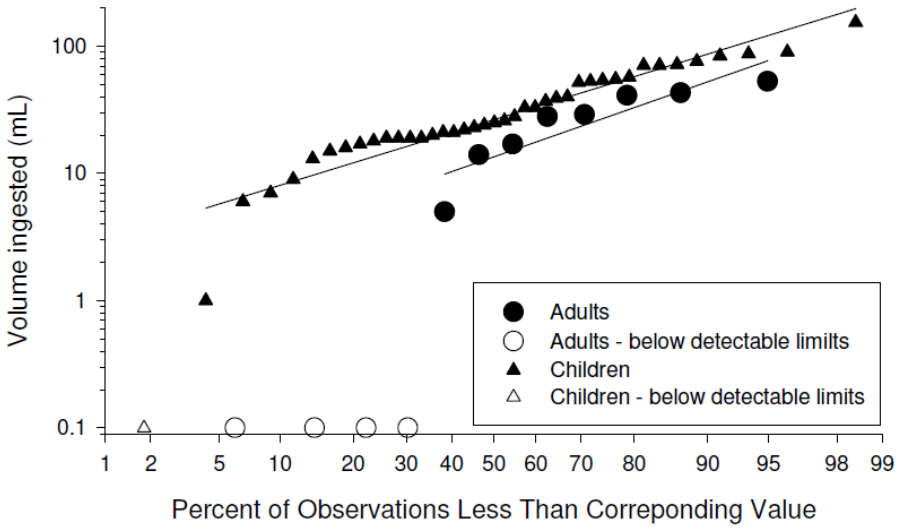

Figure 13. Ingested volumes, child and adult data separated

2.10.6. Sub-tier model: secondary infections

In this context, secondary transmission is fecal-oral transmission of enteric pathogens from an infected individual (person infected during primary contact recreation in surface water) to a susceptible individual. Symptomatic or asymptomatic individuals can infect others. Accounting for secondary transmission or immunity depends on many factors, including the goal of the QMRA (Soller and Eisenberg, 2008). Because QMRAs that evaluate risks from livestock-impacted water will use individual level, static models as the primary analysis tool, the model will not include secondary transmission or immunity.

Using a static model in this QMRA is appropriate because we expect the static model to be parsimonious under the anticipated conditions (Soller and Eisenberg, 2008). Specifically, we assume that (1) the proportion of the exposed population will be relatively low and that average frequency of exposure will be weekly or less, and (2) the average dose of pathogens in the exposure will be relatively low. Under these conditions, we expect the results from a static model to agree reasonably well with those from a dynamic model (Soller and Eisenberg, 2008).

2.11. Summary of QMRA Methods

As discussed above, the QMRA is designed to address two specific questions: (1) What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, and chicken) sources of fecal contamination? and (2) How do those risks compare to risks associated with freshwater beaches impacted by human (POTW) sources of fecal contamination? We use two complementary approaches—forward QMRA and relative QMRA— to answers these questions. The sections that follow describe how the QMRA analyses were conducted to answer the questions of interest.

2.11.1. Risk of illness associated with recreation at a beach impacted by agricultural animal sources of fecal contamination

We use a traditional forward QMRA approach to characterize the risk of illness associated with recreation at a freshwater beach. The methodology for the QMRA analyses is a Monte Carlo-based approach with model parameters characterized as statistical distributions, whenever possible. Separate Monte Carlo analyses were conducted for each agricultural animal source.

The analysis begins with literature review-based data (Appendix B) to characterize the densities and prevalences of reference pathogens in fecal waste for each animal source (solid fresh cattle feces, liquid fresh swine feces, and fresh poultry litter). We assume that these materials are applied to land at agronomic rates[14] and are mobilized during a 100-year return period storm (referred to hereafter as intense rain event) for the Piedmont region of Georgia.[15] The results from the EPA Office of Research and Development's environmental monitoring program (Section 2.12 and Appendix D) characterize the proportion of the land-applied pathogens that run-off following this type of rain event. Specifically, the density of FIB and reference pathogens in water running off is proportional to the number of land-applied organisms with a variable proportionality constant for different manure types and conditions (e.g., plot slope, antecedent soil moisture). Mathematically,

$$N_i V_{RO} = f_{i,RO} n_i M_{manure} \qquad [7]$$

where

N_i is density of organism i in runoff water (organisms/volume);

V_{RO} is net runoff during the event (volume);

$f_{i,RO}$ is the proportion of organisms mobilized during the entire event;[16]

n_i is the density of organism i in the land-applied manure; and

M_{manure} is the mass of manure applied to the plot generating runoff volume $V_{RO.}$

Thus, for each of the animal sources, the density of reference pathogens in the runoff is calculated as the product of the reference pathogen density in land applied fecal waste, the

[14] Manures were applied at agronomic rates based on measured nutrient concentrations in samples of the land-applied manure. These application rates are specific to the manures and ground cover used in the study and may differ from other manures whose nitrogen densities are different or for other plots where the nutrient requirements are different, either because of residual nutrients in the soil or because the ground cover has a different nutrient uptake. Assuming pathogens and indicator organisms are well-mixed in manures, the pathogen and indicator loads scale linearly with manure application rate and other sites may have manure indicator and pathogen loads signficantly different from those in the current study.

[15] In the literature, mobilization is often assumed to be a function of runoff, not rainfall. In this study, the rainfall applied to the plots was fairly uniform and based on a 100-year return period storm event. The runoff was variable between plots and was a function of location of the plot, slope, soil characteristics, etc.

[16] The term $f_{i,RO}$ is a random variable with range based on mobilization fractions in the EPA plot-scale experiments. Because the EPA experiments used a single rainfall intensity and rate, the dependence of $f_{i,RO}$ on event characteristics is unknown.

prevalence of infection (percent of animals shedding), the human infectious potential of the pathogen, and the proportion of the applied reference pathogens that run-off following a rain event divided by volume of the runoff for the event. In this exposure scenario, recreation is assumed to occur at the edge of the recreational waterbody, where the runoff enters the waterbody. Therefore, the dose of pathogens for this exposure scenario is the product of the volume of water ingested during recreational activities and the density of each pathogen in the runoff. That dose is input to the appropriate dose-response relationship resulting in a probability of infection. The probability of infection is multiplied by a morbidity factor to produce a probability of illness. This scenario is intentionally conservative (i.e., developed to produce health-protective estimates of risk), including an intense runoff event, no attenuation of pathogens between runoff and entry into receiving water, and ingestion of undiluted runoff.

The risk associated with each fecal contamination source is characterized as the total probability of GI illness, P_{ill}^{S}, using the probability of illness from each source-specific pathogen in a manner that is parallel to computing annual risks of infection by combining daily risks (Regli et al., 1991):

$$P_{ill}^{S} = 1 - \prod_{rp} (1 - P_{ill_{rp}}^{S}) . \qquad [8]$$

This process is repeated 10,000 times for each fecal contamination source to generate a distribution of risk.

2.11.2. Comparison of animal-impacted water risks with POTW-impacted water

The second analysis, which uses the relative QMRA approach, provides a relative comparison of the estimated risks from recreation in water impacted by agricultural sources of fecal contamination to those associated with human-impacted water. Previously developed methods (Schoen and Ashbolt, 2010; Soller et al., 2010b) form the basis for this analysis, but were extended by including land-application of FIB and reference pathogens, and mobilization (proportion of FIB and reference pathogens that run-off) during rainfall events based on the results of the EPA environmental monitoring studies (Section 2.12).

In this analysis, the estimated risks are calculated for a hypothetical waterbody that contains geometric mean FIB densities at the current (USEPA, 1986) RWQC for freshwater (33 CFU enterococci/100 mL and 126 CFU *E. coli* /100 mL, respectively). We conduct separate calculations for each source of fecal contamination (cattle, pigs, and chicken). The current RWQC were established to provide a level of health protection equivalent to approximately 8 cases of HCGI per 1000 recreation events for water impacted by treated effluent. As noted previously, recent recreational water epidemiology studies use a definition of GI illness that excludes fever as a required symptom (Colford et al., 2007; Wade et al., 2006, 2008). This more recent health metric occurs more frequently than GI illness. In this QMRA, we use an benchmark risk of 30 cases of GI illness per 1000 recreation events as an estimate of the

equivalent level of GI illness to the currently acceptable level of 8 cases of HCGI per 1000 recreation events. This estimate is based on a preliminary evaluation of the data reported by Wade et al. (2006).

Reference pathogen doses are derived from the density of the FIB from each source (Schoen and Ashbolt, 2010; Soller et al., 2010b). Pathogen dose is calculated based on independent Monte Carlo samples from the observed ranges of pathogen and FIB densities in fecal waste and the proportion of these organisms that mobilize during a rain event. Note that this sampling scheme does not require a specific relationship between the FIB and pathogen in the fecal waste or in the receiving water. The dose of each reference pathogen from each source is calculated as follows:

$$\mu_{rp}^{S} = \frac{C_{FIB}}{R_{FIB}^{S} \times 100} \times R_{rp}^{S} \times p_{rp}^{S} \times I_{rp}^{S} \times V \qquad [9]$$

where

S is the fecal contamination source;

C_{FIB} is the waterbody density of enterococci or *E. coli* (CFU/100 mL);

R_{FIB}^{S} is the density of FIB in runoff from plots with land-applied manure (CFU/100 mL) or in sewage (CFU/L);

R_{rp}^{S} is the density of pathogen species in runoff from plots with land-applied manure number of pathogens or genomes 100 mL^{-1}) or in sewage (number of pathogens or genomes L^{-1});

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 [17] (proportion of animals shedding the pathogen); and

V is the volume of water ingested (mL).

This relation is similar to previously cited methods (Schoen and Ashbolt, 2010; Soller et al., 2010b), except here, the densities of reference pathogens and FIB in water ingested during recreation are based on the density of the organisms in the land-applied manure and the proportion of the organisms running off during rain events.[18]

[17] For previous work conducted on human sources, I_{rp}^{S} was assumed to be 1.0 because the FIB and pathogen data are from sewage not individual fecal samples, and therefore already accounts for the pathogen prevalence.

[18] In these analyses, we assume that the FIB and reference pathogens derive from the source being evaluated. In reality, there can be numerous sources of FIB in a waterbody, including sources that do not contribute pathogens. The relative QMRA analyses developed in this report are conservative for waterbodies that also contain non-pathogenic sources—non-pathogenic sources would cause FIB levels to be relatively higher compared to pathogen levels.

Similar to the forward QMRA described in the previous section, doses are input to the appropriate dose-response relationship resulting in a probability of infection. The probability of illness is conditional on infection and is calculated via a morbidity fraction for each reference pathogen. The total probability of illness for each fecal contamination source is computed as described above. This process is repeated 10,000 times for each source to generate a distribution of risk. Those distributions of risk are then compared to the benchmark risks for human-impacted water described above.

2.12. Environmental Sampling

The objective for EPA's environmental monitoring and sampling study was to generate primary data to characterize zoonotic enteric pathogens and FIB densities in surface water affected by agricultural activities. In addition, the study emphasized overland transport inputs and processes.

The monitoring design included rain simulation experiments in small plots amended with solid cattle manure, swine slurry from a lagoon, and un-composited litter from a chicken operation. These matrices were selected because higher pathogen densities are associated with fresh fecal material, and pathogen removal efficiencies vary both between and within treatment processes (Bicudo and Goyal, 2003; Goss and Richards, 2008; Heinonen-Tanski et al., 2006; Larney and Hao, 2007; Letourneau et al., 2010; Martens and Böhm, 2009; Peu et al., 2006; Topp et al., 2009; Vanotti et al., 2005; Vinnerås, 2007; Wong and Selvam, 2009; Ziemer et al., 2010). Using fresh fecal material promoted conservative modeling (because of the assumption that relatively high pathogen densities are present in land-applied material) and robust results (because the model does not rely on assumptions regarding the degree of removal during treatment prior to land application).

The rainfall simulation experiments were designed to

1. estimate pathogen and FIB mobilization rates from manure-impacted plots to surface water, and
2. provide data to characterize pathogen and FIB densities in overland runoff.

The monitoring study used rainfall simulators instead of natural rainfall events to enhance reproducibility, allow greater control over important independent variables, and better characterize mobilization and loading rates of pathogens and FIB through greater sample size. Previous experiments targeting microbial transport from land-applied manure focused on the behavior of FIB from cattle manure and produced limited pathogen information (Collins et al., 2004; 2005; Guber et al., 2007a; Muirhead et al., 2006; Sinton et al., 2007).

The rainfall simulation experiments were held at 36 plots (0.75 × 2 m) on U.S. Department of Agriculture (USDA)-owned land in Oconee County, Georgia (33° 47'N, 83°23'W) (Butler et al., 2008). The experimental plots were delineated with galvanized sheet metal (23 cm width) placed into the ground to a depth of 18 cm. Consistent with previous work, Tlaloc 3000 rainfall

simulators (Joern's Inc., West Lafayette, IN) were used (Soupir, 2003; Soupir et al., 2006). Simulated rainfall was applied to the plots at a set rate intended to simulate both an intense rain event for the Georgia Piedmont region and to be sufficient to produce surface runoff. Runoff was collected at the lower end of each plot. Baseline simulations were conducted to determine background pathogen, FIB, and nutrient levels. Histograms were used to identify frequency distributions of baseline runoff volumes and to select plots within a specific range of volumes.

Manure applications followed a randomized split plot design. The treatments consisted of manure applications from three animal types—pigs (liquid manure), beef cattle (solid manure), and broiler poultry (litter)—and a control treatment (no manure application). Each treatment had three replicates (plots) and three manure applications timed relative to rainfall simulations. The timing for rainfall application was one hour, one week, and two weeks after manure application. The type (mixed fescue/Bermuda crop) and height (10 cm) of the vegetation cover was the same for all plots. Each type of manure was analyzed for pathogen and FIB loading prior to application via randomized composite samples. During each rainfall simulation run, samples were collected every five minutes for the duration of the event to account for the cumulative runoff volume. Six runoff samples from selected intervals (5, 10, 20, 30, 60 minutes, and total composited) were analyzed for both *E. coli* and enterococci total densities. Samples were split into separate containers for non-microbial analyses, including total suspended solids, dissolved organic carbon, and nutrients. Two composited samples (10 L) were collected per run for pathogen analysis (30-minute composite and total composite). Samples were analyzed for *Cryptosporidium*, *Giardia*, *Salmonella*, *E. coli* O157, and *Campylobacter*, depending on the type of manure applied. Manure from the various sources was applied at agronomic rates following USDA Natural Resources Conservation Services (NRCS) guidelines (Midwest Plan Service, 2004), and based on the nitrogen requirement of the type of crop and the nutrient concentration in the manure being applied.

In the plots where rainfall was not applied immediately after manure application (1-week and 2-week treatments), plastic covers were placed on the plots to protect them from natural rain events. These covers were placed well above the vegetation cover to allow air circulation and heat exchange. The type of plastic selected allowed for 75 to 80% of the UV light to penetrate. This experiment was conducted three times, (October 2009, March 2010, and June 2010) to obtain sufficient data points and to account for varying climatic conditions.

During the first simulation, it was determined that the levels of pathogens of interest in the applied material were too low to detect in the runoff. Therefore, for the second and third rainfall simulation runs, the manure was seeded with surrogate pathogens to determine the mobilization rates of pathogens. Surrogate pathogens were all non-virulent species that did not pose an environmental risk or of infection to project personnel. The following surrogate pathogens were used: (1) *E. coli* O157:H7 B6914 #87, which was added to cattle feces, and swine slurries, and poultry litter (the latter only during the March simulation); (2) UV-inactivated *Giardia* and *Cryptosporidium*, which were added to cattle manure and swine slurries; and (3) *Salmonella*

X3985, which was added to cattle manure, swine slurries, and poultry litter. Preliminary calculations determined the concentration of surrogate pathogens needed for each type of manure to increase the likelihood of detection in runoff. The final surrogate pathogen concentrations considered recovery methodology, decay of the organisms in manure and during transport, literature for leaching rates for pathogens or FIB from livestock manure, maximum pathogen levels observed in livestock manure, and maximum number of organisms that could be produced to use for spiking. Mixtures containing the different combination of surrogate pathogens were then prepared and seeded in the appropriate type of manure. Manure seeding was conducted in the laboratory the same day the manure was applied to the plots and transported to the field on ice. Analysis of both surrogate pathogens and wild-type pathogens was conducted in all manures and runoff as described above.

2.13. Tools Used in the QMRA

The software used to implement the forward QMRA is MathCad (Mathsoft Corp.). A previously developed MathCad worksheet, the Microbial Risk Assessment Interface Tool (MRAIT) was used as a base package and modified to accept appropriate input (Soller et al., 2007a). The R programming language (R Development Core Team, 2009) and the Python programming language (Python Software Foundation, 2009) were used for the relative QMRA analyses. Previous code in R (Schoen and Ashbolt, 2010; Soller et al., 2010b) was adapted to account for FIB and reference pathogen mobilization during rain events. The Analytica™ computational environment (Lumina Decision Systems) was used to develop initial QMRA models for livestock-impacted sites.

2.14. Summary of Assumptions

Assumptions underlying the QMRA include the following:

- GI illness is the health outcome of primary concern in this QMRA. Infection from the reference pathogens and subsequent illness result in GI illness. Based on epidemiological investigations, skin infection and disease, conjunctiva infection and disease, and ear infections and disease are assumed not to be correlated with FIB (Prüss, 1998; Wade et al., 2003; Zmirou et al., 2003). Although FIB might predict respiratory infection and illness (Fleisher et al., 1996), GI illness occurs more frequently; therefore, GI illness rates predicted by the QMRA are assumed to be conservative and protective for respiratory illness.
- Water ingestion during recreational activities is the exposure route of interest. Other routes of exposure, such as inhalation and dermal contact, do not substantially add to the risk associated with ingestion.

- The risk of illness at a freshwater beach impacted by agricultural animal sources of fecal contamination is adequately characterized by the risk associated with the bacterial and parasitic protozoan reference pathogens.
- Loss of pathogen virulence due to passage through non-human hosts or exposure to a non-enteric environment can be characterized as "high," "medium," and "low" in the QMRA based on the relative occurrence of species that infect humans and strains and serotypes present in typical livestock wastes.
- Human dose-response models adequately predict infection or illness risks for reference pathogens, regardless of the source (though variability in host-pathogen system response can be included in dose-response modeling).
- Data collected from the EPA environmental monitoring program in conjunction with data from the peer-reviewed literature can be used to estimate microbial water quality at a freshwater beach impacted by agricultural animal sources of fecal contamination.
- Use of a static, individual-level QMRA model is reasonable—secondary transmission and immunity do not substantially modify risks.
- For the general population, body contact recreation (as self-reported in water epidemiology studies) involves water ingestion volumes consistent with the recreational activities reported by Dufour et al. (2006).
- Removal or die-off of reference pathogens and FIB after mobilization from fields and prior to ingestion by swimmers is limited.
- Recreation at the assumed point of exposure produces a conservative estimate of risk and is protective compared to other potential exposure points (downstream, diluted, or contamination scenarios that are older).
- The mobilization fractions observed during the EPA simulated rain events are representative of the highest mobilization fractions realized during actual rain events.

2.15. Sources of Variability and Uncertainty

One particularly attractive attribute of QMRA is its ability to account for both variability and uncertainty. In this QMRA, we use a probabilistic framework and characterize each model parameter using a statistical distribution[19] where the parameters of those distributions account for variability and/or uncertainty. Although it is desirable to treat variability separately from uncertainty in QMRAs (USEPA, 2006), the available data were insufficient to do this for this risk assessment.

[19] In cases where data are sparse, we use a uniform distribution and specify lower and upper feasible bounds. If those bounds span more than two orders of magnitude, we use a log-uniform distribution.

2.15.1. Variability

Substantial variability is anticipated in both the QMRA exposure assessment and health effects components. We account for the known variability to the extent possible and reasonable. Table 4 summarizes the variable parameters and the underlying causes of the variation.

Table 4. Variable parameters and underlying causes for their variations

QMRA Component	Variable Parameter	Causes
Exposure Assessment	FIB and pathogen density	• Temporal and spatial heterogeneity • Sporadic loading (epidemics, super-shedders) • Rainfall and runoff (intensity, depth, antecedent conditions) • Waves and currents • Solar radiation • Tides • Season
	Ingested volume	• Exposure duration • Age • Gender
Health Effects	Dose-response	• Differences in immune system competency • Prior exposure • Vaccination • Age • Other heterogeneity in host response • Intra-species, intra-strain, intra-serotype, and intra-isolate heterogeneity in pathogen virulence • Health end-point measured
	Secondary transmission and immunity	• Population-level immune status and background infection rate • Heterogeneous contact patterns • Age

The most significant variability in the exposure assessment is due to temporal and spatial heterogeneity in reference pathogen and FIB densities. FIB and pathogen densities change by orders of magnitude over short time periods (Boehm et al., 2002, 2007; Curriero et al., 2001). Detection methods also impart variability—selective media and injured cells are important issues underlying heterogeneity. In addition to variability during nominal conditions, non-standard events can cause extreme variability. Such events include super-shedding of pathogens, combined sewer overflows, and extreme rainfall/runoff. When extreme variability exists in pathogen or FIB densities, traditional statistical fitting to distributions such as the log-normal distribution may be inappropriate (Petterson et al., 2007, 2009; Pouillot et al., 2004; Signor et al., 2007).

In health effects modeling, variation among pathogens occurs in their ability to infect humans and variation among humans occurs in their susceptibility to infection. The choice of dose-response model can account for these variations to some extent; for example, the beta-Poisson model accounts for heterogeneity in the ability of pathogens to initiate infection. However, because dose-response models are based on studies typically performed with healthy adult volunteers and with a limited number of isolates, the available models might not capture the full variability in human response or health endpoint. To address this variability, dose-response model parameters can be treated as random variables or as part of a meta-distribution that can be estimated via Bayesian inference (Englehardt and Swartout, 2004; Messner et al., 2001; Teunis et al., 2008b). Susceptible sub-populations include immunocompromised and elderly, individuals with prior exposure to a pathogen or related microorganism (Balbus et al., 2004; Balbus and Embrey, 2002; Gerba et al., 1996), or, as in the case of norovirus, persons lacking a specific antigen (Lindesmith et al., 2003).

2.15.2. Sources of uncertainty

The primary sources of uncertainty in QMRAs include

- enumeration (through microbiological analyses) estimation (through modeling) of pathogen densities,
- choice of distributional form for FIB and pathogen densities,
- choice of distributional form and range of mobilization fractions (fraction of organisms applied in manure that run-off during a rain event),
- uncertainty in dose-response model parameters,
- intensity of secondary infections, and
- model uncertainty.

The uncertainty in exposure assessment has two components: (1) FIB and pathogen density estimates, and (2) volume of water ingested. For example, MPN estimates of microorganism density are far more uncertain than those from membrane filtration techniques (Gronewold et al., 2008; Gronewold and Wolpert, 2008). Membrane filtration results can be interpreted as Poisson-distributed estimates around the true mean density (Gronewold and Wolpert, 2008). When pathogens aggregate (e.g., via clumping or attaching to particles), this assumption is not valid, and a Poisson-stopped logarithmic distribution (Teunis et al., 2008a) or discrete growth distribution (Englehardt et al., 2009) may be more appropriate. Although not well characterized in the literature, enumerations from qPCR methods are associated with uncertainty because of the small volumes amplified, cycle-to-cycle variations in amplification efficiency, inhibition, and other matrix effects (Ruijter et al., 2009; Rutledge and Côté, 2003). Uncertainty also arises when comparing FIB and pathogen density estimates from qPCR and membrane filtration methods because of differences in their ability to detect viable, viable but non-culturable cells (VBNC),

dead cells, or extra-cellular DNA (Haugland et al., 2005; Liu et al., 2009; Nocker and Camper, 2006).

Dose-response models are developed based on data from (usually healthy) humans or animals exposed to a known or estimated number of pathogens of a particular strain or subgroup. Uncertainty arises for several reasons. First, the parameter estimates are based on limited data. Second, sub-populations that were not represented in the studies might respond differently. Third, the responses of homogenous groups of volunteers might differ from those of the general population. Fourth, it is assumed that ingested doses, both in the dose-response experiments and during recreation are homogenously (Poisson) distributed, but pathogens may clump, resulting in differing actual ingested doses.

2.16. Factors and Data not Included in the QMRA

Factors not included in the QMRA include the following:

- Illnesses other than GI infections. Most other potentially water-related adverse health outcomes do not correspond with FIB in recreational waters.
- Routes of exposure other than ingestion. Rates of inhalation and hand-to-mouth activities are expected to be much lower than ingestion for swimming.
- Pathogens other than the reference pathogens. However, reference pathogens account for the majority of potentially waterborne illnesses and are representative of other pathogens that could potentially be in agricultural-animal impacted water.
- Potential loss of pathogen virulence during extra-enteric transport. Because of a lack of data, assuming no loss is conservative and health protective.
- Growth of FIB and pathogens during transport. Growth of FIB and (bacterial) pathogens is variable. A data-rich site-specific assessment would need to account for these factors.
- Die-off or attenuation of FIB and pathogens is beyond the scope of the scenario.

2.17. Identified Gaps in the Knowledge Base

Through extensive literature reviews and the EPA environmental monitoring studies (Section 2.12), we have assembled sufficient data to conduct a QMRA to estimate illness at a freshwater beach impacted by agricultural animal sources of fecal contamination. Outstanding gaps in the data include the following:

- Fate and transport of FIB and pathogens to estimate risks downstream (temporally and spatially) from the source. This data gap results from a lack of understanding of transport and survival processes for extra-enteric organisms and the variety of sites and conditions under which pathogens and FIB move from fecal pollution sources to receiving water.

- Effects of best management practices (BMPs). Basic research on the efficacy of best management practices to remove pathogens from receiving water is ongoing. How well BMPs perform is expected to vary between operations, seasons, and loading conditions. Furthermore, new practices are in development and will likely be implemented at some livestock operations. Although EPA has collected data on the efficacy of manure treatment systems and BMPs, such as the presence of vegetative filter strips and fenced areas for calves, those data cannot be used to characterize general conditions. This risk assessment assumes that there is no treatment or other attenuation of pathogens in livestock waste other than retention in manure matrixes and soil.
- Dose-response relationships. The dose-response for *Salmonella* likely does not account for variability between the environmentally-relevant strains. The existing dose-response relationship that accounts for strain variability is unstable at the low pathogen densities that are relevant in recreational water. The dose-response for adenovirus[20] is based on an inhalational route of exposure for adenovirus 4; whereas, waterborne GI illness probably results from ingested adenovirus 40/41.
- Animal-impacted recreational waters could contain pathogens of public health concern that were not evaluated. As described previously, we selected our reference pathogens based on robust criteria. The reference pathogens for agricultural animal-impacted waters are assumed to be bacterial and protozoans as human infectious virus are typically not associated with agricultural animals. However, Hepatitis E virus is associated with livestock operations (Banks et al., 2004; Legrand-Abravanel et al., 2009; Rutjes et al., 2009; Sinclair et al., 2009; Takahashi et al., 2009) and pigs shed rotavirus, but those strains appear to be host-adapted and not likely to pose a significant risk to humans (Martella et al., 2010). Although using bacterial and protozoan reference pathogens to evaluate livestock-impacted water with QMRAs is appropriate at this stage of our understanding, it is possible that future research could provide sufficient information that a reference virus could also be included for agricultural animal-impacted water QMRAs.

[20] Adenovirus is not used in this animal-impacted waters QMRA as it is generally species-specific. It was, however, used in the reverse QMRA that was previously conducted to determine which pathogens are the most likely to cause illnesses in human-impacted recreational waters (Soller et al., 2010a).

3. Analysis

The analysis phase of a QMRA is the technical evaluation of data related to the potential exposure to microbial contaminants, host characterization, and human health effects. It also includes quantification of the dose-response relationship for contaminants in water media. Although the problem formulation phase may partially address these issues, the analysis phase provides more detail and quantitative analysis (USEPA, 2010).

The two components of the analysis phase are exposure characterization and human health effects characterization of (ILSI, 1996). Characterization of exposure and human health effects are iterative and interrelated processes because they must be compatible with the risk characterization phase of the QMRA (Chapter 4). The analysis phase culminates with an exposure profile (Section 3.1.8) and a host-pathogen profile (Section 3.2.4). Calculations using these data are conducted within the risk characterization phase of the risk assessment (Chapter 4).

3.1. Exposure

Figure 14 illustrates the processes leading to human exposure to pathogens at a freshwater beach impacted by fecal contamination from agricultural sources. EPA conducted a literature review to characterize the parameters associated with these processes (Annex 3) (see also Soller et al., 2010b). The sections that follow highlight this literature review. Appendix B provides a tabular summary of the literature review for FIB and reference pathogen levels in cattle, pig, and chicken fecal source materials, as well as for chlorinated secondary effluent. Additional data that may be useful to characterize reference pathogens levels in shorebird feces and urban runoff is summarized in Appendix C.

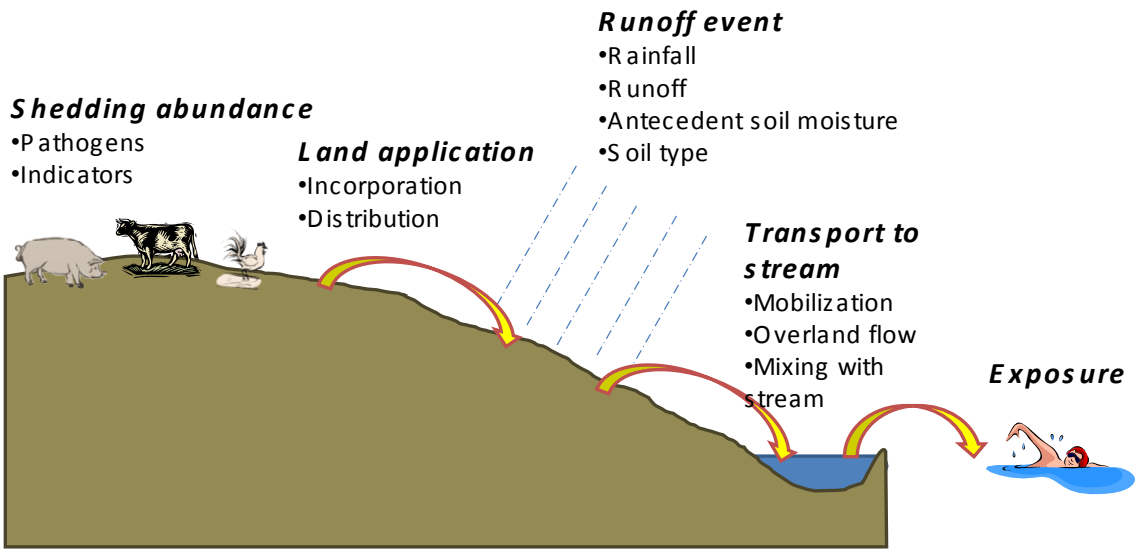

Figure 14. Schematic exposure diagram for recreation at agricultural animal-impacted waterbody

3.1.1. Prevalence and abundance of reference pathogens in livestock

Based on the systematic literature searches conducted as part of this report, we collated the prevalence and abundance ranges for this risk assessment. Two types of prevalence data are relevant to QMRAs for livestock-impacted water—sample-level prevalence and herd-level prevalence. *Sample-level prevalence* is the proportion of fecal samples from a specific operation or group of operations where a specific microorganism is detected. *Herd-level prevalence* is the proportion of herds studied in which at least one sample is positive for a specific microorganism. Because these QMRAs explore representative risks that animal operations pose to swimmers, sample-level prevalence was used to calculate risk.

Abundance is the number of organisms per mass or volume of applied manure. The abundance data used in this risk assessment were based on reported average pathogen densities for fresh solid cattle manure, solid poultry litter, and liquid pig manure. Note that the use of average values to characterize pathogen abundance is different than in prior work (Soller et al., 2010b), which used abundances from individual fecal samples. Those abundances were used for an exposure that was assumed to be in close proximity to manure deposited directly into recreational water, which was deemed appropriate for that context. The use of average values in this current risk assessment is appropriate because the land-applied material is effectively a composite sample from multiple individual samples. Thus, the average value represents an unbiased estimate for the expected value of pathogen density in the land-applied material. The land-applied composite material is comprised of fecal material from shedding and non-shedding animals, with the proportion of manure containing pathogens determined by the sample-level prevalence of a particular organism. Therefore, the average density of a given pathogen in land-applied fecal material is the average abundance scaled by the sample-level prevalence.

3.1.1.1. *Salmonella*

Large-scale studies of *Salmonella* prevalence in pigs exhibited high year-to-year and herd-to-herd variability, with reported prevalence generally falling in the 8 to 15% range (Foley et al., 2008; Hutchison et al., 2004); however, prevalence among pigs appears to increase with age (Dorr et al., 2009). *Salmonella* infection in cattle differed between dairy and beef cattle, as well as with age, season, and herd size (Callaway et al., 2005; Edrington et al., 2004; Huston et al., 2002; Kunze et al., 2008; Warnick et al., 2003; Wells et al., 2001). Large-scale studies of *Salmonella* infection in both dairy and beef cattle (Fossler et al., 2005; Hutchison et al., 2004) indicate prevalence in the 5 to 18% range, with higher prevalence reported for some herds. Prevalence in chicken flocks (both layers and broilers) was highly variable and dependent on the age of the chickens (Byrd, 1998; Martin et al., 1998) and possibly on the geographic region (Ebel et al., 1992; Garber et al., 2003). Based on the high variability of *Salmonella* observed in these studies, we selected a prevalence range of 0 to 95% as representative of *Salmonella* shedding among chickens.

Among pig manure samples positive for *Salmonella*, two studies (Boes et al., 2005; Hutchison et al., 2004) indicate a range of *Salmonella* fecal abundance from $10^{2.8}$ to $10^{4.9}$ organisms g^{-1} feces. Slurries differ from fresh fecal deposits because the conditions under which wastes are stored impact density range. The range 10^5 to $10^{6.5}$ organisms/100 mL for abundance in swine slurry was selected based on reported densities from a study with relatively high abundance taken from a lagoon with fresh manure (Vanotti et al., 2005). *Salmonella* abundance in cattle feces was reported in the range of $10^{0.6}$ to $10^{5.8}$ organisms g^{-1} feces. The range used for average abundance in solid cattle feces is $10^{2.6}$ to $10^{4.6}$ organisms g^{-1} feces based on the findings of Fegan et al. (2004) and Hutchison et al. (2004). Cattle from different production systems (grass vs. concentrate fed) did not exhibit significantly different shedding densities (Fegan et al., 2004). Average abundance of *Salmonella* in feces of chickens appears to be independent of bird age and inoculation/ingestion dose (Byrd, 1998), with representative average densities in the range of 10^{-1} to$10^{4.4}$ organisms g^{-1} of fresh chicken excrement (Kraft et al., 1969). Both studies used to establish the poultry *Salmonella* density range based density estimates on multiple samples taken from each house.

3.1.1.2. *Campylobacter*

Campylobacter spp. are frequently found in pig slurry lagoons (McLaughlin et al., 2009) and pig feces (Dorner et al., 2004; Weijtens et al., 1997), with prevalence generally increasing with the age of the animal. Given the high prevalence and increased prevalence with age, the pig *Campylobacter* prevalence is estimated to be in the range of 46 to 98%. Cattle *Campylobacter* prevalence differs among beef and dairy cattle, with feedlot cattle generally exhibiting higher prevalence than cattle on pasture, and with prevalence increasing with the length of time cattle occupy feedlots (Besser et al., 2005). Considering the different prevalence among operations and between age cohorts, a representative range of prevalence for *Campylobacter* among all

cattle is 5 to 38% (Hoar et al., 2001; Wesley et al., 2000). Chicken-shedding prevalence for *Campylobacter* also tends to increase with age (Luangtongkum et al., 2006), and flocks frequently approach 100% infection rates (Cox et al., 2002). *Campylobacter* shedding is nearly universal among chicken houses and within-house rates are high and increase with bird age. A representative range of *Campylobacter* prevalence in chickens is 57 to 69% (Cox et al., 2002; El-Shibiny et al., 2005).

Studies reporting *Campylobacter* abundance in solid pig fecal samples (Hutchison et al., 2005; Weijtens et al., 1999) suggest a representative density range of $10^{2.0}$ to $10^{5.7}$ organisms g^{-1} feces; whereas, a single study of slurry densities reported the range of $10^{3.3}$ to $10^{3.7}$ organisms/100 mL (McLaughlin et al., 2009). Studies of cattle *Campylobacter* abundance (Hutchison et al., 2005; Inglis et al., 2004; Moriarty et al., 2008; Stanley et al., 1998) reported diverse results. The range of average abundance we selected ($10^{1.8}$ to $10^{4.5}$ organisms g^{-1} feces) spanned the averages in all reported studies and fell within the full range of abundances observed in individual samples in the study reporting the greatest variability ($10^{1.2}$ to $10^{7.3}$ organisms g^{-1} feces). Studies on *Campylobacter* abundance in chicken feces (Bull et al., 2006; Cox et al., 2002; Hutchison et al., 2005; Whyte et al., 2001) were in general agreement, with a representative range of $10^{2.8}$ to $10^{6.5}$ organisms g^{-1} feces. As with poultry *Salmonella* abundance data, the poultry *Campylobacter* studies reported average abundances of groups of samples taken from floors of individual houses.

3.1.1.3. E. coli O157:H7

E. coli O157:H7 infection and shedding occurs frequently among cattle and pigs, but is very uncommon in chickens (Doane et al., 2007). Several studies report relatively low infection rates among pigs (Chapman et al., 1997; Cornick and Helgerson, 2004; Feder et al., 2003; Hutchison et al., 2004) with prevalence differing among types of operations and ages of animals—typically in the range of 0.1 to 12%. Cattle *E. coli* O157:H7 prevalence and shedding are difficult to characterize, given wide differences among age cohorts and animals on different types of operations. *E. coli* O157 prevalence appears to differ between calves and adult cattle and between cattle before and after their arrival on feedlots. *E. coli* O157 infection peaks in young cattle between 3 to 18 months of age, and declines thereafter (Ellis-Iversen et al., 2009). In a large study of beef cattle, LeJeune et al. (2004) observed a general increase in prevalence of *E. coli* O157:H7 among animals with increased time spent in the feedlot.

Pig shedding of *E. coli* O157:H7 is highly variable, and a representative range of abundances among all feces appears to go from none detected to 10^{7} organisms g^{-1} feces (Cornick and Helgerson, 2004), with animals shedding more intensely during early infection. We found no data that estimated average density in swine slurry, so we conservatively estimated an average density based on the reported density range and assuming feces were diluted to a slurry with a 4% solids fraction.

Assessment of the available studies on *E. coli* O157:H7 in cattle (e.g., Berry et al., 2007; Hutchison et al., 2004) led to estimates of prevalence and abundance ranges of 9.7 to 28% and $10^{3.1}$ to $10^{8.4}$ organisms g^{-1}, respectively. The high end of the cattle *E. coli* O157:H7 abundance range is very high and was taken from a large, systematic study that did not account for animal age or super-shedding. To avoid biasing our estimate of cattle *E. coli* O157:H7 range by including data from super-shedders or other samples that are not representative of land-applied manure, we estimated the range of average cattle *E. coli* O157:H7 densities based on analysis by Hutchison et al. (2004). Using their reported geometric mean and maximum densities, and assuming abundances were log-normally distributed, the log-mean and standard deviation of the average abundance of *E. coli* O157:H7 were estimated at 3.08 and 1.49, respectively.

3.1.1.4. Cryptosporidium

Estimates of ranges of prevalence and abundance of *Cryptosporidium* in livestock and other wastes are based on a comprehensive review by Ferguson et al. (2009) and supplemented with additional studies. *Cryptosporidium* shedding is sporadic among pigs, and individual herd prevalence may be low with a characteristic range of 0 to 45% (Heitman et al., 2002; Hutchison et al., 2005; Xiao et al., 2006). As for *E. coli* O157:H7, young cattle (<3 months) exhibit much higher prevalence of *Cryptosporidium* than older cattle (Wade et al., 2000), as well as the prevalence of genotypes that are more infectious to humans (Chalmers and Giles, 2010). A representative range for *Cryptosporidium* prevalence in cattle, inclusive of all age groups, is estimated to be 0.6 to 23%. *Cryptosporidium* shedding has been observed among chickens, though the species excreted are generally not infectious to humans (Xiao et al., 2004). An older study by Ley et al. (1988) reported *Cryptosporidium* prevalence among chickens to be between 6 to 27%.

For abundance in solid manure, a representative range of *Cryptosporidium* shedding rates among pigs is $10^{1.7}$ to $10^{3.6}$ oocysts g^{-1} (Hutchison et al., 2004). Reinoso and Becares (2008) reported the range of *Cryptosporidium* densities in swine slurry to be $10^{4.2}$ to $10^{5.4}$ oocysts/L. Cattle-shedding rates for *Cryptosporidium* vary for calves and adults, with adults sporadically shedding low densities of oocysts and calves shedding very high densities. To avoid making unnecessarily subjective assumptions about the proportion of animals that are calves and the management practices associated with calves and their manure, we excluded densities that were reported based only on calf samples from the range of averages for cattle *Cryptosporidium*. Notably, this choice led to the exclusion of data from the study by Wade et al. (2000), in which average density among samples from calves positive for *Cryptosporidium* was 21,090 oocysts/g. A representative range for average manure oocyst density of 10^{-1} to $10^{3.2}$ oocysts g^{-1} was selected based on data from Sturdee et al. (2003), where the low end of the range is based on a low detection limit for *Cryptosporidium* in manure and the known tendency of adult cattle to shed oocysts at low densities (Atwill et al., 2006). No studies allowed for the estimation of a range of abundances of *Cryptosporidium* in chicken feces, though Hutchison et al. (2004) searched

unsuccessfully for *Cryptosporidium* in fresh chicken manure as part of a large-scale study of pathogens in livestock manure.

3.1.1.5. Giardia

Estimates for the prevalence of *Giardia* in pig feces are primarily drawn from Heitman et al. (2002), Xiao et al. (2006), and Hutchison et al. (2004). The range of *Giardia* prevalence in pig manure is estimated to be 3.3 to 18%. In cattle, *Giardia* prevalence varies with animal age, with infection peaking when calves are relatively young and the probability of infection of an individual within its lifetime approaching 100% in some operations (Olson et al., 1997; Ralston et al., 2003; Wade et al., 2000). Two large-scale studies (Fayer et al., 2000; Wade et al., 2000) indicate a prevalence range for *Giardia* among cattle of 0.2 to 37%.

Wide ranges of shedding densities of *Giardia* among both pigs and cattle were observed, with pig feces abundance in the range 10^{0} to $10^{6.8}$ cysts g^{-1} (data presented graphically in Maddox-Hyttel et al., 2006). A single study reporting a slurry density of $10^{3.5}$ cysts/L was provided by Reinoso and Becares (2008). The range of average *Giardia* density for cattle was selected to be $10^{0.18}$ to $10^{3.5}$ cysts g^{-1} (Heitman et al., 2002; Wade et al., 2000).

3.1.2. Abundance of reference pathogens in disinfected secondary effluent

While the scenario evaluated in this QMRA does not cover human-impacted water specifically, the results from the agricultural animal-impacted water QMRA are compared to waters impacted by disinfected secondary effluent, as well as EPA's literature review and preliminary QMRA work that included human-impacted sites (Schoen and Ashbolt, 2010; Soller et al., 2010a; 2010b). Estimating ranges of pathogen abundance in human fecal pollution is complicated by the episodic occurrence of pathogens in sewage, large differences in removal of the pathogens for different wastewater treatment processes, and differences in disinfection doses and contact times. A summary of the literature review is provided below and a tabular summary is provided in Appendix B.

None of the bacterial reference pathogens (*E. coli* O157:H7, *Campylobacter*, *Salmonella* spp.) reportedly appear in significant densities in chlorinated secondary effluent (Garcia-Aljaro et al., 2005; Lemarchand and Lebaron, 2003; Stampi et al., 1993) as they are Gram-negative species that are very susceptible to disinfection. Reported densities of *Cryptosporidium* in secondary effluent are relatively low, even in the absence of disinfection (Bonadonna et al., 2002; Bukhari et al., 1997; Castro-Hermida et al., 2008; Payment et al., 2001; Scott et al., 2003). A representative range of *Cryptosporidium* densities in secondary effluent that accounts for episodes of natural variability in raw sewage and treatment process performance is $10^{-1.0}$ to $10^{1.5}$ oocysts L^{-1} (Rose et al., 2004).

Reported *Giardia* densities in wastewater treatment plant effluent are somewhat higher than *Cryptosporidium* densities, though *Giardia* is also subject to episodic loading and variations in

removal depending on treatment processes (Bukhari et al., 1997; Carraro et al., 2000; Castro-Hermida et al., 2008; Payment et al., 2001; Scott et al., 2003). Similar to the approach used for *Cryptosporidium*, we selected the range of *Giardia* abundance in chlorinated secondary effluent based on the widest reported range and estimate it to be $10^{-1.0}$ to $10^{2.1}$ cysts L^{-1} (Rose et al., 2004), not accounting for method recovery. *Giardia* cyst levels in chlorinated secondary effluent are only slightly higher than *Cryptosporidium* levels despite substantially higher densities in raw sewage and undisinfected secondary effluent because *Giardia* is inactivated to a greater degree with chlorine than *Cryptosporidium* (USEPA, 2005a). A wide range of norovirus densities in secondary effluent has been reported (Haramoto et al., 2006; Katayama et al., 2008; Laverick et al., 2004; Lodder et al., 1999; Lodder and de Roda Husman, 2005; Pusch et al., 2005; van den Berg et al., 2005). Based on these data, the range of norovirus abundance in chlorinated secondary effluent is in the range 10^{-2} to 10^{6} genomic copies L^{-1} (Haramoto et al., 2006; Katayama et al., 2008) (triangular distribution with mode of 4 logs). We estimate the removal range from treatment to be 1.0 to 4.0 logs (triangular distribution with mode of 2.5 logs).

3.1.3. Abundance of FIB in livestock manures

The FIB *E. coli* and enterococci are members of the normal intestinal microbiota of cattle, pigs and poultry and are assumed to be present in 100% of their fecal samples.

For cattle, an important determinant of the shedding intensity for *E. coli* is diet. Berry et al. (2006) observed different shedding intensities for cattle fed grass and cattle fed concentrate, with the cattle on concentrate shedding *E. coli* at a significantly higher density. Other large studies (e.g., Moriarty et al., 2008; Sinton et al., 2007; Thurston-Enriquez et al., 2005; Weaver et al., 2005) reported *E. coli* fecal densities consistent with the range reported by Berry and colleagues (including cattle on both grass and concentrate). Based on those studies, we use the full range of *E. coli* fecal densities reported by Berry et al. (2006), $10^{5.0}$ to $10^{6.7}$ CFU/g, for the *E. coli* density in solid cattle manure QMRA simulations.

Reported pig slurry *E. coli* abundances fall within a narrow range (Coehlo et al., 2007; Hill and Sobsey, 2003; Marti et al., 2009; Peu et al., 2006), given the variety of holding times, lagoon designs, and environmental conditions associated with pig slurries at different farms. For the average density, we used the highest and lowest average slurry densities reported in the literature, resulting in a range of densities of $10^{5.0}$ to $10^{6.7}$ CFU/100 mL. Poultry *E. coli* densities reported in the literature span a much wider range than those for solid cattle feces and pig slurries, likely because chicken litter is a mixture of bedding, feathers, feces, and other materials, and is more heterogeneous than solid cattle manure and swine slurry. Further, significant time may pass between excretion of chicken feces and sampling of the litter from the chicken house floor (not direct fecal deposits). The chicken litter *E. coli* density range for the relative QMRAs was based on those observed by Terzich et al. (2000), because that study was large (operations in 12 states) and included assays of litter, not feces. The chicken litter *E. coli* density range was $10^{5.0}$ to $10^{10.9}$ CFU/g.

Solid cattle manure enterococci densities appear more variable than those of *E. coli*, with shedding density differing by season (Moriarty et al., 2008), type of operation (Weaver et al., 2005), and other factors. The range of $10^{2.4}$ to $10^{6.8}$ CFU/100 mL was selected for solid cattle manure average enterococci density based on data from Moriarty et al. (2008) and Thurston-Enriquez et al. (2005). Average densities reported in other studies of solid cattle manure fall within this range (Sinton et al., 2007). As for *E. coli*, the range of reported average enterococci densities in swine slurry fell within a relatively narrow range (Bradford et al., 2008; Coehlo et al., 2007; Hill and Sobsey, 2003; Peu et al., 2006; Vanotti et al., 2005). The average range used in the relative QMRAs is $10^{5.0}$ to $10^{5.9}$ CFU/100 mL, based on the studies by Peu et al. (2006) and Bradford et al. (2008). Only two studies of chicken litter enterococci density were identified (Brooks et al., 2009; Kelley et al., 1995). Those studies yielded a chicken litter enterococci density range of 10^4 to 10^6 CFU/100 mL, with the upper end of the range estimated based on data presented graphically by Brooks et al. (2009).

3.1.4. Ability of livestock-derived reference pathogens to infect humans

The relative fraction of human infectious strains in each reference pathogen in non-human sources is important but highly uncertain. The available data are insufficient to quantitatively characterize this attribute within a QMRA context. Thus, we assign categorical values of low (L), medium (M), or high (H) to describe the ability of the livestock-derived reference pathogens to infect humans based on (1) the overlap of species, strains, and serotypes known to infect humans and to be present in the manure of the livestock species; (2) the prevalence of the pathogen species and types most likely to infect humans as a proportion of the overall prevalence of the pathogen in manure of a specific livestock host; and (3) review articles describing disease transmission and host-specificity for the diseases associated with each pathogen. The mid-points of the ranges of 0 to 33% for L, 33 to 66% for M, and 67 to 100% for H, were then used as point estimates in this analysis.

3.1.4.1. Campylobacter spp.

Ketley (1997) designated *C. jejuni* and *C. coli* as the species playing a major role in human infections (80 to 90% of *Campylobacter* infections), but notes that other species have the potential for initiating human infections. For all livestock hosts, the prevalence of *Campylobacter* species or subtypes of species varies between farms and regions, with age of animal, season, between isolates from fecal samples and isolates from other environmental reservoirs (e.g., trough water), and probably with other factors (El-Shibiny et al., 2005; Hakkinen and Hänninen, 2009; Minihan et al., 2004; Weijtens et al., 1999; Wesley et al., 2000). *C. jejuni* and *C. coli* are prevalent among cattle, pigs and chickens, with chickens exhibiting higher incidence of *C. coli* shedding (as a percentage of all *Campylobacter*-positive samples) than that of cattle and pigs (El-Shibiny et al., 2005).

C. jejuni and *C. coli* are also the most often isolated from humans and their feces, animal hosts and their feces, and environmental samples. Devane et al. (2005) reported a ratio of 90:10 for *C. jejuni* isolates to *C. coli* isolates in human feces samples from New Zealand. These researchers also observed that the two most common human isolates accounted for 43.6% of isolates from beef cattle feces, 32.2% of isolates from dairy cattle feces, and lesser fractions of isolates from other animals. The concordance between subtypes observed in humans and those observed in beef cattle, dairy cattle, and sheep feces and sheep offal was confirmed in subsequent work by Garrett et al. (2007). Furthermore, the dose-response characteristics *C. jejuni* appear to differ among fresh cultures and laboratory cultures (Chen et al., 2006).

Given this lack of species-specific prevalence data and the absence of a general dose-response model for human infection with *C. coli*, we know little about the potential for *C. coli* to infect humans. Based on these observations, cattle and swine *Campylobacter* were assessed as having high infectious potential for humans, while chicken *Campylobacter* were assessed to have medium human infectious potential.

3.1.4.2. Salmonella

The relative risk posed by *Salmonella* serotypes in animals is inferred by comparing the serotypes prevalent in different animal hosts and humans. The U.S. Centers for Disease Control and Prevention (CDC, 2006) identified the serotypes from human *Salmonella* isolates between 1996 and 2006. The USDA Food Safety and Inspection Service (USDA FSIS, 2009) identified the serotypes for *Salmonella* isolates identified in broilers, market hogs, steer and heifers, and cows and bulls between 1998 and 2007. Collectively, these data indicate that the prevalence of serotypes within a given host changes significantly from year to year, though for humans, the serotypes typhimurium and enteriditis were consistently among the top three isolated. The overlap between serotypes prevalent in humans and in livestock is used to estimate the potential transmission of human-infectious *Salmonella* from livestock.

Table 5 and Figure 15 summarize the 24 most common serotypes of non-typhoid *Salmonella* from human isolates. Serotype prevalence (as a percent of total isolates) for broilers, steers/heifers, cows/bulls, and market hogs are also presented. They also show the overlap between the most common human and animal *Salmonella* serotypes, with all animals exhibiting relatively high prevalence of human-infecting serotypes Typhimurium, Newport, Saint-Paul, Infantis, Anatum, and Mbandaka, and all hosts except pigs subject to infection with the Montevideo serotype.

Table 5. *Salmonella* serotype prevalences

Serotype	Human	Broiler	Steer/ Heifer	Cow/Bull	Market Hog
Typhimurium (w/ var. Copenhagen)	21.6	10.6	2.3	9.8	14.0
Enteriditis	17.8	6.8		0.7	
Newport	8.4		5.8	13.5	
Heidelberg	5.2	17.4	3.5	1.1	3.2
Javiana	3.4				
Montevideo	2.4	2.4	5.8	8.4	
Muenchen	2.0		1.2	1.1	0.3
Oranienburg	1.7		2.3		
Saintpaul	1.6		4.6	0.4	4.5
Infantis	1.5	0.9	2.3	3.6	7.4
Thompson	1.5	1.2			
Braenderup	1.0				
Agona	1.0		2.3		1.4
I, 4, [5], 12:i-	1.2	2.2			
Hadar	1.1	1.2		0.4	1.3
Mississippi	1.0				
Typhi	1.0				
Paratyphi B var L(+) tartrate (+)	1.0		2.3		
Poona	0.8		2.3		
Berta	0.6	0.3			
Stanley	0.6				
Anatum	0.6		4.6	5.8	9.5
Bareilly	0.5			0.4	
Mbandaka	0.5	0.8	1.2	2.6	0.4
Other or not identified	20.5	56.1	59.8	52.4	58.2

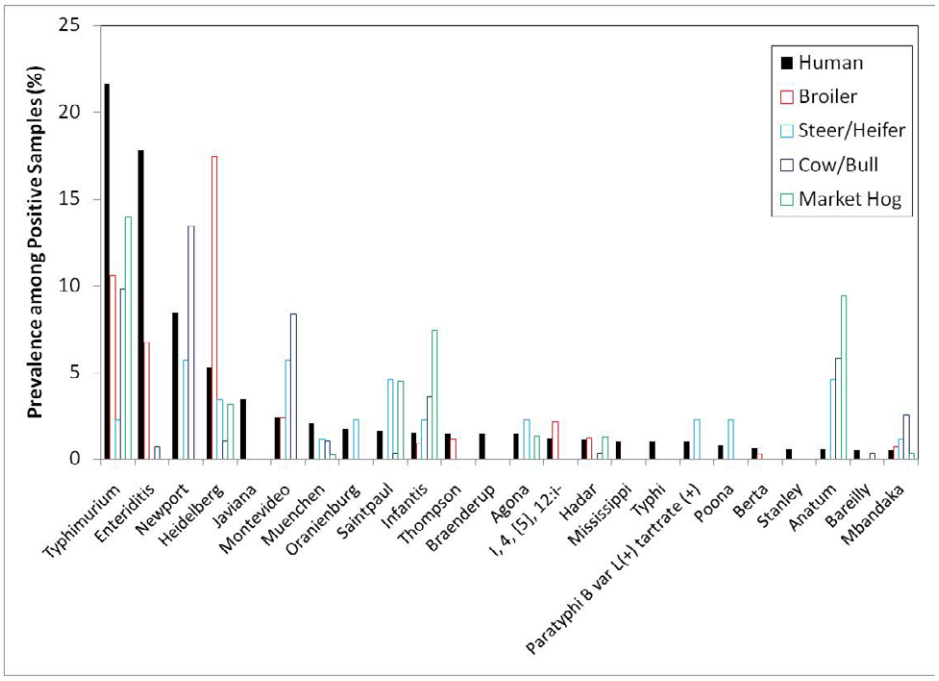

Figure 15. *Salmonella enterica* prevalence in humans and livestock

The 24 serotypes most commonly isolated from humans account for 79.5% of all isolates. The prevalence of the 24 most common human serotypes among livestock samples ranges from 52.5 to 59.8% of isolates. Because *Salmonella* infections are sporadic (Callaway et al., 2008), and serotype prevalence may change dramatically from year to year (USDA FSIS, 2009), the possibility exists that a relatively uncommon or an unknown serotype associated with animals can cause an outbreak in exposed persons.

Based on the overlap of livestock *Salmonella* serotypes with the serotypes most commonly implicated in human illness, the fecal pollution for chickens, cattle, and swine were assigned a level of medium human infectious potential.

3.1.4.3. E. coli O157

The apparent ability of Shiga toxin-negative *E. coli* O157 to acquire a *stx* virulence gene in different hosts and settings (Wetzel and LeJeune, 2007), and the potential for different virulence in isolates from humans and other sources (Lenahan et al., 2009), make it difficult to assess the potential for cattle and other animals to generate virulent *E. coli* O157. Therefore, we conservatively assume that *E. coli* O157:H7 from any source poses the same hazard to humans and assign a high human infectious potential to *E. coli* O157:H7 to pathogens from all sources.

3.1.4.4. *Cryptosporidium spp.*

Cryptosporidium species have widely varying public health significance and appear to have adapted to specific hosts or groups of hosts. For example, Xiao et al. (2004) associated *Cryptosporidium* species to major and minor hosts (Table 6). Among the more than 16 species of *Cryptosporidium* identified to date, *C. parvum* and *C. hominis* are believed to cause the majority of human infections among immunocompetent hosts. Other animals considered major hosts for *C. parvum* and *C. hominis* include cattle, sheep, goats, and monkeys (Xiao et al., 2004, 2006). Humans are minor hosts for other *Cryptosporidium* species, including *C. muris*, *C. meleagridis*, *C. felis*, and *C. canis* (Table 7). Even among *C. parvum*, however, the ability of individual isolates to infect varies as illustrated in Table 8 (Messner et al., 2001).

Among livestock species, cattle more often carry *Cryptosporidium* species that infect humans, while swine *Cryptosporidia* less often infect humans, and poultry *Cryptosporidia* appear to infect humans rarely (Xiao et al., 2006). Consequently, the human infectious potential of cattle and swine *Cryptosporidia* is assessed as high (given the occurrence of human infectious *Cryptosporidia* in swine, but not the occurrence of *C. suis* in humans), and the human infectious potential of chickens is considered as low.

Table 6. Valid *Cryptosporidium* species and associated major and minor hosts (SOURCE: adapted from Xiao et al., 2004)

Species	Major Host	Minor Host
C. muris	Rodents, Bactrian camels	Humans, rock hyrax, mountain goats
C. andersoni	Cattle, Bactrian camels	Sheep
C. parvum	Cattle, sheep, goats, humans	Deer, mice, pigs
C. hominis	Humans, monkeys	Dugongs, sheep
C. felis	Cats	Humans, cattle
C. canis	Dogs	Humans
C. meleagridis	Turkeys, humans	Parrots
C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches, duck
C. galli	Finches, chicken, capercalles, grosbeaks	—

Table 7. *Cryptosporidium* spp. of humans and domestic animals (SOURCE: adapted from Xiao et al., 2004, 2006)

Host	Major Parasites	Minor Parasites
Human	*C. hominis*, *C. parvum*	*C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, corvine genotype, pig genotype I
Cattle	*C. parvum*, *C. andersoni*	Bovine genotype B, deer-like genotype, *C. bovis*, *C. felis*
Pig	Pig genotype I	Pig genotype II
Chicken	*C. baileyi*	*C. meleagridis*, *C. galli*

Table 8. *Cryptosporidium parvum* dose-response parameter estimates (SOURCE: adapted from Messner et al., 2001)

	Parameter Estimate (*r*)	
Isolate	**Traditional**	**Bayesian (80% Credible Interval)**
UCP	0.000336	0.000339 (0.000231, 0.000556)
IOWA	0.00526	0.00488 (0.00342, 0.00752)
TAMU	0.0571	0.0370 (0.0208, 0.0833)

3.1.4.5. Giardia spp.

Different researchers have called the species of *Giardia* that cause the majority of human illnesses *G. lamblia*, *G. duodenalis*, or *G. intestinalis* (e.g., Adam, 2001; Thompson et al., 2004). Thompson and colleagues noted that *Giardia* isolates from humans fall into one of two major genotype assemblages, and that some *Giardia* genotypic groupings are confined to specific animal hosts. Based on a listing of the most important *Giardia* species and genotypes and their associated hosts (Adam, 2001), cattle and pigs appear to have the potential for shedding *Giardia* that pose risks to humans, while chickens do not appear to be a significant source of human-infectious *Giardia* cysts. Therefore, cattle and swine *Giardia* are assigned a high human infectious potential and chicken *Giardia* are assessed as low.

3.1.5. Mobilization of reference pathogens and FIB

The mobilization of reference pathogens and FIB due to rainfall is estimated based on data from the EPA environmental monitoring program (see Section 2.12 and Appendix D[21]).

The fraction of microorganisms mobilized during a rain event is primarily a function of the following:

- The organism, soil type, particle size distribution, and the strength of attachment of organisms to soil or manure matrices (Bradford and Schijven, 2002; Gargiulo et al., 2008; Guber et al., 2005; Guber et al., 2007b; Guzmán et al., 2009; Hodgson et al., 2009; McLaughlin et al., 2003);
- The rainfall intensity and duration (Davies et al., 2004; Trask et al., 2004); and
- Groundcover, tillage, and slope (Davies et al., 2004; Guber et al., 2006; Harrigan et al., 2004; Trask et al., 2004).

[21] Mobilization fractions were computed based on data from the October 2009 and March 2010 Runs. As of September 2010, the June 2010 run was complete, but the data were not yet available for these analyses. All of the raw data are available upon request from Dr. Marirosa Molina, EPA.

Models to estimate mobilization fractions are generally of the following form (Benham et al., 2006; Pachepsky et al., 2009):

$$\Delta M_R \sim M_S a \left(\Delta Q\right)^b \qquad [10]$$

where ΔM_R is the number of organisms released during some period Δt (e.g., over a specified runoff event); M_S is the number of bacteria in the manure storage layer prior to the runoff event; a and b are empirically derived constants; and ΔQ is runoff yield during the time interval Δt. This general model form uses the ratio of the number of organisms appearing in the runoff during a defined event and the number of organisms applied during the event.

In this risk assessment, we calculate the mobilization fraction for rain events using manure densities from samples taken prior to simulated rain events, and FIB and pathogen densities in composite samples collected during similar rain/runoff events, as follows:

$$f = \frac{\text{Number of organisms occurring in the runoff from the plot during an event}}{\text{Number of organisms applied to the plot in manure}} \qquad [11]$$

This approach is similar to that of Miller and Beasley (2008), who assessed mobilization based on flow weighted mean runoff density. Spiked and unspiked manures were sampled prior to application on experimental plots, and manure pathogen and FIB densities were determined in the manures with the methods described in Appendix D. For pathogens, composite runoff samples were assembled by compositing all runoff originating from each plot and sampling the composited runoff at 30 and 60 minutes after the initiation of runoff. For FIB, grab samples were collected in addition to the 60-minute composite samples. The grab sample densities were not used in mobilization fraction estimates in this risk assessment. Using these parameters, the mobilization fraction (equation 11) is calculated as follows:

$$f_i = \begin{cases} \dfrac{m_{manure} D_i}{V_{RO} C_i} & \text{solid manures} \\ \dfrac{V_{manure} C_{m,i}}{V_{RO} C_i} & \text{manure slurries} \end{cases} \qquad [12]$$

where, m_{manure} is the mass of solid manure applied to the plot; V_{manure} is the volume of slurry applied to the plot; D_i is the density of microorganism i in the solid manure (number of organisms/g); V_{RO} is the cumulative runoff volume for the rain event; and C_i is the density of organism i in a composite sample of all the runoff from the site (i.e., an event flow-weighted average concentration, dimensions of number organisms per unit volume).

Appendix E describes the data and approach used to compute the pathogen and FIB mobilization fractions for this risk assessment.

3.1.6. Factors used to convert densities of pathogens on land to densities in runoff

As described above, mobilization fraction is the proportion of organisms applied to plots in manure that is mobilized and transported in runoff. In the QMRA, FIB density in runoff water is used with ingested volume to compute doses of pathogen exposure to use in dose-response models to estimate risk. To estimate the density in the runoff water, the mobilization fraction needs to be scaled as follows to ensure consistency of units:

$$C_{i,RO} = \begin{cases} f_i \times \left(m_{manure} / V_{RO}\right) & \text{solid manures} \\ f_i \times \left(V_{slurry} / V_{RO}\right) & \text{liquid manures} \end{cases} \qquad [13]$$

where $C_{i,RO}$ is the density of organism i in runoff water (dimensions of organisms/L^3); f_i is mobilization fraction for organism I; m_{manure} is the per-plot manure application rate (dimensions of mass per plot); V_{slurry} is the per-plot slurry application rate (dimensions of L^3); and V_{RO} is the cumulative runoff volume from the plot for the rain event (dimensions of L^3).

This expression is used in the forward QMRA calculations in which we estimate risk associated with a land application and runoff event (Section 4.1). Note that this expression is not needed in the relative QMRA approach (Section 4.2) because the FIB and pathogens mobilized from manure are both diluted by the same volume of runoff water.

The average runoff volume and per-plot manure application rates used are as follows:

- average cumulative runoff volume: 57.7 L;
- cattle manure application rate: 1600 g/plot;
- swine slurry application rate: 2670 mL/plot; and
- poultry litter application rate: 670 g/plot.

These conversions result in runoff densities with units of organisms per L of runoff.

3.1.7. Volume of water ingested

The volume of water ingested during recreational activities is characterized as a log-normal distribution with a geometric mean of 18.5 mL and standard deviation of the $\log_{10}$ transformed data of 0.628 (Dufour et al., 2006; Soller et al., 2007b). This distribution is based on the reported combined data for children and adults. These data are the most quantitative data available for characterizing the volume of water ingested during recreational activities.

For comparison, previous QMRAs for recreational exposure have used ingestion volumes of 100 mL (Gerba et al., 1996; Steyn et al., 2004; Wong et al., 2009), 50 mL (Ashbolt and Bruno, 2003), 30 mL (van Heerden et al., 2005a), and an empirical distribution of ingested volumes with a range of 0 to 190 mL (specific to sports divers) (Schijven and de Roda Husman, 2006). Alternative ingestion values were evaluated via sensitivity analyses in this QMRA.

3.1.8. Exposure profile

The exposure profile distills the most important information and data developed during the exposure component of the analysis phase. Each component of the exposure analysis describes the information available on that specific topic. The exposure profile includes only the information that will be used in conjunction with the human health characterization for the risk characterization.

For this risk assessment, the exposure-related data that will be used in the calculations include the following:

- literature-based data characterizing the densities of reference pathogens in fecal source material (abundance) for each animal source (solid fresh cattle manure, fresh swine slurry, and fresh poultry litter);
- literature-based and EPA environmental monitoring program-based data characterizing the FIB densities (abundance) in each animal source (solid fresh cattle manure, fresh swine slurry, and fresh poultry litter);
- literature-based data characterizing the prevalence of infection from reference pathogens in each animal source (cattle, pigs, and chicken);
- a qualitative interpretation of the literature-based data describing the relative fraction of human infectious strains of each of the reference pathogens in non-human sources;
- EPA environmental monitoring program-based data characterizing the proportion of the land-applied FIB and pathogens that mobilize (mobilization fraction) following a rain event and runoff to a recreational waterbody; and
- literature-based data characterizing the volume of water ingested during recreational activities.

Tabular summaries of the specific data that are used in the QMRA calculations are provided in Sections 4.1 and 4.2.

3.2. Health Effects

3.2.1. Health endpoint

The health effect of interest in this QMRA is GI illness. Other health outcomes have been excluded for reasons described previously. Thus, the reference health outcomes in the QMRA analyses are

- Infection through ingesting surface water contaminated with reference pathogens during recreation, and
- GI illness conditional on infection.

As described in Section 2.3, the reference pathogens for this risk assessment are *Cryptosporidium* spp., *Giardia lamblia*, *Campylobacter* spp., *Salmonella enterica*, and *E. coli* O157:H7.

3.2.2. Dose-response relationships

As described previously, dose-response relationships for the reference pathogens are taken from the peer-reviewed literature and are for an infection endpoint. Definitions of infection most often used in dose-response models were seroconversion and/or shedding of pathogens in feces. Likewise, the definition of reference pathogen illness varied (summarized in Section 3.2.3 below), but was generally related to the incidence of diarrhea, and/or vomiting. The following are descriptions of and justifications for the reference pathogen dose-response relationships for this recreational water QMRA effort. Again, only bacterial and protozoan reference pathogen are used in this QMRA.

3.2.2.1. Cryptosporidium dose-response model

The dose-response model for *Cryptosporidium* in the QMRA is based on analysis for the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) (USEPA, 2005a). In the experimental dose-response studies, human response varied widely to different isolates of *Cryptosporidium parvum* (Messner et al., 2001; Okhuysen et al., 1999, 2002). With analyses based on those of Messner et al. (2001), the LT2ESWTR *Cryptosporidium* dose-response model was developed using Bayesian analyses of individual and combined data sets for different isolates and outbreak data. The LT2ESWTR dose-response model is exponential with model parameter $r = 0.09$. Uncertainty within the dose-response model is evaluated by allowing the model parameter to vary uniformly across the range of 0.04 to 0.16, consistent with the range reported in the LT2ESWTR (USEPA, 2005a).

3.2.2.2. Giardia dose-response model

The *Giardia* dose-response model was developed based on data from human feeding studies with *Giardia lamblia* dose over a range of 1 to 10^6 cysts (Rendtorff, 1954a, 1954b). Response data corresponding to infection (endpoint was shedding cysts in feces) were fit to an exponential dose-response model with parameter $r = 0.0199$ (Rose et al., 1991).

3.2.2.3. Campylobacter spp. dose-response model

We evaluated two dose-response models for *Campylobacter*. The first is based on a feeding study conducted by Black et al. (1988). The resulting dose-response relationship is fit to a beta-Poisson dose-response relationship with parameters $\alpha = 0.144$ and $\beta = 7.59$ (Medema et al., 1996). The second is based on outbreak data associated with exposure to contaminated milk (Teunis et al., 2005). An exact beta-Poisson dose-response model with parameters $\alpha = 0.024$ and $\beta = 0.011$ provided the best fit to the outbreak data.

3.2.2.4. *E. coli O157:H7 dose-response model*

The *E. coli* O157:H7 dose-response model was derived using data from eight outbreaks (Teunis et al., 2008b) and from an assumption that doses ingested in each of those outbreaks were Poisson-gamma distributed. The exposure model was refined by adjusting the gamma-distribution parameter for exposure to reflect the dispersion associated with each outbreak. An exploration of various models led Teunis and colleagues to select a beta-Poisson dose-response model (infection endpoint). Dr. Teunis developed and made available 10,000 pairs of dose-response parameters. We use two approaches with these data. In the first, median values from those pairs are used as point estimates ($\alpha = 0.4$ and $\beta = 37.6$). In the second approach, uncertainty in the dose-response parameter space is evaluated through the use of the individual dose-response parameter pairs in the Monte Carlo simulations.

3.2.2.5. *Salmonella dose-response model*

Salmonella occurrence and infectivity varies widely with serotype (McCullough and Eisele, 1951a, 1951b). To account for this, the dose-response model for *Salmonella* was chosen to be representative of the overall incidence of infection when individuals are exposed to the range of serotypes that could reasonably occur in recreational water. We evaluated two *Salmonella* dose-response models, a beta-Poisson model (Haas et al., 1999) and a Gompertz-log model (Coleman and Marks, 1998, 2000; Soller et al., 2007b). The Haas and colleagues dose-response model is based on infection data for multiple serotypes of *Salmonella*, with outlier data excluded from analysis. The best fit model for the pooled data set is the beta-Poisson model, with parameters $\alpha = 0.3126$ and $\beta = 2884$. The log-Gompertz model (for an illness endpoint) evaluation showed that the model parameters took on a range of values for the serotypes for which human dose-response data were available. Assuming that the infectivity of environmentally relevant serotypes are uniformly distributed over the observed range from the feeding study, the dose-response parameter ln (*a)* is estimated to vary uniformly between 29 and 50, and $b = 2.148$.

3.2.2.6. *Rotavirus dose-response model*

The rotavirus dose-response model was developed using data from human feeding studies (Ward et al., 1986). Volunteers in the study were adult males, 18 to 45 years old. Overall, the ratio of ill-to-infected individuals was 0.67, and the progression of infection to illness did not appear to be dose-dependent. The approximate beta-Poisson model with parameters $\alpha = 0.2531$ and $\beta = 0.4265$ (Haas et al., 1993) provided the best fit to the data. An issue unresolved in the peer-reviewed literature is that the viral units used in the feeding studies were reported as focus forming units rather than individual viral particles. It is, therefore, possible that the most commonly used assumption—that PFUs of rotavirus are equivalent to the focus forming units from the feeding study—results in an overestimation of risk associated with rotavirus.

3.2.2.7. Norovirus dose-response model

The dose-response model (infection endpoint) for the QMRA studies is an exact beta-Poisson model with parameters $\alpha = 0.04$ and $\beta = 0.055$. The norovirus dose-response model was developed from human feeding studies conducted with healthy adult volunteers (Teunis et al., 2008a). In this volunteer study, the virus was aggregated in the inoculum and so the dose-response model had to be flexible enough to account for this aggregation. Because norovirus particles are expected to be dilute in recreational water, this QMRA assumes that aggregation of viral particles will be minimal. Given this assumption, the aggregated dose dose-response model (Teunis et al., 2008a) simplifies to an exact beta-Poisson model.

3.2.2.8. Adenovirus dose-response model

The adenovirus dose-response model is based on dose-response data of adult human exposure to aerosols of adenovirus type 4 (Couch et al., 1966, 1969). For aerosol exposure and an infection endpoint, the best fit dose-response model for adenovirus is an exponential model with parameter $r = 0.4172$ (Crabtree et al., 1997). Use of the inhalation dose-response model yields conservative estimates for infection rates, because infection among adults is initiated with higher probability at lower doses via aerosol exposure than via other routes. The use of the inhalation adenovirus 4 dose-response model for predicting GI infection via oral exposure is established in the literature (Crabtree et al., 1997; Teunis et al., 1999; van Heerden et al., 2005a, 2005b).

The mismatch between this dose-response model and an ingestion route of exposure is likely to make risk predictions from adenovirus more uncertain than those for other reference pathogens. A significant fraction of the non-infant population may have a level of immunity to GI infection with adenovirus.

3.2.3. Morbidity

For this analysis, morbidity refers to the proportion of infections that progress to a symptomatic response (illness). For each of the reference pathogens, morbidity is expressed as a range to the extent that supporting data are available. Justification is provided below for the morbidity ranges used in the QMRA analyses.

In the dose-response study for *Campylobacter*, the proportion of infections progressing to illness was dose-dependent with best fit parameter estimates of $\kappa = 3.63 \times 10^9$ and $\eta = 2.44 \times 10^8$ (refer to equation 6). In a human feeding study (Black et al., 1988), there was no apparent trend with dose for the proportion of infections progressing to symptomatic illness, and approximately 18% of infected volunteers became symptomatic (fever, diarrhea, or both). In this QMRA, the morbidity ratio is assumed to be dose-independent because that assumption yields more conservative estimates of illness at low doses and reflects the uncertainty we believe is present for the *Campylobacter* dose-response model for low doses. Based on the data from the feeding

study, the progression from infection to symptomatic illness for *Campylobacter* is assumed to occur in the range of 0.1 to 0.6.

The progression from infection to symptomatic illness for *E. coli* O157:H7 is assumed to be in the range of 0.2 to 0.6 based on outbreak data (Bielaszewska et al., 1997); the percentage of symptomatic and asymptomatic individuals who were household contacts of hemolytic uremic syndrome patients (Werber et al., 2008); and the occurrence of anti-Stx2 IgG (Ludwig et al., 2002). This range is consistent to the proportion of illnesses reported in an analysis of an *E. coli* O157:H7 outbreak (Teunis et al., 2004).

The progression from infection to symptomatic illness for *Salmonella* varied from zero to one during the feeding studies, with low morbidity (0%) in most cases (McCullough and Eisele, 1951a, 1951b). Given the wide variability and high proportion of relatively low morbidity in the feeding studies, a point estimate of 20% is used to characterize the progression from infection to symptomatic illness.

The progression from infection to symptomatic illness for *Cryptosporidium* is based on EPA's research from development of the LT2ESWTR (USEPA, 2006). In that analysis, EPA analyzed available literature and identified studies with applicable data. DuPont et al. (1995) found that 39% of those infected had clinical cryptosporidiosis. Haas et al. (1996) provided information based on the same data also suggesting a morbidity rate of 39%, but computed 95% confidence limits of 19% and 62%. More recently, a study found that after repeated exposure to *C. parvum* (IOWA strain), the morbidity rate was the same as for the initial exposure in re-infected subjects (Okhuysen et al., 1998). Okhuysen et al. also found that 58% of their subjects who received *Cryptosporidium* doses developed diarrhea, which is an underestimate of morbidity because symptoms other than diarrhea contribute to the morbidity rate. Based on these data, the progression from infection to symptomatic illness for *Cryptosporidium* is assumed to range from 0.2 to 0.7.

Giardia infection is often asymptomatic, with asymptomatic cases representing as much as 50% to 75% of infected persons (Mintz et al., 1993). In a study at the Swiss Tropical Institute, 27% of 158 patients who had *Giardia* cysts in their feces exhibited symptoms (Degremont et al., 1981). Based on these data, the progression from infection to symptomatic illness for *Giardia* is assumed to be in the range of 0.2 to 0.7.

The progression from infection to symptomatic illness for norovirus is assumed to be in the range of 0.3 to 0.8 based on feeding study data (Teunis et al., 2008a). In that study, the conditional probability of illness among infected subjects appears to show dose dependence. However, dose independence is assumed using the lowest and highest proportion of ill patients for the various doses studied as the lower and upper bounds of the morbidity range, respectively.

3.2.4. Health effects profile

Similar to the exposure profile, the health effects profile is a distillation of the most important information and data that are developed during the health effects component of the analysis phase. The health effects profile is a relatively brief summary of only those pieces of information that will be used in conjunction with the exposure characterization for the risk characterization phase of the assessment.

For this risk assessment, the health effects-related data that will be used in the calculations include the health endpoint of interest (GI illness), the dose-response relationships for the reference pathogens, and the fraction of infections that lead to illness (morbidity). Sections 4.1 and 4.2 provide tabular summaries of the specific data used in the QMRA calculations. The interaction between these components and the risk characterization phase of the assessment is illustrated schematically in Figure 16.

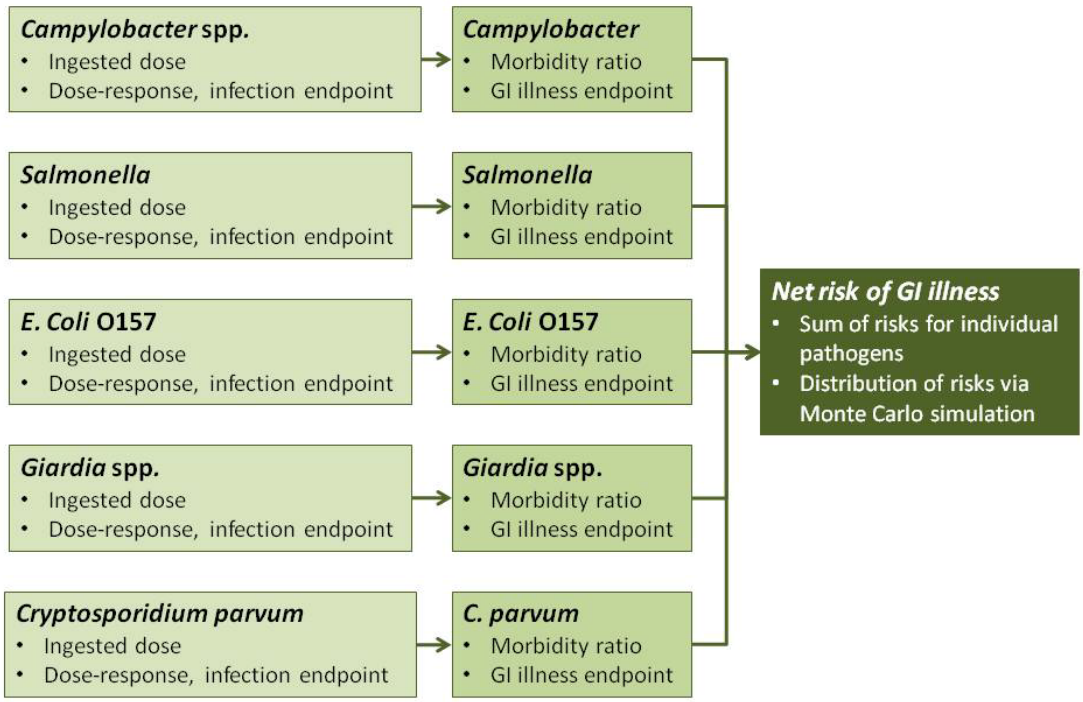

Figure 16. Interaction between health effects and risk characterization components

4. Risk Characterization

Risk characterization combines the methods outlined in the problem formulation phase and the data compiled in the analysis phase to compute and convey the overall potential risk to humans for the scenario(s) under consideration (USEPA, 2010). EPA's policy statement on risk characterization prescribes a clear, transparent, and reasonable process that is consistent with other assessments of similar scope prepared across EPA programs (USEPA, 2000). This phase of the assessment identifies and discusses all the major issues associated with determining the nature and extent of the risk. It also provides commentary on any constraints limiting interpretation of the results. The nature of a risk characterization depends on the data, information, and resources available and the regulatory application of the assessment.

Risk characterization, which can include both qualitative and quantitative data, summarizes the extent and weight of evidence and the results, major points of interpretation, and rationale. It also describes the strengths and weaknesses of the evidence, and discusses uncertainties, variability, and potential effects of alternative assumptions. Scenarios, model parameters, and analysis options that deserve further consideration are identified, so that assessment results can inform decision-making.

As described in the problem formulation chapter (2), this QMRA addresses the following two questions:

1. What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, and chicken) sources of fecal contamination? and
2. How do those risks compare to risks associated with freshwater beaches impacted by human (POTW) sources of fecal contamination?

A described below, we used two distinct QMRA risk characterization approaches to answers these questions. The first question is addressed through forward QMRA and the second question by relative QMRA. The forward QMRA provides a conservative estimate of risk associated with each of the fecal pollution sources based on the scenario. The relative approach normalizes risks to a specific FIB level to allow a direct comparison of risks among sources.

4.1. Risk of Illness Associated with Recreation at a Beach Impacted by Agricultural Animal Sources of Fecal Contamination

To characterize the risk of illness associated with recreation at a freshwater beach impacted by cattle, pig, and chicken sources of fecal contamination, we used a traditional forward QMRA approach. The general methodology for the QMRA is a Monte Carlo simulation-based approach with model parameters characterized as statistical distributions. The primary benefit of the Monte Carlo simulation approach compared to a simpler point-estimate approach is that the

inherent variability in the modeled system is accounted for explicitly and the resulting distribution provides a more nuanced view of predicted risks. Separate Monte Carlo analyses are conducted for each agricultural animal source. Sensitivity analyses evaluate the effects of alternative assumptions and parameter values on the model outputs and complement the base QMRA results.

4.1.1. Methods

A detailed schematic diagram for the forward QMRA is presented in Figure 17.

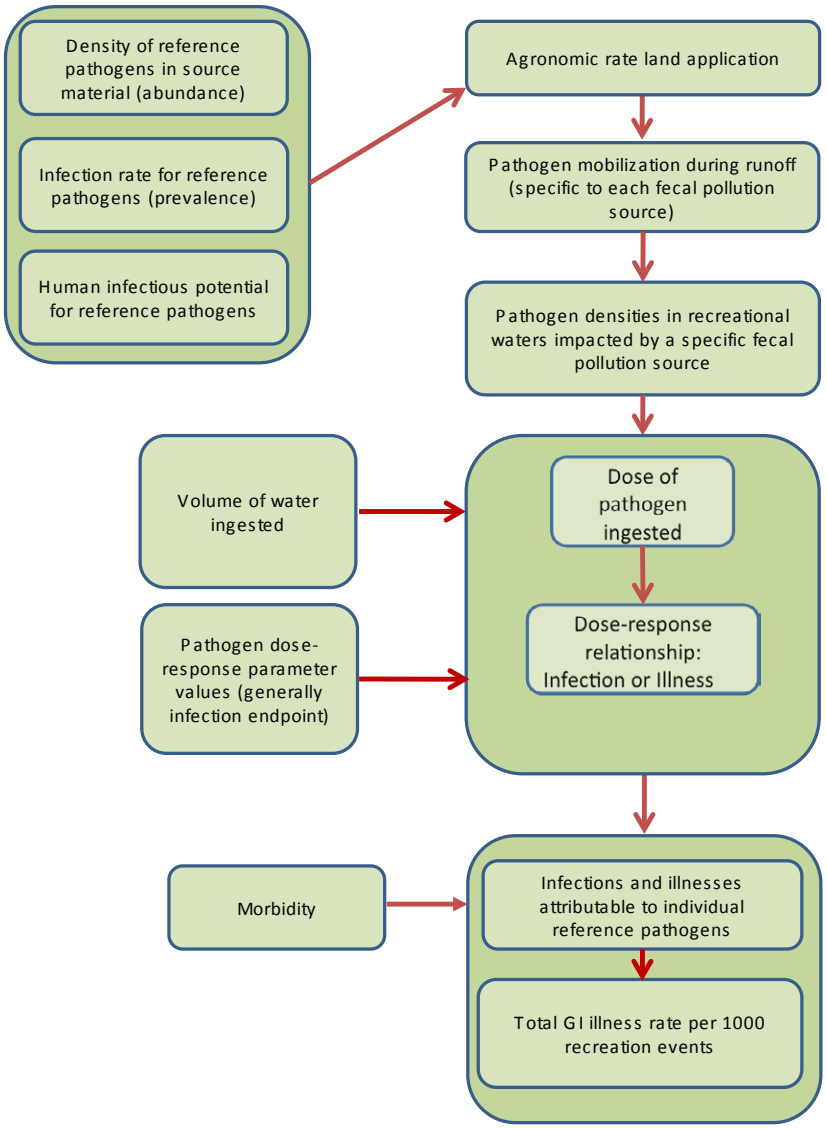

Figure 17. Detailed conceptual model for forward QMRA

The risk characterization begins with literature-based data describing the average densities of reference pathogens (abundance) in each of the animal sources under consideration (solid fresh cattle manure, fresh pig slurry, and fresh poultry litter). Table 9 summarizes of the data used for this purpose (see Chapter 3 for further information about the data). We selected cattle manure, pig slurry, and chicken litter abundance data to represent average values (typified by land-applied composited manure) from operations in the United States. The abundance data are also based on studies with wide geographic range, long duration, and large numbers of samples, where possible. This approach resulted in narrower abundance ranges than those based on individual fecal samples (Soller et al., 2010b) or uncommon events (e.g., super-shedding cattle, abundance ranges based on samples collected from young animals, operations without shedding animals). This approach minimizes potential bias in interpreting the literature-based data describing the number and ages of animals producing manure, or animal/manure management practices such as handling calf manure separately from adult animal manure.

Table 9. Abundance of reference pathogens in agricultural animal sources

	Cattle[1]		**Pigs**[2]		**Chicken**[1]	
Pathogen	Low	High	Low	High	Low	High
E. coli O157:H7 [3]	Log-normal (3.08, 1.49)		0	5.6	0	0
Campylobacter	1.8	4.5	3.3	3.7	2.0	6.3
Salmonella	2.6	4.6	5	6.8	0.5	4.4
Cryptosporidium	-0.3	3.2	4.2	5.4	0	0
Giardia spp.	0.2	3.5	3.5	3.5	0	0

1. Density in solid manures (cattle manure and chicken litter): units of $\log_{10}$ organisms/g wet weight
2. Density in liquid manures (pig slurry): units are $\log_{10}$ organisms/100 mL
3. Log-normal distribution used in place of log uniform to account for low probability events with very high abundances. Values shown are log mean and log standard deviation values

Abundance ranges from the literature for reference pathogens are characterized by log-uniform distributions in this analysis. *E. coli* O157:H7 in cattle had to be treated differently because the available abundance data indicate that average abundances are strongly influenced by infrequent shedding of high levels of pathogens. A log-normal distribution was used to account for this characteristic because a log-uniform distribution would have over-estimated the likelihood of an extreme event.[22]

We also used literature-based data to characterize the prevalence of infection in each of the animal sources (cattle, pigs, and chicken). In this analysis, prevalence represents the average proportion of animals that are shedding the reference pathogens at any point in time. As

[22] In a uniform distribution, all values between the minimum and maximum are equally likely (the log-uniform refers to the fact that values shown are $\log_{10}$ values; i.e., a value of 3 corresponds to 1000). For *E. coli* O157:H7 in cattle, use of a uniform distribution (for the $\log_{10}$ values) would have resulted in too many values at the high end of the range, therefore an alternative distribution was used that fit the literature-based data more closely.

described in Chapter 3, this prevalence is different than herd-level prevalence, which quantifies the fraction of herds that have at least one shedding individual during a specified period. In this report, we conservatively assume that shedding is occurring (i.e., herd-level prevalence is 100%). For all of the reference pathogens, relatively high herd-level prevalences are reported, particularly for *Campylobacter* in all of the livestock types and for *E. coli* O157:H7 and *Cryptosporidium* among cattle. A summary of the data employed for this purpose is provided in Table 10.

Table 10. Prevalence of infection (% of animals shedding reference pathogens at any point in time[1]

	Cattle		Pigs		Chicken	
Pathogen	Low	High	Low	High	Low	Ligh
E. coli O157:H7	9.7	28	0.1	12	0	0
Campylobacter	5	38	46	98	57	69
Salmonella	5	18	7.9	15	0	95
Cryptosporidium	0.6	23	0	45	6	27
Giardia	0.2	37	3.3	18	0	0

1. The apparent mismatch between chicken *Cryptosporidium* abundance in Table 9 and prevalence in Table 10 results from the enumeration of all *Cryptosporidia* in the study on prevalence and only specific species in the study on abundance. As noted in Section 3.1.1.4, there is no overlap in the *Cryptosporidium* species for which humans and chickens are major hosts.

The relative fraction of human infectious strains of each of the reference pathogens from non-human sources is highly uncertain, and the literature did not have sufficient data to confidently assign quantitative values or ranges to this model parameter. However, not all strains of pathogens that animals shed infect humans. Section 3.1 describes attempts to quantify the overlap in pathogenicity of animal and human strains. Those data indicate variation in the human health risk posed by pathogens originating from cattle, swine, and chickens. Values of low (L), medium (M), or high (H) human infectious potential are assigned to each reference pathogen for each fecal source based on the prevalence of known human-infectious species/strains/serotypes/isolates in animal feces (Soller et al., 2010b). The mid-points of the ranges of 0 to 33% for L, 33 to 66% for M, and 67 to 100% for H, were then used as point estimates in this analysis (Table 11).

Table 11. Human infectious potential

Pathogen	Cattle	Pigs	Chicken
E. coli O157:H7	H	H	—
Campylobacter	H	H	M
Salmonella	M	M	M
Cryptosporidium	H	L	—
Giardia	H	H	—

The fresh fecal materials are assumed to be applied to land at agronomic rates. This choice is consistent with common practice and provides a conservative estimate of the pathogen load available for mobilization, because the agronomic rate is the highest rate at which manures should be applied. The results from the EPA environmental monitoring program (summarized in Section 2.12) are used to characterize the proportion of the land-applied pathogens that mobilize (mobilization fraction) following an intense rain event and runoff to a recreational waterbody. The density of organisms (FIB and pathogens) in water running off manure-applied plots is proportional to the number of land-applied organisms, with a different proportionality constant for each organism/manure type combination. Appendix E describes the specific methods used to derive these mobilization fractions. Table 12 summarizes the mobilization fractions.

Table 12. Mobilization fractions for land applied fecal wastes (log_{10} values)

	Cattle		Pigs		Chicken	
Pathogen	Low	High	Low	High	Low	High
E. coli O157:H7	-3.65	-0.20	-3.01	-1.50	-4.01	-2.21
Campylobacter	-4.85	-1.46	-2.20	-1.01	-8.60	-1.74
Salmonella	-5.57	-1.26	-3.85	-2.40	-3.68	-2.65
Cryptosporidium	-4.46	-0.18	-3.90	-1.48	Not tested	
Giardia spp.	-6.40	-0.39	-4.58	-0.06	Not tested	

For the risk characterization, it is assumed that mobilization of pathogens due to a specific runoff event are correlated to each other; that is, an event which mobilizes one pathogen to a relatively high degree (within its observed mobilization range) also mobilizes other pathogens to a similar degree (within the observed range for those pathogens). This approach is implemented numerically by generating a random number between zero and one (for each iteration in the simulation) and treating that number as a percentile of the mobilization distributions for each reference pathogen present in the land-applied manure (each of which is log-uniform). In each simulation iteration, the mobilization fractions for each pathogen are computed from that percentile of the corresponding distribution for the microorganism-manure combination. This process is repeated for each of 10,000 iterations in each simulation.[23]

Using the data summarized above, we calculate (1) the density of each reference pathogen in runoff water as the product of the reference pathogen abundance in land-applied fecal waste from infected animals; (2) the prevalence of infection in each animal source; (3) the human infectious potential of each pathogen, (4) a proportionality constant (specific to the fecal source type and the rain event to which the calculations are referenced) that is used to convert organisms applied to organisms in runoff water; and (5) the proportion of the applied reference pathogens that run-off following a rain event (i.e., the mobilization fraction).

[23] For example, for a particular iteration a random number between 0 and 1 is drawn—assume 0.15. Next, for that iteration, we assume that the "rain event" causes runoff at the 15th percentile of each of reference pathogens. Although the mobilization fractions will vary from pathogen to pathogen depending on the reported ranges (low and high in Table 12), the relative fraction mobilized for each event is driven by the intensity of the event (as determined by the random number).

The recreational waterbody is assumed to be adjacent to the plots where the fresh fecal material is applied and has a small water volume relative to the volume of runoff it receives during the rain event. Recreation is assumed to occur at the point where the runoff meets the adjacent recreational waterbody. Therefore, the dose of pathogens for this exposure scenario is the product of the volume of water ingested during recreational activities and the density of each pathogen in the runoff. This scenario yields a conservative estimate of risk in that dilution would occur to some degree when the runoff meets the receiving water, and the dilution would reduce the density of the pathogens in the water ingested the further downstream that ingestion occurs.

The volume of water ingested in the base analysis is modeled as a log-normal distribution with a log mean and log standard deviation of 2.92 and 1.43 mL, respectively (equivalent to a GM of 18.5 mL) (Dufour et al., 2006). Sensitivity analyses evaluate the implications of alternative volumes of water ingested—specifically, evaluations of 1 mL and 50 mL ingestion values consider minimal- and high-intensity water contact activities, respectively.

The computed doses to the appropriate dose-response relationships were calculated and resulted in a probability of infection. Table 13 summarizes the dose-response relationships used in the base analyses. Sensitivity analyses evaluate alternative dose-response relationships that account for uncertainty. The alternative dose-response relationships were selected because they represent the best available alternatives that allow consideration of uncertainty based on available information from the literature (USEPA, 2010). Specifically, the exponential dose-response model parameter r is varied uniformly between 0.04 and 0.16 for *Cryptosporidium* spp. (USEPA, 2005a, 2006). The hypergeometric dose-response model parameters of $\alpha = 0.011$ and $\beta = 0.024$ are evaluated for *Campylobacter jejuni* (Teunis et al., 2005); note that individual beta-Poisson alpha and beta pairs supplied by Dr. Teunis were used for *E. coli* O157 (Teunis et al., 2008b) rather than the median of those values (as used in the base analyses). Lastly, a Gompertz-log distribution was evaluated for *Salmonella enterica* (illness) with a uniformly distributed ln(a) parameter with values ranging from 29 to 50 and parameter b equal to 2.148 (Coleman and Marks, 1998, 2000; Soller et al., 2007b).

The probability of infection from each reference pathogen is multiplied by a pathogen-specific morbidity ratio (Table 13) to produce a probability of illness. The risk associated with each fecal contamination source (cattle, pigs, and chicken) is then characterized as the total probability of GI illness, based on the probability of illness from each of the reference pathogens, as described previously.

The forward QMRA risk calculations are conducted with a modified version of MRAIT (Soller et al., 2007a), which was originally designed to characterize risks associated with exposure to pathogens in reclaimed water. It was modified for use in this risk assessment to accept input parameters consistent with this exposure scenario. The MRAIT dose-response section was also updated for *E. coli* O157:H7 to accommodate new peer-reviewed information (Teunis et al., 2008b).

Using the parameters and values described above, MRAIT generated 10,000 iterations for each reference pathogen in each fecal contamination source. This approach required 12 simulations for the base analysis (5 for cattle, 5 for pigs, and 2 for chicken because *E. coli* O157, *Cryptosporidium,* and *Giardia* are not found in chicken litter) and 6 simulations for the sensitivity analyses (alternative dose-response for *Campylobacter*, *E. coli* O157, *Cryptosporidium*, and *Salmonella,* and two alternative ingestion values to represent minimal- and high-intensity water contact activities). Thus, a total of 18 simulations (each of which comprised 10,000 iterations) were performed. Appendix F illustrates a MRAIT screen image from one of the simulations. Results were saved, exported to text files, and subsequently used to generate tabular and graphical summaries.

Table 13. Dose-response models and morbidity

Reference Pathogen	Published Dose-Response Model	Model Parameters	Infectious $Dose_{50}$	Morbidity (% of Infections Resulting in Illness)	Health Endpoint
Cryptosporidium spp.	Exponential (USEPA, 2005a, 2006)	0.09	8 oocysts	20–70%	Infection
Giardia lamblia	Exponential (Haas et al., 1999; Rose et al., 1991)	0.0199	35 cysts	20–70%	Infection
Campylobacter jejuni	Beta-Poisson (Medema et al., 1996; Teunis et al., 1996; 2005)	0.145 7.59	800 CFU	10–60%	Infection
E. coli O157:H7	Beta-Poisson (Teunis et al., 2008b)	0.4 37.6	207 CFU	20–60%	Infection
Salmonella enterica	Beta-Poisson (Haas et al., 1999)	0.3126 2884	23,600 CFU	20%	Infection

4.1.2. Base analysis cattle results

The base analysis QMRA simulation results for fresh cattle manure based on all five of the bacterial and protozoan reference pathogens are summarized and presented in Table 14, Figure 18 (boxplot format), and Figure 19 (cumulative probability format). In Figure 18 (and subsequent boxplots), the edges of the box represent the 25th and 75th percentiles of the simulation results (probability of infection or illness), the line in the center of the box is the median value, the whiskers represent the 10th and 90th percentiles, and the diamonds below and above the whiskers represent the 5th and 95th percentiles, respectively.

Table 14. Summary of infection and illness risks from recreation in cattle manure-impacted water

	Infection Risks					Illness Risks				
Pathogen	5th %ile	10th %ile	Median	90th %ile	95th %ile	5th %ile	10th %ile	Median	90th %ile	95th %ile
E. coli O157	3.9E-05	1.3E-04	1.6E-02	6.2E-01	8.1E-01	1.5E-05	4.8E-05	5.8E-03	2.3E-01	3.2E-01
Campylobacter	7.4E-06	2.1E-05	1.6E-03	7.9E-02	1.6E-01	2.3E-06	6.6E-06	5.0E-04	2.7E-02	5.4E-02
Salmonella	7.5E-08	2.3E-07	2.6E-05	2.5E-03	7.1E-03	1.5E-08	4.6E-08	5.1E-06	4.9E-04	1.4E-03
Cryptosporidium	6.0E-07	2.6E-06	5.2E-04	9.7E-02	3.5E-01	2.6E-07	1.1E-06	2.2E-04	4.1E-02	1.5E-01
Giardia spp.	1.3E-08	5.2E-08	3.7E-05	2.4E-02	9.8E-02	5.6E-09	2.2E-08	1.5E-05	1.0E-02	4.3E-02
Cumulative	2.2E-04	6.4E-04	4.6E-02	7.7E-01	9.1E-01	8.4E-05	2.3E-04	1.8E-02	3.3E-01	4.5E-01

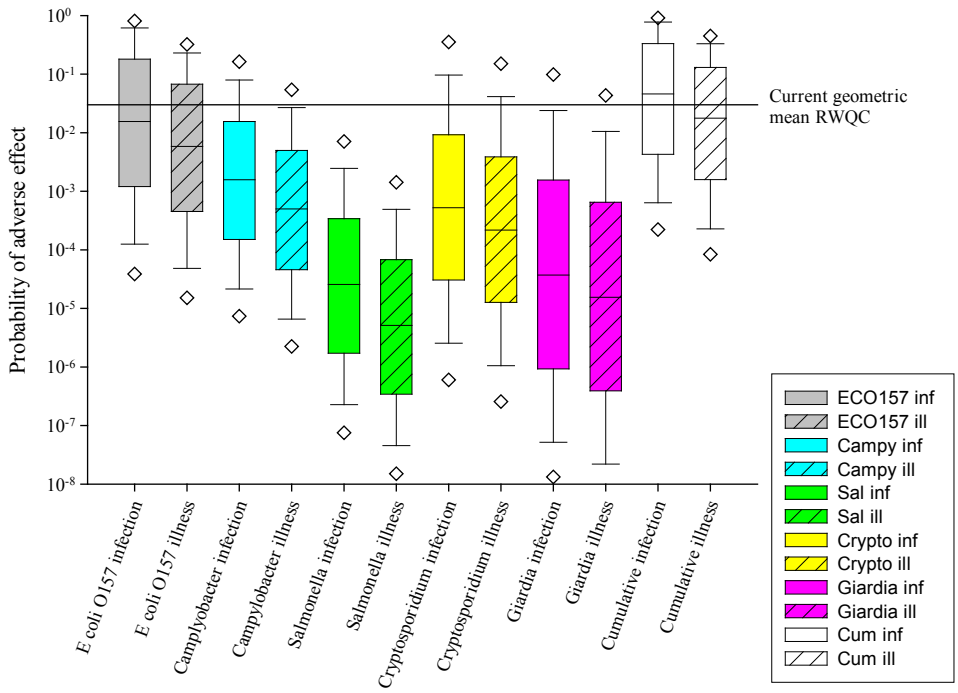

Figure 18. Probability of infection and illness from recreation in cattle-impacted water

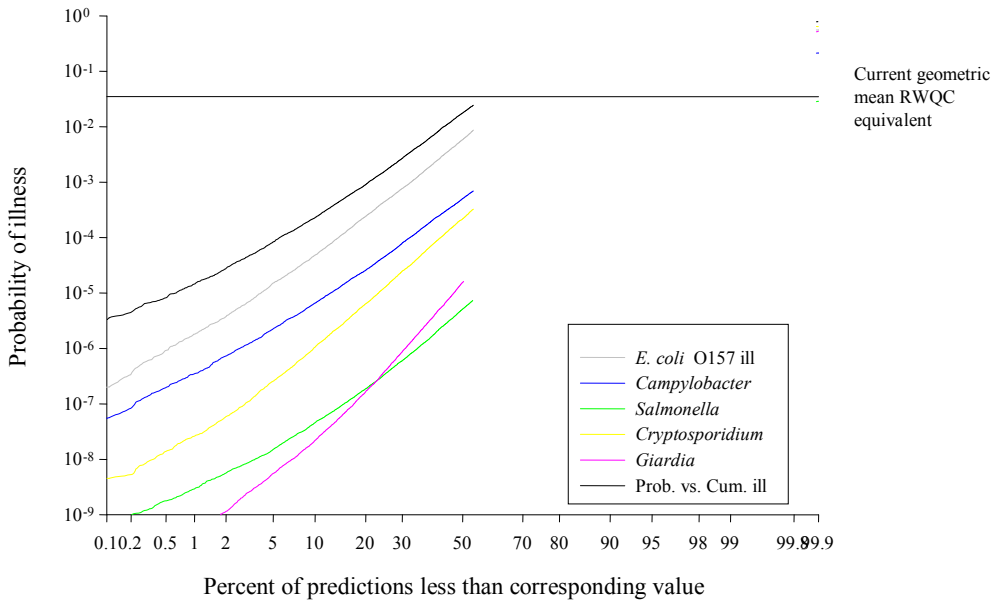

Figure 19. Cumulative probability illness risk plot for cattle manure-impacted water

Collectively, the data presented in Table 14, Figure 18, and Figure 19 indicate the following:

- The greatest predicted median risk of illness from recreational exposure to the hypothetical cattle-impacted waterbody is associated with *E. coli* O157.
- The predicted median risks of illness associated with *Campylobacter* and *Cryptosporidium* are approximately an order of magnitude below that of *E. coli* O157.
- The predicted median risks of illness associated with *Giardia* and *Salmonella* are approximately two to three orders of magnitude below that associated with *E. coli* O157.
- The predicted cumulative median risk of illness from recreational exposure to the cattle-impacted waterbody, as specified in the QMRA scenario, is slightly lower than, but effectively equivalent to the risk of illness that is associated with the current geometric mean RWQC based on water impacted by human sources of contamination (USEPA, 1986).[24]
- The predicted 90th percentile risk of illness associated with *E. coli* O157 is the highest of the reference pathogens, followed by *Cryptosporidium*, *Giardia*, and *Campylobacter*—each of which are approximately one order of magnitude lower.

[24] This can be seen from the data presented in the following two ways: (1) compare RWQC geometric mean GI illness equivalent risk (0.03) to the cumulative risk from Table 14 (0.018), and (2) compare the 50th percentile of the cumulative risk line in Figure 19 to the geometric mean RWQC line.

- The predicted 95th percentile risk of illness associated with *E. coli* O157 and *Cryptosporidium* are the highest of the reference pathogens, followed by *Campylobacter* and *Giardia*, which are approximately one half of an order of magnitude lower.

4.1.3. Base analysis pig results

The base analysis QMRA simulation results for pig slurry based on all five of the bacterial and protozoan reference pathogens are presented in Table 15, Figure 20, and Figure 21.

Table 15. Summary of infection and illness risks from recreation in pig slurry-impacted water

	Infection Risks					Illness Risks				
Pathogen	5th %ile	10th %ile	Median	90th %ile	95th %ile	5th %ile	10th %ile	Median	90th %ile	95th %ile
E. coli O157	2.2E-08	6.9E-08	1.7E-05	4.7E-03	1.4E-02	7.7E-09	2.4E-08	6.0E-06	1.7E-03	4.9E-03
Campylobacter	7.2E-04	1.3E-03	1.1E-02	7.3E-02	1.1E-01	2.0E-04	3.7E-04	3.5E-03	2.5E-02	3.8E-02
Salmonella	7.1E-06	1.5E-05	2.4E-04	3.9E-03	8.1E-03	1.4E-06	6.9E-08	1.7E-05	4.7E-03	1.4E-02
Cryptosporidium	6.5E-05	1.6E-04	4.4E-03	9.7E-02	2.0E-01	2.6E-05	6.7E-05	1.9E-03	4.3E-02	9.0E-02
Giardia spp.	1.4E-06	3.3E-06	3.2E-04	2.6E-02	6.5E-02	5.6E-07	1.4E-06	1.3E-04	1.2E-02	2.9E-02
Cumulative	1.0E-03	1.9E-03	2.1E-02	2.1E-01	3.5E-01	3.4E-04	6.4E-04	7.6E-03	8.5E-02	1.6E-01

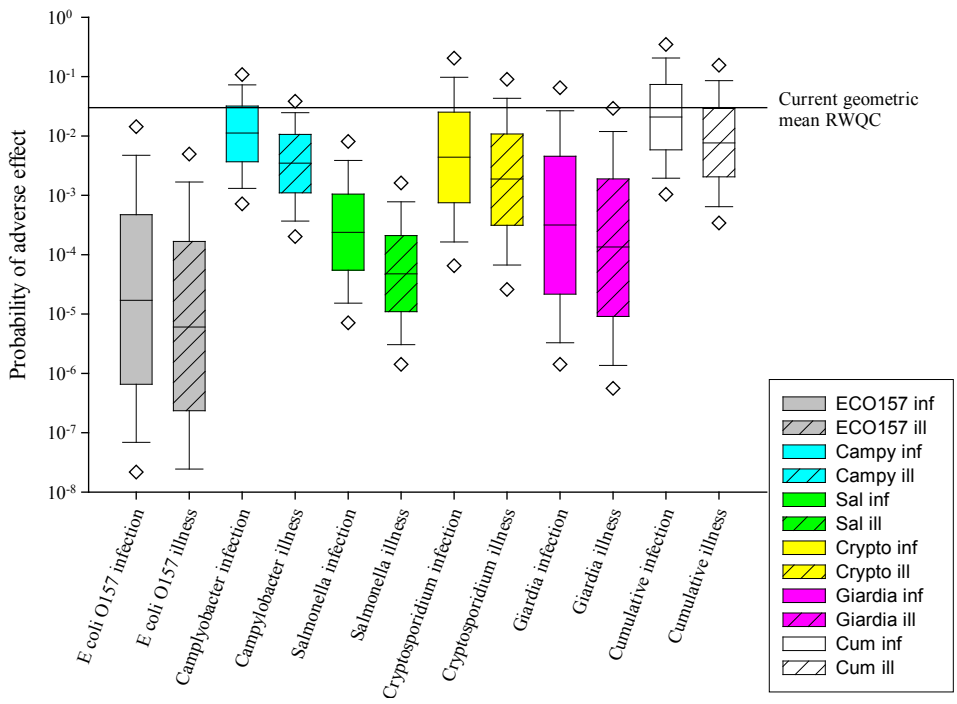

Figure 20. Probability of infection and illness from recreation in pig slurry-impacted water

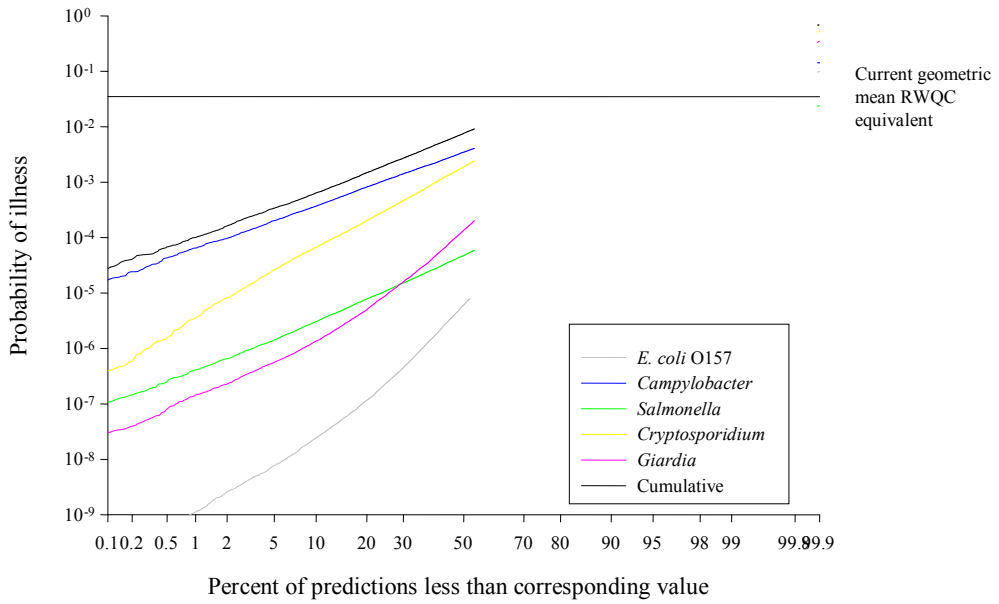

Figure 21. Cumulative probability illness risk plot for pig slurry-impacted water

The data in these tables and figures reveal the following:

- The greatest predicted median risks of illness from recreational exposure to the hypothetical pig-impacted waterbody are associated with *Campylobacter* and *Cryptosporidium*.
- The predicted median risk of illness associated with *Giardia* is approximately an order of magnitude below that of *Campylobacter* and *Cryptosporidium*.
- The predicted median risks of illness associated with *Salmonella* and *E. coli* O157 are approximately two to two-and-a-half orders of magnitude below those of *Campylobacter* and *Cryptosporidium.*
- The predicted cumulative median risk of illness from recreational exposure to the hypothetical pig-impacted waterbody is approximately four-times lower than the risk of illness that is associated with the current geometric mean RWQC (0.0076 compared to 0.03).
- The predicted 90th percentile risk of illness associated with *Cryptosporidium*, *Campylobacter*, and *Giardia* are the highest of the reference pathogens, followed by *E. coli* O157 and *Salmonella*, which are approximately one order of magnitude lower.
- The predicted 95th percentile risk of illness associated with *Cryptosporidium*, *Campylobacter*, and *Giardia* are the highest of the reference pathogens. The 95th percentile risk of illness associated with *Salmonella* is slightly lower, followed by *E. coli*

O157, which is approximately one order of magnitude lower than the reference pathogens exhibiting the highest risks.

4.1.4. Base analysis chicken results

The base analysis QMRA simulation results for fresh chicken litter based on two of the bacterial reference pathogens are presented in Table 16, Figure 22, and Figure 23. Inspection of those data highlights that chicken litter simulations were not conducted for *E. coli* O157, *Cryptosporidium* or *Giardia* (the literature review described in Section 3.1.3 and summarized in Table 9 indicated that the abundance of these references pathogens is minimal or zero, thus simulations were not conducted for these reference pathogens).

Table 16. Summary of infection and illness risks from recreation in chicken litter-impacted water

	Infection Risks					Illness Risks				
Pathogen	5th %ile	10th %ile	Median	90th %ile	95th %ile	5th %ile	10th %ile	Median	90th %ile	95th %ile
E. coli O157	-	-	-	-	-	-	-	-	-	-
Campylobacter	2.2E-08	1.2E-07	1.9E-04	1.7E-01	3.3E-01	6.9E-09	3.6E-08	6.0E-05	5.2E-02	1.1E-01
Salmonella	2.9E-08	8.0E-08	5.1E-06	3.4E-04	9.0E-04	7.2E-09	2.0E-08	1.3E-06	8.5E-05	2.2E-04
Cryptosporidium	-	-	-	-	-	-	-	-	-	-
Giardia spp.	-	-	-	-	-	-	-	-	-	-
Cumulative	4.1E-07	1.5E-06	3.5E-04	1.7E-01	3.3E-01	1.1E-07	4.4E-07	1.0E-04	5.2E-02	1.1E-01

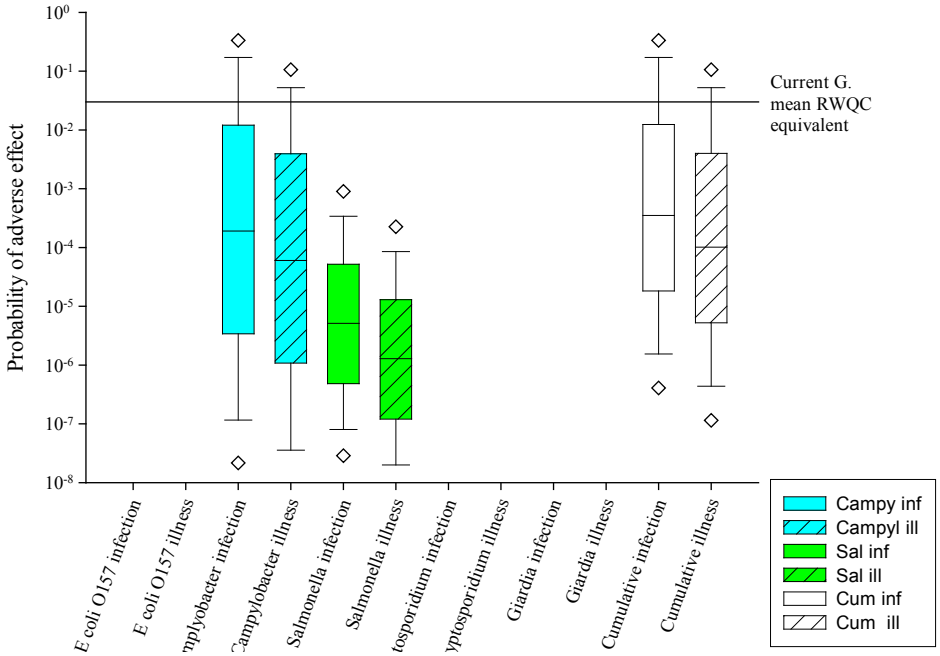

Figure 22. Probability of infection and illness from recreation in chicken litter-impacted water

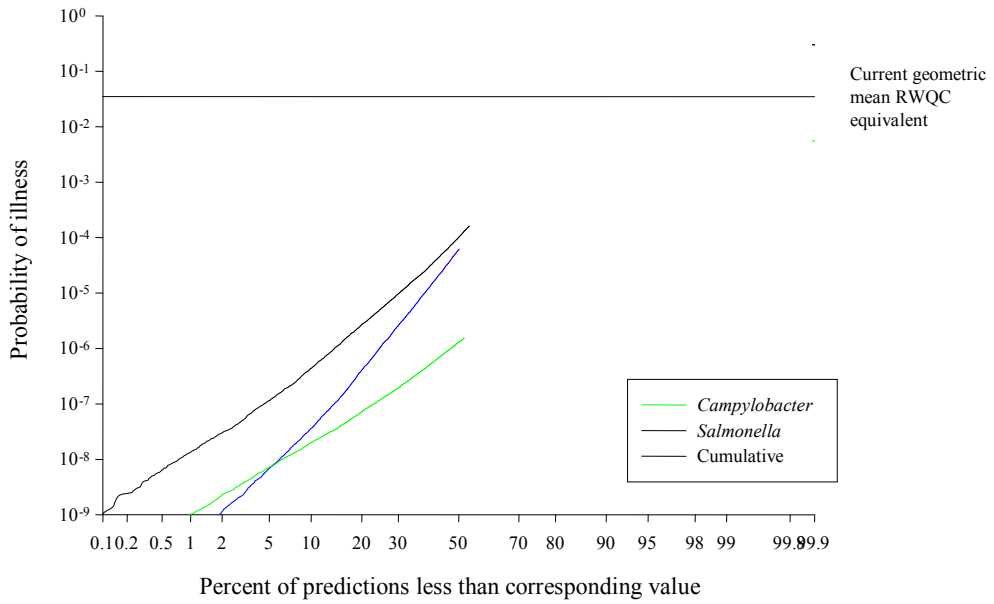

Figure 23. Cumulative probability illness risk plot for chicken litter-impacted water

The data presented in Table 16, Figure 22, and Figure 23 indicate the following:

- The predicted median *Campylobacter* risk of illness from recreational exposure to the chicken litter-impacted waterbody is greater than that associated with *Salmonella* by approximately one-and-a-half orders of magnitude.
- The predicted cumulative median risk of illness from recreational exposure to the hypothetical chicken-impacted waterbody is approximately 300-times lower than the risk of illness that is associated with the current geometric mean RWQC (0.0001 compared to 0.03).
- The predicted 90th and 95th percentile risks of illness associated with *Campylobacter* are approximately two to three orders of magnitude greater than those associated with risks from *Salmonella*.

4.1.5. Base analysis comparison of results

Comparisons of the QMRA simulation results for the cattle manure, pig slurry, and chicken litter-impacted recreational water are presented in Figure 24 and Figure 25. Note that Figure 24 consolidates the cumulative illness risks from Figure 18, Figure 20, and Figure 22 into a single boxplot. Figure 25 presents the probability densities for the simulation results.

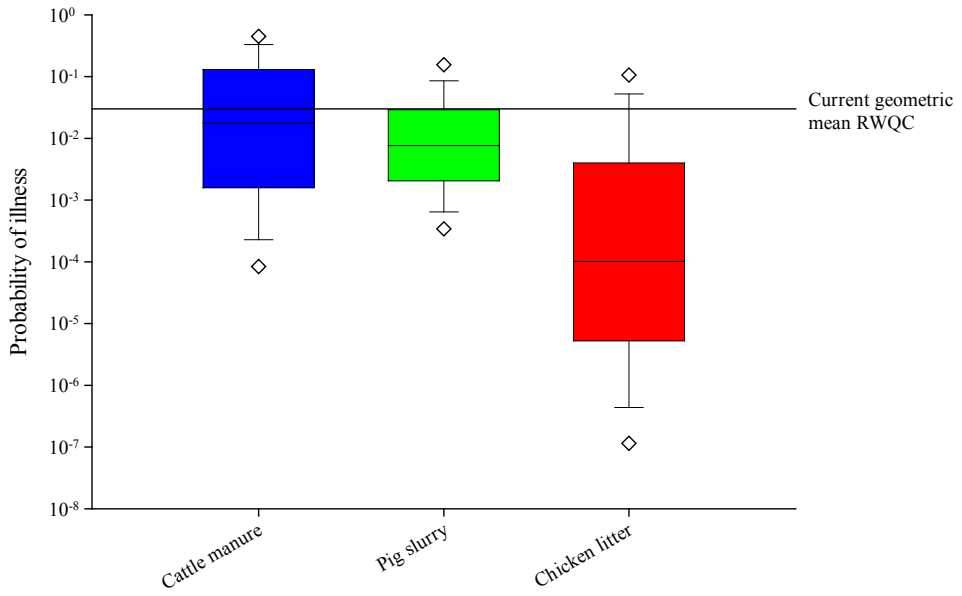

Figure 24. Comparison of illness risks from recreation in agricultural animal-impacted runoff

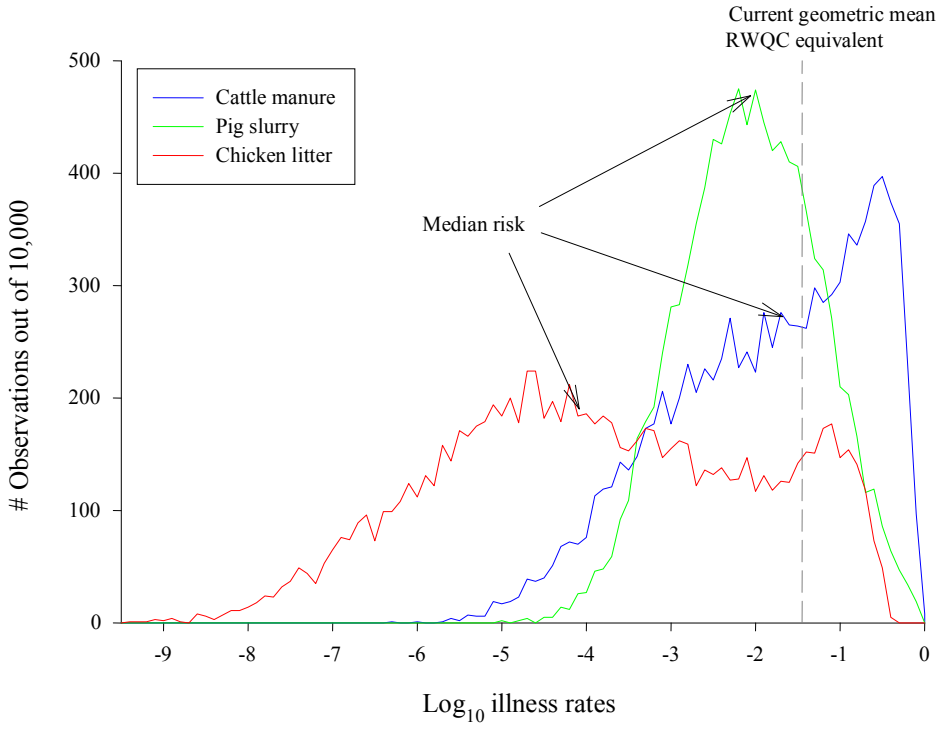

Figure 25. Probability density for illness from recreation in animal-impacted water

Figure 24 and Figure 25 reinforce the following interpretations of the base QMRA simulation results:

- The predicted median cumulative risk of illness from recreational exposure to the cattle-impacted waterbody is effectively equivalent to the risk of illness that is associated with the current geometric mean RWQC based on water contaminated by human sources.
- The predicted median cumulative risk of illness from recreational exposure to the pig-impacted waterbody is approximately four-times lower than the risk of illness that is associated with the current geometric mean RWQC.
- The median cumulative risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 300-times lower than the risk of illness that is associated with the current geometric mean RWQC.
- The predicted variability is greatest for chicken-impacted water and least for pig-impacted water.
- A substantial portion of the simulations for cattle-impacted water resulted in risks that appear to be relatively high (for example, greater than 100 illnesses per 1000 recreation events). A smaller but still substantial fraction of the simulations for pig and chicken-impacted water also resulted in apparently high risks.[25]

4.1.6. Sensitivity analysis results for alternate dose-response relationships

We used pig slurry-impacted water to conduct the sensitivity analysis for alternative dose-response relationships to represent all three sources and to maximize the likelihood that any differences would be apparent (because cattle-impacted water risks are higher, the potential to observe substantial changes in simulation output is lower). Similarly, because chicken-impacted water risks are substantially lower, changes in simulation output may not represent changes in simulation output for cattle-impacted water. Finally, pig slurry risks include all reference pathogens, whereas, chicken litter risks include only a subset of the reference pathogens.

As indicated in Section 4.1.1, alternative dose-response simulations were conducted for *Cryptosporidium*, *Campylobacter*, *E. coli* O157, and *Salmonella enterica*. The results from those simulations are presented in probability plot format in Figure 26 (*Cryptosporidium*), Figure 27 (*Campylobacter*), and Figure 28 (*E. coli* O157). The alternative simulations for Salmonella enterica resulted in illness risks that were extremely low (below 10^{-9}), so are not presented graphically.

[25] The parameter combinations causing these high risk outcomes are discussed in Section 4.3.

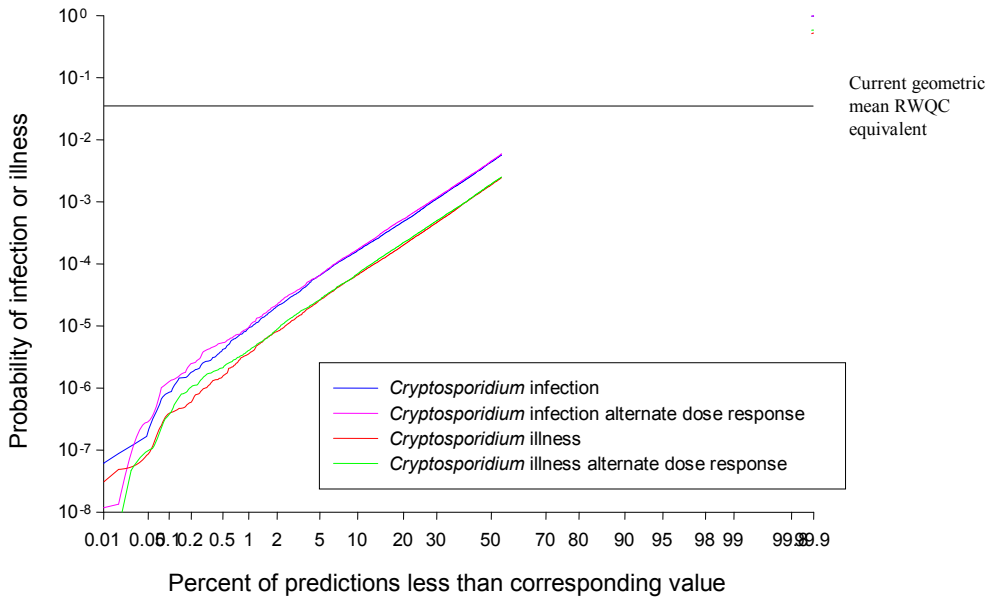

Figure 26. Cumulative probability plot: evaluation of alternative dose-response for *Cryptosporidium*

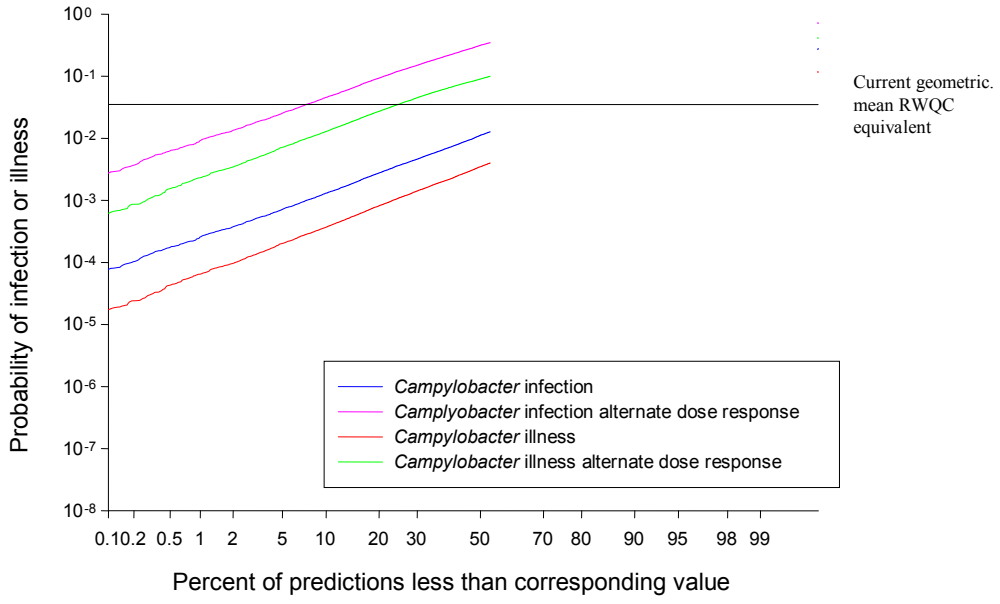

Figure 27. Cumulative probability plot: evaluation of alternative dose-response for *Campylobacter*

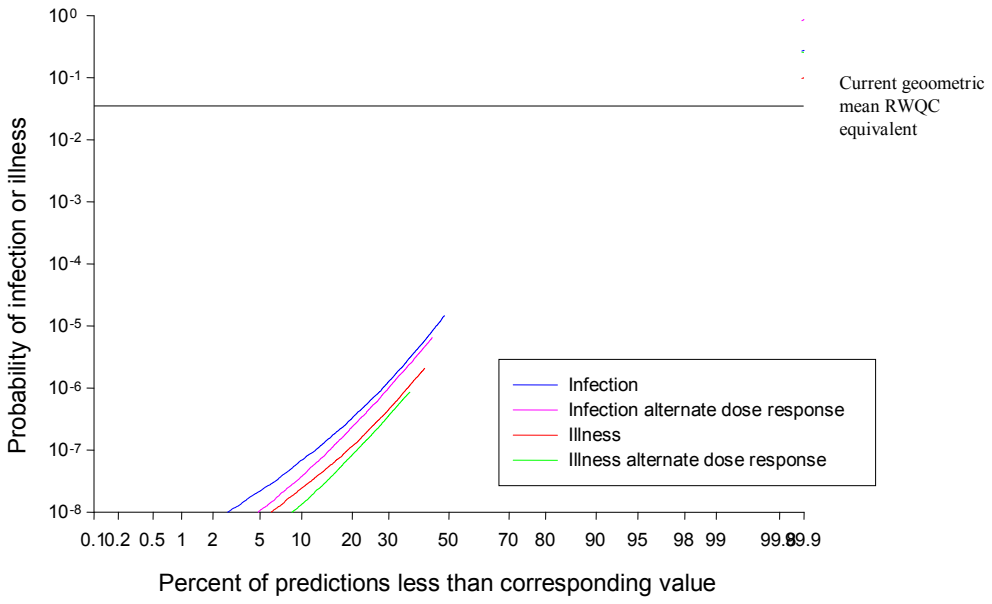

Figure 28. Cumulative probability plot: evaluation of alternative dose-response for *E. coli* O157

The sensitivity analysis indicates that the QMRA results are not substantially impacted by including uncertainty in dose-response *for Cryptosporidium* (Figure 26) or *E. coli* O157 (Figure 28). This can be seen by comparing the "illness" to "illness alternative dose-response" curves in the corresponding figures. The results of the QMRA simulations are, however, sensitive to the selection of dose-response relationships for Campylobacter jejuni and Salmonella.

The alternative dose-response relationship for *Salmonella* was a Gompertz-log relationship that was developed to account for strain variability (Coleman and Marks, 1998, 2000; Soller et al., 2007b). The Gompertz-log model is an empirical dose-response model that is based on a good fit to the experimental data collected in volunteer feeding studies from the 1940s (McCullough and Eisele, 1951a, 1951b). Those studies, however, used doses of *Salmonella* that were several orders of magnitude greater than the predicted doses used in this QMRA. Because the Gompertz-log model is an empirical model, and the doses under consideration are outside of the range that provided a good fit, the extent to which this dose-response relationship may be used to extrapolate to low-dose risk predictions is not known.

The alternative dose-response for *C. jejuni* is a hypergeometric (exact beta-Poisson) function (Teunis et al., 2005). This dose-response relationship amends the previous dose-response relationship by Medema et al. (1996) to account for low-dose human response to *C. jejuni* exposure shown in two contaminated milk outbreaks. This relationship exhibits higher levels of infection at low doses and a steeper increase with dose than the previous function, which was

based only on the human feeding study. However, other outbreak-based dose-response studies indicate that the dose-response relationship can shift based on the type of contaminated media (e.g.,type of food, water) (Bollaerts et al., 2008). Given that this alternative dose-response is based on exposure (primarily in children) to *C. jejuni* in milk, and this risk assessment is for the general population exposed to *Campylobacter* spp. via animal-impacted recreational water, use of the base analysis *Campylobacter* dose-response is reasonable. However, the additional uncertainty associated with the applicability of the alternative dose-response relationship should be taken into consideration during the risk management process.

4.1.7. Sensitivity analysis results for alternate ingestion

Sensitivity analysis simulations were also conducted using alternative rates of water ingestion of pig slurry-impacted water—1 mL and 50 mL (compared to the base analysis that used a log-normal distribution of water ingestion with geometric mean of approximately 18 mL). For these simulations, *Cryptosporidium* was the model reference pathogen.

The 1 mL ingestion is used to evaluate the potential risks associated with low-contact activities such as wading, beachcombing, fishing, and others. Similarly, the 50 mL ingestion is used to evaluate the potential risks associated with prolonged exposure to water or vigorous water play. The base analysis was designed to be consistent with the self-reported body-contact recreation in EPA's water epidemiology studies.

The results from these simulations are summarized in Table 17 and presented in boxplot format in Figure 29. These alternative ingestion volume simulations indicate that the median risks scale linearly with volume ingested, within the evaluated ranges. Furthermore, the 5th and 10th percentiles of the risk distributions are impacted to a lesser degree than the median risk values by the selection of a point estimates rather than the use of a log-normal distribution. The 90th and 95th percentiles of the risk distributions are impacted to a greater degree than the median risk values by the selection of a point estimate rather than the use of a log-normal distribution.

Table 17. Alternate ingestion: *Cryptosporidium* infection and illness from pig-impacted runoff

	Infection Risks					Illness Risks				
Ingestion	5th %ile	10th %ile	Median	90th %ile	95th %ile	5th %ile	10th %ile	Median	90th %ile	95th %ile
1 mL Point estimate	7.0E-06	1.5E-05	2.4E-04	3.4E-03	6.3E-03	2.9E-06	6.2E-06	9.9E-05	1.5E-03	2.8E-03
Lognormal distribution	6.5E-05	1.6E-04	4.4E-03	9.7E-02	2.0E-01	2.6E-05	6.7E-05	1.9E-03	4.3E-02	9.0E-02
50 mL point estimate	3.5E-04	7.4E-04	1.2E-02	1.6E-01	2.7E-01	1.5E-04	3.1E-04	4.9E-03	6.9E-02	1.2E-01

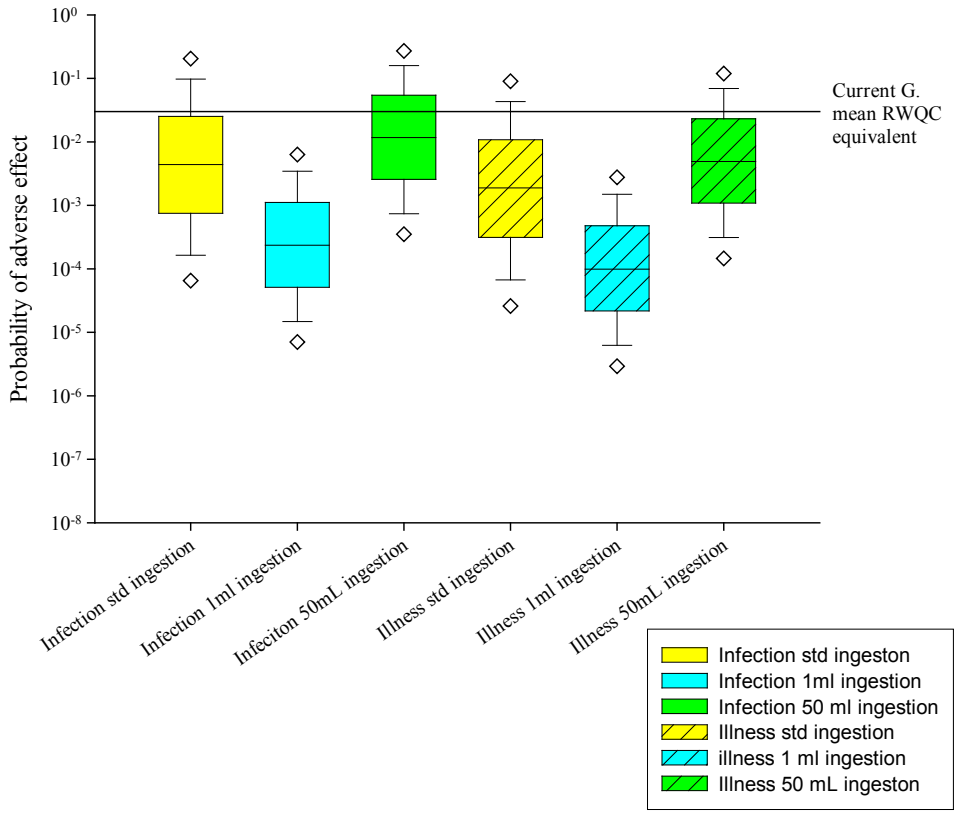

Figure 29. Alternate ingestion: *Cryptosporidium* infection and illness from pig-impacted runoff

4.2. Relative QMRA for Animal-Impacted Water and Human-Impacted Water

A relative QMRA approach was used to compare the risk of illness associated with recreation at a freshwater beach impacted by cattle, pig, and chicken sources of fecal contamination and human-impacted recreational water. This approach complements the forward QMRA approach by normalizing risks from specific livestock fecal pollution sources to a specified FIB density and by facilitating a comparison of risks between different fecal pollution sources. However, this approach requires more assumptions and data than used in the forward QMRA approach, such as the range of FIB densities in fecal pollution sources and an assumption that FIB mobilization has the same driving forces as pathogen mobilization.

For animal-impacted water, FIB and pathogen loading to a recreational waterbody can occur via direct or indirect (runoff) contamination. Previous studies developed a methodology, model, and set of literature to evaluate the estimated human health risks from exposure to recreational waters impacted by human and direct fresh non-human sources of fecal contamination (Schoen and Ashbolt, 2010; Soller et al., 2010b). The results from those relative QMRA studies indicate that at a given level of FIB in a waterbody, the GI illness risks associated with recreational exposure impacted by direct cattle contamination might not be substantially different from those impacted by human sources. However, the risks associated with exposure to recreational water impacted

by direct gull, chicken, and pig fecal wastes appear to be substantially lower than those impacted by human sources (Soller et al., 2010b).

The relative QMRA conducted for this study extends the previous work by considering indirect loading of pathogens and FIB via land application of livestock waste to a waterbody. Rather than assuming livestock wastes are deposited directly into receiving water, the revised model assumes that fresh livestock wastes are applied to land at agronomic rates, and pathogens and FIB are mobilized and transported to receiving water during an intense rain event. Although manure handling practices differ between operations, we assume land application, which reflects national practices. This approach does not preclude a QMRA for sites that handle manure differently. In addition, down-slope processes from land-applied wastes also vary between sites. Here, we conservatively assumed that runoff is introduced into the receiving water with no buffer strips or other best management practices in place. Finally, estimates of FIB and pathogen mobilization during and subsequent to rainfall events are based on the results of the EPA environmental monitoring studies.

Similar to the forward QMRA described above, the general methodology for this relative QMRA is a Monte Carlo simulation-based approach with model parameters characterized as statistical distributions. The simulation code used by Schoen and Ashbolt (2010) and Soller et al. (2010b) was adapted for this relative QMRA to include mobilization of land-applied pathogens and FIB due to a rainfall event.

4.2.1. Methods

In these analyses, the estimated risks are calculated for a hypothetical waterbody that contains FIB densities from fresh cattle manure, fresh pig slurry, and fresh poultry litter at the current geometric mean RWQC (USEPA, 1986) for freshwater (33 CFU /100 mL enterococci and 126 CFU /100 mL *E. coli*, respectively). Separate analyses were performed for each source of fecal contamination based on each of the FIB.

The conceptual diagram for the relative QMRA was presented previously (see Figure 8). Reference pathogen doses are derived as a function of the density of the FIB from each of the specific sources (Schoen and Ashbolt, 2010; Soller et al., 2010b). Specifically, pathogen dose is calculated based on independent Monte Carlo samples from observed or literature-based ranges of pathogen and FIB densities in fecal waste, the prevalence of infection, the fraction of human-infectious strains, and the proportion of the FIB and pathogens that mobilize during a rain event. This sampling scheme does not require a specific relationship between the FIB and pathogens in the fecal waste or in the receiving water. However, the mobilization of pathogens and FIB are related to each other, as Section 3.1.6 describes. The dose of each reference pathogen from each source is calculated as follows:

$$\mu_{rp}^{S} = \frac{C_{FIB}}{(R_{FIB}^{S} \times M_{FIB}^{S}) \times 100} \times (R_{rp}^{S} \times M_{rp}^{S}) \times p_{rp}^{S} \times I_{rp}^{S} \times V \qquad [14]$$

where

S is the fecal contamination source;

C_{FIB} is the waterbody density of enterococci or *E. coli* (CFU 100 mL^{-1});

R_{FIB}^{S} is the density of FIB in land-applied manure (g or CFU $100mL^{-1}$);

M_{FIB}^{S} is the mobilization fraction of the FIB for the fecal source (#/100mL runoff)/(#/g manure) or (#/mL manure runoff/#/mL manure slurry);

R_{rp}^{S} is the density of pathogen species in land-applied manures with pathogens (number of pathogens or genomes (g or 100 mL)$^{-1}$);

M_{rp}^{S} is the mobilization fraction of the pathogen species for the fecal source;

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 (proportion of animals shedding the pathogen); and

V is the volume of water ingested (mL).

Although this equation is similar to that used in previous related QMRAs (Schoen and Ashbolt, 2010; Soller et al., 2010b), except here the pathogen and FIB densities in water ingested during recreation are a function of the organisms in the land-applied manure and the mobilization fractions of the organisms during rain events. In the previous studies, (1) mobilization fractions of the organisms during rain events were not included, and (2) direct contamination occurred from cattle, pig, and chicken feces rather than indirect contamination from cattle manure, pig slurry, and chicken litter.

Similar to the forward QMRA described above, doses are input to the appropriate dose-response relationship resulting in a probability of infection. Probability of illness is computed using the morbidity fractions for each reference pathogen. The total probability of illness for each contamination source is computed as described previously (i.e., one iteration). Each simulation includes 10,000 iterations for each fecal contamination source/pathogen combination. The resulting distributions of risk are compared to a benchmark risk for human-impacted water (based on the current geometric mean RWQC) and to the risk results for direct agricultural contamination as reported by Soller et al. (2010b).

The animal source pathogen abundance, prevalence, human infectious potential, mobilization fractions, morbidity fractions, and dose-response models used in this analysis are the same as were used in the forward QMRA presented above (Table 9, Table 10, Table 11, Table 12, and Table 13, respectively). Table 18 summarizes the literature-based and observed data used to characterize the FIB densities (abundance) in solid fresh cattle manure, fresh pig slurry, and fresh poultry litter.

Table 18. Abundance of fecal indicator bacteria in fecal sources

		Cattle[1]		Pigs[2]		Chicken[1]	
Indicator		Low	High	Low	High	Low	high
Enterococci							
	Literature	2.4	6.8	5.0	5.9	4.0	6.0
	Observed	4.7	5.5	0.2	2.0	3.8	5.8
E. coli							
	Literature	5.0	6.7	5.0	6.7	5.0	10.9
	Observed	6.7	8.3	0.7	3.1	2.7	4.4

1. Density in solid manures: units of $\log_0$ (#/g wet weight)

2. Density in liquid manures: units are $\log_{10}$ (#/100 mL)

Similar to the forward QMRA, we used EPA environmental monitoring program (see Section 2.12 and Appendix D) results to characterize the proportion of the land-applied FIB that mobilize and run-off to a recreational waterbody (mobilization fraction) following a typical rain event. The density of FIB (*E. coli* and enterococci) in water running off manure-applied plots is assumed to be proportional to the number of land-applied organisms, with a different proportionality constant for each organism/manure combination.

Several alternative indicator organism-detection method combinations were used to monitor indicator density in manure and runoff in the EPA environmental monitoring program. Those alternatives included an *E. coli* O157 surrogate with soil and manure matrix affinities and runoff characteristics that were assumed to be similar to those of generic *E. coli*. The alternatives with sufficient data for characterizing mobilization of FIB were enterococci via culture on membrane-enterococus indoxyl-ß-D-glucoside (mEI) agar, *E. coli* via the Colilert MPN method, and *E. coli* O157 surrogate strain via membrane filtration. The observed mobilization fractions for *E. coli* via Colilert and enterococci via culture on mEI ager were analyzed to determine whether the mobilization distributions appeared uniform or triangular (described in Appendix E). Table 19 summarizes the mobilization distributions used for FIB in the relative risks analyses.

Table 19. Mobilization of fecal indicator bacteria for animal fecal sources

	Cattle		Pigs		Chicken	
Organism	Distriubtion	Values	Distriubtion	Values	Distriubtion	Values
Enterococci	Uniform	(-2.8, 0.3)	Uniform	(-1.0, 2.5)	Triangular	(-1.25, -0.25, 0.32)
E. coli	Triangular	(-5.0, -2.75, -2.0)	Uniform	(-2.0, 1.0)	Triangular	(-2.75, 0.25, 1.25)
E. coli O157 surrogate	Uniform	(-3.7, -0.20)	Uniform	(-3.0, -1.5)	Uniform	(-4.0, -2.2)

1. Mobilization fractions reported as $\log_{10}$ values
2. Parameters of the Uniform distribution are (min, max)
3. Parameters of the Triangular distribution are (min, mode, max)
4. Mobilization values greater than 0 represent an increase in indicator in runoff compared to the land applied material

For the relative QMRA, we conducted two complementary sets of analyses using the data described above. In the first approach (Approach 1), the enterococci and *E. coli* abundance and mobilization distributions were based on observed data from the EPA environmental monitoring program ("Observed" data in Table 18; "enterococci" and "*E. coli*" data in Table 19). The results from these analyses are likely specific to the observed abundances of the FIB in the land-applied materials and site features such as the densities of the FIB in the soil (before land application).

The mobilizations reported for enterococci and *E. coli* depend on their abundance in the land-applied material. As described in Appendices D and E, use of the pig slurry data in Approach 1 is highly questionable because the manure pathogen densities are drawn from distributions developed based on a literature review and intended to reflect typical conditions in the United States. In contrast, manure FIB densities were selected from manures specific to the EPA environmental monitoring program experiments. Furthermore, the FIB densities in runoff from control plots were high relative to runoff from pig manure-treated plots and some chicken-manure-treated plots—particularly for enterococci (values substantially greater than 0 in Table 19).

In Approach 2, the enterococci and *E. coli* abundances were literature-based (Table 18), the *E. coli* mobilization distributions were based on the *E. coli* O157 surrogate data, and the enterococci mobilization distributions were based on the observed data from the EPA environmental monitoring program (Table 19). The use of the pig slurry enterococci mobilization data is inappropriate in this case because the mobilizations reported for enterococci and *E. coli* are dependent on their abundance in the land-applied material. The abundance and mobilization data for this approach represent average values that are substantially less dependent on the enterococci and *E. coli* levels observed during the environmental monitoring program.

4.2.2. Relative QMRA results

The relative QMRA simulation results are summarized in Table 20 and shown in boxplot format in Figure 30 for Approach 1 and Figure 31 for Approach 2. A probability density plot for the *E. coli*-based results from Approach 2 is presented in Figure 32.

The predicted Approach 1 enterococci and *E. coli*-based results for swine slurry, and *E. coli*-based results in chicken litter were driven by the low observed levels of FIB and observed mobilization fractions greater than one. As indicated above, use of the pig slurry data in this analysis is questionable because the pig slurry used in the EPA environmental monitoring program had significantly lower counts of enterococci and *E. coli* than those reported in the literature and is likely substantially different than the pig slurries used to estimate pathogen abundances. For these reasons, we believe that the Approach 2 results are more robust.

Table 20. Relative QMRA illness risks from exposure to agricultural animal-impacted water

			Illness Risks				
Data Used	**Indicator**	**Fecal Source**	5th %ile	10th %ile	Median	90th %ile	95th %ile
Approach 1	Enterococci	Cattle manure	9.1E-06	1.9E-05	3.2E-04	5.8E-03	1.6E-02
		Pig slurry	2.2E-01	3.3E-01	6.7E-01	8.2E-01	8.4E-01
		Chicken litter	7.8E-06	2.1E-05	2.3E-03	6.8E-02	9.6E-02
	E. coli	Cattle manure	9.7E-08	2.3E-07	4.9E-06	1.2E-04	3.4E-04
		Pig slurry	1.2E-01	2.0E-01	6.3E-01	8.1E-01	8.4E-01
		Chicken litter	6.1E-04	1.7E-03	5.7E-02	1.6E-01	1.8E-01
Approach 2	Enterococci	Cattle manure	2.6E-06	8.0E-06	1.1E-03	8.0E-02	1.6E-01
		Pig slurry	Not conducted				
		Chicken litter	4.7E-06	1.4E-05	1.4E-03	5.3E-02	8.0E-02
	E. coli	Cattle manure	4.0E-06	9.6E-06	2.1E-04	5.4E-03	1.4E-02
		Pig slurry	4.7E-05	9.1E-05	1.0E-03	1.1E-02	2.0E-02
		Chicken litter	1.2E-09	6.7E-09	5.2E-06	4.5E-03	1.7E-02

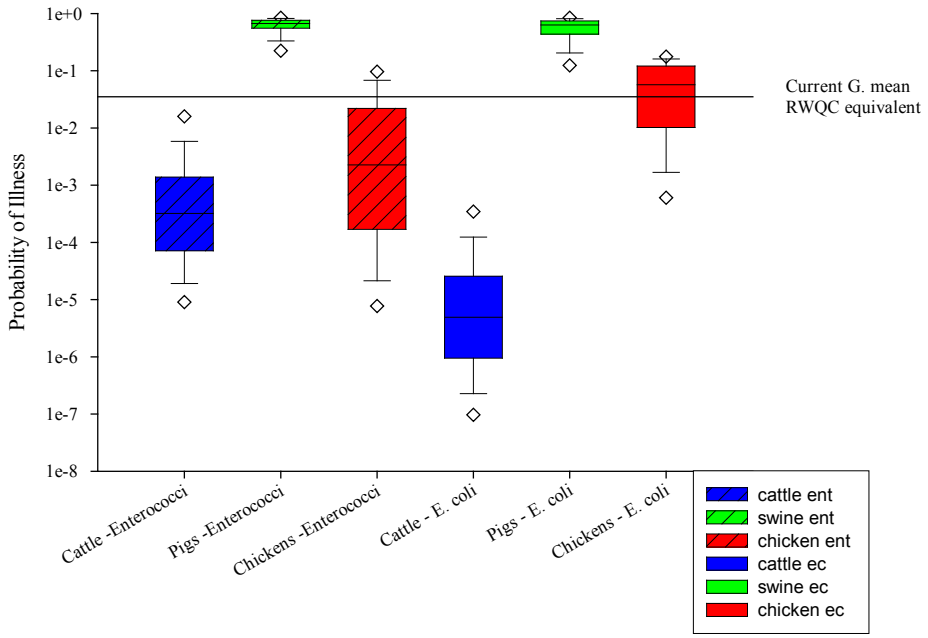

Figure 30. Relative QMRA approach 1 probability of illness boxplot

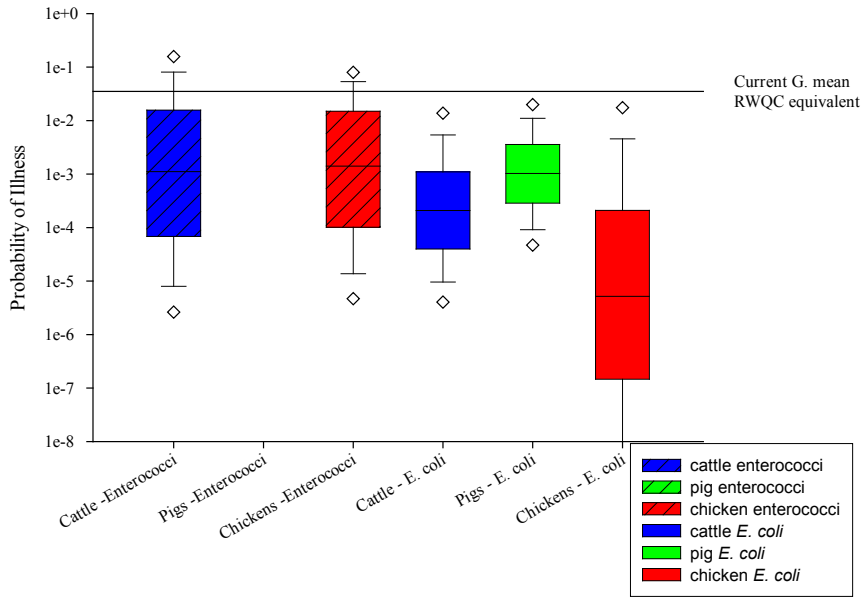

Figure 31. Relative QMRA approach 2 probability of illness boxplot

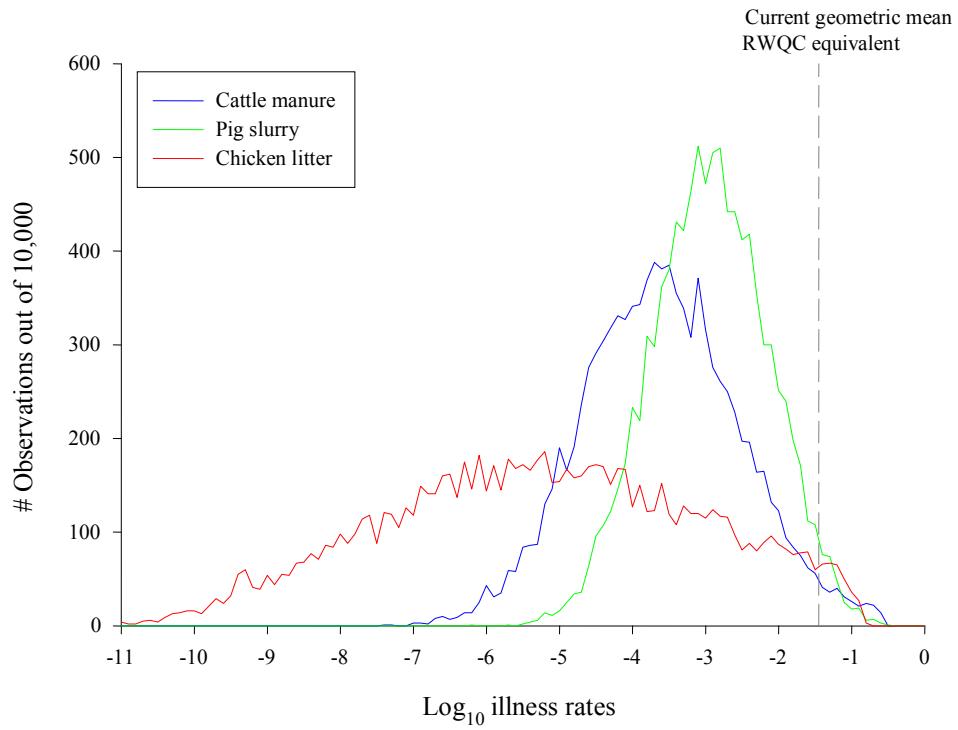

Figure 32. Probability density for illness risks from *E. coli* relative QMRA approach 2

Inspection of the data presented in Table 20, Figure 30, Figure 31, and Figure 32 indicates the following:

- The enterococci-based and *E. coli*-based results for Approach 2 indicate that the predicted median risk of illness from recreational exposure to the cattle-impacted waterbody is 25 to 150-times lower than risk of illness associated with human sources of contamination at the current geometric mean RWQC (1.1×10^{-3} and 2.1×10^{-4} respectively compared to 0.03).
- The *E. coli* -based results for Approach 2 indicate that the predicted median risk of illness from recreational exposure to the pig-impacted waterbody is approximately 30-times lower than the risk of illness associated with human sources of contamination at the current geometric mean RWQC (1.0×10^{-3} compared to 0.03).
- The enterococci-based and *E. coli*-based results for Approach 2 indicate that the predicted median risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 20- to 5000-times lower than the risk of illness associated with human sources of contamination at the current geometric mean RWQC (1.4×10^{-3} and 5.2×10^{-6}, respectively compared to 0.03), depending on the FIB used.

- The predicted variability is greatest for chicken-impacted water and least for pig-impacted water.
- A small portion of the simulations resulted in risks that were greater than the current geometric mean equivalent risk under Approach 2.

For cattle-impacted water, the enterococci-based results from Approach 1 are similar to those from Approach 2. The Approach 1 *E. coli*-based results are lower than those from Approach 2 by two to three orders of magnitude. This result likely occurred because the *E. coli* densities observed during the EPA environmental monitoring program were well above the literature-based results.

For chicken-impacted water, the enterococci-based results from Approach 1 are similar to those from Approach 2. The Approach 1 *E. coli*-based results are less credible, as indicated above.

The predicted relative risks of illness are highly dependent on the FIB used. Relative QMRA results are generally higher for enterococci than for *E. coli.* The median Approach 2 risks of illness for cattle-impacted water based on enterococci are higher than those for *E. coli* by approximately one order of magnitude. The median Approach 2 risks of illness for chicken-impacted water based on enterococci are higher than those for *E. coli* by approximately two-and-a-half orders of magnitude.

4.3. Discussion

4.3.1. Interpretation of results

The purpose of this QMRA was to estimate the human GI illness risk associated with recreation at a freshwater beach impacted by fecal contamination from agricultural animal sources. Again, the analysis addresses the following two questions: (1) What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, and chicken) sources of fecal contamination?, and (2) How do those risks compare to risks associated with freshwater beaches impacted by human sources?

Two complementary QMRA approaches were used. A traditional forward QMRA approach characterizes the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal sources of fecal contamination. A relative QMRA compares the estimated risks from recreation in water impacted by agricultural sources of fecal contamination to those associated with human-impacted water.

The forward QMRA results estimate risk of illness in runoff within the context of the exposure scenario evaluated. We made several simplifying assumptions to limit the scope of the exposure scenario and ensure that the evaluation results would protect health relative to uninvestigated conditions. Some of the most important assumptions were

- primary contact recreation occurs at a freshwater beach adjacent to land that has fresh cattle manure, pig slurry, or chicken litter applied at agronomic rates;
- GI illness is the health outcome of primary concern and GI illness rates are protective for respiratory illness;
- ingestion of water is the primary exposure route of interest;
- FIB and pathogens reach the beach via runoff from an intense rainfall event;
- results of the EPA environmental monitoring program can be used to estimate mobilization fractions of FIB and pathogens to the recreational water; and
- recreation at the assumed point of exposure is health protective compared to other potential exposure points (downstream, diluted, or aged contamination scenarios).

The forward QMRA base analyses indicate that the predicted median risk of illness from recreational exposure to the cattle-impacted waterbody during and immediately after an intense rain event is effectively equivalent to the risk of illness associated with the current geometric mean RWQC (USEPA, 1986). The predicted median risk of illness from recreational exposure to the pig-impacted waterbody is approximately 4-times lower than the risk of illness that is associated with the current geometric mean RWQC (i.e., 0.03/0.0076 = 4), and the predicted median risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 300-times lower than the risk of illness associated with the current geometric mean RWQC. *E. coli* O157 is the predicted dominant risk agent in cattle-impacted water, followed by *Campylobacter* and *Cryptosporidium*. For pig-impacted water, *Campylobacter* and *Cryptosporidium* are the predicted dominant risks agents, followed by *Giardia*. For chicken-impacted water, *Campylobacter* is the predicted dominant risk agent. To anchor the results, we compared these QMRA results to a summary of the literature on recreational water outbreaks with animal-related sources (USEPA, 2009a). The outbreak literature indicates that the pathogen source in the majority of recreational water-related outbreaks remains unknown. However there are several examples of recreational water outbreaks where cattle were the principal source of contamination (Cransberg et al., 1996; Feldman et al., 2002; Ihekweazu et al., 2006). In those outbreaks, *E. coli* O157 was the etiologic agent, which is consistent with the QMRA results. No outbreak reports are available for pig- or chicken-impacted waters.

For all three animal sources, there were combinations of model parameters resulted in predicted risks that are substantially higher than the median risks (refer to Figure 25). At first glance, this observation may appear to suggest that risks from animal-impacted waters may be of greater concern than the median risk values suggest. However, this trend is not specific to agricultural-animal impacted water, and in fact, the same observation may be made about predicted risks from recreational exposure to pathogens in disinfected secondary effluent (Figure 33) (Soller et

al., 2010a, 2010b).[26] These high-risk parameter combinations warrant careful risk management consideration, as they may represent specific environmental conditions under which the risk of illness may be unacceptably high. Moreover, understanding the drivers of these high risk conditions could provide opportunities for meaningful risk reductions. For instance, some pathogens exhibit seasonality or life-cycle dependencies, which could be exploited by targeting the high-prevalence time periods through best management practices.

The relative QMRA compares the estimated risks from recreation in water impacted by agricultural sources of fecal contamination to those associated with human-impacted water. In these analyses, we assume that sufficient agricultural animal-impacted runoff occurs so that the freshwater beach contains geometric mean FIB densities (enterococci and *E. coli*) equivalent to the current RWQC. In essence, this approach considers the relative level of risk from the various fecal sources at a fixed level of FIB. By selecting the current geometric mean RWQC FIB levels as the comparison point, risks in human impacted waters inherently serve as a reference, because the current RWQC were established to provide a known level of public health protection in human-impacted water (i.e., 8 cases of HCGI per 1000 recreation events, or in this risk assessment, an equivalent risk of 30 cases of GI illness per 1000 recreation events).

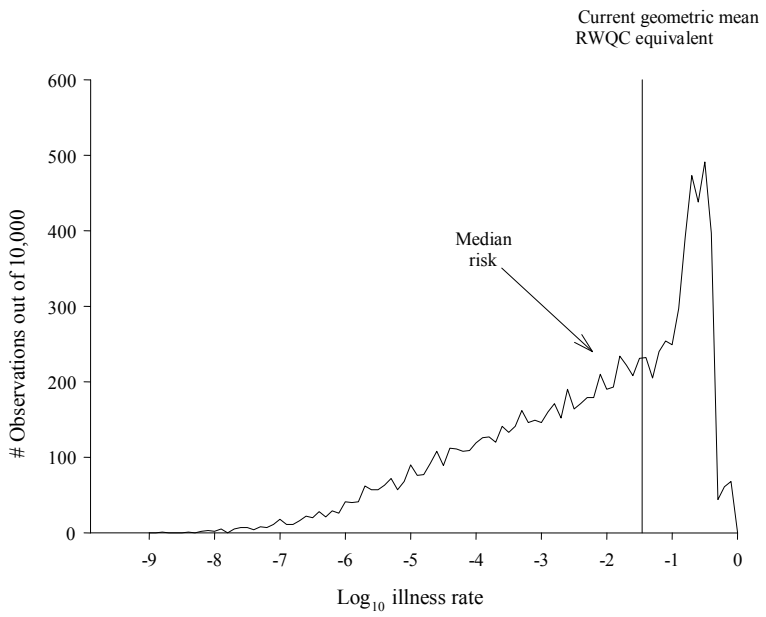

Figure 33. Probability density for illness from recreation in disinfected secondary effluent

[26] For disinfected secondary effluent, the density in the tail of the distribution likely occurs when norovirus densitites in the raw wastewater are high and attenuation through wastewater treatment is low. Refer to Section 3.1.2 and Appendix B for further information.

The results from the relative QMRA reflect both specific conditions from the EPA environmental monitoring study and average values that are substantially less dependent on the FIB levels observed during the EPA studies. In general, we believe the most representative results of the relative QMRA studies are those from Approach 2. The principal findings from the Approach 2 relative QMRA are that at the current geometric mean RWQC:

- the predicted median risk of illness from recreational exposure to the cattle-impacted waterbody is 25- to 150-times lower than the risk of illness associated with human sources of contamination;
- the predicted median risk of illness from recreational exposure to the pig-impacted waterbody is approximately 30-times lower than the risk of illness associated with human sources of contamination; and
- the predicted median risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 20- to 5000-times lower than the risk of illness associated with human sources of contamination.

The results from Approach 1 indicate clearly that FIB levels in fecal material from a specific location can have a strong influence on the relative QMRA. In Approach 1, several combinations of input parameters resulted in output that likely is not widely representative. This set of conditions highlights the need to carefully and appropriately match FIB and pathogen datasets in relative QMRA studies. In Approach 1, using pathogen abundance data in swine slurry from the literature and FIB data from aged slurries resulted in output that diverged from previous and current work.

The relative QMRA presented in this report uses the same methodology and set of peer-reviewed literature that was developed to evaluate risks from exposure to recreational water impacted by direct non-human contamination (Schoen and Ashbolt, 2010; Soller et al., 2010b). This work extends those previous related QMRAs to include land application of fecal material, and FIB and pathogen mobilization during and after rainfall events. These additions generally resulted in decreased risks for cattle, but not for pigs or chicken.

The EPA environmental monitoring program data indicate that environmental conditions underlying the data (nature, age, and level of treatment of the source material and levels of native *E. coli* and enterococci in soil, etc.) can strongly influence FIB densities in the source material (Table 18). We can only speculate on the extent to which the same conditions influence reference pathogen densities; future monitoring and modeling studies could address this knowledge gap.

In comparing our results from this risk assessment (Approach 2) to the results from the direct-contamination scenario (Soller et al., 2010b), the risks associated with indirect contamination are decreased for cattle and essentially unchanged for pig- and chicken-impacted water. This

comparison is analogous to one that can be made for waters impacted by POTW effluent compared to raw or poorly treated sewage. For the indirect contamination scenario, risks in cattle-impacted water appear to be similar to those from pig or chicken-impacted waters. In the case of direct contamination, risks in cattle-impacted waters are higher than those from pig or chicken-impacted waters (Soller et al., 2010b).

Finally, similar to the forward QMRA results, combinations of model parameters for all three animal sources result in predicted risks that are substantially higher than the median risks (Figure 32), highlighting the need for careful risk management of the types of conditions that could lead to these high-risk outcomes.

4.3.2. Considerations and caveats

Like any scientific study, this work has a number of important considerations and conceptual constraints. In this report, we compiled a vast range of disparate data and information to provide an improved understanding about risks that would be difficult or impossible to characterize through an observational study. Risk assessment is used in this way by governmental and regulatory agencies worldwide to protect public health from exposure to a myriad of contaminants through numerous routes of exposure (e.g., air pollution, food protection, drinking water). To facilitate the conduct of this risk assessment, we necessarily made several simplifying, health-protective assumptions to limit the scope of the assessment. In this regard, several of the most important considerations and conceptual constraints are discussed below.

<u>Exposure scenario is limited</u>. The analyses only considered one exposure scenario, and which was intentionally limited. Several important attributes of the exposure scenario might make it difficult to extend the results from these analyses to a diverse range of recreational sites and situations. The chain of events leading to human exposure to agricultural animal-derived pathogens from recreational water is complex, and numerous processes can impact the predicted risks. For example, manure handling practices before land application can greatly influence FIB and pathogen levels in the land-applied material. This risk assessment evaluated the most common minimum manure handling processes used in the United States; however, the pig slurry FIB data from the EPA environmental monitoring program indicated the potential for substantial variability. Similarly, BMPs (e.g., post-land application, pre-runoff) could greatly change the abundance of FIB and pathogens in runoff, and dilution of the runoff water with uncontaminated water would change the relative abundance of FIB and pathogens in recreational water.

Given the myriad exposure-related conditions that could reasonably occur in agricultural animal-impacted water and the substantial variability related to exposure, we chose a relatively simple and health protective exposure scenario for this analysis. If a more comprehensive exposure model was implemented that included manure treatment, attenuation of pathogens and FIB prior to and after runoff, and dilution of runoff water, the resulting forward QMRA would certainly yield lower risk estimates. For example, our exposure scenario specifies recreation in undiluted

runoff that is impacted by land applied manure at current U.S. agronomic rates. Risks would certainly be lower in surface waters that are less impacted (due to less loading, dilution, or attenuation due to die-off between runoff and recreation).

On the other hand, the impact of a more comprehensive model on the relative QMRA is less certain. Pathogens and FIB are attenuated by the same processes, though by different amounts and at different rates. This differential attenuation could produce fewer FIB than pathogens and result in a higher risk associated with a given FIB level than if no treatment or best management practices were undertaken. This possibility does not indicate that treatment or BMPs are undesirable—just that the interpretation of FIB must consider all relevant processes in the exposure profile.

We did not account for super-shedding exposure scenarios in this analysis (Arthur et al., 2009; Chase-Topping et al., 2008). Risks to human health would be greater than those reported here if super-shedding increased levels of pathogens in feces compared to a relatively constant level of indicator data (see Annex 2). For example calves shed high levels of *Cryptosporidium* during defined periods (Bryan et al., 2009; Chase-Topping et al., 2008). The general approach in this QMRA could be used to evaluate specific conditions that could lead to higher risks such as defined animal populations, soil types, rainfall patterns, dilution of receiving water, or the presence of super-shedding animals.

<u>FIB and pathogen mobilization</u>. FIB and pathogen mobilization was modeled on a simulated intense rain event in a single location (Georgia, USA). The experimental work produced the first reported estimates for mobilization of both *Campylobacter* and *Salmonella* and valuable data for assessing the runoff of the other organisms, but the extent to which the mobilization results apply to other types of rain events at this location is not known. Furthermore, because soil characteristics vary substantially across the United States, the mobilizations are likely specific to the soil at the study location. FIB in soil can strongly influence mobilization rates, depending on the relative levels of the FIB in the applied source material and FIB already present in the soil. For example, during the EPA environmental monitoring study, the densities of enterococci and *E. coli* in the pig slurry and chicken litter were relatively low (compared to levels reported in the literature), but some levels of these FIB in the runoff were greater than the levels in the source material. The predicted mobilization fraction of the FIB, therefore, was reported as greater than one ($\log_{10}$ value of 0), indicating that the FIB in the runoff water originated from the soil rather than the source material. These observations have clear implications for interpreting FIB data that are soil-based as compared to fecal source-based for agricultural animal-impacted water.

Furthermore, the prevalence of infection and the associated implications on pathogen abundance in land-applied fecal source material is undoubtedly more complicated than this model addresses. Because a given animal is either infected or not at any point in time, the variability in fecal source abundance could be greater than this analysis suggests because we used average abundances and assumed that at least one animal contributing to land-applied manure is shedding

at any given time. A more rigorous characterization of this variability would mostly result in lower risks, but would result in greater risk when infection is present. Identifying the frequency and conditions leading to those high-risk periods could present opportunities for risk reductions.

Other zoonotic pathogens. Animal-impacted water could contain pathogens of public health concern that were not evaluated and that might not fit into the FIB paradigm that is used to regulate recreational water quality in the United States. We selected our reference pathogens because they comprise an overwhelming proportion of all known pathogens that cause non-foodborne illness in the United States (Mead et al., 1999), are representative of the fate and transport of other pathogens of potential concern from a waterborne route of exposure (Ferguson et al., 2009), are present in human and animal waste and recreational water (USEPA, 2009b), possess the potential for extra-enteric survival, and have corresponding dose-response relationships in the peer-reviewed literature (USEPA, 2010). The scientific understanding of zoonotic pathogens is continually evolving, and based on evolving information, recreation in agricultural animal-impacted water could cause illnesses that would otherwise be considered rare. For example, Hepatitis E virus, *Listeria monocytogenes*, or *Leptospira* are pathogens that are present in agricultural animal waste but which are thought to cause few illnesses from recreational water exposure.

- Hepatitis E is a virus that can cause serious liver disease. Although, Hepatitis E is uncommon in the United States,[27] it is associated with livestock operations (Banks et al., 2004; Legrand-Abravanel et al., 2009; Rutjes et al., 2009; Sinclair et al., 2009; Takahashi et al., 2009). Further, the presence of Hepatitis E in pigs (Feagins et al., 2007; Meng et al., 1999; Smith, 2001) and an emerging virus related to Hepatitis E in chickens (Haqshenas et al., 2001) are of particular note.
- *Listeria monocytogenes* can cause a serious disease mainly in elderly persons, pregnant women, newborns, and immunocompromised adults.[28] *Listeria monocytogenes* is found in soil and water, and animals can carry the bacterium without appearing ill. In the United States, an estimated 2500 persons become seriously ill with listeriosis each year (Mead et al., 1999).
- *Leptospira* occurs worldwide and is an important zoonosis, in part due to its prolonged survival in water (Levett, 2001; Meites et al., 2004). Zoonotic reservoirs include livestock (pigs and cattle), domestic pets (dogs), and wildlife (Levett, 2001). The source of *Leptospira* infection in humans usually results from dermal contact with the urine of an infected animal.

Therefore, if exposure to animal-impacted water was widespread, illnesses from non-reference zoonotic pathogens could occur at higher rates than would otherwise be expected.

[27] http://www.cdc.gov/hepatitis/HEV/index.htm

[28] http://www.cdc.gov/nczved/divisions/dfbmd/diseases/listeriosis/

Low probability events. In assessing the relative risks associated with fecal pollution sources, we used median and other percentile values for describing risks. This choice is appropriate for the purpose, but does not describe the risks associated with extreme, rare, or low probability events. Although risks associated such types of extreme events are difficult to characterize, they are important in the overall risk management context.

4.4. Conclusions

The risk assessment described in this report addresses two questions: (1) What is the risk of illness associated with recreation at a freshwater beach impacted by agricultural animal (cattle, swine, and chicken) sources of fecal contamination?; and (2) How do those risks compare to risks associated with freshwater beaches impacted by human sources?

For our exposure scenario (runoff-induced pathogen mobilization from land-applied fecal material) the median risk of illness from recreational exposure to the cattle-impacted waterbody is equivalent to the risk of illness associated with the current (1986) geometric mean RWQC; the median risk of illness from recreational exposure to the pig-impacted waterbody is approximately four-times lower than the risk of illness associated with the current geometric mean RWQC; and the median risk of illness from recreational exposure to the chicken-impacted waterbody is approximately 300-times lower than the risk of illness associated with the current geometric mean RWQC.

In comparing animal-impacted water to human-impacted water, the most representative results come from literature-based FIB and pathogen abundances combined with mobilizations from the EPA environmental monitoring program. These results indicate that at the current geometric mean RWQC, the predicted median risk of illness from recreational exposure to each of the animal-impacted water are at least 20 to 30-times lower than risk of illness associated with human-impacted water. These risks are similar to or lower than those associated with direct agricultural animal contamination.

Risk assessment is widely used by governmental and regulatory agencies worldwide to protect public health from exposure to a myriad of contaminants through numerous routes of exposure. Air pollution regulations, protection of the food supply chain, and drinking water regulations are large-scale examples that illustrate the effective use of risk assessment methodologies within a environmental regulatory context. To date, epidemiology studies have been the primary tool used to characterize human health risks from exposure to recreational water. Those epidemiology studies have generally focused on waters impacted by wastewater effluent (i.e., human sewage-impacted waters). Substantial progress has been made in improving the quality of wastewater effluent in the United States in recent decades. Now more attention is being paid to other sources of fecal contamination. In fact, non-point fecal contamination is one of the most common reasons that waterbodies in the United States are classified as impaired with respect to their use as recreational waters. Epidemiology studies are not likely to be effective in

characterizing risks in many waters of this type due to technical, logistical and/or financial constraints. As illustrated in this report, QMRA is a viable and valuable complement to epidemiology studies for waters where epidemiology studies are not available, do not apply, or are impractical. Finally, the data, results, and caveats of this study provide context for an improved understanding of recreational risks in diverse waterbodies, and could help to facilitate implementation of upcoming new or revised RWQC.

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APPENDICES

Appendix A. Selected Peer-Reviewed QMRAs for Recreational Water Exposure

Table 21. Synopsis of selected peer-reviewed QMRAs of recreational water exposure

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Ashbolt and Bruno (2003)	Risk of GI illness and respiratory illnesses associated with recreational water	Enteric viruses Adenovirus	Ratio of pathogens to enterococci assumed relatively constant – data on enterococci collected during the study and reported as number of samples meeting a compliance criterion	50 mL fixed volume assumed	Exponential dose-response model with r = 1 for enteric viruses. Adenovirus dose-response model (r = 0.417) for respiratory illness associated viruses	Not considered	Not reported
Gerba et al. (1996)	Risk of rotavirus infection from recreational and drinking water exposures	Rotavirus	Drinking water concentrations estimates assumed to be 0.004 PFU/L and 100 PFU/L, based on review of the occurrence of rotavirus in drinking water and surface water and assuming 99.99% removal in treatment. Surface water concentrations estimated to be 0.24/L and 29/L (the occurrence range).	Ingested volumes used were 100 mL for recreational exposure, 2 L for child and adult drinking water exposure, and 4 L for elderly drinking water exposure.	Beta-Poisson dose-response model (α = 0.26, N_{50} = 5.62) used for risk of infection. Risk of clinical illness assumed 0.5× risk of infection. The fraction of illnesses progressing to mortality assumed 0.1% for the general population and 1.0% for the elderly.	Secondary transmission rates discussed, but details on calculations not provided	Risks corresponding to high and low concentrations in drinking water and recreational water presented
Jolis et al. (1999)	Risk of cryptosporidiosis associated with exposure at parks and golf courses irrigated with tertiary reclaimed water.	*Cryptosporidium parvum*	Concentration of *Cryptosporidium parvum* in tertiary effluent set to the arithmetic mean of six samples (variability not reported or considered). Concentration in treated secondary effluent taken as 2 logs less than the mean of three samples of secondary effluent.	Assumed golfer and park user ingested volume of 1 mL per outing	Exponential *Cryptosporidium parvum* model (r = 0.00467, 95% confidence interval <0.00195, 0.0962>, no information on distributional form assumed for r) Ratio of illness to infection set at 0.5.	Not considered	Not reported. Authors critically assessed findings in their study and characterized the study as preliminary.

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Julian et al. (2009)	Risk of rotavirus infection from multiple exposure routes for a child 6 years of age or younger; exposure routes were fomite-to-mouth, fomite-to-hand, and hand-to-mouth	Rotavirus	Virus density on fomite assumed uniformly distributed (0.001–10 virus/cm^2). Inactivation rate on fomite and hands assumed normally distributed (different mean and standard deviation for fomite and hand distributions)	Transfer efficiency from fomite to mouth and hand-to-mouth assumed normally distributed with a mean of 41% and a SD of 25%. Transfer efficiency from fomite to hand assumed normally distributed with a mean of 36% and SD deviation of 26%.	Beta-Poisson dose-response model (α = 0.26, N_{50} = 5.62) used for risk of infection	Not considered	Model was run with a parameter set to either the 25th or 75th percentile value of its distribution and all other parameters at the median value. Sensitivity to a parameter is assessed based on the ratio of the p_{25} to the p_{75} estimated risks.
Parkin et al. (2003)	Risk of enterovirus infection to sensitive population via recreation in water receiving WWTP effluent; study was a data collection and problem formulation effort	Coxsakievirus A and B Echoviruses Human enteroviruses Polioviruses	Anecdotal data on virus occurrence in swimming water reported, but no characterizations of temporal variation in viruses found in a literature search	Not considered	Epidemiology studies indicate that children at greater risk than adults for enterovirus infection; the effects of dose-response and exposure not differentiated; authors noted there are no known dose-response relationships for children	Not considered	Not relevant
Roberts et al. (2007)	Risk of cryptosporidiosis associated with fishing in an urbanized stream reach	*Cryptosporidium*	Number of oocysts ingested per month via hand-to-mouth transmission or in consumption of fish was assumed Poisson-distributed; distribution parameters estimated using occurrence of oocysts in hand-washings and on fish	Not calculated separately from pathogen concentration estimate	Exponential (r = 0.00419). The dose-response parameter was treated as a random variable, although the distributional form used is not reported.	Not considered	Sensitivity analysis results reported, but details of the method not provided

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Schijven and de Roda Husman (2006)	Risk of infection for occupational and sport divers in fresh and marine water	*Campylobacter jejuni* Enteroviruses	Both pathogens assumed log-normally distributed, with the reported lowest and highest values (in the literature) assumed to be the 99% confidence interval values.	Ingested water depended on diver status (recreational vs. occupational), setting (marine vs. fresh vs. swimming pool) and on equipment used, especially mask type. Reported ingested volumes ranged from 0–190 mL. Number of dives per year drawn from an empirical distribution.	Hypergeometric (exact beta Poisson) model with $\alpha = 0.145$ and $\beta = 8.007$ was used for dose-response for *C. jejuni* The rotavirus hypergeometric model with $\alpha = 0.167$ and $\beta = 0.191$ used for dose-response for enteroviruses; note that this is an extremely conservative assumption	Not considered	Annual risk of infection differed significantly with diver status (occupational vs. recreational), equipment used, and setting
Schoen and Ashbolt (2010)	Risk of GI illness from swimming in human- and gull-impacted surface water	*Salmonella* *Campylobacter* *Giardia* *Cryptosporidium* Norovirus	Study introduced the relative risks QMRA approach in which pathogen densities are drawn from distributions based on reports for specific fecal pollution sources and referenced to indicator levels for the same fecal pollution sources. Pathogens in gull wastes were *Campylobacter* and *Salmonella*. All pathogens assumed present in human sewage.	Relative doses of pathogens and indicators are used	Norovirus: Poisson-stopped logarithmic series *Salmonella*: Gompertz model for serotype Bareilly *Campylobacter*: two alternative parameterizations of the exact beta-Poisson model *Cryptosporidium*: exponential model *Giardia*: exponential model	Not considered	Stochastic framework used

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Signor and Ashbolt (2006)	Human exposure to pathogens via drinking water when routine pathogen monitoring is conducted	*Cryptosporidium* spp.	During base flow conditions, untreated water *Cryptosporidium* density is log-normally distributed with mean and standard deviation of log-transformed densities equal to 3.11 and 1.28, respectively. During event (rainfall) conditions, untreated water *Cryptosporidium* density is log-normally distributed with mean and standard deviation of log-transformed densities equal to 5.27 and 0.61, respectively.	Ingested (oral) volume log-normally distributed with mean and standard deviation of log-transformed densities equal to -0.046 and 0.535, respectively	Exponential model, r = 0.00419	Not considered	Model sensitivity was assessed via comparison of three sampling scenarios
Soller at al. (2003)	Risk of viral gastroenteritis associated with recreational and non-recreational use of a river downstream of a wastewater treatment plant discharge. Two wastewater treatment scenarios were compared.	Model enteric virus with clinical features of rotavirus	Bacteriophage concentration in raw wastewater assumed uniformly distributed in the range 1×10^4–5×10^4. Removal modeled for treatment and removal and mixing processes modeled for discharged effluent. The ratio of model enteric virus concentration to bacteriophage concentration assumed log-uniform distributed in the range 0.001–1.0.	Exposure factor was a random variable chosen from uniform distributions whose ranges were selected based on observed recreational use by month and day of the week (weekday v. weekend).	Beta-Poisson (presented in study in modified form) with α assumed uniformly distributed in the range 0.15–0.42 and β in the range 0.3–2.3.	Dynamic population-based model, including individuals infected from activities other than use of river for recreation	Univariate sensitivity analyses for input parameters

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Soller et al. (2006)	Risk of infection during full-body contact recreation in a non-POTW impacted estuarine recreational waterbody	Rotavirus as a representative pathogen	Rotavirus density was based on a model calibrated with empirical coliphage data. The relationship between coliphage density, expected rotavirus density, and fraction of total pathogen load comprised by rotavirus not presented explicitly.	Hourly rate of water ingestion assumed; swimmers were in the water at different times and for different durations	Beta-Poisson (presented in study in modified form) with α assumed uniformly distributed in the range 0.125–0.5 and β in the range 0.21–0.84; probability of symptomatic response range 0.1–0.45	Secondary transmission modeled via a deterministic time-dependent transmission model accounting for the immune status of the population	Sensitivity analyses performed for several variables; variables set to low, medium and high values to determine whether their variation changed the study findings
Soller et al. (2010a)	Risk of GI illness during primary contact with water during recreation	*Salmonella* *Campylobacter* *Giardia* *Cryptosporidium* *E. coli* O157:H7 Norovirus Adenovirus Rotavirus	Study introduced the reverse QMRA approach in which pathogen densities are inferred from QMRA conducted with known sources and illness rates. Pathogen densities relative to each other based on (1) observed relative densities in POTW effluent and (2i) the proportion of U.S. non-food GI illness	Point estimate based on arithmetic mean of log-normal distribution of values reported by Dufour et al. (2006)	Adenovirus: Exponential model, r = 0.4172 Rotavius: beta-Poisson model, α = 0.2531, β = 0.4265 Norovirus: beta-Poisson model, α = 0.04, β = 0.055 *Salmonella*: approximate beta-Poisson model, α = 0.04, β = 2884 *E. coli* O157:H7: approximate beta-Poisson model, α = 0.4, β = 45.9 *Campylobacter*: exact beta-Poisson model, α = 0.024, β = 0.011 *Cryptosporidium*: Exponential model, r = 0.09 *Giardia*: exponential model, r = 0.0199	Not considered	Stochastic framework used and model results validated via comparison of modeled time to illness onset distribution with observed time to illness onset distribution

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Soller et al. (2010b)	Risk of GI illness during primary contact with water during recreation	*Salmonella* *Campylobacter* *Giardia* *Cryptosporidium* *E. coli* O157:H7 Norovirus Adenovirus Rotavirus	Study used the relative risks QMRA approach in which pathogen densities were drawn from distributions based on reports for specific fecal pollution sources and referenced to indicator levels for the same fecal pollution sources	Relative doses of pathogens and indicators used	Adenovirus: Exponential model, r = 0.4172 Rotavius: beta-Poisson model, α = 0.2531, β = 0.4265 Norovirus: beta-Poisson model, α = 0.04, β = 0.055 *Salmonella*: approximate beta-Poisson model, α = 0.04, β = 2884 *E. coli* O157:H7: approximate beta-Poisson model, α = 0.4, β = 45.9 *Campylobacter*: exact beta-Poisson model, α = 0.024, β = 0.011 *Cryptosporidium*: exponential model, r = 0.09 *Giardia*: exponential model, r = 0.0199	Not considered	Stochastic framework used and model results validated via comparison of modeled time to illness onset distribution with observed time to illness onset distribution
Steyn et al. (2004)	Risk of infection via drinking water or water-borne recreation	*Salmonella*	*Salmonella* density determined during monitoring; calculations performed for the GM value (167 CFU/100 mL), the minimum value (36) and the maximum value (883)	For full contact recreation, ingested volume assumed 100 mL	Approximate beta Poisson dose-response, with α = 0.3126 and N_{50} = 23,600	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
van Heerden et al. (2005a)	Risk of human adenovirus infection via drinking water or recreational water exposure	Adenovirus	Adenovirus density assumed Poisson-distributed (in time, not space) with the distribution mean determined from frequency of positive determinations among drinking water and surface water samples. Mean adenovirus densities (in viruses per 100 mL) were 0.0014 and 0.00245 for two drinking water, 0.0546 for a river water, and 0.0097 for water behind a dam.	Drinking water consumption rate fixed at 2 L per capita p er day and recreational water consumption rate fixed at 30 mL per capita per day	Exponential model was used for adenovirus dose-response; the model parameter was not explicitly provided, although based on the citation provided in the study, it can be inferred to be that for inhalation of adenovirus aerosols, r = 0.417	Not considered	Univariate sensitivity analyses conducted to assess the impact of consumption rates, dose-response parameters and recovery rates on risk estimates
Wong et al. (2009)	Risk of enteric virus infection associated with swimming at coastal beaches impacted by POTW discharges	Adenovirus	Experimental distribution for adenovirus occurrence based on Regression on Order Statistics to account for non-detect observations.	100 mL/day	Exponential model, r = 0.417 (based on data for inhalation of adenovirus aerosols)	Not considered	Not reported

Appendix B. Data Summary Reference Pathogens in Livestock and Human Waste

B.1 Reference Pathogens in Livestock Manure

Table 22. Reported *Salmonella* densities in livestock feces and other matrices

Study	Sournce/Media	Serotype	Description	Abundance	Notes
Boes et al. (2005)	Swine manure slurry from 62 herds; Danish farms	Typhimurium	Samples drawn from swine manure slurry and from soil after application of swine manure slurry	Salmonellae detected in all slurry samples. Average *Salmonella* Typhimurium density was 0.2 CFU/g (note – not $\log_{10}$ CFU); maximum density estimated to be 2500 CFU/g for a sub-clinically-infected herd; observed abundance among 112 slurry samples 33% of samples with < 0.1 MPN, 13% of samples between 0.1 and 1 MPN, 28% between 1 and 10 MPN, 12% between 10 and 110 MPN, and 14% > 100 MPN.	Authors proposed a polynomial survival model for *Salmonella* in soil
Byrd (1998)	Fecal material and poultry litter from hatcheries	Typhimurium	Day-old chicks challenged with 100, 10^4 or 10^6 *Salmonella typhimurium* by gavage; litter and cecal contents monitored for 17 days	Pens containing chicks inoculated with 100 Salmonellae: 2.05 to 3.03 $\log_{10}$ CFU/g litter (*n = 10*) Pens containing chicks inoculated with 10^4 *Salmonella*: 2.39 to 4.55 $\log_{10}$ CFU/g litter (*n = 10*) Pens containing chicks inoculated with 10^6 *Salmonella*: 3.65 to 4.42 $\log_{10}$ CFU/g litter (*n = 10*)	Fecal colonization rate and *Salmonella* count in fecal contents varied according to challenge dose; the number of chicks inoculated (5%, 10%, 25%, and 50% of chicks in a pen) did not influence the overall incidence of infection in the pen
Haley et al. (2009)	Stream water	spp.	Water samples from a mixed use (livestock, on-site septic system, small community) watershed sampled for *Salmonella*	Geometric mean of *Salmonella* in water did not vary greatly among sampled sites; the highest and lowest mean densities were 0.746 MPN/100 mL and 0.496 MPN/100 mL	
Hutchison et al. (2004)	Fresh pig manure	spp.	Multiple commercial farms	Geometric mean of 600 CFU/g (*n* = 10); maximum observation of 78,000 CFU/g	Wastes taken from farms throughout Great Britain and results are believed representative of overall prevalence in the region
Hutchison et al. (2004)	Fresh chicken manure	spp.	Multiple commercial farms	Geometric mean of 220 CFU/g (*n* = 12); maximum observation of 22,000 CFU/g	Wastes taken from farms throughout Great Britain and results believed representative of overall prevalence in the region

Study	Sournce/Media	Serotype	Description	Abundance	Notes
Hutchison et al. (2004)	Fresh cattle manure	spp.	Multiple U.K. commercial farms	Geometric mean of 2100 CFU/g (n = 62); maximum observation of 580,000 CFU/g	Wastes taken from farms throughout Great Britain and results believed representative of overall prevalence in the region. *Salmonella* density higher in stored manure than fresh manure

Table 23. Reported *Campylobacter* spp. densities in livestock manure and other matrices

Study	Source/Media	Strain	Description	Abundance	Notes
Blaser et al. (1980)	Human feces	*jejuni*	*C. jejuni* recovered and enumerated from stools from 8 persons with suspected campylobacteriosis; *C. jejuni* identified an all 8 samples	Median: 2.8×10^8 CFU/g. Range: 6×10^6–1×10^9 CFU/g	
Cox et al. (2002)	Chicken feces	spp.	Results are the composite of samples taken from 35 commercial broiler farms; results segregated by age of chicken	Breeders: 2.8–3.9 $\log_{10}$ CFU/g feces Broilers: 3.5–6.5 $\log_{10}$ CFU/g feces	*Campylobacter* less prevalent in broilers (offspring) than breeders, but shedding (colonization) higher in broilers than breeders
Dorner et al. (2004)	Poultry (broiler) feces	spp.	Abundance data from multiple studies were pooled and fit to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β) = (27.78, 0.2558)	
Dorner et al. (2004)	Nursing or weaner pigs	spp.	Abundance data from a single study (Weijtens et al., 1999) fit to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β) = (4.419, 0.6319)	
Dorner et al. (2004)	Sows and gilts	spp.	Abundance data from two studies (Weijtens et al., 1997; Weijtens et al., 1999) fit to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β) = (4.207, 0.8859)	
El-Shibiny et al. (2005)	Poultry	spp.	Estimates based on multiple published studies	10^6–10^9 CFU/g excreta	
Hutchison et al. (2005)	Cattle	spp.	Composite samples of manure from pens collected	320 CFU/g for fresh feces 530 CFU/g for stored feces	
Hutchison et al. (2005)	Swine	spp.	Composite samples of manure from pens collected	310 CFU/g for fresh feces 1600 CFU/g for stored feces	
Hutchison et al. (2005)	Poultry	spp.	Composite samples of manure from pens collected	260 CFU/g for fresh feces 590 CFU/g for stored feces	
Moriarty et al. (2008)	Dairy cattle	spp.	Samples taken from 4 farms considered to span conditions in New Zealand	For all seasons: median 430 CFU/g, range 15–1.8×10^7 CFU/g.	Prevalence of *C. jejuni* and *C. coli* reported, but not related to abundance in manure; *Campylobacter* abundance bi-modally distributed among samples

Study	Source/Media	Strain	Description	Abundance	Notes
Stanley et al. (1998)	Beef cattle feces	spp.	Fresh beef cattle sampled at slaughter	610 MPN/g feces	
Stanley et al. (1998)	Dairy cattle feces	spp.	Fresh dairy cattle manure samples collected in pens of 4 dairy herds in the United Kingdom	Adult cows: 69.9 MPN/g feces (SD 3) Calves: 33,000 MPN/g (SD 170)	Two peak periods (seasonal) of shedding noted
Weijtens et al. (1997)	Sow feces at one week prior to delivery	spp.	Sow feces sampled and bacteria enumerated 1 week prior to delivery	5.0±1.1 $\log_{10}$ CFU/g (farm 1, n = 5) and 3.6±0.4 $\log_{10}$ CFU/g (farm 2, n = 5)	Prevalence data for sows and piglets also collected at 1 week, 4 weeks and 8 weeks post-delivery
Weijtens et al. (1999)	Fattening pig feces from 10 weeks of age to 25 weeks	spp.	For each sampling event, six feces samples were collected per pig; pigs were monitored from birth and housed with 16 pigs each on an experimental farm	At 13 weeks: mean fecal *Campylobacter* density 4.1±0.7 $\log_{10}$ CFU/g (n = 8 pigs, average of 6 fecal samples per sampling event per pig) At 19 weeks: mean fecal *Campylobacter* density 3.3±1.0 $\log_{10}$ CFU/g (n = 8 pigs, average of 6 fecal samples per sampling event per pig) At 25 weeks: mean fecal *Campylobacter* density 2.0±0.1 $\log_{10}$ CFU/g (n = 8 pigs, average of 6 fecal samples per sampling event per pig)	The abundance (and prevalence) of *Campylobacter* varied weekly and between fecal samples on a given sampling event. Several pigs had periods of non-detectable fecal *Campylobacter* between periods of high fecal *Campylobacter* abundance. Abundance was highest shortly after colonization and generally decreased with age.
Whyte et al. (2001)	Poultry feces	spp.	Fecal samples from sacrificed chickens from 10 Irish farms enumerated for *Campylobacter*; though samples were analyzed before, during, and after transport to a processing facility, the only values quoted are for before transport; studies conducted in Ireland	6.11±0.37 $\log_{10}$ CFU/g feces for 5 farms and 6.61±0.38 $\log_{10}$ CFU/g feces for 5 additional farms	

Table 24. Reported *Cryptosporidium* spp. densities in livestock manure and other matrices

Study	Source/Media	Species	Description	Abundance	Notes
Atwill et al. (2003)	Beef cow (>24 months) feces, California	*C. parvum*	Manure samples from preparturient and postparturient beef cows on three California farms sampled and *C. parvum* was enumerated via a sensitive method	For samples positive for *C. parvum*, the arithmetic mean oocyst density was 3.38 oocysts/g feces and SD 2.64 oocysts/g feces	No significant difference in prevalence or shedding of *C. parvum* between preparturient and postparturient cows
Atwill et al. (2006)	Beef cattle feces from feedlot	*C. parvum*	Manure from 22 feedlots in 7 western and central states sampled in the period 8/2000 to 1/2002	Among samples positive for C. parvum, the geometric mean was 447 oocysts/g manure (range 203–7702 oocysts/g)	*C. parvum* detected in only 0.2% of samples; abundance data fit with a negative binomial distribution
Berry et al. (2007)	Beef cattle feces from feedlot	spp.	Manure from beef feedlots was sampled (composite samples) each 4 weeks during a 26 month study	Average: 14 oocysts/g Range: 0.5 oocysts/g manure to 1510 oocysts/g manure	*Cryptosporidium* spp. identified in 58% of composite manure samples collected over a 26-month study
Heitman et al. (2002)	Manure from dairy cattle	*C. parvum*	Manure from two dairy operations collected from pasture	Mean densities in manure from the two farms were 18.8 and 490 oocysts/g (considering only positive samples)	*C. muris* not detected in any fecal samples
Hutchison et al. (2004)	Cattle manure	*C. parvum*	Manure samples collected from throughout Great Britain	For fresh manure GM density 19 oocysts/g (n = 44) Maximum density 3500 For stored manure, GM density 10 oocysts/g (n = 12) Maximum density 480	
Hutchison et al. (2005)	Fresh and stored pig manure	*C. parvum*	Composite samples from fresh and stored manure were collected between April 2000 and December 2002	GM densities 58 for fresh manure, and 33 for stored manure	
Hutchison et al. (2004)	Fresh and stored chicken manure	*C. parvum*	Composite samples from fresh and stored manure collected between April 2000 and December 2002	No *C. parvum* identified in any chicken samples	
Moriarty et al. (2008)	Dairy cattle manure	Spp.	Samples taken from freshly-deposited manure	Among positive samples, *Cryptosporidium* density ranged from 1–25 oocysts/g feces.	Prevalence low in the herds studied

Study	Source/Media	Species	Description	Abundance	Notes
Sturdee et al. (2003)	Cattle feces	*C. parvum*	Rectal and recently-deposited fecal samples collected at a farm with beef and dairy cattle and calf rearing operations	Description: Mean (oocysts/g) Bull beef: 1371 Dairy cow: 1778 Calf, home-bred: 107,025 Calf, bought-in: 24,448	Highest observed density was 280,000 oocysts/g feces for a home-bred calf
Wade et al. (2000)	Dairy cattle feces	*C. muris*	Fecal samples collected rectally from dairy cattle at 109 farms in southeastern New York; data were stratified by cattle age	Mean: 24,413 oocysts/g feces Range: 1 to 100,000 oocysts/g feces	*C. muris* recovered from animals with a wide range of ages
Wade et al. (2000)	Dairy cattle feces	*C. parvum*	Fecal samples collected rectally from dairy cattle at 109 farms in southeastern New York	Mean: 21,090 oocysts/g feces Range: 1 to 79,040 oocysts/g feces	*C. parvum* was recovered only from calves <30 days of age

Table 25. Reported *Giardia* spp. densities in livestock manure and other matrices

Study	Source/Media	Species	Description	Abundance	Notes
Hutchison et al. (2004)	Cattle farmyard manures and slurries	*G. intestinalis*	Resultsfor samples collected throughout Great Britain	Geometric mean and maximum cyst densities 10 and 5000 cysts/g, respectively	
Hutchison et al. (2004)	Swine farmyard manures and slurries	*G. intestinalis*	Results for samples collected throughout Great Britain	Geometric mean and maximum cyst densities 68 and 160,000 cysts/g, respectively	
Ralston et al. (2003)	Range beef calf and dam manures	spp.	Fecal samples collected from calves and dams from range operations in Canada	*Giardia* abundance in feces varied with animal age group. Density ranged from 0 at 1 week of age to a maximum of 2230 cysts/g (range 0–574,933 cysts/g of feces) of feces at 5 weeks of age. The geometric mean decreased after week 5 to a low of 2 cysts/g at 25–27 weeks of age	
Heitman et al. (2002)	Dairy cattle manure	spp.	Fecal samples collected from farms in Canada	Mean cyst range 1.5–29.9 cysts/g	
Heitman et al. (2002)	Pig manure	spp.	Fecal samples collected from farms in Canada	Mean cyst density 16.1 cysts/g	
Wade et al. (2000)	Dairy cattle manure	spp.	Fecal samples collected from 212 farms in southeastern New York	1–85,217 cysts, mean of 3039 cysts/g feces	

Table 26. Reported *E. coli* O157:H7 densities in livestock manure and other matrices

Study	Animal	Source/Media	Description	Abundance	Notes
Cornick and Helgerson (2004)	Swine	Feces	3-month old pigs challenged with graded doses of *E. coli* O157:H7; pigs housed indoors on concrete floors or decks; experiments were conducted in Iowa	Shortly after inoculation fecal *E. coli* density ranged between 10^3 and 10^7 CFU/g. Two weeks after inoculation, fecal *E. coli* O157:H7 density ranged from 50 to 1000 CFU/g. Two months after inoculation, fecal *E. coli* density ranged from non-detect to 10^4 CFU/g.	Swine infectious dose of *E. coli* O157:H7 is higher than that of cattle, resulting in lower incidence of transmission of *E. coli* O157:H7 between pigs than between cattle. Shedding duration was dose-dependent, with shedding lasting at least 2 weeks for all challenged animals and for >2 months for some animals.
Hutchison et al. (2004)	Swine	Manure	Samples were collected from multiple commercial farms in the UK	Geometric mean of 3900 CFU *E. coli* O157/g (n = 15). Highest observed density was 750,000 CFU *E. coli* O157/g	
Kudva et al. (1998)	Sheep	Manure pit	Composite samples (from manure pits receiving waste from multiple animals) collected and enumerated for *E. coli* O157:H7; experiments were conducted in Idaho	1.15×10^8 CFU/g feces from a composite sample	Prior to shedding, sheep experimentally inoculated with *E. coli* O157:H7; some of the animals contributing to the manure pit were not infected.
Kudva et al. (1998)	Cattle	Manure	Composite samples (from manure pits receiving waste from multiple animals) were collected and enumerated for *E. coli* O157:H7; experiments conducted in Idaho	Two samples yielded 2.04×10^7 CFU/g feces and 4.35×10^8 CFU/g feces	Prior to shedding, cattle experimentally infected with *E. coli* O157:H7; some of the animals contributing to the manure pit not infected
Kudva et al. (1998)	Cattle	Manure slurry	Untreated slurries and treated slurries (the retentate post-storage and separation) were sampled and enumerated for *E. coli* O157:H7; experiments conducted in Idaho	Two samples of untreated slurry yielded 1.02×10^6 CFU/mL and 2.36×10^6 CFU/mL A single sample of treated slurry yielded 2.35×10^6 CFU/mL	Prior to shedding, cattle were experimentally infected with *E. coli* O157:H7; some of the animals contributing to the manure pit not infected

B.2 Reference Pathogens in Treated Sewage

Table 27. Reported rotavirus densities in treated sewage

Setting	Range	Study
Activated sludge effluent	0–1500 /L (average 740)	Bates et al. (1984)
Secondary effluent	1–21 fluorescent foci/L (GM = 9.8)	Hejkal et al. (1984)
Unchlorinated secondary effluent	48–3228 (average 1012)/L	Rao et al. (1987)
Chlorinated secondary effluent	0–32 (average 9.6)/L	Rao et al. (1987)
Secondary sewage effluent	7.5–374 (GM = 41)/L	Smith and Gerba (1982)

Table 28. Reported adenovirus densities in treated sewage

Setting	Range	Study
Secondary effluent	594–9030 genome copies/L	Bofill-Mas et al. (2006)
Treated wastewater	2400 genome copies/mL (relatively stable with season)	Carducci et al. (2008)
Secondary effluent	1×10^3–4×10^4 genome copies/L	Fong et al. (2010)
Secondary effluent	ND–54000 PCR detection units/L (mean = 390)	Haramoto et al. (2007)
Unchlorinated secondary effluent	0–600 infectious units(IU)/L (GM=250)	Irving and Smith (1981)
Secondary effluent	6.1×10^5–1.4×10^6 viral genome copies/L (0 genome copies in chlorinated secondary effluent)	He and Jiang (2005)
Chlorinated secondary effluent	0–1150 IU/L (GM = 300)	Irving and Smith (1981)
Chlorinated secondary effluent, multiple plants	Mean of 7000 reverse transcription-(RT-PCR) units/L	Katayama et al. (2008)
WWTP effluent	0–4000 MPN/L (estimated from results presented graphically)	Sedmak et al. (2005)
Lake Michigan water	7–3800 viral particles/L	Xagoraraki et al. (2007)
Secondary effluent	ND–2.5 MPN/L	MWRDGC (2008)

Table 29. Reported norovirus densities in treated sewage

Setting	Range	Study
Treated wastewater	ND–0.64 copies/mL for genotype I; below dectection–2.6 copies/mL for genotype 2	Haramoto et al. (2006)
Chlorinated secondary effluent, multiple plants	47–2900 RT-PCR units/L	Katayama et al. (2008)
Treated wastewater	2.2–3.0 logs of removal for secondary treatment	Haramoto et al. (2006)
Treated sewage	0–1,650,000 DNA copies/L	Laverick et al. (2004)
Treated sewage	896–7499 PCR-detectable units/L	Lodder and de Roda Husman (Lodder and de Roda Husman, 2005)
Treated sewage and river water samples	1.8×10^4–9.7×10^7 genetic equivalents/L	Pusch et al. (2005)

Table 30. Reported *Salmonella* densities in treated sewage

Setting	Range	Study
Chlorinated secondary effluent	7.5×10^5–8.5×10^6 (viable only) MPN	Desmont et al. (1990)
Treated sewage disinfected with peracetic acid	30 CFU/100 mL	Jiménez-Cisneros et al. (2001)
WWTP effluent	43–460 MPN/100 mL	Koivunen et al. (2001)
Treated wastewater	ND–9 MPN/L	Langeland (1982)
Secondary effluent	3–573 MPN/L (mean 110)	Lemarchand and Lebaron (2003)
Treated wastewater	0–60 MPN	Teltsch et al. (1980)

Table 31. Reported *Campylobacter* spp. densities in treated sewage

Setting	Range	Study
WWTP effluent	262–79,000 organisms/100 mL (paper reviews data from other studies and enumeration technique is not stated)	Jones (2001)
WWTP effluent	ND–3000 MPN/100 mL (estimated based on graphical data)	Koenraad et al. (1994)
WWTP secondary effluent	0–9MPN/100 mL	Stampi et al. (1993)
WWTP disinfected secondary effluent	0	Stampi et al. (1993)
Receiving water for WWTP effluent	ND–0,500 CFU/100 mL	Vereen et al. (2007)

Table 32. Reported *Cryptosporidium* spp. densities in treated sewage

Setting	Range	Study
Tertiary effluent	Mean 37 oocysts/L (SD = 9)	Bonadonna et al. (2001)
Treated wastewater	<10–60 oocysts/L	Bukhari et al. (1997)
Teritary effluent	Mean = 0.21 oocysts/L (SD = 0.06)	Carraro et al. (2000)
Secondary effluent	2–390 oocysts	Castro-Hermida et al. (2008)
Secondary effluent	4–8 oocysts/L	Cheng et al. (2009)
Secondary effluent	0.03–9.6 oocysts/L	Ferguson et al. (2009)
Secondary effluent	ND–209 oocysts/L (mean = 0.91)	Lemarchand and Lebaron (2003)
Secondary effluent (multi-region study)	< 0.1 to 40.8 oocysts/L	McCuin and Clancy (2006)
Treated wastewater	1–120 oocysts/L (GM = 4)	Payment et al. (2001)
Secondary effluent	ND–343 oocysts/L	Robertson et al. (2000)
Secondary effluent	100–44,500 oocysts/L	Robertson et al. (2006)
Tertiary effluent	Mean of 0.0003 oocysts/L	Rose et al. (2001)
Treated wastewater	0.06–1.15 oocysts/L	Suwa and Suzuki (2001)
Treated wastewater	8.3–8.05 oocysts/L (n = 3)	Zuckerman et al. (1997)

Table 33. Reported *Giardia* spp. densities in treated sewage

Setting	Range	Study
Secondary effluent from 7 wastewater treatment plants in England	<10–720 cysts/L	Bukhari et al. (1997)
Secondary effluent from a large Italian WWTP	0.77–2.4 cysts/L	Carraro et al. (2000)
Non-disinfected secondary effluent numerous Spanish WWTPs	2–6000 cysts/L	Castro-Hermida et al. (2008)
Settled non-disinfected secondary effluent from 4 plants in Ireland	0–3 cysts/L (mean densities from 4 plants)	Cheng et al. (2009)
Effluent from a large Canadian WWTP employing phyisco-chemical treatment	2–898 cysts/L	Payment et al. (2001)
Effluent from multiple Norwegian WWTPs	100–51,333 cysts/L	Robertson et al. (2006)
Chlorinated secondary effluent from multiple plants in the United States	$0.1–1.4\times10^2$ cysts/L (mean = 12.8 cysts/L)	Rose et al. (2004))
Combined data for raw sewage and WWTP effluent for Israeli plants	0–300 cysts/L	Zuckerman et al. (1997)

Table 34. Reported *E. coli* O157:H7 densities in treated sewage

Setting	Range	Study
Influent, primary effluent, and secondary effluent (PCR of EHEC toxicity factor)	0–1 (volume tested not available, only 1 sample [influent] had one PCR positive signal)	Grant et al. (1996)
Cow waste lagoon water (PCR of 3 EHEC toxicity factors)	68–2.3×104 MPN/100 mL	Chern et al. (2004)
Municipal sewage treatment plant serving, 400,000 (stx_2-carrying bacteria)	1.6(±0.3) log(MPN + 1)/mL	García-Aljaro et al. (2004)
Municipal sewage treatment plant serving 5000 (stx_2-carrying bacteria)	2(±0.4) log(MPN + 1)/mL	García-Aljaro et al. (2004)
Municipal sewage treatment plant serving1,400,000 (stx_2-carrying bacteria)	1.9(±0.4) log(MPN + 1)/mL	García-Aljaro et al. (2004)
Municipal sewage treatment plant serving 1500 (stx_2-carrying bacteria)	2.3(±0.2) log(MPN + 1)/mL	García-Aljaro et al. (2004)
Municipal sewage treatment plant serving 1500 (stx_2-carrying bacteria)	1.2(±0.2) log(MPN + 1)/mL	García-Aljaro et al. (2004)
Raw sewage (stx_2-carrying bacteria)	2.6 log(MPN + 1)/mL	García-Aljaro et al. (2004)
Secondary effluent and tertiary effluent (stx_2-carrying bacteria)	Below detection limit	García-Aljaro et al. (2004)
Human wastewater (*E. coli* O157)	10 to 100 CFU/100 mL	García-Aljaro et al. (2004)

Appendix C. Shorebirds and Stormwater Reference Pathogen Literature Review

Although not modeled in this QMRA study, shorebirds and stormwater are other non-point sources of fecal pollution posing risks to humans. In this appendix, the routes by which fecal pollution from these sources reaches recreation sites are described and the hazards posed by those fecal pollution sources are summarized.

C.1 Overview of Pathogen and FIB Loads Attributable to Shorebirds

Pathogen and indicator loads attributable to waterfowl can reach recreational water via multiple routes and in significant densities. Routes by which waterfowl fecal indicators and pathogens may reach the waters at recreational sites include the following (Figure 34):

- direct deposition as feces into the water column;
- direct deposition via mechanical transfer (e.g., carried to receiving water on the legs of birds wading in sewage or sewage-impacted water) into the water column;
- resuspension of deposited organisms from sediment or suspension of organisms growing in sediment;
- runoff of organisms (either deposited or progeny of deposited organisms) from soil, vegetation or impervious areas near the recreation area; and
- advection of bird-origin FIB or pathogens from stocks (e.g., in wetlands hydraulically connected to recreation site waters during high tides, as noted by He et al., 2007) when the stocks become hydraulically connected to the recreational water during tides or flooding.

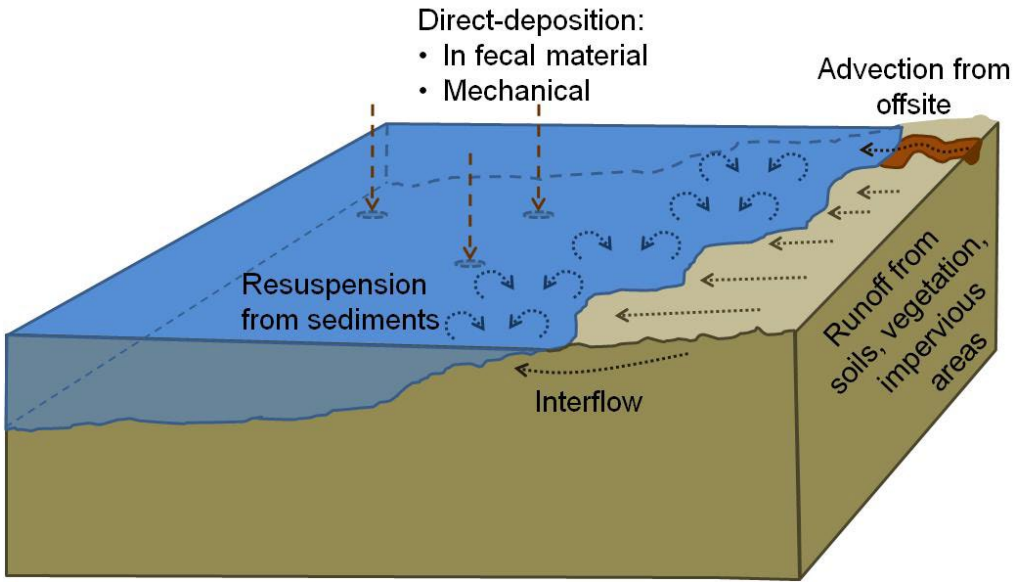

Figure 34. Routes by which bird-origin FIB and pathogens reach recreation sites

The densities of FIB and pathogens in bird feces depend upon whether or not birds are infected (prevalence) and the abundance of the FIB and pathogens in the feces. Assuming that the contamination is reasonably fresh, the ratio of pathogen to FIB densities in recreational water primarily impacted by birds is roughly the same as that in the bird feces. This assumption does not account for temporal variations in the FIB to pathogen ratio that might arise from

- growth of FIB (but not pathogens with the known exception of *E. coli* O157, and the possible exceptions of *Salmonella* or *Campylobacter*, though no reports of growth of the latter two pathogens were identified during preparation of this report) in sands and sediment;
- different die-off rates for specific pathogens and FIB; and
- differences in FIB to pathogen ratio among the rainfall-driven loads (advection, runoff) and loads that are relatively steady (direct deposition and resuspension).

In general, these effects are relatively minor compared to the impact of loading rates of pathogens and FIB.

C.2 *Cryptosporidium* and *Giardia* in Shorebird Feces

To identify the pathogens found in bird feces, EPA conducted an initial literature review. The results of that review indicate that protozoan and bacterial reference pathogens have been isolated from birds frequently (e.g., see Hubálek, 2004) and viral reference pathogens have not been reported in bird feces as summarized below.

Many waterfowl are known to carry *Cryptosporidium* and *Giardia*. Graczyk et al. (2008) reviewed the open literature and reported numerous avian species known to harbor human-infectious *Cryptosporidium* and *Giardia*. Theyreported the density of the oocysts and cysts in some bird feces (Table 35) (based on Table 1 in Graczyk et al., 2008). Clearly, *Cryptosporidium* and *Giardia* including species implicated in human infections, are prevalent and abundant in gulls (*Larus* sp.), ducks (*Anas* sp.), Canadian geese (*Branta canadensis*), and other bird species known to be prevalent near recreational water sites.

Table 35. Avian species associated with *Cryptosporidium* and *Giardia* (SOURCE: adapted from Graczyk et al., 2008)

Pathogen Species	Avian Species	Comments	Reference
Cryptosporidium spp.	*Larus* spp.	5% of fecal and 22% of cloacal lavage samples positive; 64% and 83% of oocysts, respectively, were viable	Smith et al. (1993)
Cryptosporidium spp.	*Anas discour* *A. cerca carolinensis* *A. platyrhynchos* *A. americana* *Lophodytes cucullanus* *Mergus merganser*	Migratory ducks; 49% of birds positive; PCR did not confirm *C. parvum*; oocyst concentration range 0–2182/g feces; mean 47.53±270 oocysts/g	Kuhn et al. (2002)
Cryptosporidium spp.	*Branta canadensis*	Residential and migratory geese; 81 and 90% of fecal samples from collection sites positive	Kassa et al. (2004)
C. parvum *C. hominis*	*B. canadensis*	Residential and migratory geese; 2.4% of samples positive; novel avian genotypes identified; oocysts acquired from local unhygienic sources	Zhou et al. (2004)
C. parvum	*B. canadensis*	Migratory geese; oocysts infectious to neonatal geese; oocyst concentration range 670–6900/g feces; mean 3700 oocysts/g feces	Graczyk et al. (1998)
Giardia spp.	*Anas discour* *A. cerca carolinensis* *A. platyrhynchos* *A. americana* *Lophodytes cucullanus* *Mergus merganser*	Migratory ducks; 49% of birds positive; PCR did not confirm *G. lamblia*; cyst concentration range 0–29,293/g feces; mean 436±3525 oocysts/g	Kuhn et al. (2002)
Giardia spp.	*B. canadensis*	Migratory geese; cyst concentration range 750–7900 cysts/g feces; mean 4100 cysts/g feces	Graczyk et al. (1998)

C.3 Pathogenic Bacteria in Shorebird Feces

Campylobacter species including the human-infectious *C. jejuni* and *C. coli* have been reported for gulls (Hubálek, 2004; Kinzelman et al., 2008; Quessy and Messier, 1992); crows, magpies and starlings (Ito et al., 1988); and domestic pigeons (Ito et al., 1988; Lillehaug et al., 2005), They are likely common in other bird species. Reported prevalences are as high as 25%, indicating the likelihood that large numbers of birds might be infected simultaneously and that those birds have the potential to generate sufficiently high densities of *Campylobacter* to pose a credible human health hazard.

Salmonella has been documented to occur in many birds in many settings (Alley et al., 2002; Berg and Anderson, 1972; Butterfield et al., 1983; Casanovas et al., 1995; Cornelius, 1969; Cruickshank and Smith, 1949; Duncan et al., 1983; Fenlon, 1981; Fricker, 1984; Girdwood et al., 1985; Kapperud and Rosef, 1983; Karaguzel et al., 1993; Kirk et al., 2002; Kirkpatrick, 1986; Lévesque et al., 1993; Locke et al., 1973; McDonough et al., 1999; Mitchell and Ridgwell, 1971; Palmgren et al., 2006; Quessy and Messier, 1992; Wobeser and Finlayson, 1969). As with Salmonellae from animal operations, the hazard these bird-origin pathogens pose to humans is

related to the serotypes that are present. A brief survey of the literature on bird-borne *Salmonella* is presented below, with the primary intent of demonstrating the carriage of human infectious serotypes of *Salmonella* by birds that might be present at recreational water sites.

Similar to *Salmonella* in livestock and humans, serotype prevalence among birds appears to vary temporally and spatially. Fenlon (1983) found that gulls nesting near a sewage treatment plant and feeding on sewage had a 55% carriage rate of *Salmonella.* There was general (though not perfect) concordance between serotypes present in the raw and treated sewage and the serotypes found in the gull feces. Interestingly, given practical limits on sampling frequency for sewage effluent and the likelihood that prevalence of different *Salmonella* serotypes vary with time, the gull feces may yield a more complete picture of *Salmonella* serotype presence in sewage than individual samples drawn from the effluent. Palmgren et al. (2006) observed a 2.7% prevalence of *Salmonella* spp. in Black-headed gulls at a site in Sweden, with the dominant serotype (> 50% of isolates) being Typhimurium—a serotype important in human salmonellosis. The authors found the *S*. Typhimurium DT195 isolates from gulls were related to those isolated from domestic animals and humans, and hypothesized that Black-headed gulls might play a role in the spread of *S*. Typhimurium in Sweden. Shorebirds other than gulls may be sources of human-infectious *Salmonella*, as shown by Kirkpatrick (1986), who isolated *S*. Newport and *S*. Typhimurium var Copenhagen from droppings in black-crowned night heron nests. The author noted that, during the time period of the study, *S*. Newport and *S*. Typhimurium were the two most common serotypes in human infections in the vicinity of the study site (Ocean County, New Jersey, USA) and speculated that the herons were infected via sewage-impacted marine water.

An estimate for density of *Salmonellae* in gull droppings is provided by Lévesque et al. (1993). Among ring-billed gulls (*Larus delawarensis*) nesting along the St. Lawrence River in the vicinity of Quebec City, mean concentration of *Salmonellae* on 3 different sampling days was 150, 230, and 12,000 CFU/g feces; the ratios of *Salmonellae* to fecal coliforms on those three days were 6.25×10^{-5}, 2.09×10^{-4}, and 2.31×10^{-3}, respectively. Among typed *Salmonella* isolates, several serotypes potentially pathogenic in humans (brandenberg, agona, hadar, Stanley, and Typhimurium) were identified.

Few studies have shown a connection between birds and *E. coli* O157 contamination of recreational water, though two routes—mechanical transmission (attached to birds) and transmission via fecal material of infected birds are possible. Hubálek (2004), in a review of literature on pathogens in birds, noted that pathogenic strains of *E. coli*, such as *E. coli* O157:H7, have been isolated from both healthy and diseased birds (both resident and migrant) including *Ardea cinerea* (the grey heron), *Branta canadensis* (Canadian geese), *Cygnus columbianus* (tundra swans), *Uria aalge* (the common murre), and *Columba palumbus* (wood pigeons).

Ćížek et al. (2000) achieved experimental infection of pigeons with *E. coli* O157. The infected pigeons appeared asymptomatic, yet shed the pathogens for 14.8 ± 3.4 days when infected with a

dose of 10^5 CFU and 20.2 ± 5.2 days when infected with a dose of 10^9 CFU. Based on this finding, the authors considered it credible that pigeons may play a role in *E. coli* O157 infection transmission. Shere et al. (1998) also hypothesized that birds may play a role in *E .coli* O157:H7 on dairy farms, based on genetic similarity between *E. coli* O157:H7 isolated from cattle and a pigeon found on the same dairy farm. Foster et al. (2006) isolated STEC O157 from droppings at a bird feeding station in Scotland. Potential hosts (known to feed at the station) include blackbirds, greenfinches, chaffinches, house sparrows, or unobserved species. STEC O157 was isolated from only 1 of 231 composite samples, which indicates that STEC O157 occurrence in birds is relatively rare.

In summary, all of the bacterial reference pathogens occur in feces of birds. *Campylobacter* and *Salmonella* of strains and types pathogenic to humans are prevalent in a variety of bird species. *E. coli* O157:H7 has been observed in bird feces, but appears much less prevalent than *Campylobacter* and *Salmonella.* The *Campylobacter* species and *Salmonella* serotypes observed in bird feces often are similar to those prevalent in adjacent human populations.

C.4 Reference Pathogens in Stormwater

In conducting the literature review for reference pathogens in animal and human-impacted water, numerous articles were obtained with information describing the occurrence and densities of reference pathogens in stormwater. Although these data are not used explicitly in the QMRAs described in this report, these data are potentially valuable for future consideration. A summary of the data that were found are summarized below (Table 36).

Table 36. Reported reference pathogen densities in stormwater-dominated water

Study	Pathogen	Prevalence	Abundance	Notes
Arnone et al. (2005)	*Cryptosporidium* spp.	0–100%	0–31 oocysts/100 L	Samples taken from five locations, with features ranging from urban high-density to wooded/pervious; highest prevalence and abundance of *Cryptosporidium* was in runoff from the wooded area
Betancourt and Rose (2005)	*Cryptosporidium* spp.	25% (1/4 samples)	<2–287 oocysts/100L (GM = 72)	Samples collected in Florida and designated as “stormwater”; drainage not described
Ćížek et al. (2008)	*Cryptosporidium* spp.	NA	50–180 oocysts/100L (based on arithmetic means of samples)	Samples collected in five tributaries to a drinking water reservoir; data presented graphically as densities in stormwater
Jiang et al. (2005)	*Cryptosporidium* spp.	88% (determined by PCR) or 56% (determined by microscopy	Not determined	Samples collected from streams during rain events; genotypes indicated nearly all isolates likely of non-human origin

Study	Pathogen	Prevalence	Abundance	Notes
Till et al. (2008)	*Cryptosporidium* spp.	3% and 5% of samples from municipal and forested drainages, respectively	Data only presented graphically	
Arnone et al. (2005)	*Giardia* spp.	0–100%	0–377 oocysts/100L	Samples taken from five locations; with features ranging from urban high-density to wooded/pervious; highest prevalence and abundance of *Giardia* in runoff from the wooded area
Betancourt and Rose (2005)	*Giardia* spp.	0% (0/4 samples)	—	Samples collected in Florida and designated as "stormwater"; drainage not described
Till et al. (2008)	*Giardia* spp.	7% of samples from forested and municipal drainages	Data reported graphically	*Giardia* occurrence appeared relatively insensitive to land use
Betancourt and Rose (2005)	Enteric viruses	100% (4/4 samples)	0.48–4.4 MPN/100 L (GM = 2)	Samples collected in Florida and designated as "stormwater"; drainage not described
Rose et al. (1987)	Rotavirus	2 out of eight sites studied	0.237–0.25 MPN PFU/L	Samples collected from recreational water in regions without suspected impacts from POTWs or animal operations; authors speculated that pathogens may have been of swimmer origin
Rajal et al. (2007)	Adenovirus 40/41	1 out of 61 samples (2%)	230 genomes/L	The authors speculated that the estimated adenovirus density is an underestimate.
Rajal et al. (2007)	Enteroviruses	0 out of 61 samples (2%)	ND	
Till et al. (2008)	Adenovirus	31% of samples from a forested drainage and 28% of samples from a municipal drainage	Not determined	High adenovirus occurrence in the forested drainage attributed to a single known source
Claudon et al. (1971)	*Salmonella*	4/12 samples (33%)	—	Sample sites loctaed in a separate storm sewer system upstream of discharge from an experimental animal operation; serotypes were, in general, consistent with those commonly causing human infection

Appendix D. EPA Environmental Monitoring Program

The objective for EPA's environmental monitoring and sampling effort was to generate primary data to characterize recreator exposure to fecal pathogens and FIB in surface water impacted by agricultural activities through the analyses of overland transport inputs and in-stream processes. The study design includes conducting rain simulation experiments in small plots amended with either beef cattle manure, swine slurries, or poultry broiler litter. This appendix provides a detailed description of the experiments, which are summarized in Section 2.12.

D.1 Rainfall Simulation

D.1.1 Experimental design, plot, and event description

The rainfall simulation experiments were conducted on 18 1.5 × 2 m plots divided in halves, providing for a total of 36 0.75 × 2 m treatment plots. The plots located in USDA-owned land in Oconee County, GA (33° 47'N, 83°23'W) are described in Butler et al. (2008). Each treatment plot was delineated with galvanized sheet metal (23 cm width) placed into the ground to a depth of 18 cm. The vegetation cover was maintained at 10 cm in height and consisted of a mixed crop of fescue and bermuda grasses. The slopes for the treatment plots ranged from 8 to 12%. Two rainfall simulators (Tlaloc 3000 type, Joern's Inc., West Lafayette, IN), were placed each on top of one double plot. This type of rainfall simulator has been commonly used for nutrient and pathogen transport studies (Soupir, 2003; Soupir et al., 2006). Baseline simulations were conducted to determine background pathogen, FIB, and nutrient levels. Histograms were initially used to identify frequency distributions of baseline runoff volumes and allowed us to select plots within a specific range of volumes. During the rainfall simulation event, rainfall was applied to 4 plots per day, 3 days per week, for a total of 12 plots per week for 3 consecutive weeks after manure application.

Treatments consisted of manure applications from the following three animal types: swine (liquid manure), beef cattle (solid manure), and poultry (broiler litter)—and a control treatment (no manure application). Each treatment had three replications (plots) and three manure application timings relative to rainfall simulation time. The manure was applied to the plots in a completely randomized split plot design taking into consideration the type of manure and the rainfall application regime (1 hour, 1 week, and 2 weeks after manure was applied to the plots). The rainfall application rate was set at 6.125 pounds per square inch, which resulted in 2 to 4 inches of rain per hour. This rate was equivalent to a precipitation return period of <100 years for the Georgia piedmont area and sufficient to produce a surface runoff event in a reasonable timeframe (30 minute to 3.5 hours, depending on the moisture conditions of the soil). After runoff was produced, rainfall continued to be applied for 60 minutes. In the plots where rainfall was not applied immediately after manure application (1-week and 2-week treatments), plastic

covers were placed on the plots to protect against natural rain events. These enclosures were placed well above the vegetation cover to allow for air circulation and heat exchange. The type of plastic selected allowed for 75 to 80% of the UV light to penetrate. This experiment was conducted three times, (October 2009, March 2010, and June 2010) to obtain sufficient data points and to account for varying climatic conditions. Soil moisture was determined during the March and June simulations. During the March 2010 simulation (Run B), it varied from 0.271 ± 0.042 m^3/m^3 prior to start the simulation to 0.466 ± 0.032 m^3/m^3 during the simulation.

D.1.2 Sample collection

Runoff was collected at the lower end of each plot by means of a stainless steel flume, at 5-minute intervals for the duration of the event and was composited in a 40 gallon container to determine cumulative runoff volumes. The color of the runoff varied depending on the type of manure applied. Poultry and cattle produced runoff of a deep brown (poultry) to greenish color (cattle), very high in suspended solids, while swine and control treatments produced light brown runoff. Five samples from selected intervals (5, 10, 20, 30, and 60 minutes) were collected directly from the flume (~500 mL) to determine *E. coli* and enterococci total densities. An extra sample (1 L) was collected at 15 minutes for *Clostridium* analysis. After the microbial sample was obtained at the selected time point, the remainder of the runoff was added to the 40 gallon container. After each five minute addition, the container was weighed to determine the cumulative runoff volume. Two composited samples (10L) were collected per run from the 40 gallon container for pathogen and FIB analysis (30-min composite and total composite). These samples were analyzed for *E. coli*, enterococci, *Clostridium* spp., *Cryptosporidium*, *Giardia*, *Salmonella*, *E. coli* O157, and *Campylobacter*, depending on the type of manure applied. Composited samples (10 L bladders) and individual *Clostridium* samples (1 L) were shipped the same day of collection to an independent laboratory on ice by overnight courier. Temperature inside the coolers was monitored during transport with individual digital thermometers (i-buttons).

D.2 Manure Description and Plot Application

As noted previousloy, manures were obtained from cattle, swine, and poultry. The total amount of manure to be used on all plots was collected directly from farms a day in advance of the first day of the study. Cattle manure was obtained from a beef cattle farm operated by USDA/Agricultural Research Service (ARS) in Watkinsville, GA, by collecting fresh pats from the pasture site where cattle were grazing. Broiler litter was obtained from a poultry farm operator. The litter was obtained directly from the inside of the chicken house from the top layer of litter. Litter composition was considered to be typical of this type of operation, and consisted of a mixture of chicken manure, wood chips, and feathers. Swine manure was obtained from two different sources because the first operator (University of Georgia) temporarily discontinued swine operations during the course of the study. During the first two simulations, swine manure

was obtained from a lagoon receiving manure flushed from the swine pits. During the third simulation, the swine manure was obtained from a commercial facility housing over 2500 pigs. The manure was obtained directly from the pipe as the house was being flushed before it actually mixed with the lagoon material. Once collected, all manures were transported to the laboratory and stored at 4 °C until the day of application, which consisted of 24 hours, 1 day, or 2 days. Holding times depended on the experimental design. The solids fraction of the applied solid manures is provided in Table 37.

Table 37. Percent solids of poultry and cattle manure applied to experimental plots

Type of Manure	Simulation Run	% Solids
Poultry	A	69.3
Cattle	A	11.7
Poultry	B	75.6
Cattle	B	11.9

Each type of manure was sampled for pathogen and FIB loadings prior to application via randomized composite samples. Manures were weighed in the laboratory into individual containers on the day of application and transported to the field on ice. Application of manures was scheduled for Tuesday, Wednesday, and Thursday during the first week of the event. Rainfall was applied to the plots accordingly to the day of application following 1 hour, 1 week, and 2 weeks after manure was applied. Cattle manure was applied in small pats evenly distributed across the plots. Poultry litter and swine slurry were poured directly and evenly across the plots.

Poultry and swine manures were applied at agronomic rates (100 and 300 lb/acre, respectively) following USDA Natural Resources Conservation Service (NRCS) guidelines based on the nitrogen requirement of the type of crop and the nutrient concentration in the manure being applied. Cattle manure was applied at 10% of the total daily manure produced by grazing beef cattle.

D.3 Seeding of Manures with Surrogate Pathogens

During the first simulation (Run A), it was determined that the natural concentration of the pathogens of interest was too low in the manure being applied to detect in the runoff water. Therefore, it was decided to seed the manures with surrogate pathogens to determine the leaching rates of pathogens from the applied manures. The surrogate pathogens selected were all non-virulent species that did not pose a risk of infection to project personnel or the environment. Manures were spiked for both Run B and Run C.

D.3.1 Surrogate pathogens description

The surrogate organisms used to seed the manures, as well as the type of manure that they were added to, is provided below.

- *E. coli* O157:H7 B6914 #87 was added to cattle feces, poultry litter (only during the March 2010 simulation), and swine slurries;
- *Salmonella* X3985 was added to cattle manure, poultry litter, and swine slurries; and
- UV-inactivated *Cryptosporidium* and *Giardia* were added to cattle manure and swine slurries.

D.3.2 Stock surrogate cocktails

Seeding experiments were conducted by an external laboratory to determine the concentration of surrogate pathogens to add to the different types of manures to increase the likelihood of detection in runoff water. The calculation for the final surrogate pathogen concentrations took into consideration the recovery of the methodology used for analysis, the decay of the organisms in manure as well as during transport, values from literature for previously observed leaching rates for pathogens or FIB from livestock manures, maximum pathogen levels observed in livestock manure, and maximum number of organisms that could be produced to use for spiking. Stock surrogates suspensions were shipped by overnight courier to EPA and stored at 4 ± 1° C until the day of use. Each suspension was vortexed for 2 minutes before removing an aliquot for the stock enumeration or preparing the spike cocktail.

On each day of spiking, aliquots (500 µL) of each individual surrogate suspension were aseptically removed from the stock tubes after 2 minutes of vortexing. Each suspension volume was transferred to an individual labeled, sterile 2 mL tube with a screw-cap. Vials were stored in the refrigerator until analysis.

Spike cocktails were prepared each day for each manure type. A chart designating the volumes of each surrogate suspension to be used to prepare the cocktail for each manure type was provided with the stock surrogates that were shipped to the EPA laboratory. Enough volume of each surrogate cocktail was provided so that a 1.5 mL (1500 µL) subsample was removed from each cocktail for enumeration. The 1.5 mL spike cocktail was aseptically transferred to a 2 mL labeled, sterile tube with a screw cap.

Individual plots to be seeded with swine slurry received 1×10^{9} UV-irradiated *Cryptosporidium parvum* oocysts, 1×10^{7} UV-irradiated *Giardia lamblia* cysts, 1×10^{10} *E. coli* O157:H7 strain B6194, and 1×10^{10} *Salmonella* X3985. For plots to be amended with cattle manure, 5×10^{7} *C. parvum* oocysts, 1×10^{7} *G. lamblia* cysts, 1×10^{10} *Salmonella* X3985, and 1×10^{10} *E. coli* O157:H7 strain B6194 was applied. Surrogate spiking levels for each poultry litter plot received 1×10^{10} *E. coli* O157:H7 strain B6194 (March 2010 only) and 1×10^{10} *Salmonella* X3985.

D.3.3 Manure spiking procedure

Swine. Each day the total number of plots to have swine slurry was determined using the experimental design diagram. The total volume of slurry applied to plots was calculated and was aseptically transferred to a sterile 20 L carboy containing a sterile Teflon-coated stir bar. After adequate mixing, volumes of the un-spiked slurry required for background analysis were removed and transferred to sterile containers that were stored at 4 °C until analysis. The spike cocktail was then aseptically added to the container, under continuous stirring. The container housing the spike cocktail was rinsed with sterile phosphate buffered saline (PBS) and added to the container. The spiked slurry was stirred on a stir plate for 30 minutes. After mixing, spiked manure subsamples were removed. The remaining slurry was measured into individual 4 L sterile containers for application to the plots. After application to the plots, the container was rinsed with sterile PBS and this rinse water was also applied to the plots to ensure a complete transfer of the spiked manure.

Poultry. Poultry litter was measured into 5 gallon plastic pails equipped with a cover (rinsed with 70% ethyl alcohol [EtOH], inverted on clean foil or bench protectors, and air-dried overnight). For each plot that received poultry litter, 1.05 kg was weighed and transferred to an individual 2.5 kg container. Before adding the spike cocktail, un-spiked subsamples were removed for analyses. The spike cocktail was then added in three portions to each container using a sterile pipette and shaking for 5 minutes after the addition of each portion. After the final portion was added, the spike cocktail container was rinsed with PBS and added to the poultry litter. The covered pail was shaken for 5 minutes and allowed to stand for 30 minutes. Spiked subsamples were removed from the container for additional analyses. Spiked subsamples were directly added to the fields. Containers were rinsed at the end as described above.

Cattle. The total amount of cattle manure was measured into a 5 gallon plastic pail (rinsed as for poultry litter above). For each plot that receives cattle manure, 2.4 kg was applied. On each day of manure application, the total mass of cattle manure needed for the day was calculated and additional amounts were included for additional assays. The total amount of cattle manure was added to a clean, sterile container and un-spiked samples were removed for subsequent analyses. The spike cocktail was added in three portions using a sterile pipette and then mixed with a clean mixing device after the addition of each portion. After the final portion was added, the spike cocktail container was rinsed with PBS and added to the cattle manure. The manure was mixed a final time for 5 minutes and allowed to stand for 30 minutes. Spiked manure subsamples were removed for analysis. Subsamples for plot application were weighed into sterile containers using disposable sterile scoops or equivalent.

D.4 Microbial Analysis

Samples were coded as C for cattle, S for swine, P for poultry, and X for control. Samples were analyzed for the presence and concentration of *E. coli* O157:H7, *Salmonella* spp.,

Campylobacter spp., *Clostridium* spp., *Giardia* cysts, and *Cryptosporidium* oocysts and infectious *Cryptosporidium* spp. In addition, the sample were also analyzed for the presence and concentration of the surrogate pathogens *E. coli* O157:H7 B6914 #87 and *Salmonella* X3985. Percent solids in cattle and poultry manure samples were determined using Standard Methods 2540B. The swine manure was treated as a water sample. A 1:10 dilution (5 g or mL of samples in 45 mL phosphate buffered water [PBW]) was prepared for all manure samples. Each volume was analyzed in triplicate for the MPN and *Clostridium* assays. A summary of the organisms analyzed and the methods employed is provided in Table 38.

Table 38. Organisms and methods used for analysis of water and manure samples

Organism	Method	Description
E. coli	Culture	(Colilert)
Enterococcus	Culture	Method 1600
E. coli 0157	Culture	Broth tube enrichment MPN
E. coli 0157	qPCR	Gene targets: *stx1*, *stx2*, *eae*
Salmonella	Culture	Broth tube enrichment MPN
Salmonella	qPCR	
Salmonella X3985	Culture	Broth tube enrichment MPN
E. coli B6-194	Culture	Direct plating onto TSA-A.
Crypto/Giardia	Microscopy	EPA Method 1623
Campylobacter	Culture	Broth tube enrichment MPN
Campylobacter	qPCR	
Clostridium	Culture	Modified TSCF

D.4.1 qPCR Assays

Each cubitainer containing runoff water was shaken to re-suspend settled particles and then aliquoted to individual sterile containers for various analyses. For quantification of bacterial pathogens using qPCR, 400 mL of each water sample was centrifuged at 4000× g at 4 °C for 30 min, the supernatant was discarded and the pellet was re-suspended in PBS. Concentrated samples were stored at -80 °C until DNA purification and qPCR assays.

D.4.2 Fecal indicator assays

Enterococci concentrations were determined in both manure and runoff samples by membrane filtration following EPA Method 1600. A 1:10 dilution using PBW was prepared for all solid manures (cattle and poultry), while swine was analyzed as a water sample. For manure, dilutions from 10^{-2} to 10^{-5} (g or mL) were prepared. For runoff samples, dilutions ranged from 1 mL to 10^{-3} mL, depending on the type of manure. Defined substrate technology (Colilert®, Idexx) was used to determine concentrations of *E. coli* in both manure and runoff water. For manure, the same dilution range as used for enterococci was used; for runoff water, the volumes used

included 1, 10, 50 and 100 mL, depending on the type of manure or fecal aging time. Samples were analyzed in duplicate.

D.4.3 MPN assays

Concentrations of *E. coli* O157:H7, *Salmonella* spp., and *Campylobacter* spp. in runoff samples were determined using the MPN technique. Samples were analyzed in triplicate, at volumes of 10, 1, and 0.1 mL in the enrichment step by pipeting the volume directly to the tubes. In subsequent weeks, the highest volume analyzed for each sample was first concentrated by membrane filtration onto a 0.45-µm cellulose nitrate membrane and subsequently transferred to the enrichment medium. The volume assayed by membrane filtration varied (between 30 and 100 mL), depending on the amount of particulates present in each sample. The other volumes analyzed, 5 and 0.5 mL, were pipetted directly into the tubes.

For *E. coli* O157:H7 and *Salmonella* spp. MPN assays, buffered peptone water (BPW) was inoculated and incubated at 35 to 37 °C for 20 to 24 hours. A 10 µL portion of each BPW bottle/tube enrichment was then streaked onto HardyCHROM™ O157 agar. Presumptive *E. coli* colonies were tested for a positive indole reaction and a negative fluorescence. Colonies with the appropriate response were then tested with antiserum against *E. coli* O157 antigen using a commercially available latex agglutination kit. For *Salmonella* spp., 100 µL from each BPW bottle/tube enrichment was inoculated into a 10 mL tube of Rappaport-Vassiliadis enrichment broth and incubated for 24 hours at 43°C. A 10 µL portion of each enrichmentbroth tube was then streaked on *Salmonella* and *Shigella* xylose lysine deoxycholate agar biplate and incubated for 18 to 24 hours at 35 to 37°C. Isolated presumptive positive colonies were inoculated to Enterotubes and incubated at 35 to 37 °C for 18 to 24 hours for biochemical confirmation of *Salmonella* spp.

The enrichment step for samples analyzed for *Campylobacter* spp. included the same volumes as analyzed for *E. coli* O157:H7 and *Salmonella* spp. Water or membrane filters were inoculated in Bolton Broth and incubated at 35 to 37 °C for 4 hours and then transferred to 42 ± 1°C for 20 to 44 hours. After the incubation period, a 10 µL portion from each enrichment tube/bottle was streaked for isolation onto *Campylobacter* blood free selective agar. The plates were incubated in a microaerophilic atmosphere (5 to 6% oxygen, 10% carbon dioxide and 85 to 85% nitrogen) at 37°C for 48 hours. Following incubation, each plate was inspected for presumptive-positive *Campylobacter* growth. Next, each plate was tested for positive latex agglutination using a commercially available kit. Plates with colonies resulting in autoagglutination were scored positive for MPN calculations and retained for qPCR assay.

D.4.4 Surrogate pathogens assays

E. coli O157:H7 strain B6914 was analyzed by direct plating onto trypticase soy agar. Colonies that fluoresced green under UV light were enumerated; positives were confirmed with *E. coli*

O157 latex agglutination kit. *Salmonella* surrogate was determined using the same MPN assay described above.

D.4.5 EPA Method 1623: Giardia and Cryptosporidium

For enumeration of *Giardia* cysts and *Cryptosporidium* oocysts, 2 to 9 L of each sample was analyzed using EPA Method 1623 with samples concentrated by centrifugation or filtration. Packed pellet volumes were measured and no more than 0.5 mL packed pellet volume was analyzed in one immunomagnetic separation (IMS) reaction as prescribed in the method. Isolated cysts and oocysts were enumerated as prescribed in the method using epifluorescence microscopy.

In cattle manure samples, 10 mL of the diluted manure sample (5 g wet weight in 45 mL PBW) was analyzed in one IMS reaction. Recovered cysts and oocysts were enumerated using epifluorescence microscopy. For the swine manure, 50 mL was concentrated by centrifugation and analyzed as described above. Poultry manure samples were not analyzed for *Giardia* cysts and *Cryptosporidium* oocysts.

D.4.6 Infectious Cryptosporidium oocysts by foci detection method

Cryptosporidium oocysts were isolated from interfering debris using IMS as described in EPA Method 1622. The isolated bead-oocyst complex was rinsed with 10 mL PBS to remove the IMS buffers, which are toxic to the human ileocaecal adenocarcinoma (HCT) monolayers. The rinsed bead-oocyst complex was quantitatively transferred to 1.5-mL Eppendorf tube using PBS. The bead-oocyst complex was rinsed and the supernatant discarded. The bead-oocyst complex was re-suspended in 150 µL of Hank's balanced salts solution (HBSS) or PBS and an equal volume of Hank's Balanced Solution, pH 2.0, containing 2% trypsin and incubated at 37 °C for 1 hour. Every 15 minutes, the tubes were vortexed for 10 seconds. After 1 hour, the tubes were prepared for magnetic particle concentration. Inoculation medium (300 µL) was added to each tube, gently mixed, and centrifuged at 10,000× g for 2 minutes. The supernatant was aspirated to 50 µL and a fresh 500 µL aliquot of inoculation medium was added and gently mixed. The tube was centrifuged at 10,000× g for 2 min and the supernatant aspirated to 50 µL. Each sample concentrate was re-suspended in 350 µL of inoculation medium and inoculated to a single well of an 8-welled chamber slide containing a monolayer of HCT-8 cells (ATCC CCL-244) and 100 µL of inoculation medium. Chamber slides were incubated for 65 to 72 hours at 35° C in a humid (5% CO_2) atmosphere. After the incubation period, the growth medium was aspirated from each well and the monolayers were rinsed with pre-warmed PBS to remove unattached oocysts. Wells were then fixed with absolute methanol for 8 minutes and then rehydrated for 30 minutes with PBS containing 2% goat serum and 10% of a 0.002% solution of Tween 20. Infections were detected by staining monolayers with a fluorescein labeled polyclonal rat immunoglobulin G antibody for detection of the intracellular reproductive stages of

Cryptosporidium parvum oocysts. Enumerations of infection sites were observed using epifluorescence microscopy.

D.4.7 Microscopy for Giardia and Cryptosporidium assays

A Zeiss Axioskop fluorescence microscope, equipped with a blue filter block (excitation wavelength, 490 nanometer (nm); emission wavelength, 510 nm) was used to detect labeled oocysts at a magnification of 360×. DAPI staining characteristics were observed at 640× magnification using a UV filter block (excitation wavelength, 400 nm; emission wavelength, 420 nm). The internal morphology of oocysts and intracellular reproductive stages of *C. parvum* oocysts was observed by using Nomarski DIC microscopy at 640 to 1600× magnification.

D.4.8 Clostridium spp. assays

Samples were analyzed for *Clostridium* spp. densities using a modification of the SCA/NHS method for detection of *Clostridium* spp. on tryptose sulfite cycloserine (TSC) agar. Water samples were filtered through 0.45-µm cellulose nitrate filters and aseptically applied to agar plates. Plates were incubated at 44.5 °C for 48 hours. Brown to black colonies were counted as *Clostridium* spp. Due to the presence of high levels of particulate matter, fluorescence was not assessed on these samples.

Appendix E. Pathogen and FIB Mobilization Fractions Due to Rainfall

The degree to which microorganisms run-off from soil depends on myriad factors. Rather than attempt characterizing each of those factors separately, all experiments and analyses were conducted for a defined typical rain event. Experimental conditions for plot-scale runoff experiments were selected based on an intense (< 100 year return period) rain event for the Georgia Piedmont region.

In plot-scale experiments rainfall was applied to plots at an average rate of 6.89 cm/hourr (σ = 0.62 cm/hour) for a rain event duration of 60 minutes. Average cumulative runoff volume from plots was 57.7 L (σ = 16.1 L). A histogram showing the distribution of runoff volumes for individual plots is presented in Figure 35. The wide variability in runoff volume despite the low variability in rain intensity and duration arises from differences between plots including antecedent soil moisture, slope, location, soil type and grain size distribution and other factors.

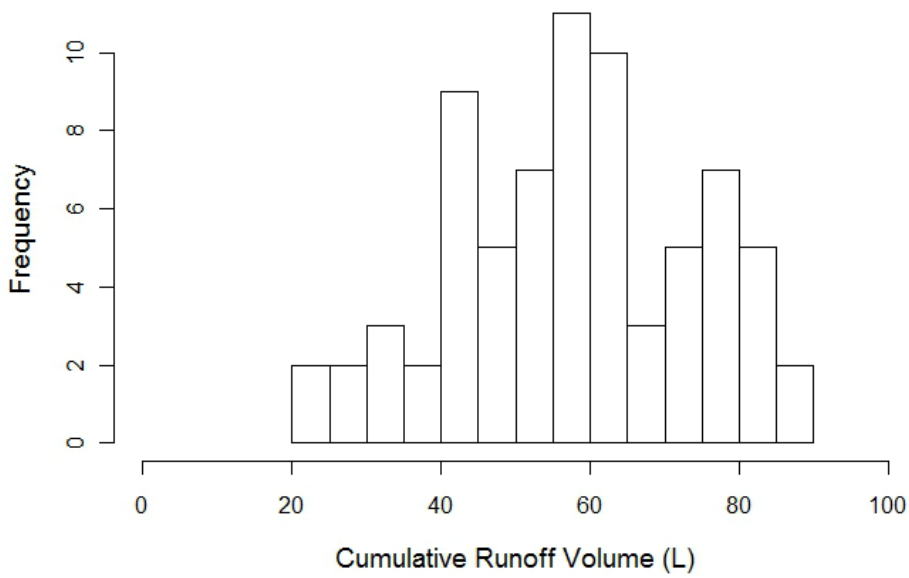

Figure 35. Histogram of cumulative runoff volumes from plots subject to the design rain event

In addition to application of a prescribed rainfall at a typical intensity, the design event simulated in plot-scale experiments entailed land application of manures to plots at an agronomic rate. The agronomic application rate is the mass or volume per unit area with a nutrient content equal to the nutrient requirement for the vegetation on the plot (Midwest Plan Service, 2004). The agronomic rate accounts for the type of vegetation on the plot, the nutrient content of the soil prior to application of the manure, and the nutrient content of the manure. The application rates selected for solid cattle manure, swine slurry, and poultry litter are presented in Table 39.

Table 39. Manure application rates

Manure	Application Rate per Plot	Application Rate per Area (plot dimensions: 2 m × 0.75 m)
Solid cattle manure	1.6 kg	1.07 kg/m^2
Swine slurry	2.7 L	1.78 L/m^2
Poultry litter	0.667 kg	0.445 kg/m^2

Data for both manure density and runoff density are required to calculate mobilization fraction via equation 12. As described in Appendix D, manures and runoff composite samples were assayed for numerous pathogens and FIB, in some cases via multiple methods. For use in equation 12, there must be sufficient data for a given organism-method combination for both manure and runoff samples. Criteria for selecting data for use in equation 12 included the following:

- only plots with manure sample densities above detection limits were used;
- data indicating more pathogens running off than applied were assumed anomalous (perhaps resulting from contamination of plots or cross-contamination in the laboratory) and excluded; and
- at least 5 paired data for runoff and manure samples were available for a particular manure-organism combination.

FIB data yielding mobilization fractions greater than one were not excluded from analyses, since background levels of FIB were relatively high on control plots. However, two alternative methods for estimating indicator runoff fraction were used so that the impact of background indicator runoff could be assessed. The occurrence of FIB in control plots is described below and implications with respect to QMRA modeling are described. For *Campylobacter*, there were insufficient culture data to estimate the range of mobilization fractions; thus, it was assumed that mobilization fraction calculated using qPCR manure and runoff density is equivalent to that calculated using MPN counts in the manure and runoff. This assumption is consistent with the correlation in qPCR and culture counts of organisms in fresh manures as observed by Klein et al. (2010). The methods employed and data sets with data meeting the criteria for use in estimating mobilization fraction are summarized in Table 40.

Table 40. Method-organism combinations and data availability

Organism	Method	Data for Manure?	Data for Runoff?
Pathogens			
Campylobacter	MPN	☑	☑
	Simplates	☑	
	qPCR	☑	☑
Cryptosporidium	EPA 1623	☑	☑
Giardia	EPA 1623	☑	☑
E. coli O157	MPN	☑	
E. coli O157 surrogate	Membrane filtration	☑	☑
E. coli stx1	qPCR	☑	
E. coli stx2	qPCR	☑	
E. coli eae	qPCR	☑	
Salmonella (wild type)	MPN		
Salmonella surrogate	MPN	☑	☑
Salmonella	qPCR		☑
Fecal indicator bacteria			
Clostridium perfringens	Culture (mCP)	☑	☑
	Culture (TSC)	☑	☑
E. coli	MPN (Colilert)	☑	☑
Enterococci	Culture (mEI)	☑	☑
Enterococcus	qPCR		
Total coliforms	MPN (Colilert)	☑	☑

Equation 12 was evaluated using the manure application rate data (Table 39), cumulative runoff volumes for each plot, and the manure and runoff organism densities (Table 40) to determine the mobilization fraction for each plot. Results for pathogens are summarized in Table 41. For all pathogens except *Campylobacter* mobilization fractions are based on a single organism-method combination. For *Campylobacter*, MPN data were insufficient to develop mobilization fractions for poultry litter and qPCR data were used. For nearly all pathogens the mobilization fraction ranges spanned several orders of magnitude, despite the relatively uniform rainfall treatment applied to each plot. The *E. coli* O157 surrogate was observed in control plot runoff. This observation indicates the potential for contamination of control plots or runoff water samples from control plots.

Table 41. Mobilization fraction ranges and means for pathogens

Pathogen	Manure	Log_{10} of the Minimum Mobilization Fraction	Log_{10} of the Minimum Mobilization Fraction	Geometric Mean of Mobilization Fraction	Basis/Method
Campylobacter	Cattle	-4.85	-1.46	0.000373	MPN
	Swine	-2.20	-1.01	0.0495	MPN
	Chicken	-8.60	-1.74	8.52E-07	qPCR
E. coli O157	Cattle	-3.65	-0.200	0.0159	CFU, surrogate
	Swine	-3.01	-1.501	0.00664	CFU, surrogate
	Chicken	-4.01	-2.214	0.00118	CFU, surrogate
Salmonella	Cattle	-5.57	-1.26	000235	MPN, surrogate
	Swine	-3.85	-2.40	0.000781	MPN, surrogate
	Chicken	-3.68	-2.65	0.000556	MPN, surrogate
Cryptosporidium	Cattle	-4.46	-0.179	0.00272	EPA 1623
	Swine	-3.90	-1.48	0.00201	EPA 1623
	Chicken	Not tested			EPA 1623
Giardia	Cattle	-6.40	-0.387	4.72e-05	EPA 1623
	Swine	-4.58	-0.0617	0.00481	EPA 1623
	Chicken	Not tested			EPA 1623

FIB mobilization fractions were generally calculated as described above, but details of the calculations are presented below separately to address mobilization fractions greater than one. *E. coli* and enterococci densities observed in manures used in plot-scale experiments are compared to "typical" ranges derived from reports in the literature in Table 42. The distributions of experimental FIB densities appear skewed for both FIB and for all three manure types. The range of cattle manure densities in manures used in the plot experiments is higher than that from literature studies. Because fresh manures from an operational cattle facility were used in experiments, we believe the experimental manures are typical of manures in the United States for similar types of operations and manure handling practices. Therefore, the range of manure FIB densities considered "typical" may be too narrow. Both enterococci and *E. coli* were much less abundant in the poultry litter than in values reported in the literature. Because poultry litter is a heterogeneous mixture of feces and other materials, it less clear how typical the experimental manure densities are. Swine slurry FIB densities are much lower than typical values for both enterococci and *E. coli*. A plausible explanation for those low densities and indication that these are not typical that the slurries were taken from an operation with very few pigs contributing to the slurry lagoons during the second round of experiments.

Table 42. Comparison of typical and experimental manure FIB densities

		Log_{10}(min)	Log_{10}(max)	Arithmetic Mean
Enterococci				
Cattle	Literature	2.0	5.1	
	Experiment	4.70	5.46	147,000
Swine	Literature	5.3	7.2	
	Experiment	0.176	2.02	46.5
Chicken	Literature	5.0	7.0	
	Experiment	3.78	5.81	219,000
E. coli				
Cattle	Literature	5.0	6.7	
	Experiment	6.69	8.32	55,600,000
Swine	Literature	6.1	7.3	
	Experiment	0.70	3.07	546
Chicken	Literature	5.1	10.9	
	Experiment	2.69	4.36	9012

Runoff indictor densities from plots with manures and control plots are summarized in Table 43. Densities in control plot runoff were variable, and in some instances high relative to swine and poultry runoff densities. Other mobilization studies have also reported significant densities in runoff from control plots (Miller and Beasley, 2008; Thurston-Enriquez et al., 2005). *E. coli* O157 surrogate data are included in Table 43 because mobilization association with that organism is similar to that of generic *E. coli* and there is no background *E. coli* O157 surrogate in the soil to confound estimation of mobilization fraction from manure-borne organisms. There were several instances of *E. coli* O157 occurrence in runoff from control plots, potentially due to transport of *E. coli* O157 from up-slope to down-slope plots.

Histograms of the FIB mobilization fractions for enterococci and *E. coli* for each manure type are presented in Figures 36 to 41. Cattle mobilization fractions are within anticipated ranges, though mobilization fractions greater than one (more enterococci running off the plot than applied in the manure) occurred for three plots. Mobilization fraction was significantly greater than for many of the plots treated with swine slurry. This, along with the very low densities of FIB in swine slurries applied to the plots and relatively high densities of FIB in the runoff from the control plots, indicates that the majority of *E. coli* and enterococci in the runoff from plots with swine slurry applied did not originate from the swine slurry. These findings indicate a disconnection between the FIB and pathogen densities in the runoff. Poultry litter FIB mobilization results are between those for cattle and swine. Mobilization fractions from plots with applied poultry litter are generally less than one and in the instances in which mobilization fraction exceed one the fraction is not as high as those observed for runoff from plots treated with swine manures.

Table 43. Runoff FIB densities for plots with and without manure application

Enterococci				
Parameter	**Cattle**	**Swine**	**Chicken**	**Control**
Log_{10}(Minimum mobilization fraction)	-2.784	-0.694	-1.168	—
Log_{10}(Maximum mobilization fraction)	0.262	2.315	1.141	—
Arithmetic mean of mobilization fraction	0.336	30.2	1.316	—
Geometric mean of mobilization fraction	0.0480	7.13	0.497	—
Minimum runoff density (CFU/100 mL)	580	0.5 (DL)	500	0.5
Maximum runoff density (CFU/100 mL)	560,000	56,000	3,600,000	192,000
Arithmetic mean of runoff density (CFU/100 mL)	100,200	8440	378,000	27,280
Geometric mean of runoff density (CFU/100 mL)	20,050	782	45,500	1313
E. coli				
Parameter	**Cattle**	**Swine**	**Chicken**	**Control**
Log_{10}(Minimum mobilization fraction)	-5.13	-1.55	-2.94	
Log_{10}(Maximum mobilization fraction)	-1.98	0.972	1.17	
Arithmetic mean of mobilization fraction	0.001988	2.62	3.02	
Geometric mean of mobilization fraction	0.000575	0.917	0.711	
Minimum runoff density (CFU/100 mL)	520	0.5	0.5	0.5
Maximum runoff density (CFU/100 mL)	1203300	310.6	54,750	6630
Arithmetic mean of runoff density (CFU/100 mL)	317,000	65.7	9390	553
Geometric mean of runoff density (CFU/100 mL)	208,500	9.17	1298	12.6
***E. coli* O157 surrogate**				
Parameter	Cattle	Swine	Chicken	Control
Log_{10}(Minimum mobilization fraction)	-3.65	-3.01	-4.01	
Log_{10}(Maximum mobilization fraction)	-0.20	-1.50	-2.21	
Arithmetic mean of mobilization fraction	0.141	0.0116	0.0026	
Geometric mean of mobilization fraction	0.0159	0.00664	0.00118	
Minimum runoff density (CFU/100 mL)	230,700	2130	118	
Maximum runoff density (CFU/100 mL)	47,600,000	508,000	1,402,000	
Arithmetic mean of runoff density (CFU/100 mL)	8,560,000	147,550	334,000	1429
Geometric mean of runoff density (CFU/100 mL)	2,760,000	32,100	7590	

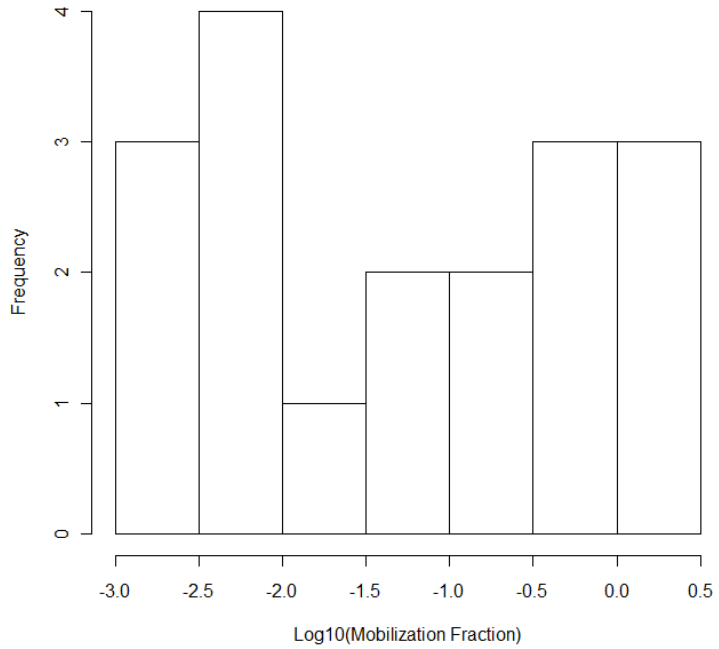

Figure 36. Histogram of mobilization fractions for enterococci from plots treated with cattle manure

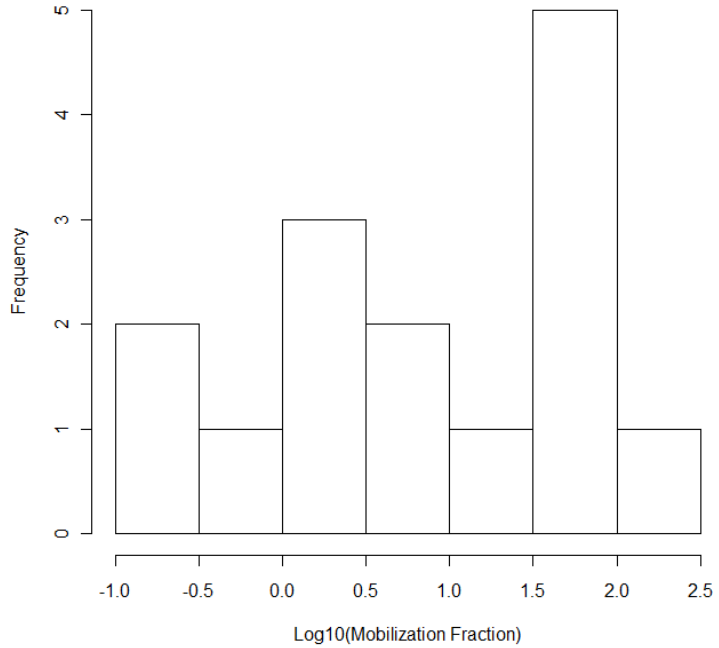

Figure 37. Histogram of enterococci mobilization fractions from plots treated with swine slurry

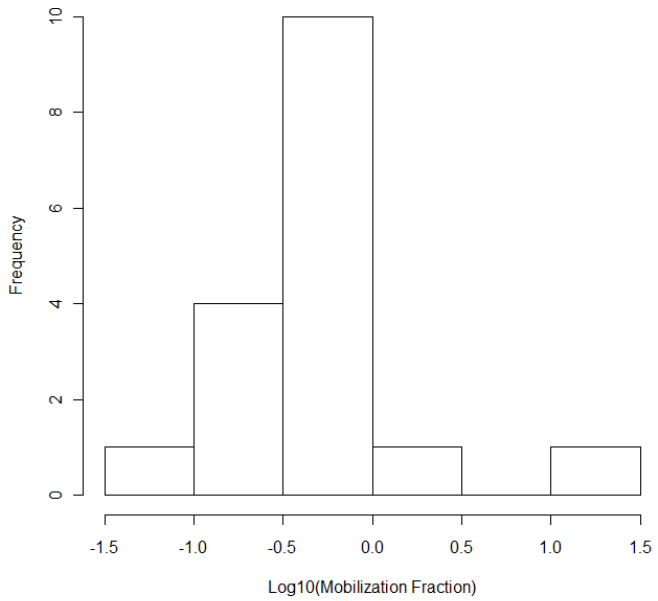

Figure 38. Histogram of mobilization fractions for enterococci from plots treated with poultry litter

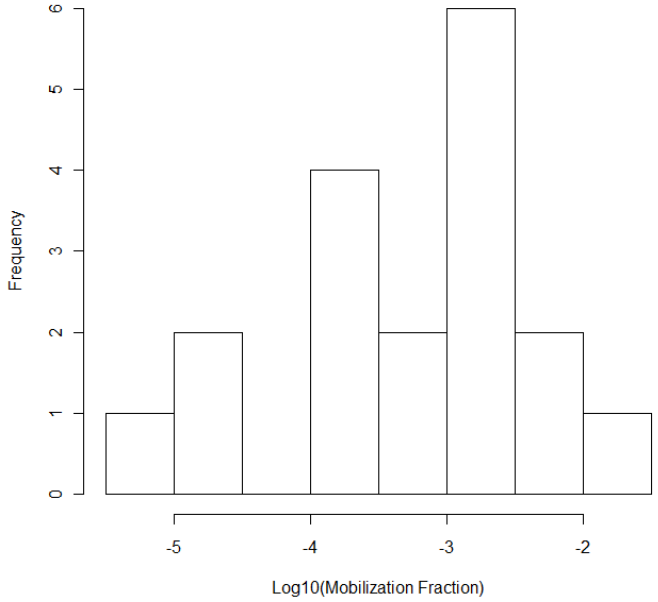

Figure 39. Histogram of *E. coli* (via Colilert) mobilization fractions for cattle manure plots

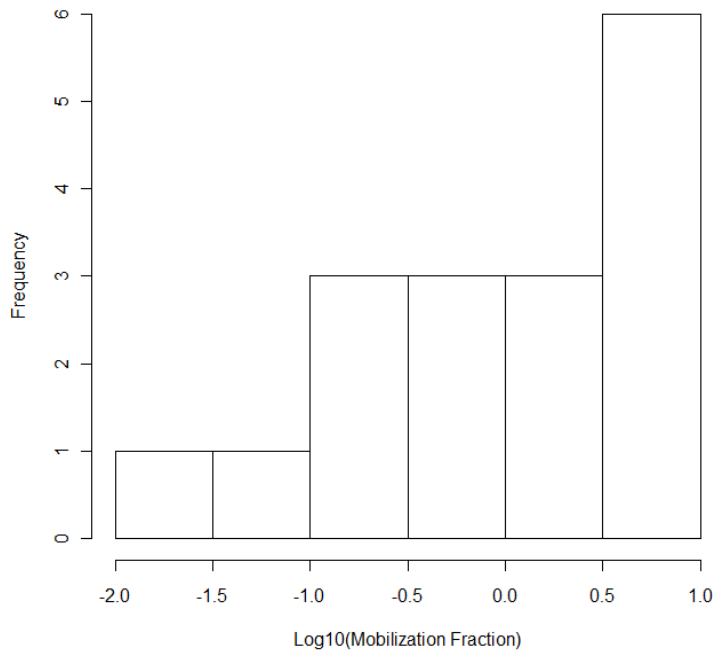

Figure 40. Histogram of *E. coli* (via Colilert) mobilization fractions for swine slurry plots

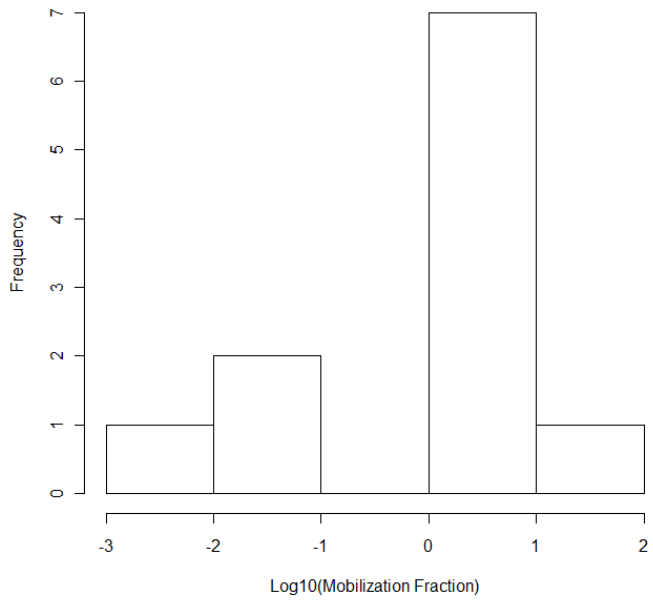

Figure 41. Histogram of *E. coli* (via Colilert) mobilization fractions for poultry litter plots

Inspection of the mobilization histograms suggests two alternative treatments for modeling mobilization and runoff of FIB. In the first alternative, manure FIB abundances and mobilizations are based on observations from the experiments, with mobilization fraction distributions based on inspection of the histograms in Figures 36 to 41. Parameters corresponding to this alternative are presented in Table 44. In QMRA calculations conducted

using these parameters (see Section 4.2), pathogen abundances drawn from the literature are paired with FIB abundances taken from experimental data. This mismatch renders results from alternative 1 specific to the experimental conditions of the mobilization experiments and not necessarily representative of general livestock runoff occurrences.

Table 44. Mobilization and abundance distributions, alternative 1

	Mobilization (Experimental)				**Manure Abundance (Experimental)**	
	Distribution	Min	Max	Mode	Min	Max
Enterococci						
Cattle	Uniform	-2.8	0.26		4.7	5.5
Swine	Uniform	-1.0	2.5		0.176	2.0
Chicken	Triangular	-1.25	0.32	-0.25	3.8	5.8
E. coli						
Cattle	Triangular	-5.0	-2.0	-2.75	6.7	8.3
Swine	Uniform	-2.0	1.0		0.70	3.1
Chicken	Triangular	-2.75	1.25	0.25	2.7	4.4

In the second alternative, *E. coli* O157 surrogate mobilization is used instead of mobilization distributions observed for *E. coli* and Experimental mobilization distributions are used for *Enterococcus* for cattle and poultry. Because no suitable data were available for characterizing *Enterococcus* mobilization in swine slurry no calculations were performed for that manure-inidcator combination. In this alternative, indicator abundances are based on the observations presented in the literature. Parameters corresponding to this alternative are presented in Table 45. This alternative has the advantage over alternative 1 of using consistent sets of abundances for pathogens and FIB and of basing mobilization fractions on only the organisms originating in the manures.

Table 45. Mobilization and abundance distributions, alternative 2

	Mobilization (Experimental for ENT, *E. coli* O157 surrogate values for *E. coli*)				**Manure Abundance (Literature)**	
	Distribution	Min	Max	Mode	Min	Max
Enterococci						
Cattle	Uniform	-2.8	0.26		2.0	5.1
Swine					4.6	4.8
Chicken	Triangular	-1.25	0.32		5.0	7.0
E. coli						
Cattle	Uniform	-3.65	-0.20		5.0	6.7
Swine	Uniform	-3.0	-1.5		6.1	7.3
Chicken	Uniform	-4.0	-2.2		5.1	10.9

Appendix F. Microbial Risk Assessment Interface Tool Simulation Images

The forward QMRA calculations were performed using MRAIT, a tool originally developed for estimating risks related to biosolids application. The tool was substantially modified for use in estimating risks associated with runoff from land-applied agricultural wastes and is in development for use in additional QMRAs. This appendix provides the output of MRAIT. These results are included in this report both to demonstrate the methodology used in the forward QMRA calculations and as an illustration of MRAIT.

Compared with other QMRA tools, MRAIT is intended for relatively easy use by users informed in QMRA methodologies but without extensive programming experience. As illustrated in the information below, users may rely on default assumptions for dose-response model parameters, source prevalences and abundances, and mobilization parameters. More advanced users may choose parameters based on additional data or on site-specifc data and knowledge.

Microbial Risk Assessment Interface Tool

Developed by EOA, Inc. through
WERF projects 00-PUM-3 and 04-HHE-3

Version 1.0

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MRAIT modifed September 2010 by Soller Environmental, LLC for US EPA to conduct P4 QMRA

Introduction

The Microbial Risk Assessment Interface Tool esimates the population risk of microbial infection or illness associated with ingestion of reclaimed water from various exposure scenarios.

This modified version is configured to estimate risk of infection or illness associated with recreational activities in agricultural impacted waters and POTW impacted recreational waters. The mobilization of microbes is based on field studies conducted by EPA in Athens, GA.

To run the model, below you will be asked to provide pathogen concentration data, and to specify a set of parameters that define the model. For an example case study and guidance in specifying the parameters, please refer to the user documentation that accompanies this tool.

A. Specify pathogen. The risk assessment will be performed for the following pathogen:

pathogen :=

- Norovirus
- Cryptosporidium
- Giardia
- Salmonella
- E. coli 0157

Note that MRAIT was not set up to run **Campylobacter or Norovirus.**

To run Camplylobacter select "E coli O157:H7" and change the dose response parameters below in section F.

MRAIT has been modified to run Norovirus. To run norovirus, select it from the list to the left, then follow instructions below in Section C.

B. Input data. Specify an input file with concentration data for the above selected pathogen.
The file must be a text file with no header, and contain one column of concentrations (**units of pathogens per liter**).

Abundance of reference pathogens in fecal sources (logs #g or #/100mL)

Pathogen	Cattle		Pigs		Chicken		Secondary Effluent	
	low	high	low	high	low	high	low	high
E. coli O157:H7	0.5	5.65	ND	5.6	0	0	-	-
Campylobacter	0	5.176	3.3	3.7	2.8	6.5	-	-
Salmonella	0.9	5.76	5	6.8	0.3	4.8	-	-
Cryptosporidium	0	3.9	4.2	5.4	0	0	-1	1.5
Giardia spp.	0	4.93	3.5	3.5	0	0	-1	2.1
Norovirus [1]	-	-	-	-	-	-	-2	6
Norovirus attenuation [2]	NA	NA	NA	NA	NA	NA	1	4

1. Density in raw sewage: triangular distribution with min, mode and max = (-2,4,6) logs
2. Attenuation through secondary disinfected effluent: triangular distribution with min, mode and max = (1,2.5,4) logs
3. Density in solid manures: units of log(#/g wet weight)
4. Density in liquid manures: units are $\log_{10}$(#/100 mL)

Cattle abundace		Pig abundance		Chicken abundance	
AC_EC_l := 0.5	AC_EC_h := 5.65	AP_EC_l := 0	AP_EC_h := 5.6		
AC_Camp_l := 0	AC_Camp_h := 5.176	AP_Camp_l := 3.3	AP_Camp_h := 3.7	ACh_Camp_l := 2.8	ACh_Camp_h := 6.5
AC_S_l := 0.9	AC_S_h := 5.76	AP_S_l := 5	AP_S_h := 6.8	ACh_S_l := 0.3	ACh_S_h := 4.8
AC_Cryp_l := 0	AC_Cryp_h := 3.9	AP_Cryp_l := 4.2	AP_Cryp_h := 5.4		
AC_G_l := 0	AC_G_h := 4.93	AP_G_l := 3.45	AP_G_h := 3.5		

Prevalence (%)						
Pathogen	Cattle		Pigs		Chicken	
	low	high	low	high	low	high
E. coli O157:H7	9.7	28	0.1	12	-	-
Campylobacter	5	38	46	98	57	69
Salmonella	5	18	7.9	15	0	95
Crypto	0.6	23	0	45	6	27
Giardia	0.2	37	3.3	18	-	-

Cattle prevalence

PC_EC_l := 9.7 PC_EC_h := 28

PC_Camp_l := 5 PC_Camp_h := 38

PC_S_l := 5 PC_S_h := 18

PC_Cryp_l := 0.6 PC_Cryp_h := 23

PC_G_l := 0.2 PC_G_h := 37

Pig prevalence

PP_EC_l := 0.1 PP_EC_h := 12.0

PP_Camp_l := 46 PP_Camp_h := 98

PP_S_l := 7.9 PP_S_h := 15

PP_Cryp_l := 0 PP_Cryp_h := 45

PP_G_l := 3.3 PP_G_h := 18

Chicken prevalence

PCh_Camp_l := 57 PCh_Camp_h := 69

PCh_S_l := 0 PCh_S_h := 95

PCh_Cryp_l := 6 PCh_Cryp_h := 27

Human Infectious Potential			
Pathogen	Cattle	Pigs	Chicken
E. coli O157:H7	H	H	-
Campylobacter	H	H	M
Salmonella	M	M	M
Crypto	H	L	L
Giardia	H	H	-

Cattle Infectious potential

HC_EC := 0.835

HC_Camp := .835

HC_S := 0.5

HC_Cryp := .835

HC_G := .835

Pig Infectious potential

HP_EC := .835

HP_Camp := .835

HP_S := 0.5

HP_Cryp := .165

HP_G := .835

Chicken Infectious potential

HCh_Camp := 0.5

HCh_S := 0.5

HCh_Cryp := .165

Pathogen	Morbidity (%)	
	low	high
E. coli O157:H7	20	60
Campylobacter	10	60
Salmonella	20	
Cryptosporidium	20	70
Giardia	20	70
Norovirus	30	80

MEC_l := 0.2 MEC_h := 0.6 MN_l := 0.3 MN_h := 0.8

MCamp_l := 0.1 MCamp_h := 0.6 MS := 0.2

MCryp_l := 0.2 MCryp_h := 0.7

MG_l := 0.2 MG_h := 0.7

Mobilization fraction of pathogens (log10 mobilization fractions)

Pathogen	Cattle		Pigs		Chicken	
	low	high	low	high	low	high
E. coli O157:H7	-3.65	-0.2	-3.01	-1.5	-4.01	-2.21
Campylobacter	-4.85	-1.46	-2.2	-1.01	-8.6	-1.74
Salmonella	-5.57	-1.26	-3.85	-2.4	-3.68	-2.65
Cryptosporidium	-4.46	-0.18	-3.9	-1.48	Not tested	
Giardia spp.	-6.4	-0.39	-4.58	-0.06	Not tested	

Note that mobilizations for each simulation are generated in a separate worksheet based on the above data. Those values are written to a file and read in below. It is assumed that pathogens are mobilized for each simulation at a random percentile of the log uniform distribution

Conversion factors to convert percent mobilizations to organisms per liter

$cat_convert := 27.64$ $pig_convert := .6909$ $chick_convert := 17.27$

Use input variables from above for these definitions highlighted in yellow

$Vals := 10000$

Number of random input values ("Vals") specified here is set at 10000 to be at least as great as the number of simulations that will be run.

$land_applied_abun := runif(Vals, AC_EC_l, AC_EC_h)$

$$Prevalence := runif\left(Vals, \frac{PC_EC_l}{100}, \frac{PC_EC_h}{100}\right)$$

$Human_inf := HC_EC$

$Morbidity := runif(Vals, MEC_l, MEC_h)$

For Salmonella, set Morbidity = 1 because dose response is based on illness not infection

$cattle_mobil :=$ $pig_mobil :=$ $chick_mobil :=$

$convert := cat_convert$

$mobilization := submatrix(cattle_mobil, 0, 9999, 0, 0)$

Need to select appropriate variable (cattle_mobil, pig_mobil, or chick_mobil, and set the third andfourth values to
0, 1, 2, 3, or 4 for
EC, Campy, Salmonella, Crypto Giardia, respectively

$$runoff := \overrightarrow{\left(10^{land_applied_abun} \cdot Prevalence \cdot convert \cdot Human_inf \cdot 10^{mobilization}\right)}$$

$conc := runoff$

Pathogen Details

C. Wastewater treatment. Specify what treatment processes will be applied. If the input data correspond to final effluent concentrations (i.e., no additional wastewater treatment applied), select "None" below:

treatmenttype :=

- Secondary treatment (activated sludge), filtration, and disinfection
- Filtration and disinfection
- Disinfection (combined chlorine)
- None - Effluent concentrations provided as input
- Other - User provides log reduction

For the animal impacted waters QMRA set the WWTP to **"None".**

For human impacted water norovirus assessment select **"Other"** and use a triangular distribution with values of (1, 2,5, 4) logs

The default inactivation and/or removal distribution for this treatment - pathogen combination is as follows in units of log reduction:

Distribution

Check below if you wish to override the defaults to the left:

☐ override defaults

Distribution:

- Normal
- Uniform
- Triangular
- Point Estimate
- Log Normal
- Gamma
- Negative Binomial

log removal:

D. Fit concentration data to a statistical distribution. Specify the form of the statistical distributional that will be used to fit the input concentration data specified above in Section A (default is Lognormal) OR specify that the input data are to be used directly

conc_choice :=

- Lognormal
- Weibul
- Use input data directly

For the animal impacted waters QMRA set "conc_choice" to "**Use input data directly**"

Exposure Details

E. Specify an exposure scenario: There are three alternative exposure scenarios built into this interface:

1. Crop irrigation assumes that exposure to pathogens occurs via ingestion of crops irrigated via reclaimed water.
2. Recreation assumes that exposure to pathogens occurs via ingestion of reclaimed water through recreational activities in an unrestricted impoundment.
3. Golf Course/Landscape Irrigation assumes that exposure to pathogens occurs via incidental or accidental ingestion of reclaimed water from a golf course or park.

exp_scenario :=

- Recreation
- Crop Irrigation
- Golf Course/Landscape Irrigation

For the animal impacted waters QMRA set exposure to "recreation"

Parameters for Recreation:

Recreation Exposure Pathway: (Note if recreation is not selected above as the exposure route of interest the following section is inactive and cannot be changed)

Specify the volume that is ingested per exposure event: (units of ml)

The default ingestion volume for an exposure event associated with recreational exposure:

Distribution: lognormal

log	2.92
log	1.43

Check below if you wish to override the defaults to the left:

☐ override defaults

Choose a distribution:

Normal
Uniform
Triangular
Point Estimate
Log Normal
Gamma
Negative Binomial

distribution values in units of ml:
(or ln(ml) if log normal distribution)

Other Exposure Routes

Dose Response Details

F. Specify dose-response function:

Reference Pathogen	Published dose-response model	Model parameters	ID_{50}	Morbidity (% of infections resulting in illness)	Health Endpoint
Cryptosporidium spp.	Exponential (U.S. EPA, 2005a, 2006)	0.09	8 oocysts	20–70%	Infection
Giardia lamblia	Exponential (Haas et al., 1999; Rose et al., 1991)	0.0199	35 cysts	20–70%	Infection
Campylobacter jejuni	Beta-Poisson (Medema et al., 1996; Teunis et al., 1996; 2005)	0.145 7.59	800 CFU	10–60%	Infection
E. coli O157:H7	Beta-Poisson (Teunis et al., 2008b)	0.4 37.6	207 CFU	20–60%	Infection
Salmonella enterica	Beta-Poisson (Haas et al., 1999)	0.3126 2884	23,600 CFU	20%	Infection

Note that the dose response parameters that were in the orignial MRAIT have been updated to reflect those shown to the left

default functional form and paramaters for this pathogen are:

Functional form: Beta-Poisson

a:	point	value:	0.37
b:	point	value:	37.65

Check below if you wish to override the defaults to the left:

☐ override defaults

Choose a functional form:

Exponential
Beta-Poisson
Hypergeometric
Gompertz-log

If you are overriding the function above you need to provide dose-response parameters:

Read from file (to the right)
Specify parameters manually (below)

If reading dose-reponse parameters from a file, specify the file here (see user documentation for guidance on the formating of this file):

(right-click on disk icon, and select properties to change the path and filename)

If specifying dose-response parameters manually, set them here:

Choose a distribution:

Specify parameters:

Normal
Uniform
Triangular
Point Estimate
Log Normal
Gamma
Negative Binomial

Choose a distribution:

Specify parameters:

Normal
Uniform
Triangular
Point Estimate
Log Normal
Gamma
Negative Binomial

G. Model selection suggestion: Based on values for specified exposure, dose, the dose-response function, and the following tolerance for error, the program will suggest either the static or dynamic model.

Specify your tolerance for error: Difference in predicted incidence between static and dynamic models

<10/100,000
<1/100,000
<0.01/100,000

per year

For the animal impacted waters QMRA this does not need to be specified. This is only needed if a dynamic model is being considered. The animal impacted QMRA uses a static model

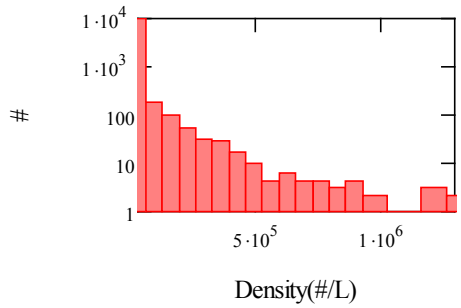
Specify the number of simulations you wish to run:

numsims :=

100
500
1000
5000
10000

I. Results

Histogram of runoff density for: E. coli 0157



Summary statistics for run off density:

Number of input points: conc_n = 10000

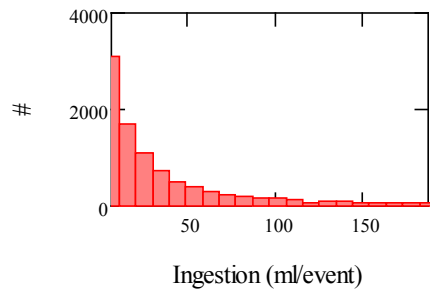
mean(conc) = 13075.96 min(conc) = 0.002

median(conc) = 62.24 max(conc) = 1.32×10^6

Histogram of exposure:

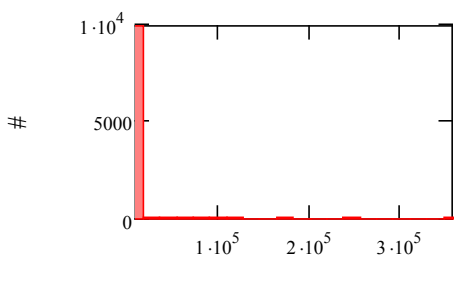
Recreation

ingestion: lognormal 2.92 1.43

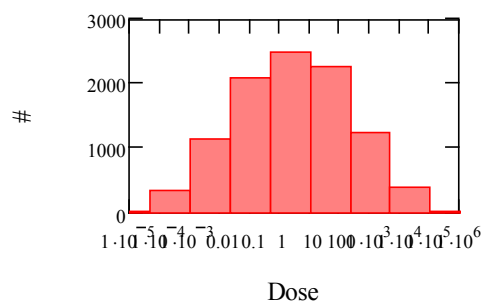


Histogram of dose:

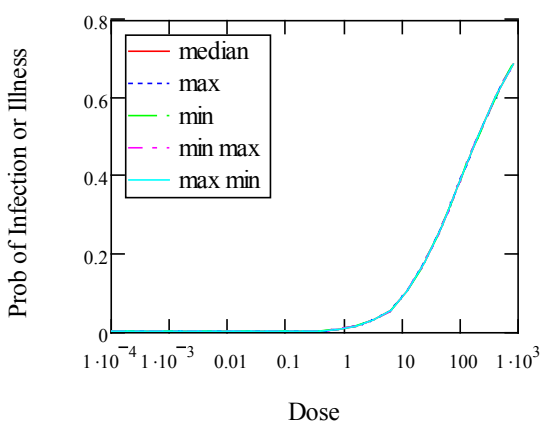
Linear scale



Log scale



Dose-response curve:



beta-poisson

point

value

$$\text{exponential_DR}(d, r) := 1 - \exp(-r \cdot d)$$

$$\text{betapoisson_DR}(d, \alpha, \beta) := 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}$$

$$\text{hypergeom_DR}(d, \alpha, \beta) := 1 - \text{mhyper}(\alpha, \alpha + \beta, -d)$$

$$\text{gompertz_log_DR}(d, x, y) := 1 - \exp(-\exp(-x + y \cdot \ln(d)))$$

Risk result:

Based on the Static model the distribution of the probability of infection per exposure event is:

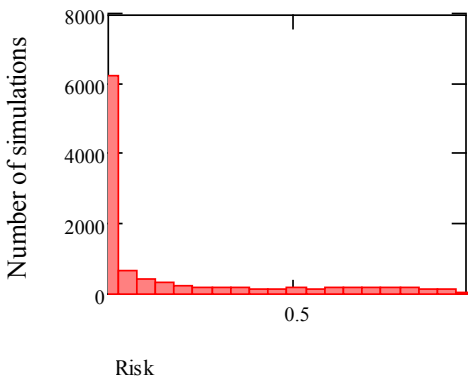
number

Note: Be careful in comparing static vs dynamic, and individual vs population-based results, as the description of risk shown in the box above will vary by scenario, depending upon static vs dynamic, individual vs population-based, and in some cases whether the dose-response for the pathogen of interest is expresed in terms of probability of illness or infection.

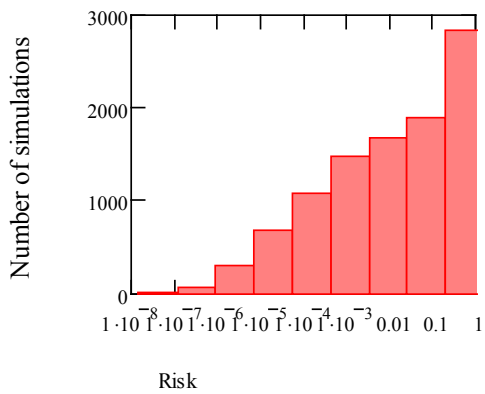
10th %ile:	3.2e-5
Median:	1.2e-2
Mean:	1.5e-1
90th %ile:	6.0e-1

Note: if the risk estimates are below 1e-9 per event (or per 100,000 for a population level assessment), they are simply reported as <1e-9.

Linear scale



Log scale



Illness Risk

$$\text{percentile}(\text{illness_prob}, 0.05) = 2.9 \times 10^{-6}$$

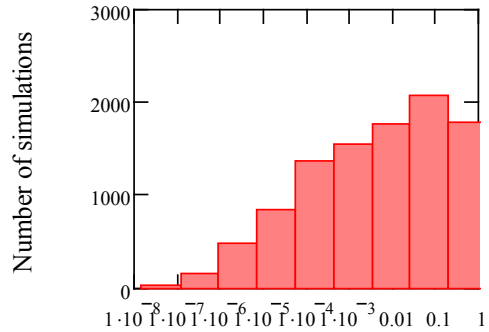
$$\text{percentile}(\text{illness_prob}, 0.1) = 1.2 \times 10^{-5}$$

$$\text{median}(\text{illness_prob}) = 4.388 \times 10^{-3}$$

$$\text{mean}(\text{illness_prob}) = 0.059$$

$$\text{percentile}(\text{illness_prob}, 0.9) = 0.221$$

$$\text{percentile}(\text{illness_prob}, 0.95) = 0.301$$



infection_prob

illness_prob

These commands send the simulation output to a file for archiving

ANNEX 1

State-of-the-Science Review of Quantitative Microbial Risk Assessment: Estimating Risk of Illness in Recreational Waters (Final Report – August 2010)

For

Quantitative Microbial Risk Assessment to

Estimate Illness in Freshwater Impacted by

Agricultural Animal Sources of Fecal Contamination

U.S. Environmental Protection Agency

December 2010

STATE-OF-THE-SCIENCE REVIEW OF QUANTITATIVE MICROBIAL RISK ASSESSMENT: ESTIMATING RISK OF ILLNESS IN RECREATIONAL WATERS

U.S. Environmental Protection Agency

Office of Water

Office of Science and Technology

Health and Ecological Criteria Division

August 2010

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ACRONYMS

ΔLOR	difference in log odds ratio
Ab+	antibody positive
AD	automatic differentiation
ANOVA	analysis of variance
AWQC	ambient water quality criteria
BEACH Act	Beaches Environmental Assessment and Coastal Health Act of 2000
BIC	Bayesian information criterion
CART	classification and regression tree
CDC	U.S. Centers for Disease Control and Prevention
CFU	colony forming units
CI	confidence interval
CSA	conditional sensitivity analysis
CSO	combined sewer overflow
CWA	Clean Water Act
DALY	daily adjusted life years
DEC	diarrhegenic (*E. coli*)
DGD	discrete growth distribution
DIC	deviance information criterion
DSA	differential sensitivity analysis
EHEC	enterohemorrhagic *E. coli*
EPA	U.S. Environmental Protection Agency
FIB	fecal indicator bacteria
FMD	foot and mouth disease (virus)
GI	gastrointestinal
HAV	Hepatitis A virus
ILSI	International Life Sciences Institute
mL	milliliters
MCMC	Markov Chain Monte Carlo
MRA	microbial risk assessment
NEEAR	National Epidemiological and Environmental Assessment of Recreational (Water Study)
NRC	National Research Council
NRSA	nominal range sensitivity analysis
PBBK	physiologically-based biokinetic
PCR	polymerase chain reaction
PFU	plaque forming units
POTW	publicly owned (sewage) treatment works
QMRA	quantitative microbial risk assessment
RWQC	recreational water quality criteria
TCID	tissue culture infectious dose
TMDL	total maximum daily load
TSS	total suspended solids
USDA	U.S. Department of Agriculture
UV	ultraviolet (light)

WHO	World Health Organization (United Nations)
WWTP	wastewater treatment plant
WQS	water quality standard(s)

EXECUTIVE SUMMARY

This report provides a "state-of-the-science" review of quantitative microbial risk assessment (QMRA) techniques for estimating the risk of illness from exposure to pathogenic microorganisms in recreational waters. QMRA is one component in a comprehensive toolbox being developed by U.S. Environmental Protection Agency (EPA) to support the implementation of new or revised recreational water quality criteria (RWQC).

INTRODUCTION

The Beaches Environmental Assessment and Coastal Health Act of 2000 (BEACH Act) requires EPA to publish new or revised RWQC. Historically, RWQC have been based on the results of epidemiological studies. These studies provide quantitative relationships between indicator organism densities and adverse health outcomes at those locations. To meet the requirements of the BEACH Act, EPA conducted several new epidemiological studies in coastal marine and freshwaters. EPA is evaluating the extent to which the relationships from these studies broadly apply to other waters covered under the Clean Water Act (CWA).

QMRA is one tool EPA could use to evaluate the applicability of the new epidemiology studies to other waters. To date, a limited number of QMRAs have been performed specifically for recreational waters. EPA's Office of Science and Technology (Health and Ecological Criteria Division) and Office of Research and Development requested this state-of-the-science review to consolidate and summarize the scientific literature on QMRA techniques applicable for recreational waters.

REPORT CONTENT

This report provides a detailed review of the technical literature associated with QMRA emphasizing recreational waters impacted by pathogens from cattle, swine, and/or poultry waste, including

- An overview of QMRA including a description of the features that differentiate microbial risk analysis from chemical risk analysis.
- A summary of the literature search strategy.
- A summary and comparison of the available QMRA studies of waterborne pathogens for recreational water exposures. These studies establish the current state-of-the-science with respect to QMRA for waterborne contaminants and provide insights into the techniques currently available for use in a QMRA of animal-impacted waters.
- A description of cutting-edge or novel techniques for use in exposure assessment, health effects modeling, and risk characterization. These techniques could expand the boundaries of the current state-of-the-science for QMRA in the near to mid-term future.

LITERATURE REVIEW RESULTS

Approximately 300 QMRA studies, review papers, and related literature were identified. Studies of limited relevance to recreational settings or of lower quality were excluded from detailed review. Drinking water studies focusing on the role of treatment process efficacy for determining relative risks were also excluded. This process resulted in 40 key studies that

- establish a list of key QMRA elements for supporting RWQC;
- identify the pathogens most commonly addressed in QMRAs;
- identify how variability has been addressed in QMRA studies;
- assess the frequency that secondary transmission is included in QMRAs; and
- compare the methods used for sensitivity analyses and risk characterization.

Additionally, the literature indicates that there are numerous techniques specific to exposure assessment, health effects modeling, and risk characterization that are novel or beyond the approaches typically used in QMRA studies. For each of these areas, this report provides an overview of "traditional" QMRA approaches followed by a summary and comparison of these techniques.

KEY FINDINGS

QMRA has been used under a wide variety of settings and scenarios and is useful in conditions where epidemiological studies are difficult, impractical, or cost-prohibitive. Moreover, QMRAs typically consider variability more comprehensively than other techniques for evaluating potential health hazards. Collectively, the following key findings can be drawn from these studies:

1. Most QMRAs focus on a limited number of waterborne pathogens. The two pathogens analyzed most frequently, human enteric viruses and *Cryptosporidium*, are both believed to be major contributors to risk of waterborne GI illness.
2. Temporal and spatial variability of pathogen density is difficult to characterize because of limited data.
3. A limited number of dose-response models are available and most studies do not account for variability and uncertainty in their dose-response models.
4. Secondary transmission of infection and immunity are typically not accounted for in risk estimates.
5. Most QMRAs do not differentiate between average (nominal) conditions and rare events. Rare events can be associated with higher levels of human health risk than nominal conditions.

I INTRODUCTION

I.1 BACKGROUND

A central goal of the Clean Water Act (CWA) is to protect and restore waters of the United States for swimming and other recreational activities. A key component in the CWA framework for protecting and restoring recreational waters is State adoption of water quality standards (WQS) to protect the public from illnesses associated with microbes in water. In this regard, one of EPA's key roles is to recommend ambient water quality criteria (AWQC) for recreational waters under Section 304(a) of the CWA) for subsequent adoption by States.

Historically, U.S. Environmental Protection Agency (EPA or the Agency) recommended AWQC have been based on fecal indicator bacteria densities. In the 1960s, the Federal government recommended fecal coliforms as the basis for AWQC for recreational waters. In 1986, EPA recommended enterococci and *E. coli* as the basis for the current criteria (U.S. EPA, 1986). These fecal indicator organisms do not cause human illness themselves (i.e., they are not human pathogens); rather, they are indicators of fecal contamination and therefore indicators of the potential presence of human pathogenic organisms (NRC, 2004).

It has been almost 25 years since EPA last issued AWQC for recreational waters. The science related to AWQC development and implementation has advanced significantly during this time. EPA believes that new scientific and technical advances need to be considered, if feasible, in the development of new or revised 304(a) criteria by 2012. To this end, EPA has been conducting research and assessing relevant scientific and technical information to provide the scientific foundation for the development of new or revised criteria. The enactment of the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 provided EPA with an opportunity to conduct new studies and provided additional impetus to issue new or revised criteria for coastal recreational waters (specifically, for Great Lakes and coastal marine waters) to replace or amend the 1986 EPA recommended criteria. EPA believes that the new or revised criteria must be scientifically sound, implementable for broad CWA purposes, and provide for improved public health protection over the 1986 criteria.

I.2 PURPOSE

As one aspect of developing new or revised AWQC, the Agency would like to consider extending the observed relationships between indicator organisms and adverse health outcomes as determined from discrete series of epidemiology studies to the broader set of waters covered under the CWA so that all waters of the United States are equivalently protective of public health. Additionally, once new or revised recreational AWQC are published, the Agency would like to provide States guidance on using quantitative microbial risk assessment (QMRA) as part of an implementation toolbox that could be used to ensure State water quality standards (WQS) are appropriately protective for local conditions and/or for developing WQS. To support that effort, the Health and Ecological Criteria Division within the Office of Water, in conjunction with the Office of Research and Development, requested the development of a "state-of-the-science" review for QMRA for estimating risk of illness resulting from exposure to fecal material of a variety of sources with a particular emphasis on animal-derived (cattle, swine, and poultry) waste. This report documents the results of that effort.

The approach employed to develop this report was to conduct a review of the technical literature to establish the state-of-the-science of QMRA with an emphasis on development of QMRAs for recreational waters impacted by pathogens from cattle, swine, and/or poultry waste.

I.3 REPORT CONTEXT

The attributes of QMRA to support the implementation of new or revised RWQC that may favor its use in conjunction with other health-based approaches include the following:

- QMRA methods explicitly account for variability and uncertainty in pathogen occurrence, exposure rates, and human health response;
- QMRA models may be used to evaluate alternative scenarios or potential management options; and
- risk estimates from QMRA models are accompanied by confidence intervals and sensitivity information that may be used to support risk management.

For the case of human exposure to animal-derived pathogens from recreational waters, the following features are consistent with the use of QMRA:

- the occurrence of pathogens varies widely with time, location in a water body, region, land use, and myriad other factors;
- the human health effects related to animal-origin pathogens may vary significantly between pathogen strains, serotypes, or isolates; and
- there are a large number of settings where exposure to waterborne pathogens of animal origin is possible and those settings may be widely diverse (e.g., some settings may be impacted primarily from dairy cattle while others by swine).

Although QMRA is a promising approach for analyzing risks associated with recreational water use, developing QMRAs of animal-impacted waters presents numerous challenges, some of which may be alleviated through application of the techniques and data assembled in this report. Challenges in developing QMRAs for animal-impacted waters arise in all aspects of the QMRA framework (ILSI, 1996, 2000), which includes exposure assessment, health effects modeling, and risk characterization.

Exposure assessment is complicated by the many and complex physical processes comprising the production and transport of animal-derived pathogens from farms to receiving waters and ultimately to humans. For example,

- pathogen production rates vary significantly between farms as well as among individual animals at a given farm;
- manure handling differs from farm to farm and from season to season—these practices have a profound effect on the availability of pathogens for transport to receiving waters;
- factors governing overland and subsurface transport of pathogens are, at present, not entirely understood, and rates are generally highly variable;

- inactivation (or growth) rates of pathogens varies with pathogen, media, and environmental conditions, and the data that are available to describe these processes are incomplete; and
- there may be multiple pathways by which pathogens reach receiving waters, some of which may be complex.

Furthermore, in developing a QMRA for animal-impacted waters, the complexity of flow paths by which pathogens reach receiving waters will require innovative modeling. Models must be sufficiently detailed to include factors to which risk estimates are sensitive, but simple enough that they can provide information to support risk management.

Complications in health effects modeling for QMRAs of animal-impacted waters relate to dose-response model data gaps and the potential secondary infections. Even among the select reference pathogens on which this report focuses, there is strain-to-strain variability, substantial uncertainty in low-dose infection rates, and variability in person-to-person sensitivity to infection.[1] It is also possible that pathogens of animal origin differ from those of human origin (e.g., in sewage) in their ability to infect humans. Although the importance of considering sensitive sub-populations in QMRA models is well recognized, dose-response models that would allow differentiation between these groups are generally lacking. While techniques for including secondary transmission in QMRA models are relatively well established, as discussed in the survey of published QMRAs, they are seldom included nor are parameter values well described.

The techniques and metrics used in risk characterization for QRMAs of animal-impacted waters will be critical for the effective use of QMRA risk estimates by risk managers and the scientific community at large. As described below, sensitivity analyses are often overlooked in risk assessments. It is expected that models for risk from animal-impacted waters will rely on many parameters and that multiple models will need to be evaluated. Studies reviewing techniques for sensitivity analysis and suggesting best practices are reviewed in this report to facilitate sensitivity analysis for animal-impacted waters.

I.4 REPORT ORGANIZATION

Section II of this report provides a brief overview of QMRA and focuses on the fundamental components of QMRA and the features that differentiate microbial risk analysis from chemical risk analysis. Section III first summarizes the literature search strategy and then reviews and compares QMRA studies related to waterborne recreational exposure. These studies establish the current state of the science with respect to QMRA for waterborne contaminants and provide insights into the techniques currently available for use in a QMRA of animal-impacted waters. Lastly, Section IV provides a description of cutting-edge or novel techniques for use in exposure assessment, health effects modeling, and risk characterization as related to QMRA. These techniques are likely to expand the boundaries of the current state of the science for QMRA in the near- to mid-term future. In this section, particular emphasis is placed on exposure assessment techniques that might be employed in the analysis of the complex and variable processes leading to ingestion of pathogens originating from animal feces.

[1] In this report, use of the term "infectious dose" is avoided because it is considered to be ambiguous. Where possible, the relative ability of pathogens to initiate infection is expressed in terms of specific doses such as ID_1 (the dose at which 1% of the exposed population is expected to become infected) or ID_{50} (the median infectious dose; the dose at which 50% of the exposed population is expected to become infected).

II QMRA BACKGROUND

Quantitative microbial risk assessment (also known as MRA and pathogen risk assessment) is a process that evaluates the likelihood of adverse human health effects that can occur following exposure to pathogenic microorganisms or to a medium in which pathogens occur (ILSI, 1996b, 2000). To the extent possible, the QMRA process includes evaluation and consideration of quantitative information; however, qualitative information is also employed as appropriate (WHO, 1999). QMRA methodologies have been applied to evaluate and manage pathogen risks for a range of scenarios including from food (Bollaerts et al., 2009; Nauta et al., 2005; Seto et al., 2007), sludge/biosolids (Dowd et al., 2000; Eisenberg et al., 2004, 2008; Flemming et al., 2009) ,drinking water (Astrom et al., 2007; Medema et al., 1995; Regli et al., 1991; Soller, 2009), recycled water (Asano et al., 1992; Westrell et al., 2003) and recreational waters (Ashbolt and Bruno, 2003; Soller et al., 2003, 2006, 2010a,b).

The principles, processes, and methods for conducting risk assessments for chemical agents were formalized in the early 1980s by the National Research Council (NRC) resulting in a four step process or framework (NRC, 1983). These are hazard identification, dose-response assessment, exposure assessment, and risk characterization. Many of the earliest MRAs employed the NRC conceptual framework to provide a structure from which the assessments could be conducted (Haas, 1983; Regli et al., 1991; Rose et al., 1991).

As the field of microbial risk assessment developed, it became clear that there were some complexities associated with modeling the infectious diseases that are unique to pathogens. Thus, there are features of microbial risk that necessitate use of techniques and data in different ways than in assessment of chemicals and other risks, and include:

- variations in the ability of individual organisms in a population of pathogens to initiate infection;
- wide variations in the susceptibility of human and animal hosts to infection—and even wider variation in expression of disease symptoms;
- risks of secondary (person-to-person) transmission of pathogens;
- growth of pathogens *in vivo* and for a smaller subset in the environment;
- high variability (spatial and temporal) in the occurrence of pathogens in the environment; and
- difficulty in recovery and enumeration of pathogens.

Therefore, the conceptual framework for chemicals may not always be appropriate for the assessment of risk of human infection following exposure to pathogens. To address this concern, EPA's Office of Water developed a conceptual framework in conjunction with the International Life Sciences Institute (ILSI) to assess the risks of human infection associated with pathogenic microorganisms (ILSI, 1996b). The Office of Water is in the process of developing a comprehensive document that describes tools, methods, and approaches for microbial risk assessment to support human health protection for water-based media. The EPA/ILSI framework emphasizes the iterative nature of the risk assessment process (Figure 1), and allows wide latitude for planning and conducting risk assessments in diverse situations. This framework consists of the following three principal components: problem formulation, analysis, and risk characterization. The analysis phase is further subdivided into the characterization of exposure and human health effects.

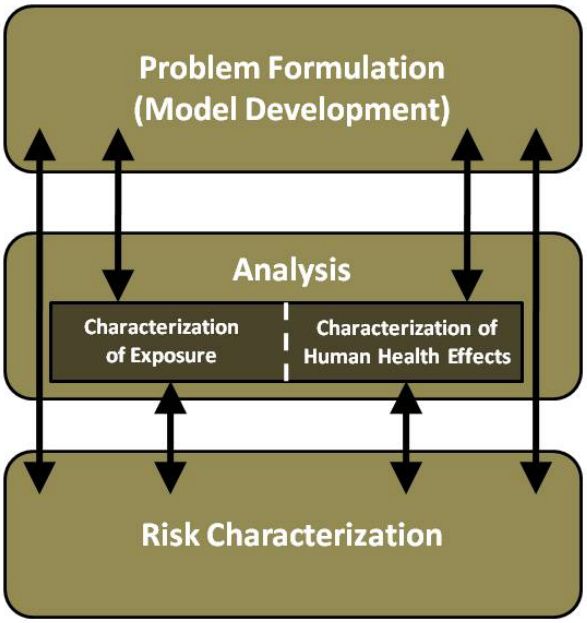

Figure 1. EPA/ILSI Generalized Framework for Assessing the Risks of Human Disease Following Exposure to Food- and Waterborne Pathogens
(SOURCE: Adapted from ILSI, 1996)

The problem formulation stage is used to identify (1) the purpose of the risk assessment, (2) the critical issues to be addressed, and (3) how the results might be used to protect public health. Once identified, initial descriptions of the exposure and potential health effects are described and then a conceptual model is developed. This conceptual model is used as a starting point for the analysis phase of the risk assessment and later as an interactive tool along with components developed in the analysis phase to initiate the risk characterization.

In the analysis stage, information about both the exposure and the health effects is compiled and summarized. This compilation of quantitative and qualitative data, expert opinion, and other information results in exposure and host/pathogen profiles that explicitly identify the data to be integrated into the risk characterization and the associated assumptions and uncertainties. These two elements, while separate, must also be sufficiently interactive to ensure that the results are compatible. Specific features of the analysis phase are shown in Figure 2.

The final stage, risk characterization, results in a statement of the likelihood, types, and/or magnitude of effects likely to be observed in the exposed population under the expected exposure scenario, including all of the inherent assumptions and uncertainties. Often, the risk characterization phase includes data integration through parameterization of a mathematical model, numerical simulation, and interpretation.

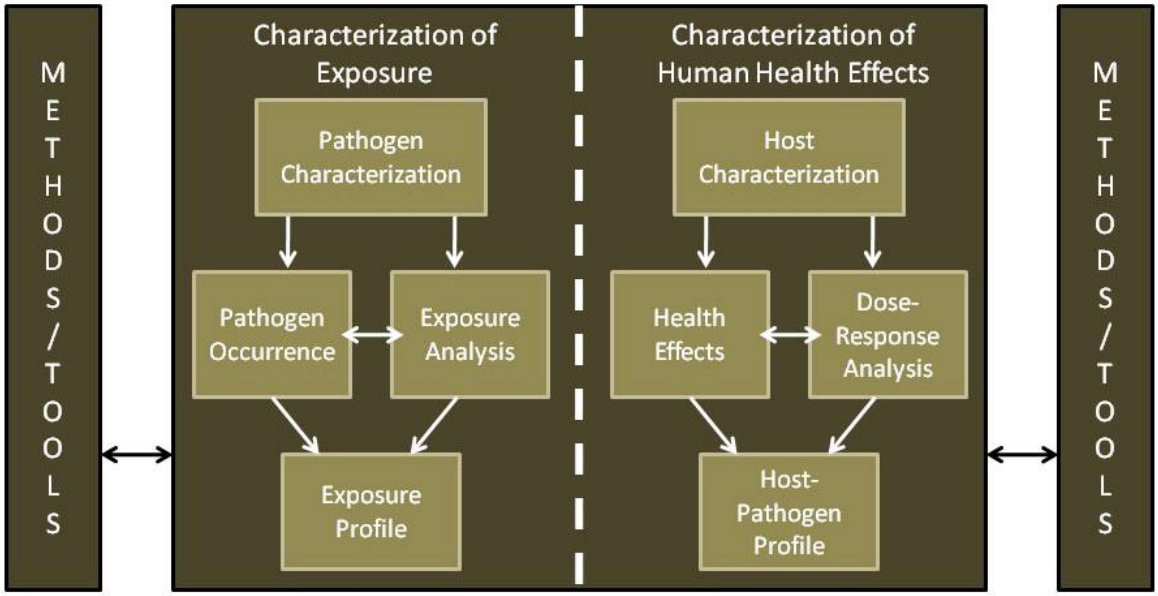

Figure 2. Elements of the Analysis Phase within the EPA/ILSI QMRA Framework (SOURCE: Adapted from ILSI, 1996)

III QMRA LITERATURE REVIEW: STATE-OF-THE-SCIENCE

A literature search (as detailed below) was performed to identify and obtain studies relevant to QMRA or its components—exposure assessment, human health effects assessment (including dose-response modeling), and risk characterization—as related to waterborne contaminants and with a particular emphasis on risks posed by animal-derived pathogens. The literature search also sought to identify the following:

- studies describing QMRAs or commonly used QMRA techniques relevant to characterizing risks from recreational water exposure; and
- studies describing novel or cutting-edge QMRA-related techniques.

The literature search strategy and results of the literature review are summarized below.

III.1 LITERATURE SEARCH STRATEGY AND SUMMARY OF RESULTS

A summary of the literature search strategy is presented in Table 1. Searches were made in the specified databases for the primary keywords with results narrowed, if necessary, by secondary keywords. Similarly, citation searches by the authors listed in Table 1 AND any one of the primary keywords were conducted in the Web of Science database, in some cases using secondary keywords.

Consistent with the approach used in a prior QMRA literature review (Soller et al., 2004), titles and abstracts of studies identified from the literature search were assessed to determine which studies were highly relevant. Highly relevant studies were assigned to the following categories according to the study type or the element(s) of QMRA studied:

- QMRA studies (i.e., a specific risk is estimated or a methodology for estimating a specific risk was explored);
- overviews and reviews;
- exposure assessment;
- dose-response modeling;
- sensitivity analysis;
- risk characterization; and
- application of Bayesian techniques.

III.2 DEVELOPING THE LITERATURE DATABASE

The literature search yielded more than 350 highly relevant studies. These studies were retrieved and reviewed. Based on review of these articles, more than 160 additional studies were identified for inclusion in the literature database. The additional studies were identified based on key references in papers reviewed during the first round of literature survey or to fill data gaps left open in the first round. Of the over 500 total studies acquired, more than 300 were related to the state-of-the-science of QMRA (QMRA studies, papers reporting new or advanced techniques, review papers, etc.). More than 200 contained data, analyses, or reviews related to the occurrence, abundance, fate, or hazard of animal-derived pathogens or to manure handling. The latter studies are not summarized in this report.

Table 1. Literature Search Strategy

Databases	Authors	Primary Keywords	Secondary Keywords
Current Contents	Ashbolt, N.	QMRA	Infection
Web of Science	Bollaert, K.	MRA	Pathogen*
Pubmed	Buchanan, R.L.	Microbial risk	Microbial*
Highwire	Cassin, M.H.	Risk assessment	Fecal
ASCE Civil Engineering Database	Edberg, S.C.	Exposure assessment	*Salmonella*
Environmental Engineering abstracts	Eisenberg, J.N.S.	Dose response	*E. coli* O157*
Water Resources abstracts	Englhardt, J.	Risk characterization	*Campylobacter*
	Gale, P.	Fecal pollution	*Cryptosporidium*
	Gerba, C.P.	Indicator bacteria	*Listeria monocytogenes*
	Haas, C.N.	Manure	
	Koopman, J.	Disease transmission	
	McBride, G.	Secondary transmission	
	Medema, G.J.	Sensitivity analysis	
	Messner, M.	Variability	
	Olivieri, A.	Susceptibility	
	Parkin, R.	Recreational water	
	Petterson, S.R.		
	Pouillot, R.		
	Regli, S.		
	Rose, J.B.		
	Roser, D.		
	Soller, J.A.		
	Teunis, P.F.M.		

III.3 QMRA: STATE-OF-THE-SCIENCE

The results of the literature review for QMRA and related studies are reported in this section. To make this task tractable, it was necessary to prioritize the review so that the most relevant and highest quality QMRA studies were examined in greatest detail. Narrowing the criteria for including studies meant excluding some high-quality studies in the food literature, such as studies primarily concerned with post-slaughter processes, the preparation of food products. For example, the large number of studies of *Listeria monocytogenes* growth in deli meat storage was not directly relevant to recreational waterborne exposure and was excluded. However, several studies providing novel techniques for incorporating growth of *Listeria monocytogenes* into exposure assessment are reviewed below (exposure assessment techniques). Drinking water studies that focused on the role of treatment process in determining risk were also excluded, although several studies that assessed the role of source water quality in finished drinking water risk were included.

The following objectives were established for reviewing the selected QMRAs:

- development of a list of QMRA studies from which to draw elements of future study designs;

- identification of the pathogens addressed in QMRAs and potential reasons the study authors selected those pathogens;
- identification how variability has been addressed in QMRA studies, particularly source variability and consumption variability;
- assessment of the tendency for risk analysts to include secondary transmission in the estimate of overall risk; and
- comparison of the practices used by different QMRA researchers and practitioners, particularly sensitivity analyses and risk characterization.

As noted in the preceding section, studies were added to the database that were cited in other studies but not identified in the initial literature survey. Doing so was important, given the tendency of authors to justify their choice of model structure and parameterization on the work and choices of prior researchers. A total of 40 studies meeting the criteria described above were selected for detailed review. The main exposure scenarios, indicators and pathogens evaluated, and dose-response models employed in these 40 studies are summarized in Table 2, while Table 3 includes synopses of these studies.

Based on the results of the literature review, it can be generally inferred that the utility of QMRA has been clearly demonstrated in a wide variety of scenarios. Moreover, (1) QMRA has been used in conditions where analysis by other techniques such as epidemiological methods would have been difficult or cost-prohibitive, and (2) QMRAs typically consider variability more comprehensively than alternative techniques for assessing potential health hazards. For example, many of the QMRAs reviewed for this report accounted for the variability in pathogen or indicator density by treating them as stochastic variables.

Several specific observations may be drawn from comparison of the QMRA studies. First, the assembled studies focused on a small subset of the pathogens potentially important in waterborne exposure during recreation. The two pathogens analyzed most frequently, rotavirus and *Cryptosporidium*, are both believed to be important contributors to risk of GI illness, primarily due to their ID_{10} (or other measure of low-dose infection), frequent occurrence in sewage, and, particularly for *Cryptosporidium*, relatively high persistence in environmental matrices. As reported in a recent review of outbreaks caused by waterborne viruses (Sinclair et al., 2009), norovirus and echovirus (along with adenovirus) have been implicated in the majority of outbreaks associated with waterborne viruses since the 1950s, making their absence from the list of pathogens analyzed in the QMRAs in Table 3 conspicuous.[2] Another potential reason for frequent selection of rotavirus and *Cryptosporidium* is the availability of peer-reviewed dose-response models based on oral ingestion. Numerous studies (Bastos et al., 2008; Eisenberg et al., 2004, 2006; Hamilton et al., 2006; Ottoson and Stenström 2003; Petterson and Ashbolt, 2001b; Soller et al., 2003, 2006) used rotavirus as a surrogate for enteric viruses. When considering the general public, this approach is conservative, given that rotavirus has a higher probability that a single organism can initiate infection than all other enteric viruses with known dose-response.[3] When considering children, use of a dose-response model developed based on experiments on adults may not yield a conservative estimate of risk.

[2] QMRAs have recently begun to address risk associated with norovirus (e.g., see Schoen and Ashbolt, 2010; Soller et al., 2010a,b). However, these studies are not summarized here as they were published after the literature review was conducted.
[3] Comparison with norovirus is not made here, as the recent dose-response model (Teunis et al., 2008) uses dose units in cell equivalents that precludes direct comparison with dose-response models based on culturable units.

Table 2. Topics Evaluated in QMRA Studies Used to Establish the State-of-the-Science

Exposure Scenario	No. of Studies	Pathogen	No. of Studies	Dose-Response Model	No. of Studies
Water, non-drinking	16	*Cryptosporidium*	17	Exponential	19
Water, drinking	15	Rotavirus	15	Exact beta-Poisson	2
Food	8	*Giardia*	8	Approx. beta-Poisson	17
Soil	8	*Campylobacter* spp.[1]	7	Beta-binomial	2
Aerosol	2	*Salmonella enterica*[2]	6	Empirical function of enterococci density	2
Fomite	1	Enterovirus	5	Log-normal	2
		Adenovirus	4		
		C. jejuni	3		
		E. coli O157	2		
		Fecal indicator bacteria[3]	2		
		E. coli[4]	2		
		Other[5]	1		

[1] Includes all *Campylobacter* studies; studies dealing specifically with *C. jejuni* are also included
[2] Includes all *Salmonella* studies; the sole study dealing with *Salmonella enterica Typhimurium* is also included
[3] Not pathogenic
[4] Excludes *E. coli* O157
[5] *Ascaris lumbricoides*, coronaviruses, diarhheagenic *E. coli*, enterohemorrhagic *E. coli*, foot and mouth diseases (FMD) virus, hepatitis A virus (HAV), *Listeria monocytogenes*, *Salmonella enterica* Typhimurium, sanitary conditions.

The mismatch between pathogens potentially present in a particular setting and those chosen for analysis in QMRA will only be resolved if the scientific community develops dose-response models for other enteric viruses. Given the costs and ethical concerns related to human (feeding trial) studies, it is unknown if additional studies will be conducted. Thus, further insights may be limited to animal feeding studies (pending improved understanding of interspecies differences in dose-response) or on the novel dose-response model development techniques described in Section IV below.

Second, modeling variability in pathogen source density appears to be hampered by scarcity of both data and analysis techniques. The two most common methods for accounting for source variability among the studies are (1) use of empirical distributions for pathogen density based on relatively short time series, and (2) assumption of log-normal distribution of pathogen densities. Drawbacks to use of empirical distributions are inconsistency in sampling strategies used to develop databases, frequent non-detects, and, most importantly, constraint of pathogen densities to those observed in a limited number of samples. In sampling from a set of observations to account for pathogen density variability, the estimates for pathogen density are constrained to the highest and lowest observed values. This constraint prevents consideration of rare events associated with potentially high risk, such as severe/chronic adverse health outcomes.

Use of distributions to describe pathogen density in sources overcomes the constraints associated with use of empirical distributions. Among the studies reviewed for this report, many studies

employed point estimates for pathogen source density. Among studies using distributions to describe pathogen variability, the following distributions were employed: normal, triangular, log-normal, negative binomial, uniform, and Poisson. Use of distributions to characterize pathogen source variability raises difficulties in accounting for frequent non-detects of pathogens. Pathogen occurrence tends to be tied to sporadic events such as rainfall events of a particular magnitude (Signor and Ashbolt, 2006) or the occurrence of outbreaks among humans or animal populations. As such, pathogen time series are characterized by frequent non-detects. One study that accounted for non-detects in development of a distribution for pathogen occurrence (Petterson and Ashbolt, 2001b) divided pathogen observations into non-detects and detects and then fit the pathogen densities from samples in which pathogens were detected separately.

Third, most of the studies reviewed in this report employ identical dose-response models but do not account for variability and uncertainty in dose-response model parameters. As with variability in exposure, this observation highlights the fact that high quality and diverse dose-response model data are not available. The use of a small number of dose-response models may indicate that some QMRA modelers choose the pathogens to model based on the availability of dose-response models. Lack of dose-response models for many pathogens of public health concern and for differing routes of exposure is a major data gap. The need for dose-response models corresponding to different exposure routes (i.e., ingestion, inhalation, etc.) arises from the ability of some waterborne pathogens (e.g., adenovirus) to initiate infection via multiple routes. The exact beta-Poisson relationship was seldom used, as were empirical models popular in food dose-response studies (e.g., as described in Buchanan et al., 2000; Moon et al., 2004). Variability in dose-response model parameters or in response of the exposed population are rarely considered or addressed. A likely cause for the latter is that dose-response model studies do not consistently provide confidence intervals for model parameters and seldom present quantitative information on the distribution form for parameter estimates. Another technique for including variability of population response into risk estimation would be to assume population response to be binomially or beta-binomially distributed. Alternatively, different dose-response models might be used for sensitive and non-sensitive populations, as demonstrated for *Cryptosporidium* by Pouillot et al. (2004).

Finally, secondary transmission and (temporary) immunity are often neglected in risk estimation. Several studies (Eisenberg et al., 2004, 2008; Soller et al., 2006; Soller and Eisenberg, 2008; Soller, 2009) have demonstrated that consideration of secondary transmission and immunity can significantly influence overall risk associated with exposure to pathogens and often in unintuitive ways.

Table 3. Summary of Select Published QMRA Studies

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
An et al. (2007)	Occupational risk associated with reuse water in rice paddies	*E. coli*	*E. coli* concentrations used in Monte Carlo simulations drawn from distributions of data from experimental studies; the authors indicate *E. coli* concentration distribution was normal without justification or details in parameter estimation. Number and timing of irrigation events not stated.	Not adequately described; based on 1000-fold and 10,000-fold reduction in volumes associated with "direct ingestion"	Beta-Poisson model for *E. coli* (Haas et al., 1999) (α = 0.1778, N_{50} = 8.60×10^{7}) 50% of infected persons assumed to develop illness	Not considered	Not reported
Ashbolt and Bruno (2003)	Risk of GI illness and respiratory illnesses associated with recreational waters	Enteric viruses and adenovirus	The ratio of pathogens to enterococci was assumed relatively constant; data on enterococci collected during the study and reported as number of samples meeting a compliance criterion.	50 mL fixed volume assumed	Exponential dose-response model with r = 1 for enteric viruses. Adenovirus dose-response model (r = 0.417) for respiratory illness-associated viruses	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Bastos et al. (2008)	Risk of infection associated with consumption of crops irrigated with reclaimed wastewater	Rotavirus, *Campylobacter*, *Giardia*, *Cryptosporidium*	Point estimates for risk made using ranges of pathogens drawn from measurement of concentration in treatment effluent and using empirical relations for retention of bacteria on food crops. Range of concentrations 0.1 to 1 organisms per 10^5 *E. coli* bacteria for rotavirus and *Campylobacter* and 0.01 to 0.1 organisms per 10^5 *E .coli* bacteria for *Giardia* and *Cryptosporidium*.	Based on statistics drawn from official Brazilian sources. The authors discriminated between low and high income persons.	Beta-Poisson model for rotavirus (α = 0.253, N_{50} = 6.17) Beta-Poisson model for *Campylobacter* (α = 0.145, N_{50} = 896) Exponential model for *Giardia* (r = 0.0199) and *Cryptosporidium* (r = 0.0042).	Not considered	Not reported
Charles et al. (2003)	Reduction in risk in occurrence of pathogens in raw drinking water associated with buffer distances between septic system and receiving waters	Enteric viruses (adenovirus, enteroviruses, reoviruses, norovirus, rotavirus, HAV virus), pathogenic protozoa	Distributions based on data from monitoring	Not considered	Not considered	Not considered	Sensitivity analyses were planned; the impact of factors such as septic system management and disinfection on risk to be evaluated.

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Diallo et al. (2008)	Risk of GI illness due to direct ingestion of canal waters during recreation or other activities, or due to consumption of raw crops irrigated with canal waters	*Cryptosporidium*, *Giardia*, and diarrhegenic *E. coli* (DEC)	8% of measured *E. coli* assumed DEC; all pathogen distributions assumed triangular, with median, lowest, and highest values based on data collected in the study	Ingested volumes of water 100 mL and 50 mL for 2 scenarios; soil ingested masses 10 and 100 mg	Beta-Poisson model for DEC (α = 1778, N_{50} = 8.60×10^{7}) Exponential model for *C. parvum* (r = 0.00467) Exponential model for *Giardia* (r = 0.0198)	Not considered	Multiple point values corresponding to different assumptions assessed
Dowd et al. (2000)	Risk of infection for workers and near-neighbors during application of biosolids	Rotavirus, coronavirus, *Salmonella* spp., *E. coli*	Pathogen concentration at point of ingestion estimated based on Gaussian dispersion models for point sources and areal sources; release rates from sources based on experimental measurements of aerosol transport and abundance of pathogens in aerosols of biosolids.	Based on an assumed normal inhalation rate of 8 m^3/day	Exponential model for Coxsackievirus B3 (r = *0.2532*) and beta-Poisson model for *Salmonella* typhi (α = 0.3126, N_{50} = 2.3×10^{4})	Not considered	Point estimates corresponding to a range of distances from the source, wind velocities, and durations of exposure

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Eisenberg et al. (2004)	Risk of infection (including secondary transmission and immunity) from direct ingestion of soils amended with biosolids	Enterovirus	Pathogen density in raw sludge was not reported; % pathogens present in biosolids (applied) ranged from 0.1% to 0.5% of pathogens in raw sludge; simulations considered enterovirus removal in treatment and subsequent survival in the environment	1 to 200 mg of soil per day or less (three point values)	Approximate beta-Poisson, with α ranging from 0.126 to 0.5 and β ranging from 0.21 to 0.84	Secondary transmission considered, including the possibility that individuals were in an immune state	Simulations run for high, medium and low values of most model parameters. Results analyzed via CART (classification and regression tree analysis)
Eisenberg et al. (2008)	Risks associated with direct ingestion, aerosol exposure, and groundwater exposure to pathogens associated with applied biosolids	Rotavirus (as a surrogate for enteric viruses)	Raw sludge enteric virus concentration assumed to be log-normally distributed; pathogen removal in treatment calculated based on models of treatment processes and found to be linearly-related to retention time	Direct ingestion rate assumed 100 mg/day. An ingestion rate of 1.L per capita per day assumed for groundwater ingestion. An average breathing rate of 0.83 m^3/hr and exposure time of 8 hours used for aerosol exposure.	Beta-Poisson (α = 0.26, N_{50} = 5.62; originally reported as β = 0.42 plaque forming units (PFU)	Secondary transmission considered; secondary transmission estimated via a deterministic compartmental transmission model	Estimated risk associated with 3 different sludge treatments compared

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Flemming et al. (2009)	Human health risk from oral or aerosol ingestion of soils amended with fresh or stored biosolids	*Campylobacter* spp., *Salmonella* spp., *Cryptosporidium* spp., *Giardia* spp., *Clostridium perfringens*	Pathogen data fit to log-normal distributions via censored regression	Soil ingestion rate for children: log-normal distribution with geometric mean of 35 mg/d and standard deviation of log-transformed ingested volumes 3.94 Aerosol ingestion based on average breathing rate of 0.83 m^3/h and aerosol concentration based on modeling	Gompertz-log model for salmonellosis (see Table 4) with β = 2.148 and α distributed uniformly from 29 to 50. Exponential model for *Cryptosporidium* infection, with *r* uniformly distributed from 0.04 to 0.16 Exponential model for Giardia infection with *r* = 0.0199 Lognormal model for *Clostridium perfringens* with mean of lognormal = -24.7 and standard deviation of the lognormal = 2.32.	Not considered	Stochastic model used and risk estimates presented with CIs Model sensitivity tested via assessment of alternate scenarios Relative importance of pathogens in overall risk assessed

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Gale (2005)	Risk of infection through consumption of root crops grown on agricultural lands where treated sewage sludge is applied	Non-typhi salmonellas, *Campylobacter jejuni* (strain A3249), *Listeria monocytogenes*, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia,* enterovirus	Arithmetic mean values for pathogen concentration in raw sewage used along with an event tree approach to estimate post-treatment pathogen concentrations. Removal in all phases of treatment assumed known and removal fractions for the processes based on data drawn from published studies. The exposure model accounts for decay after application and removal via washing prior to consumption.	Proportion of the population consuming treated sewage irrigated crops and mean consumption masses taken from European Union and United Kingdom published data	Beta-Poisson model for non-typhi *salmonellas* (α = 0.3136, N_{50} = 24,420), *Campylobacter* (α = 0.15, N_{50} = 795), *Listeria monocytogenes* (α = 0.17, N_{50} = 2.1×10^6), *Escherichia coli* O157 (α = 0.16, N_{50} = 1130), and rotavirus (α = 0.265, N_{50} = 5.6). Exponential model for *Giardia* (r = 0.0199) and *Cryptosporidium* (r = 0.00419).	Not considered	No formal sensitivity analysis performed; assessments made of the model's major uncertainties
Gerba et al. (1996)	Risk of rotavirus infection from recreational and drinking water exposures	Rotavirus	Drinking water concentrations estimates were 0.004 PFU/L and 100 PFU/L, based on review of the occurrence of rotavirus in drinking waters and surface waters and assuming 99.99% removal in treatment; surface water concentrations estimated to be 0.24/L and 29/L (the occurrence range)	Ingested volumes used 100 mL for recreational exposure, 2 L for child and adult drinking water exposure, and 4 L for elderly drinking water exposure	Beta-Poisson dose-response model (α = 0.26, N_{50} = 5.62) used for risk of infection. Risk of clinical illness was assumed 0.5 $\times$ risk of infection. Fraction of illnesses progressing to mortality assumed 0.1% for the general population and 1.0% for the elderly	Secondary transmission rates discussed, but details on calculations not provided	Risks corresponding to high and low concentration s in drinking water and recreational waters presented

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Giannoulis et al. (2005)	Risk of contamination of a groundwater drinking water source	Fecal coliforms; sanitary survey results were also factored into risk determination	Risk based on frequency distribution of combined sanitary survey and fecal coliform monitoring results	Not applicable	Risk considered low when sanitary survey score less than 3 (scale of 1 to 10, with 10 being high risk) and fecal coliform count was in category "B" or "A" (scale of A to E, with E being the highest fecal coliform count)	Not applicable	Not reported
Hamilton et al. (2006)	Risk of enteric virus infection associated with consumption of raw vegetables with non-disinfected secondary treated reclaimed water	Rotavirus (as a surrogate for enteric viruses)	Virus concentration in irrigation water assumed log-normally distributed, with the mean and standard deviation based on experimental data. Volume of irrigation water retained after irrigation was assumed log-logistic distributed for broccoli, normally distributed for lettuce and cucumbers, and based on empirical data for cabbages. Inactivation of viruses between irrigation and harvest was assumed to follow first-order kinetics; two estimates for inactivation rate parameter were used.	Ingested mass based on empirical probability density function for consumption of 4 foods drawn from U.S. Department of Agriculture (USDA) reports	Beta-binomial model (α = 0.167, β = 0.191; based on fits of data to the beta-Poisson model)	Not considered	Spearman rank correlation for input variables

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Jolis et al. (1999)	Risk of cryptosporidiosis associated with exposure at parks and golf courses irrigated with tertiary reclaimed water.	*Cryptosporidium parvum*	Concentration of *Cryptosporidium parvum* in tertiary effluent set to the arithmetic mean of 6 samples (variability not reported or considered). Concentration in treated secondary effluent taken as 2 logs less than the mean of 3 samples of secondary effluent.	Assumed golfer and park user ingested volume of 1 mL per outing	Exponential *Cryptosporidium parvum* model (r = 0.00467, 95% CI <0.00195,0.0962>, no information on distributional form assumed for r) Ratio of illness to infection set at 0.5.	Not considered	Not reported; authors critically assessed findings in their study and characterized the study as preliminary
Julian et al. (2009)	Risk of rotavirus infection from multiple exposure routes for a child 6 years of age or younger; exposure routes were fome-to-mouth, fome-to-hand, and hand-to-mouth	Rotavirus	Virus density on fomite assumed uniformly distributed (0.001 to 10 virus/cm^2); Inactivation rate on fomite and hands assumed normally distributed (different mean and standard deviation for fomite and hand distributions).	Transfer efficiency from fome to mouth and hand-to-mouth assumed normally distributed with a mean of 41% and a standard deviation of 25%. Transfer efficiency from fome to hand was assumed normally distributed with a mean of 36% and a standard deviation of 26%.	Beta-Poisson dose-response model (α = 0.26, N_{50} = 5.62) used for risk of infection.	Not considered	Model run with a parameter set to either the 25th or 75th percentile value of its distribution and all other parameters at the median value. Sensitivity to a parameter is assessed based on the ratio of the p25 to the p75 estimated risks.

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Makri et al. (2004)	Risk of cryptosporidiosis from ingestion of drinking water	*Cryptosporidium parvum*	Concentration in source water assumed Poisson-distributed. Oocyst viability assumed beta-distributed. Oocyst recovery in monitoring assumed beta-distributed.	Water consumption taken from empirical data; 5th and 95th percentiles used	Lognormal dose-response for infection endpoint (μ = -5.48, σ = 0.3502), beta-distributed probability of illness given infection, probability of prolonged illness given illness = 0.15.	Not considered	No formal analysis performed
Mara et al. (2007)	Risk of rotavirus infection associated with use of wastewater for restricted and unrestricted crop irrigation	Rotavirus, *Campylobacter, Cryptosporidium*	Concentrations used in risk estimation based on *E. coli* occurrence and ratio of *E. coli* to *Cryptosporidium* and *Campylobacter*. Low and high values for *E. coli* used for point estimates. For mechanized agriculture, low and high values 10^5 and 10^6 *E. coli* per 100 g soil and for labor-intensive agriculture, low and high values 10^4 and 10^5 *E. coli* per 100 g soil.	Ingestion assumed for soil particles from lands irrigated with reclaimed wastewater. In highly mechanized agriculture, ingestion rates assumed 1 to 10 mg / day for 150 days. In labor intensive agriculture ingestion 10 to 100 mg/day for 300 days.	Beta-Poisson model for rotavirus (α = 0.253, N_{50} = 6.17) and *Campylobacter* (α = 0.145, N_{50} = 896) and exponential model for *Cryptosporidium* (r = 0.0042).	Not considered	Variation in risk with estimated wastewater quality evaluated

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Ottoson and Stenström (2003)	Risk of infection from reclaimed water in direct consumption, use of fields irrigated with reclaimed water and use of groundwater influenced by reclaimed water	Rotavirus, *Salmonella typhimurium*, *Campylobacter jejuni*, *Giardia lamblia*, *Cryptosporidium parvum*	Concentrations of pathogens in greywater were assumed proportional to measured coprostanol concentration with the proportionality derived from epidemiology studies. Coprostanol concentration assumed log-normally distributed in greywater. Die-off of pathogens assumed first order in all pathways	Accidental ingestion of 1 mL of untreated greywater or 1 mL treated greywater; ingestion of 1mL per day for 26 days/yr; consumption of groundwater (volume not reported).	Beta-Poisson model for rotavirus (α = 0.265, N_{50} = 5.6), *Campylobacter jejuni* (α = 0.145, N_{50} = 896) and *Salmonella* typhimurium (α = 0.3126, N_{50} = 23,600). Exponential model for *Giardia* (r = 0.0199), *Cryptosporidium* (r = 0.00419) and fecal enterococci (r = 0.00565).	Not considered	Regression analysis of risks predicted using two models
Parkin et al. (2003)	Risk of enterovirus infection to sensitive population via recreation in waters receiving wastewater treatment plant (WWTP) effluent; study was a data collection and problem formulation effort.	Coxsakievirus A and B, echoviruses, human enteroviruses and polioviruses	Anecdotal data on virus occurrence in swimming waters reported, but no characterizations of temporal variation in viruses found in a literature search.	Not considered	Epidemiology studies indicate that children are at greater risk than adults for enterovirus infection. The effects of dose-response and exposure not differentiated. The authors noted there are no known dose-response relations for children.	Not considered	Not relevant

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Petterson et al. (2001a)	Risk of viruses on lettuce irrigated municipal secondary treatment effluent	Enterovirus (with rotavirus as a representative virus)	Positive (detected) secondary effluent enteric virus concentrations were fit to log-normal and Gaussian-kernel type distributions. 27% of samples were below the detection limit. Decay of viruses on lettuce assumed to follow first order decay with the inactivation rate assumed normally distributed; distribution parameters based on measurements with B40-8 phage.	Mass ingested per lettuce consumption event 100 g.	Rotavirus dose-response model used; parameters and assumptions regarding their distributional form not provided	Not considered	Not reported; stochastic analysis

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Pouillot et al. (2004)	Risk of cryptosporidiosis via consumption of treated drinking water	*Cryptosporidium parvum*	In the first scenario, *C. parvum* density in source water fixed at values reported by local health authorities (in oocysts per 100 L finished water (1, 0, 0, 0, 4, 2, 0, 1, 0, 77, 1) and density assumed Poisson distributed. In a second scenario, *C. parvum* density in source water assumed negative-binomially distributed	Distribution of ingested volumes based on empirical data drawn from a study of French participants	Immunocompetent population: exponential dose-response model, $r = 5.26\times10^{-3}$, 95% CI [2.88×10^{-3}, 10.9×10^{-3}] Immuno-compromised population: exponential dose-response model, $r = 0.354$, 95% CI [0.221, 0.612] Probability of illness given infection assumed beta-distributed, with p = beta(9,11), based on experimental data.	Not considered	A second-order Monte Carlo method used to estimate risk and confidence intervals for all risks; this allowed evaluation of sensitivity to uncertain parameters
Roberts et al. (2007)	Risk of cryptosporidiosis associated with fishing in an urbanized stream reach	*Cryptosporidium*	Number of oocysts ingested per month via hand-to-mouth transmission or in consumption of fish assumed Poisson-distributed. Distribution parameters estimated using occurrence of oocysyts in hand-washings and on fish.	Not calculated separately from pathogen concentration estimate	Exponential (r = 0.00419); the dose-response parameter was treated as a random variable, though the distributional form used is not reported	Not considered	Sensitivity analysis results reported, but details of the method not provided

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Ryu and Abbaszadegan (2008)	Risk of cryptosporidiosis and giardiasis from drinking treated surface waters	*Cryptosporidium, Giardia*	Oocyst and cyst concentrations equated to mean daily concentrations for seasonal and annual estimates	2 L per capita per day	Exponential model for *Giardia* (r = 0.0199), *Cryptosporidium* (r = 0.00419). Fraction of pathogens recovered capable of initiating infection 0.41 for *Cryptosporidium* and 0.22 for *Giardia*.	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Schijven et al. (2005)	Risk of foot and mouth disease (FMD) virus infection in cattle consuming surface waters into which partially or untreated milk contaminated with FMD has been discharged	FMD virus	The route of infection assumed discharge of contaminated milk into a sewer system, inactivation of FMD virus, discharge prior to treatment or discharge after wastewater treatment, and consumption of surface waters by cows. Inactivation rate in various media drawn from prior studies; concentration of FMD virus in untreated milk assumed 160 $TCID_{50}$/mL. Additional calculations performed at a milk FMD density of 1.6×10^{6} $TCID_{50}$/mL.	Cows assumed to drink 50 L per capita per day	Exponential models; for calves exposed to FMD aerosols, r = 0.03 (95% CI 0.017 to 0.051) For pigs exposed to aerosols, , r = 0.0016 (95% CI 0.00074 to 0.003) For pigs given oral doses, , $r = 4.1\times10^{-7}$ (95% CI 2.0×10^{-7} to 7.5×10^{-7})	Not considered	Several scenarios and dose-response models were evaluated (model sensitivity was evaluated)

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Schijven and de Roda Husman (2006)	Risk of infection for occupational and sport divers in fresh and marine waters	*Campylobacter jejuni*, enteroviruses	Both pathogens assumed log-normally distributed with the reported lowest and highest values (in the literature) assumed to be the 99% CI values.	Ingested water depended on diver status (recreational vs. occupational), setting (marine vs. fresh vs. swimming pool) and on equipment used, especially mask type. Reported ingested volumes ranged from 0 mL to 190 mL. Number of dives per year was drawn from an empirical distribution.	The exact beta Poisson model with α = 0.145 and β = 8.007 used for dose-response for *C. jejuni*. The rotavirus exact beta Poisson model with α = 0.167 and β = 0.191 used for dose-response for enteroviruses.	Not considered	Annual risk of infection differed significantly with diver status (occupational vs. recreational), equipment used, and setting.

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Seidu et al. (2008)	Risk of (1) accidental consumption of wastewater by farmers (2) accidental consumption of contaminated soils by farmers; (3) accidental consumption of both wastewater and soil by farmers and (4) consumption of wastewater-irrigated lettuce collected from the farm, wholesaler or retail market.	Rotavirus, *Ascaris lumbricoides*	Rotavirus density estimated as fecal coliform density × 10^{-5}; rotavirus assumed log-normally distributed in stream and ditch water, in soils and on lettuce; rotavirus assumed uniformly distributed in piped water. Estimates for *A. lumbricoides* density distribution in various media drawn from published studies. Like rotavirus, distribution of *A. lumbricoides* in all media, except pipe-borne water, assumed log-normal. The distribution of *A. lumbricoides* in piped water assumed uniform.	Accidental irrigation water ingestion assumed uniformly distributed from 1 to 5 mL/day for 150 d/yr; accidental ingestion of soil assumed uniformly distributed from 10 to 100 mg soil/d for 150 days/yr. Lettuce consumption assumed uniformly distributed from 10 to 12 g per serving, 208 exposures/yr	Beta-Poisson model for rotavirus (α = 0.253, N_{50} = 6.17) An exponential dose-response model with r = 1 used for *A. lumbricoides*; model chosen because there no peer-reviewed dose-response model available and because it is the most conservative estimate	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Shuval et al. (1997)	Risk of infection associated with consumption of uncooked vegetables from fields irrigated with wastewater	HAV	Fecal coliform concentration in applied wastewater is assumed to be 10^7 CFU per 100 g of vegetable. Die off before harvest assumed 3 logs and ratio of hepatitis A virus to fecal coliforms is assumed $1:10^5$.	Daily average consumption of lettuce and cucumbers assumed to be 100 g and number of days both vegetables are consumed assumed to be 150 days/yr.	Beta-Poisson model for HAV median infectious dose estimated based on unattributed data (N_{50} = 30 to 1000 PFU) and α = 0.2 (with no justification). Attack rate estimated as 0.5 for lettuce consumption and 0.25 for cucumbers.	Not considered	Risks reported for a high and low estimates of ID_{50} and for two levels of wastewater treatment

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Signor and Ashbolt (2006)	Human exposure to pathogens via drinking water when routine pathogen monitoring conducted.	*Cryptosporidium* spp.	During baseflow conditions, untreated water *Cryptosporidium* density log-normally distributed with mean and standard deviation of log-transformed densities equal to 3.11 and 1.28, respectively. During event (rainfall) conditions, untreated water *Cryptosporidium* density log-normally distributed with mean and standard deviation of log-transformed densities equal to 5.27 and 0.61, respectively.	Ingested (oral) volume log-normally distributed with mean and standard deviation of log-transformed densities equal to -0.046 and 0.535, respectively	Exponential model, r = 0.00419	Not considered	Model sensitivity assessed via comparison of three sampling scenarios

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Signor et al. (2007)	Risk of infection from drinking water following a rainfall/runoff event	*Cryptosporidium*, *Giardia*, *Campylobacter*	Drinking water reservoir influent pathogen concentration data corresponding to wet and dry weather fit to log-normal and gamma distributions. Parameter uncertainties were quantified via Markov Chain Monte Carlo (MCMC) analyses.	1.1 L/day	Beta-Poisson model for *Campylobacter* (α = 0.145, N_{50} = 896). Exponential model for *Giardia* (r = 0.0199), *Cryptosporidium* (r = 0.00419).	Not considered	Influences of uncertainties (quantified via MCMC) and variability (estimated via Bayesian techniques) on risk assessed via factor sensitivity analysis (worst-case scenario determination)
Smeets et al. (2007)	Risk of infection from *Cryptosporidium* in treated drinking water	*Cryptosporidium*	Cumulative distribution function for *Cryptosporidium* density in treated drinking water taken from empirical distribution with low concentrations extrapolated from data. Distribution of *Cryptosporidium* in finished drinking water also estimated based on stochastic model of drinking water treatment.	Number of 190 mL glasses of drinking water consumed assumed Poisson-distributed, with mean equal to 0.53 L	*Cryptosporidium* dose-response model beta-Poisson with α = 0.115 and β = 0.176	Not considered	Analysis stochastic and risk results presented as statistical distribution

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Smeets et al. (2008)	Risk of infection from *Campylobacter* in treated drinking water	*Campylobacter*	Source water *Campylobacter* density was taken from historical raw water density measurements and CIs developed using bootstrap analysis. Removal in treatment estimated via stochastic model of all treatment processes.	0.177 L unboiled water / d	beta-Poisson dose-response model with α = 0.145 and β = 7.59.	Not considered	Model sensitivity evaluated for drinking water treatment models. Risk model stochastic and risks presented with CIs
Soller at al. (2003)	Risk of viral gastroenteritis associated with recreational use of a river downstream of a WWTP discharge. Two wastewater treatment scenarios compared.	Model enteric virus with the clinical features of rotavirus	Bacteriophage concentration in raw wastewater assumed uniformly distributed in the range 1×10^4 to 5×10^4. Removal modeled for treatment and removal and mixing processes modeled for discharged effluent. The ratio of model enteric virus concentration to bacteriophage concentration assumed log-uniform distributed in the range 0.001 to 1.0.	Exposure factor was a random variable chosen from uniform distributions whose ranges were selected based on observed recreational use by month and day of the week (weekday vs. weekend)	Beta-Poisson (presented in study in modified form) with α assumed uniformly distributed in the range 0.15 to 0.42 and β in the range 0.3 to 2.3.	Dynamic model, including individuals infected in activities other than use of river for recreation	Univariate sensitivity analyses for input parameters.

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Soller et al. (2006)	Risk of infection during full-body contact recreation	Rotavirus as a representative pathogen	Rotavirus density based on a model calibrated with empirical coliphage data. The relationship between coliphage density, expected rotavirus density and fraction of total pathogen load made up by rotavirus based on literature review.	Hourly rate of water ingestion assumed. Swimmers were in the water at different times and for different durations.	Beta-Poisson (presented in study in modified form) with α assumed uniformly distributed in the range 0.125 to 0.5 and β in the range 0.21 to 0.84 Probability of symptomatic response range 0.1 to 0.45.	Secondary transmission modeled via a deterministic time-dependent transmission model accounting for the immune status of the population	Sensitivity analyses performed for several variables; variables were set to low, medium, and high values to determine whether their variation changed the study findings
Steyn et al. (2004)	Risk of infection via drinking water or waterborne recreation	*Salmonella*	*Salmonella* density determined during monitoring. Calculations performed for the geometric mean value (167 CFU/100 mL), the minimum value (36) and the maximum value (883)	For full contact recreation, ingested volume assumed 100 mL	Approximate beta Poisson dose-response, with α = 0.3126 and N_{50} = 23,600	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Strachan et al. (2002)	Risk of *E. coli* O157 infection of humans using pasture for recreational activities	*Escherichia coli* O157	Empirical data for concentration in cattle feces (range 0 to > 5 $\log_{10}$ CFU/g) provided the distribution of pathogens in feces. The number of days prior to human exposure that animals were in fields was a random variable with a uniform distribution.	Mass of soil ingested during a 24-hour camp and during an 8 hour day were random variables with triangular distributions	Dose-response assumed to follow a beta-binomial dose-response model; metrics for assessing model fit not provided. Details of the model not provided.	Not considered	Sensitivity analysis (called importance analysis) was performed and results were presented as Spearman Rank correlations
Teunis et al. (1997)	Risk of infection by *Cryptosporidium* or *Giardia* in drinking water	*Cryptosporidium parvum*, *Giardia lamblia*	Empirical data for *Giardia* and *Cryptosporidium* fit with a negative binomial distribution; because seasonal variations in both parasites noted, data sets broken into winter and summer sets and fit separately. Treatment efficiency assumed beta-binomially distributed.	Log-normal distribution of drinking water consumption, mean equal to 0.153 L/day with uncertainty factor of 8.2.	Exponential dose-response relations used for both *Cryptosporidium* and *Giardia*. Readers referred to cited studies for parameters.	Not considered	Stochastic risk assessment performed; no explicit sensitivity analysis reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
van Heerden et al. (2005)	Risk of human adenovirus infection via drinking water or recreational water exposure.	Adenovirus	Adenovirus density assumed Poisson-distributed (in time, not space) with the distribution mean determined from frequency of positive determinations among drinking water and surface water samples. Mean adenovirus densities (in viruses per 100 mL) were 0.0014 and 0.00245 for two drinking waters, 0.0546 for river water, and 0.0097 for water behind a dam.	Drinking water consumption rate fixed at 2 L per capita per day and recreational water consumption rate fixed at 30 mL per capita per day.	The exponential model used for adenovirus dose-response. The model parameter not explicitly provided, though based on the citation provided in the study, it can be inferred to be that for inhalation of adenovirus aerosols, $r = 0.417$.	Not considered	Univariate sensitivity analyses conducted to assess the impact of consumption rates, dose-response parameters and recovery rates on risk estimates
Westrell et al. (2004)	Risk of infection during treatment, handling and soil application of sludge and wastewater, and risk of infection via consumption of crops irrigated with biosolids or via recreation in waters receiving wastes from land-applied wastes	Rotavirus, adenovirus, EHEC, *Salmonella*, *Cryptosporidium*, *Giardia*.	Pathogen concentrations in untreated sewage based on measured concentrations; distributional forms and parameters not reported. Inactivation rates in anaerobic digestion drawn from the literature, but not reported. Die-off after land application assumed negligible or outpaced by regrowth.	Not reported.	Parameters and models not reported; other studies were also cited	Not considered	Not reported

Study	Risk of Interest	Microorganism(s)	Pathogen Concentration and Variability	Ingested Volume or Mass	Dose-Response	Secondary Transmission	Sensitivity Analysis
Wong et al. (2009)	Risk of enteric virus infection associated with swimming at coastal beaches impacted by POTW discharges.	Adenovirus	Experimental distribution for adenovirus occurrence based on regression on order statistics to account for non-detect observations.	100 mL/day	Exponential model, r = 0.417 (based on data for inhalation of adenovirus aerosols)	Not considered	Not reported

[1] The Gompertz dose-response model is found in Table 4.

Note that several relevant studies were also published after this review was conducted but before it was finalized. Thus, it was not feasible to include Schoen and Ashbolt (2010) or Soller et al. (2010a,b,c).

IV NOVEL AND CUTTING-EDGE QMRA-RELATED TECHNIQUES

This section summarizes and describes techniques specific to exposure assessment, health effects modeling, and risk characterization that are novel, new, or beyond the approaches that typically have been employed in QMRA studies. For each of these topical areas, an overview of "classical" approaches QMRA is provided, followed by a review of the novel techniques identified in the state-of-the-science literature review. Where possible, results of papers describing recommended practices or methods for selecting techniques are also provided.

IV.1 EXPOSURE ASSESSMENT

Exposure modeling of animal-derived pathogens is a particularly difficult component of developing QMRAs for animal-derived pathogens. Like most biological systems, there is substantial variability in many facets of exposure to animal-derived wastes. Pathogen loads are known to vary between individual animals, from farm to farm, and from region to region. Manure handling practices, too, differ greatly among farms and regions and profoundly impact persistence and abundance of pathogens that can potentially reach receiving waters. Pathogen survival (or growth) varies between pathogens, with environmental media, and with other conditions such as moisture content, temperature and pH. Uncertainty of estimates of pathogen and indicator loads may be significant, particularly for pathogens like *Cryptosporidium* spp. that are highly infectious, and for pathogens with frequent non-detects.

In this section, novel techniques for conducting exposure assessments or estimating model parameters and their variability are described. Both deterministic and stochastic models are reviewed, and the uses of Bayesian methods in exposure assessment are highlighted.

IV.1.1 General Description

Pathogen loads from animal sources differ with animal, season, region, manure management practices, and method of land application. The prevalence of pathogens differs significantly among animals, with cattle producing a high proportion of *E. coli* O157:H7 and *Cryptosporidium* loading; poultry and dairy cattle contributing significantly to *Campylobacter* loading; and swine and poultry both contributing high loads of *Salmonellae*. Manure handling varies widely between U.S. farms, with manure directly deposited on pasture, land-applied as solids, or land-applied as slurries. Solid manure and manure slurries may undergo treatment before application, though the degree of treatment for applied manure may not be even for all applied manure.

Ferguson et al. (2003) divide processes governing the relationship between watershed pathogens and concentrations in surface waters into those most important in organism inactivation (water/osmotic potential, temperature, sunlight, pH, and inorganic and organic nutrients) and those most important in transport (adsorption/desorption effects, hydrological movement, and mechanical or biological movement). These processes are illustrated in Figure 3, which shows that wastes of animal-origin reach receiving waters via multiple pathways (e.g., in surface runoff, in interflow, after adsorption/desorption to soils or vegetation) and the transport of pathogens and fecal indicator organisms is dependent on processes that are highly variable.

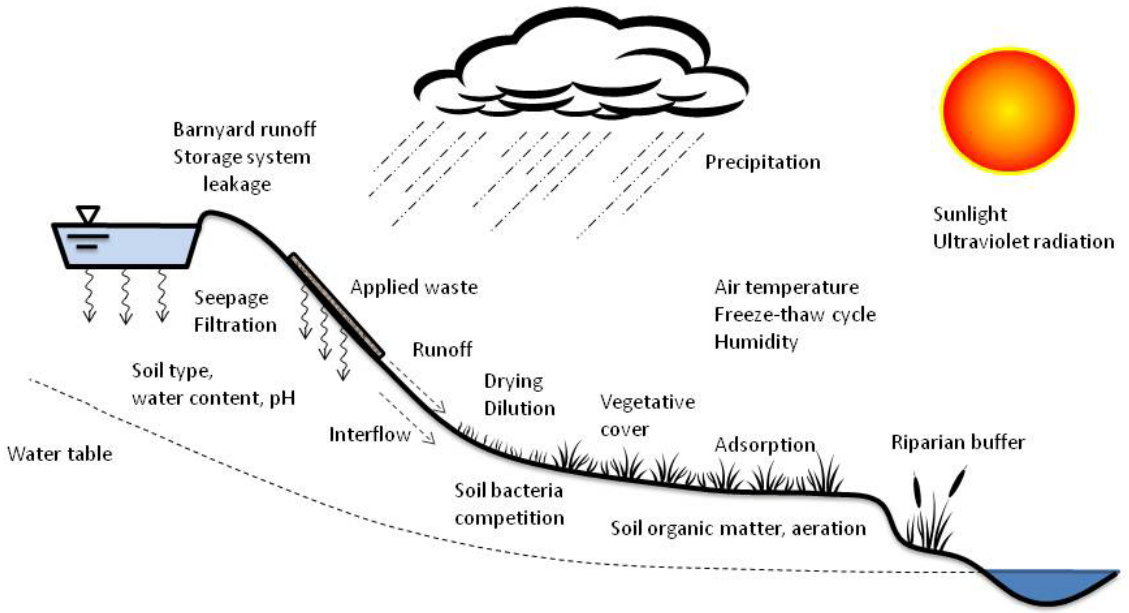

Figure 3. Factors Affecting the Viability of Pathogens and Indicators Along with Pathways
(SOURCE: Adapted from Rosen, 2000)

Atwill et al. (2002) describe the pathway followed by enteric microorganisms from fecal material to a specified downslope location (in this case a receiving stream) as comprising the following steps:

1. rainfall of sufficient intensity erodes the top layer of a fecal pat, releasing pathogenic organisms onto the wetted soil surface;
2. rainfall intensity reaches infiltration capacity and pathogenic organisms are carried downslope via sheet flow, preferential rill flow, or exfiltration in variable source areas;
3. pathogens are transported downslope or infiltrate into the subsurface; and
4. vegetative buffer strips intercept flows laden with pathogenic organisms and enhance infiltration.

Other processes not discussed by Atwill and colleagues that may be significant in determining overall loading and transport of pathogens from fecal material to receiving waters are treatment practices (for agricultural wastes), adsorption to plants and other matter, inactivation via exposure to UV radiation, desiccation or toxic materials, and predation.

A final aspect of exposure assessment that has recently received attention is the distribution of pathogens and indicators in environmental matrices (e.g., Englehardt et al., 2009; Gale, 2005) and consideration of method uncertainty and use of censored data within QMRA frameworks (e.g., Petterson et al., 2007, 2009; Signor and Ashbolt, 2006).

IV.1.2 Cutting-Edge Exposure Assessment Techniques

Cutting edge techniques for exposure assessment found from the literature search include use of Bayesian methods for leveraging data related to one component of the exposure pathway to develop knowledge about conditions in another part of the pathway where data are scarce; use of

highly resolved transport models, including the use of stochastic fate and transport models with varying degrees of resolution; and the use of alternatives to the log-normal and Poisson distribution for describing the temporal and spatial distribution of pathogens in environmental settings. Each of these types of techniques for exposure assessment is discussed below.

Application of Bayesian techniques to exposure assessment

The use of Bayesian techniques in exposure assessment was demonstrated by Eisenberg et al. (2008) in their risk analysis for exposure to amended biosolids (treated sludge) projects. The authors used prior information about pathogen concentration in raw sludge and removal efficacy of sludge treatment processes to predict post-removal pathogen concentrations in biosolids. The data for post-treatment pathogen concentrations were then used to inform the likelihood of pathogen occurrence. As described elsewhere in this report, transport of animal-derived fecal pollution is highly variable and many of the processes comprising the transport are relatively poorly characterized in the literature. Bayesian techniques such as those demonstrated by Eisenberg and colleagues offer a means for using data from better-characterized processes to improve estimates for processes associated with greater variability or for which there are fewer data. Other studies have used Bayesian techniques in exposure analysis for quantifying uncertainty associated with assumed distributional forms for *Cryptosporidium* density (Signor et al., 2007) and for quantifying uncertainty related to microbial counts (Clough et al., 2005).

In microbial risk analyses, Bayesian techniques offer the opportunity to estimate model parameters given a relatively small amount of data pertaining to a highly variable system. For example, Clough et al. (2003) used Bayesian methods to estimate herd-level prevalence from pathogen prevalence in fecal pats. Variabilities in the system included the fecal material produced per animal per day (particularly fecal production for infected and non-infected animals), farm-to-farm differences in infection rates and fecal production rates, seasonal variations in infection prevalence and fecal production rates, and number of animals in herds. Prior distributions used for number of infected animals included uniform and beta-binomial distributions. These analyses indicate that the posterior distribution is relatively insensitive to the choice of prior distribution and that the number of pat samples used influences the CI for parameter estimates.

Ranta et al. (2005) also applied Bayesian techniques to the problem of estimating *Salmonella* infection in cattle herds and animal populations. In surveillance of cattle for *Salmonella*, individual animals are either infected or not, and are either tested or not. The rate of detection of an infected cow, therefore, depends both on the rate of infection and the frequency of testing. Testing generally occurs if an animal exhibits adverse health effect symptoms but may occur for other reasons. The probability of an animal being tested given that it shows symptoms was assigned a beta prior distribution while the probability that an animal is tested given no symptoms was assigned a uniform prior distribution. Based on the model and knowledge of testing results for *Salmonella* among Finnish cattle, the authors developed an estimate for the overall prevalence of *Salmonella* infection among Finnish cattle and determined that assessment of the status of Finnish cattle could be made with a “modest” sample size.

Petterson et al. (2001b) used Bayesian methods to estimate virus inactivation rates on salad crops. Bayesian methods were chosen over other techniques such as bootstrap methods to reduce computational complexity of estimating inactivation rate parameters. Prior distributions for model parameters (some of which were log- or logistic-transformed) were normal and were

tested to ensure choice of prior distribution did not unduly influence posterior distributions. As for the previously discussed researchers, Petterson and colleagues found that Bayesian techniques allowed use of basic assumptions for certain biological processes while replacing "hidden" distributional assumptions for other processes with transparent analyses. Additionally, the outputs of Bayesian analyses were deemed to allow a more meaningful investigation of uncertainty. From their analyses, Petterson et al. (2001b) determined that a negative binomial distribution of viruses on salad crops was a better characterization than a Poisson distribution and estimated inactivation rates on multiple salad crops.

Bayesian techniques have also been used in the estimation of parameters for distributions describing variability of pathogens in environmental waters (Petterson et al., 2007; 2009; Pouillot et al., 2004). For example, Petterson et al. (2009) developed a point estimate for uncertainty in counts of *E. coli* O157:H7 using data from experiments in which the pathogen was spiked into water samples and detected via polymerase chain reaction (PCR). They then used Bayesian techniques to develop a distribution describing the temporal variability of *E. coli* O157:H7 at a raw water intake of a drinking water treatment plant. In the hierarchical model used in that study, several distributions—including a gamma distribution, log gamma distribution, and a constrained log-gamma distribution—were evaluated as potential meta-distributions for *E. coli* O157:H7 counts. Estimates of pathogen density were found to be highly dependent upon the selected model type, which indicates the need to use additional information in selection and development for models describing temporal variability in density. Improved models could be developed via inclusion of fate and transport models (Ramachandran, 2001), distributions based on analysis of additional or alternative data, or expert judgment. In a similar study of the treatment of distribution of *Cryptosporidium* oocysts in drinking water source waters, Petterson et al. (2007) showed the importance of separate analyses of method uncertainty and variability. Hierarchical Bayesian analysis was used to develop a distribution for oocyst density. Estimates for the parameters of the distribution of oocysts were dependent upon whether method uncertainty and oocyst variability were treated separately and on the assumptions made of method uncertainty.

In the food microbiology and risk literature, several studies have been published documenting use of Bayesian techniques for estimation of growth rate parameters in different matrices. These techniques could be extended to circumstances in which extra-enteric growth relevant to recreational water exposures may occur. Three studies (Albert et al., 2005; Crépet et al., 2009; Pouillot et al., 2003) have used hierarchical Bayesian models for development of growth models of *Listeria monocytogenes* in foods. These studies are an exemplary application of Bayesian techniques because of uncertainty in growth parameters for the pathogen and because of the variability in growth rate due to factors (e.g., temperature, physiological state of inocula, variability among strains, etc). In the study by Pouillot et al. (2003), expert knowledge regarding difference in growth rates among strains was required to develop parameter estimates.

Albert et al. (2005) used the growth uncertainty and variability parameters determined by Pouillot et al. (2003) within a Monte Carlo simulation of *L. monocytogenes* growth in milk during storage prior to transport. The model was developed for incorporation into a QMRA model of risks associated with exposure to *L. monocytogenes* in milk. During storage, the temperature of the milk varies. Other variable elements in the growth process are related to timing (milking time, storage time, etc.), bacterial abundance in milk added to storage, and differences among bacteria related to strain. A deterministic model of the *L. monocytogenes*

growth process was run as an element of a Monte Carlo simulation in which input variables (23 total) were drawn from appropriate distributions. Despite what the authors refer to as a "simple" process model, their findings were that the stochastic model could not succinctly characterize the growth process.

Crépet et al. (2009) performed a Bayesian analysis similar to that of Pouillot et al. (2003) to determine the variaiblity and uncertainty of *L. monocytogenes* growth parameters on fresh leafy salads. As for the studies conducted for milk, the use of Bayesian techniques allowed use of data corresponding to a range of environmental conditions in parameter estimation and accounted for uncertainty and variability more comprehensively than previously published growth models.

Use of high-resolution fate and transport models as components in QMRAs

Highly-resolved hydrodynamic and transport models have been developed for analysis of the stocks, flows, sources and sinks of pathogens in watersheds (a review of established watershed transport models may be found in Coffey et al., 2007); in coastal waters (e.g., by Liu et al., 2006); for mixing in a river reach downstream of a WWTP discharge (Soller et al., 2003); for flow in a combined sewer overflow (CSO)-impacted river and bay (King County Department of Natural Resources, 1999a); and in analysis of an impaired waterbody (Soller et al., 2006). For effective use of highly-resolved models in QMRA, two challenges must be overcome: (1) collection of data required for model development or calibration, and (2) experimental design of modeling such that conditions corresponding to conditions that can reasonably be expected to arise are considered. As for samples collected on a particular day, models predict microbial occurrence for a set of input hydrodynamic, biological and physical conditions. Modeling efforts should therefore be designed to account for variation in these conditions rather than producing a "snapshot" related to a single set of conditions.

A dated but still informative example of a watershed scale fate and transport model is provided by Walker and Stedinger (1999). Their *Cryptosporidium* source, fate, and transport model included addition of oocycts to manure on fields, storage and inactivation of oocysts after deposition, washoff of manure and oocysts into watercourse, oocyst routing in streams, oocyst fate in a drinking water source reservoir, and occurrence of oocysts in undisinfected drinking water. Calculations were based on the generalized watershed loading function model that, in turn, used a soil conservation model for erosion from fields. A single set of input parameters were used as input to a single year-long time period simulation. Their results indicated that dairy oocyst loads were small compared with those of WWTP effluents and that there is a strong seasonality in oocyst loading of the drinking water source. Using the QMRA framework, results of a single run of a model such as this with a single (albeit representative) set of input parameters are of limited value. Two approaches for using deterministic models such as that of Walker and Stedinger (1999) or results from such models within a stochastic framework more conducive to use in QMRA are described below.

A comparison and critique of two hydrologic and erosion models that have been adapted for modeling transport of bacteria is provided by Benham et al. (2006). Models described in that review for release of microorganisms from fecal material are a linear model (equation 1), an exponential model (equation 2), a power law model (equation 3), and an empirical model (equation 4).

$$\Delta N_R = N_S k_1 \Delta Q \qquad (1)$$

$$\Delta N_R = N_S(1 - e^{-k_2 \Delta Q}) \quad (2)$$

$$\Delta N_R = N_M a \left(\frac{\rho_w Q}{m_d} \right)^b \quad (3)$$

$$\Delta N_R = N_M [1 - (1 + k_3 \beta Q)^\beta] \quad (4)$$

In equations 1 to 4, ΔN_R is number of microorganisms released during time period Δt; N_S is number of organisms in the manure top layer; ΔQ is the runoff yield for the time period Δt; N_M is the total number of microorganisms in surface applied manure before a runoff event; Q is the runoff depth during the rainfall event; ρ_w is the density of water; m_d is the dry mass of applied manure; and k_1, k_2, k_3, a, b, and β are empirical parameters.

Ferguson et al. (2003) note that other factors including moisture content of the manure, whether the manure originated as diarrhea, and slope may have a strong influence on the mobilization of microorganisms from land-applied manure. For manure applied as a slurry, leaching losses of microorganisms from the applied manure may be significant and the rate of leaching is dependent on how well the soils are drained.

Downslope transport of microorganisms occurs in both surface and subsurface flows (Benham et al., 2006; Ferguson et al., 2003) and tends to follow preferential flow paths. Benham and colleagues hypothesize that larger amounts of bacteria are transported in rills than in sheetflows and that accumulation of organisms occurs in microponds. Features of subsurface soils believed to create preferential flow paths for microorganisms include groundcover (i.e., planted regions appear to enhance infiltration of microorganisms), tilling practice, and earthworm burrows (Ferguson et al., 2003).

Adsorption of organism to media (soils, vegetation) remains poorly understood and models to predict it are rudimentary. As reported by Benham et al. (2006), two commonly used watershed transport models employ a simple linear partitioning model (equation 5) to predict the fraction of bacteria sorbed to soil particles.

$$S = K_D C \quad (5)$$

In equation 5, S is the sorbed bacteria density (CFU per g), C is the bacteria concentration in suspension (CFU/mL), and K_D is a partition coefficient. Data for estimating K_D for pathogens of interest is a data gap in the knowledge of organism fate and transport in watersheds, streams, and coastal waters. Ferguson et al. (2003) suggest adhesion of bacteria to soils may play a minor role in transport during periods of high rainfall intensity, when the majority of microorganism transport occurs.

Atwill et al. (2002) evaluated the filter efficiency of vegetative buffer strips of differing soil types, slopes, and vegetative cover. In that study, vegetative buffer strips were assembled in soil boxes and subjected to artificial rainfall of varying intensities. Removal of *C. parvum* by the buffer strips was between 1-log/m for a buffer constructed with sandy loam soil to 3-log/m for a buffer constructed with a silty clay or loam soil. Pathogen removal was observed at moderate rainfall rates and for slopes as high as 20%.

Several reports of the use of stochastic models for occurrence, persistence, and growth of pathogens were found in the literature database. These reports, described in greater detail below,

generally predict distribution of pathogen or indicator occurrence based on Monte Carlo simulations of stochastic models or Monte Carlo simulations of deterministic models whose parameters are chosen from distributions for each simulation. Montville and Schaffner (2005) used a Monte Carlo analysis of pathogen growth during sprout production to evaluate monitoring schemes focused on reducing the incidence of contaminated sprouts reaching consumers. In contrast to the model of Albert et al. (2005) discussed above, their model was purely stochastic. The model inputs included pathogen prevalence on seeds (which can be surprisingly high), decay rates, growth rates, and detection probabilities for monitoring at different phases of the sprout production process. The resulting model indicated that disinfection of seeds alone could not ensure pathogen elimination and that monitoring of seeds for pathogens is an essential part of an overall risk management strategy.

Alternatives to log-normal and Poisson distributions for describing spatial and temporal variability

Most commonly, the distribution of indicators and pathogens at a particular location and time is described by a log-normal distribution or Poisson-log-normal distribution. The spatial distribution of microorganisms in a well mixed volume at a moment in time is described by a Poisson or, less commonly, by a negative binomial distribution. Although quite useful and relatively easy to manipulate, these distributions may not be appropriate for use in circumstances such as analysis of time series with frequent non-detects; analysis of systems with non-homogeneous distribution of microorganisms (e.g., due to gradients with distance source, association of microorganisms with particles or detached biofilms; clumping of microorganisms with each other or due to harboring of microorganisms in other organisms); or analysis of distribution of organisms after processes such as water treatment and wastewater treatment (Gale et al., 2002).

Englehardt et al. (2009) recently proposed and verified a theoretical distribution for describing microbial counts in water. Their model is developed based on the concept that the number of organisms present at a particular time and location is the result of some initial number of organisms at some prior time undergoing series and parallel events—each changing the number of organisms. The distribution is termed the discrete growth distribution (DGD); however, it is important to note that growth does not refer to growth of the microorganisms. Rather, it refers to a particular mathematical process. Reported advantages of the DGD over alternative models are that it has a theoretical basis and, perhaps most importantly according to the author, when fit to data it can predict the probability that zero organisms are observed. The Poisson distribution and other discrete distributions share the ability to predict the occurrence of no organisms. The DGD distribution is given by

$$p(v) = \frac{q^{v^{\eta}}}{\sum_{i=0}^{\infty} q^{i^{\eta}}}; \quad 0 \le q \le 1, \quad \eta \ge 0 \tag{6a}$$

where v is a discrete number of organisms, q is parameter of the distribution ($0 < q < 1$), and η is a parameter of the distribution related to the number of processes (called causes) leading to the distribution observed. The denominator in the expression for the DGD is denoted $D_{q,\eta}$ and can be approximated by

$$D_{q,\eta} \cong \sum_{1=0}^{M} q^{i^{\eta}} + \frac{\Gamma\left[1/\eta, (M+1)^{\eta}(-\ln q)\right]}{n(-\ln q)^{1/\eta}} \tag{6b}$$

where M is a parameter governing the accuracy of the approximation and Γ indicates the upper incomplete gamma function. Englehardt et al. (2009) did not provide guidelines on the selection of M. To estimate distribution parameters for the DGD, equation 6a can be linearized via double-log transformation, to the form

$$\log\left\{-\log\left[\frac{p(v)}{p(0)}\right]\right\} = \eta \log(v) + \log[-\log(q)] \tag{6c}$$

A plot showing the DGD probability distribution with the Poisson distribution and negative binomial distribution is presented in Figure 4. Not that the three distributions were generated for illustration of their differences (i.e., not fit to actual data). All three distributions have the same expected value and the parameters for the negative binomial distribution were chosen such that the resulting distribution is similar to the DGD distribution (though no optimization was performed). Figure 4 also shows that the DGD and negative binomial distributions are similar to each other and very different from the Poisson distribution. Both the DGD and negative binomial distributions are relatively flat at the lower range of indicator density. The DGD offers the advantage of being mechanistic (i.e., based on a series of plausible events) and therefore

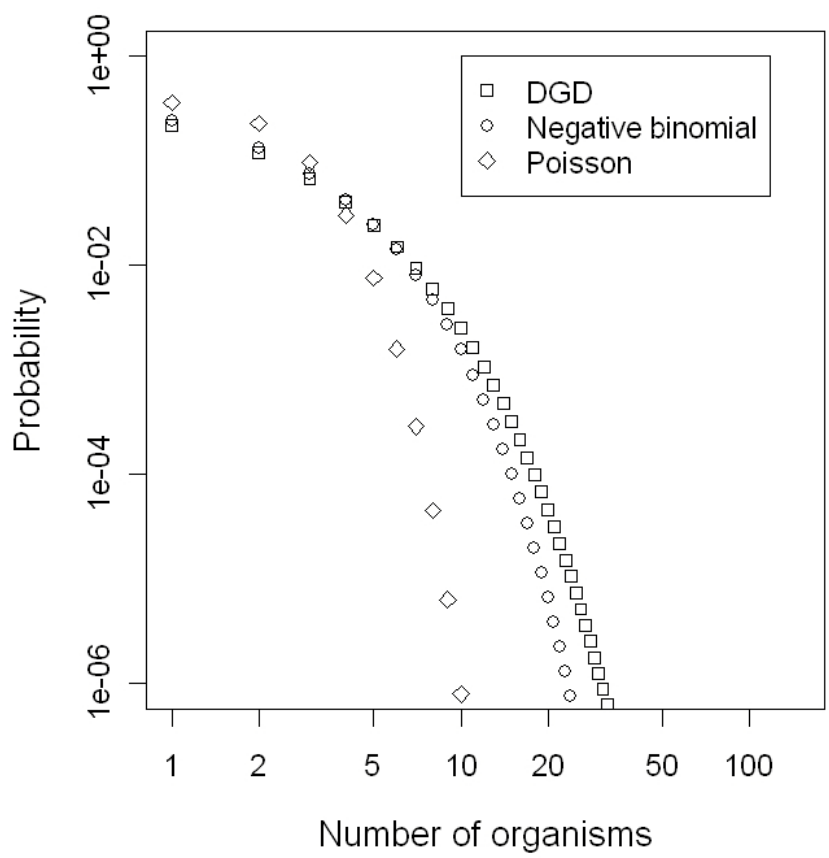

Figure 4. DGD, Negative Binomial, and Poisson Probability Distribution Illustration

appropriate for extrapolation outside the data range for which data are available. The negative binomial offer simplicity in use, including convolution with other distributions for developing analytical dose-response models.

As described above, alternatives to the log-normal and Poisson distributions for describing spatial variability in indicator density are the negative binomial and DGD. These distributions are both flatter than the Poisson distribution and are appropriate for describing the distribution of indicators at a site with known heterogeneity. Other distributions have been suggested for describing indicator and pathogen occurrence, though none of the studies reviewed for this report employed them. These alternative distributions are (Haas et al., 1999) the Poisson log-normal, the Poisson inverse Gaussian, and the Poisson Generalized Inverse Gaussian (Poisson-GIG).

IV.1.3 Summary: Cutting-Edge Techniques for Exposure Modeling

Characterization of variability and uncertainty of the complex systems giving rise to human exposure is perhaps the most pressing need in QMRA exposure assessment. Techniques in development for filling this need include the following:

- estimation of model parameters for deterministic and stochastic fate and transport models using Bayesian techniques;
- incorporation of high-resolution fate and transport models (e.g., computational fluid dynamic models, field- and drainage-scale overland fate and transport models) into QMRA models—preferably within a stochastic (Monte Carlo) framework;
- explicit treatment of both variability and uncertainty within QMRA; and
- development of novel distributions that better characterize pathogen and indicator occurrence and temporal and spatial variability.

The application of Bayesian techniques and the development and implementation of high-resolution fate and transport models are accessible to QMRA practitioners. In cases where use of these techniques is justified, analysis of complex systems that are recalcitrant to analysis with conventional techniques may be evaluated and characterized.

IV.2 HEALTH EFFECTS MODELING

IV.2.1 Dose-Response Modeling

Dose-response modeling overview

Dose-response models relate the density of a pathogen, surrogate organism, or indicator organism to the probability of a particular (adverse) response (endpoint). Endpoints for dose-response models employed in previous QMRA studies have included infection (presence of antibodies or other measureable changes such as the presence of substances in blood); illness (exhibition of symptoms such as diarrhea); mortality (especially in studies of category A biological agents[4]) (Bartrand et al., 2008); acute febrile response; and development of lesions in lung tissue, skin, or their organs.

[4] According to the U.S. Centers of Disease Control and Prevention (CDC), high-priority agents include microorganisms that pose a risk to national security because they (1) can be easily disseminated or transmitted from person-to-person; (2) result in high mortality rates and have the potential for major public health impact; (3) might

The exponential and beta-Poisson are the dose-response models most often used in QMRA performed for waterborne pathogens. Favorable properties of those two models are that they are mechanistic (can be derived based on assumptions about the infection process) and that they predict a non-zero probability of infection at low dose (Haas et al., 1999). The exponential dose-response model, presented in equation 7, is derived based on the assumptions of a Poisson-distributed dose and equal and independent probability, *r*, of each ingested organism initiating an infectious focus.

$$p(R \mid d) = 1 - e^{-rd} \tag{7}$$

In equation 7, *R* denotes response, *d* is dose, and *r* is the probability that a single organism initiates an infectious focus. The beta-Poisson dose-response model, shown in equation 8, is based on the assumptions that the ingested dose is Poisson-distributed and the probability of a response at a given dose is beta-distributed.

$$p(R \mid d) = 1 - {}_1F_1(\alpha, \alpha + \beta, -d) \tag{8}$$

In equation 8, ${}_1F_1$ denotes a confluent hypergeometric function and α and β are parameters of the distribution. An approximation to the exact beta-Poisson model, presented in equation 9a (Furumoto and Mickey, 1967), is frequently used. The approximate version is given by

$$P(R \mid d) = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha} \tag{9a}$$

that may also be reparameterized as follows:

$$P(R \mid d) = 1 - \left[1 + \frac{d}{N_{50}}(2^{\frac{1}{\alpha}} - 1)\right]^{-\alpha} \tag{9b}$$

where N_{50} is dose corresponding to median response (e.g., ID_{50}). The relation in equation 9b provides a good approximation to the exact beta-Poisson model when $\beta >> 1$ and $\alpha << \beta$.

Other dose-response models have been used in MRA studies, especially in literature pertaining to risks associated with food. Representative examples are reviewed below, with mechanistic dose-response models presented first, empirical models presented second and models developed using Bayesian techniques.

Teunis et al. (1999) proposed a dose-response model for gastroenteritis illness given infection. Assuming the duration of illness is gamma-distributed the authors derive the following dose-response model for illness given infection

$$P(\text{illness} \mid \text{infection}) = 1 - \frac{1}{(1 + \psi\lambda)^a} \tag{10}$$

In equation 10, *a* is a parameter of the distribution (the shape factor for the gamma distribution for disease duration) and $\psi\lambda$ is treated as a single dose-dependent parameter. The authors evaluated the following functional relationships for ψ: $\psi\lambda = \eta d$; $\psi\lambda = \eta / d$; $\psi\lambda = \eta$, finding

cause public panic and social disruption; and (4) require special action for public health preparedness. See http://www.bt.cdc.gov/agent/agentlist-category.asp#b for further information.

that the probability of illness given infection was not dose-dependent for *Salmonella enterica* and *Cryptosporidium*, and that the probability of becoming ill when infected decreases with increasing dose for *Campylobacter jejuni*.

Numerous empirical dose-response models have been used in prior QMRAs and related studies, particularly in food risk literature. Representative examples are listed in Table 4; however, a discussion of these dose-response models is beyond the scope of this report. Unless there are compelling reasons to the contrary, the exponential and beta-Poisson dose-response models are preferred over these alternatives because they are biologically-based and better suited to low-dose extrapolation. Although this appears to be a majority position in the literature (as evidenced by the use of the exponential and beta-Poisson or related dose-response models in the majority of studies in Table 3), there is a minority but significant opinion to the contrary. Brookmeyer et al. (2005) developed a mechanistic dose-response model (referred to as a "competing risks" model) for inhalation of anthrax. The model relies on assumptions of a constant, uniform risk of anthrax spores being cleared from the lung, and a constant, uniform risk of germination of anthrax sporesin the lung. The resulting dose-response model is

$$F(t) = 1 - \exp\left\{ \frac{-dx}{\kappa + \theta} [1 - e^{-(\kappa+\theta)t}] \right\} \tag{11}$$

where $F(t)$ is the cumulative attack probability function (probability that a spore germinates at or before time t), κ is rate of clearance of spores from the lung, and θ is spore germination rate.

Table 4. Select Empirical Microbial Dose-Response Models

Model	Equation	Parameters
Weibull-Gamma	$P(d) = 1 - (1 + d^b / \beta)^{-\alpha}$	Three parameter model: *b*, α, β
Weibull	$P(d) = 1 - \exp(-ad^b)$	Two parameter model: *a*, *b*
Gompertz[1]	$P(d) = 1 - \exp[-\exp(a + b\, f(d))]$	Two parameter model: *a*, *b*; *f*(*d*) denotes a transformation (e.g., log)
Log-normal	$P(d) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{(\ln d - \alpha)/\beta} \exp(-\frac{1}{2} t^2) dt$	Two parameter model: α, β
Log-logistic	$P(d) = 1 / \{1 + \exp[-(\ln d - \alpha)/\beta]\}$	Two parameter model: α, β
Exponential-Gamma	$P(d) = 1 - \exp(-\gamma d) / (1 + d^b / \beta)^{\alpha}$	Three parameter model: α, β, γ
Weibull-exponential	$P(d) = 1 - \exp(-\alpha d^{\gamma}) / (1 + d^{\gamma} / \beta)$	Three parameter model: α, β, γ
Shifted Weibull	$P(d) = \begin{cases} 1 - \exp\{-[(d-\alpha)/\beta]^{\gamma}\} & d \geq \alpha \\ 0 & 0 \leq d < \alpha \end{cases}$	Three parameter model: α, β, γ

[1] When the function *f* is the natural log transformation of dose, this models is referred to as an "extreme value" model in Moon et al. (2004) and can be shown to be equal to the Weibull distribution.

The two model parameters were estimated using physiological (clearance rates from lungs) and microbiological data (spore germination rates) rather than quantal response data. When the estimated parameters were used in equation 11, the model accurately predicted a median incubation period for an anthrax outbreak. Note that the exponential dose-response model results from equation 11 when $t \to \infty$. The implication of this equivalence is that, in the absence of dose-response data or a published dose-response model, there is the potential to estimate the exponential dose-response mode parameter based on physiological and microbiological data related to the pathogen of concern.

Hamilton et al. (2006) used a beta-binomial dose-response model to estimate the incidence of gastroenteritis resulting from consumption of vegetables irrigated with reclaimed water. The authors describe the beta-binomial model as an extension of the beta-Poisson model, although it is unclear whether the dose is assumed Poisson-distributed in the model presented. The dose-response model, as described in their study, is presented in equation 12. Details on the derivation of the model are not provided in the study by Hamilton et al. (2006) or the study (Cassin et al., 1998).cited as the source of the beta-binomial model.

$$P(R \mid d) = 1 - \frac{\binom{\alpha+\beta-1}{\alpha}}{\binom{\alpha+\beta+d-1}{\alpha}} \tag{12}$$

Haas et al. (1999) present three empirical dose-response models (the log-logistic model , equation 13; the log-probit model, equation 14; and the Weibull model, equation 15) for consideration, although they indicate that these models should be used with caution because their accuracy may be poor under conditions significantly different from those for which the models were developed. Additional empirical models, including a Weibull-gamma model (equation 16) were assessed by Holcomb et al. (1999), who also assessed a flexible exponential model that is not described here because details on its use and derivation were not provided.

$$P = \frac{1}{1 + \exp[q_1 - q_2 \ln(d)]} \tag{13}$$

$$P = \phi\left[\frac{1}{q_2}\ln\left(\frac{d}{q_1}\right)\right] \tag{14}$$

$$P = 1 - \exp(-q_1 d^{q_2}) \tag{15}$$

$$P = 1 - [1 + \frac{d^{\chi}}{\beta}]^{-\varepsilon} \tag{16}$$

In equations 13 to 16, q_1, q_2, β, χ, and ε are parameters and $\phi(y) = \int_{-\infty}^{y} \exp(-\frac{x^2}{2})dx$. In their comparison of dose-response models for risk of infection in food consumption, Holcomb et al. (1999) determined that the Weibull-gamma model (equation 16) yielded the best fit to data they were studying. This finding is not surprising, since the Weibull-gamma has three parameters and

all other models had two or fewer parameters. The authors did not test whether the 3-parameter model provided a statistically-significant improvement in fit over models with fewer parameters.

Cutting-edge dose-response modeling techniques

Bayesian techniques

Bayesian methods are being increasingly used in microbial risk assessment to estimate dose-response model parameters and their uncertainties. These methods, described briefly below, provide opportunities for development of dose-response models for small data sets, or when sufficient data are not available for use of frequentist (traditional) techniques, or to improve dose-response model confidence using data that might otherwise not have been used in dose-response model development.

In the simplest implementation of Bayesian inference for estimating parameters for dose-response models, an assumption is made that dose-response characteristics for related exposures belong to a distribution of responses whose precise form is unknown but whose distribution arises from the biology, pathology, and other features of the pathogen-host combinations. In the case of dose-response modeling, the parameter(s) of the dose-response model is/are assumed to be a random variable drawn from the known or unknown distribution. Bayesian inference involves making an assumption about the functional form of the distribution of dose-response parameters (referred to as the "prior" or prior distribution) and treating observed responses as conditional probabilities. Using Bayes' theorem (equation 17), if R is a set of observations of responses (each corresponding to a different dose) and θ is the set of values from which the dose-response model parameters arise, the probability that a particular parameter describes the dose-response observations, R, is given by

$$P(\theta \mid R) = \frac{P(R \mid \theta) \times P(\theta)}{\int P(R \mid \theta) \times P(\theta)\, d\theta} \tag{17}$$

The conditional probability $P(R \mid \theta)$ (i.e., the probability that the observations, R, occur given a specific set of parameters, θ) is equal to the likelihood of the observations. If host heterogeneity in response is low and response at a particular dose may be assumed binomially distributed, the likelihood of observing R given θ is

$$P(R \mid \theta, d) = \prod_{i=1}^{\#\text{doses}} \binom{n_i}{p_i} \pi(\theta, d_i)^{p_i} \left[1 - \pi(\theta, d_i)\right]^{n_i - p_i} \tag{18}$$

where n_i is the number of subjects in dose group i, p_i is the number of positive responses in dose group i, and $\pi(\theta, d)$ is the dose-response model (e.g., exponential or beta-Poisson) evaluated at parameter(s) θ and dose d_i. If host heterogeneity were high, the likelihood function in equation 2 could be replaced with one based on an alternative distribution such as a beta distribution.

The mechanics of generating estimates for dose-response model parameters then become

- selection of the most appropriate dose-response model, $\pi(\theta, d)$ (or comparison of multiple models);
- selection of a prior distribution describing the variation in dose-response model parameters, $P(\theta)$; and

- evaluation of equations 17 (using equation 18) over the range of plausible values of the model parameter(s).

The result of this procedure, termed the posterior distribution, gives the refined probability distribution for the dose-response model parameter(s) consistent with available data. The integral in the denominator of equation 17, in general, precludes analytical solutions and estimation of the posterior distribution is usually made via MCMC methods (Gilks et al., 1996).

Meta-analysis (or hierarchical modeling) of multiple data sets corresponding to differing conditions (e.g., experiments conducted using different strains of a pathogen, as explored by Messner et al. (2001) may be conducted within the Bayesian framework. In Bayesian meta-analyses, an assumption is made about the distributional form of the population from which dose-response model parameters is drawn. The parameters of the distribution assumed for the model parameters are called the hyperparameters and are estimated in the course of analysis. The posterior distribution is calculated as described above using data from all experiments included in the meta-analysis. The resulting posterior distribution may be used for evaluating parameters for conditions different from those under which data were collected (e.g., to estimate expected dose-response parameter and credible interval for an unknown strain) and as a component in microbial risk assessment.

As with frequentist approaches, model fits from Bayesian inference must be assessed prior to adoption. Alternative approaches for assessing dose-response models and application of Bayesian techniques include the following:

- use of a Q-Q plot (graphical technique for assessing whether data fit a given distribution) to verify the fit to the assumed distribution;
- use of a graphical plot of the data and the model to assess dose-response model fit as well as the classic likelihood ratio test to compare the dose-response model with the best (completely parameterized) model;
- Bayesian information criterion (BIC), deviance information criterion (DIC),or other appropriate tests of model fit;
- cross-validation (subject to availability of data);
- assessment of the sensitivity of the posterior distribution to the choice of prior distribution (relative insensitivity of the posterior distribution to choice of prior indicates the prior is not biasing the posterior distribution); and
- comparison of Bayes factors (Kass and Raftery, 1995).

Studies in which dose-response models have been developed using Bayesian techniques are described below and compared in Table 5. In Table 5, analysis type specifies whether individual data sets were analyzed separately ("individual") or whether multiple data sets corresponding to different experiments of factors were analyzed as part of a meta-analysis. When a meta-analysis was performed, the factor(s) that differed between data sets are reported.

Messner et al. (2001) used Bayesian techniques to refine dose-response parameter estimates for three isolates (IOWA, TAMU, and UCP) of *Cryptosporidium parvum* and to explore the variation of the dose-response parameter among isolates and for unknown strains. Bayesian methods were selected, in part, because of high variation in dose-response among strains. First,

Table 5. Comparison of Bayesian Dose-Response Studies

Study	Pathogen	Analysis Type	Factor Varied in Meta-Analysis	Dose-Response Model	Prior Distribution for Dose-Response Parameter(s)
Messner et al. (2001)	*Cryptosporidium parvum*	Individual models for 3 isolates	Not applicable	Exponential	Uniform log-transformed
Messner et al. (2001)	*C. parvum*	Meta-analysis	Isolate	Exponential	Log-normal
US EPA (2003, 2006)	*C. parvum*	Meta-analysis	Isolate	Exponential	Logit-normal, Logit-t, and beta
Teunis et al. (2002a)	*C. parvum*	Meta-analysis	Isolate	Generalized hypergeometric	Uniform log-transformed for all parameters
Teunis et al. (2004)	*E. coli* O157:H7	Individual models for teachers and pupils	Not applicable	Beta-Poisson with variables transformed	Uniform(0,1) for transformed variable $u = \alpha/(\alpha + \beta)$ Normal(0,10) for transformed variable $v = \log_{10}(\alpha + \beta)$
Teunis et al. (2005)	*Campylobacter jejuni*	Meta-analysis	Outbreak and feeding study data	Beta-Poisson infection model (parameters α, β) with conditional probability of illness (parameters η and r)	All dose-response parameters (α, β, η, and r) were transformed and noninformative priors were used for all transformed variables A log-normal prior was used for *C. jejuni* density in milk
Teunis et al. (2008)	*E. coli* O157:H7	Meta-analysis	Outbreak data	Beta-distributed probability that pathogens initiate an infectious focus and negative binomial distribution of dose	Priors for transformed variables were normal; hyperpriors for the mean and standard deviation of transformed variables were normal- and gamma-distributed
Engelhardt (2004)	Rotavirus	Individual	Not applicable	Exact beta-Poisson	Uniform (noninformative) priors for both α and β
Englehardt and Swartout (2004)	*C. parvum*	Meta-analysis	Isolate	Exact beta-Poisson	Not described

Study	Pathogen	Analysis Type	Factor Varied in Meta-Analysis	Dose-Response Model	Prior Distribution for Dose-Response Parameter(s)
Englehardt and Swartout (2006)	*C. parvum*	Individual	Not applicable	Modified beta-Poisson for illness endpoint, morbidity ratio independent of dose	A joint, noninformative log-normal prior used for the model parameters (α and β)
Chen et al. (2006)	*C. jejuni*	Meta-analysis	Isolate	Approximate beta-Poisson	Hyper-parameters (assumed to be the mean and standard deviation of the parameter distributions) assumed normally-distributed with an extremely wide range of parameter values; transformations used to generate hyperparameters not explicitly defined

Messner and colleagues assumed all data were best fit by an exponential dose-response model and analyzed data sets for individual strains separately using a noninformative prior ($\pi[\ln(k)]$ = constant) to develop estimates for the dose-response parameter and credible interval for each strain. Second, they conducted a meta-analysis in which the prior for dose-response model parameter, k, was assumed to be log-normal (among isolates) and hyperparameters for the prior distribution were the log-mean and standard deviation of log-transformed parameter. In that study, meta-analysis only slightly changed parameter estimates for individual strains, but provided insights into the envelope of values within which the dose-response model parameter for an unknown isolate of *Cryptosporidium parvum* is expected to fall.

In developing a dose-response model for use in evaluating criteria for *Cryptosporidium* density in finished drinking water, EPA used Bayesian techniques to account for variability in dose-response model parameter among isolates and to assess hypotheses about the distribution of the dose-response model parameter over the range of *C. parvum* isolates (U.S. EPA, 2003, 2006). As described above (see Messner et al., 2001), the exponential model dose-response parameter varied by two orders of magnitude among three isolates for which human graded dose data were available. There were also concerns that data corresponding to one of the isolates were not representative of human response because of the status of the isolate at the time it was administered to volunteers. To explore the sensitivity of the dose-response model to selection of data and meta-distribution choice for the dose-response model parameter, EPA generated and compared four dose-response models. A MCMC technique was used to estimate the parameters for the distribution of the exponential dose-response model parameter, r, and subsequently the expected value and 95% credible interval for r. The resulting credible intervals were sensitive to both model choice and data choice. Because all the models were considered equally plausible, the model selected for use in regulation was a composite of the alternative models. Additional means for comparing models would have been to assess the parsimony of the models or to compare the fits of the models (e.g., with Bayes factors).

Teunis et al. (2002a) analyzed the same *Cryptosporidium parvum* data as Messner et al. (2001) but based their Bayesian analysis on the two-parameter exact beta-Poisson dose-response models, which accounted for variability within strains and between strains. Uniform log-transformed priors were selected for all (four) dose-response model parameters. In contrast to the model developed by Messner and colleagues (2001), the model developed by Teunis et al. (2002a) accounts for heterogeneity at the pathogen level, and is more consistent with experimentally-observed dose-response. Teunis and colleagues suggest this difference makes their model a better candidate for estimating population-level risks.

Teunis et al. (2004) assessed outbreak data (number of teachers and pupils exposed, number of teachers and pupils ill, and dose) for the development of an *E. coli* O157:H7 dose-response model using Bayesian techniques. This study demonstrates the ability of Bayesian techniques to develop a dose-response model using data insufficient for development of a model using frequentist approaches. A beta-Poisson dose-response model was assumed because it accounts for heterogeneity in the host-pathogen interaction and because it has desirable behavior at low dose. The model parameters were transformed for analysis and the priors assigned to the transformed variables were a uniform distribution for $u = \alpha/(\alpha + \beta)$ and a normal distribution for $v = \log_{10}(\alpha + \beta)$. The authors note that use of only two data points is an extreme demonstration of the ability (and necessity) of Bayesian models in dose-response model development with limited data.

Subsequent work by Teunis et al. (2008) accounted for heterogeneity in dose among individuals in an *E. coli* O157:H7 outbreak using data (estimated dose, number exposed and attack rate) from nine previous outbreaks with different exposure vehicles (food, water, soil, and incidental direct ingestion). This information was used to develop a dose-response model that is consistent with variability in infection and illness arising from different conditions under which outbreaks occur. These conditions include the vehicle by which hosts are exposed to pathogens, differences in characteristics of the exposed population, genetic variations among pathogens associated with different outbreaks, and condition of the pathogen in different outbreaks. In contrast to models developed using data generated in controlled exposures, Bayesian models based on outbreak data may capture the variability associated with typical and unintended human exposures and infections. Teunis et al. (2008) proposed a novel dose-response model to account for heterogeneity in exposure among individuals for each outbreak. Assuming the number of pathogens in a given vehicle (mass of food, volume of water) is Poisson-distributed and that the Poisson mean is gamma-distributed among different food samples, water samples, (etc.), the dose associated with a particular outbreak may be expressed as Poisson-gamma mixture (i.e., negative binomially distributed). Under these assumptions, the probability of exposure is

$$P_{\exp}\left(\overline{C}V,\phi\right)=1-\left(1+\frac{\overline{C}V}{\phi}\right)^{-\phi} \tag{19}$$

where $\overline{C}$ is average density of the pathogen in a given source, V is the ingested volume of the source, and ϕ is a parameter of the distribution. Assuming the ability of pathogens to initiate infection is beta-distributed (an underlying assumption of the beta-Poisson model), the probability of infection of a negative-binomially distributed dose is given by

$$P_{\inf}\left(\overline{C}V \mid \phi,\alpha,\beta\right)=1-{}_2F_1\left(\alpha,\phi,\alpha+\beta;-\overline{C}V/\phi\right) \tag{20}$$

where ${}_2F_1$ denotes a hypergeometric function. Teunis et al. (2008) used fixed estimates of the dispersion parameter, ϕ, and estimated the dose-response parameters α and β via hierarchical Bayesian analysis. Beta-Poisson model parameters were transformed as follows:

$$\begin{aligned} w &= \log\left(\tfrac{u}{1-u}\right) \\ z &= \log\left(v\right) \end{aligned} \tag{21}$$

where

$$\begin{aligned} u &= \frac{\alpha}{\alpha+\beta} \\ v &= \alpha+\beta \end{aligned} \tag{22}$$

Priors for w and z were normal, and hyper-priors for the mean and standard deviation of w and z were normal and gamma, respectively. The dose-response model and credible interval for the parameters resulting from the analysis were suggested to be an improvement over dose-response models proposed in prior studies because it more accurately accounts for heterogeneities present in non-controlled exposures.

Outbreak data were evaluated in a Bayesian framework along with feeding study data in a study of *Campylobacter jejuni* dose-response and risk (Teunis et al., 2005). Two features of that study that distinguish it from other Bayesian dose-response studies are use of feeding study and

outbreak data for development of a single model and use of a four-parameter dose-response function developed in a prior study (Teunis et al., 1999). Outbreak data were for two outbreaks of campylobacteriosis associated with ingestion of contaminated milk. In the dose-response model, the probability of illness at a given dose is the product of the probability of infection ($P(d|\alpha,\beta)$ when the beta-Poisson model is used), and the conditional probability of illness given infection ($h(d|r,\eta)$ when the model for conditional probability of illness proposed by Teunis et al., 1999 is used). Here, r and η are models parameters:

$$P(d \mid \alpha,\beta)\times h(d \mid r,\eta)=\left[1-{}_1F_1(\alpha,\alpha+\beta,-d)\right]\times\left[1+(1+\eta d)^{-r}\right] \tag{23}$$

Estimates of dose used for outbreak data were based on reported milk consumption (data were binned as "none," "draught," "½ cup", "1 cup", and "2 cups") and density of *C. jejuni* in milk, which was considered a random variable with a log-normal prior distribution. Dose-response relations for the combined outbreak and feeding study data were markedly different from those developed using feeding study data alone. The authors noted that, as for most feeding studies, those conducted for campylobacteriosis utilized immunocompetent adult volunteers, whereas the outbreak data were mainly for children. This fact merits consideration of the modified dose-response relation from the Bayesian analysis in QMRAs, because it includes effects on a sensitive population (children) and consequently may provide a more conservative estimate of population level risk.

Englehardt (2004) proposed use of Bayesian techniques for dose-response models developed to overcome limitations of scarce data for low dose and the limited number of data sets for describing exposure and infection scenarios. Exposure and infection scenarios can be expected to be highly variable. A beta-Poisson dose-response relation was selected for evaluation and noninformative (uniform) priors were used for the dose-response model parameters (α and β). A single data set for infection of human volunteers with rotavirus was used to demonstrate the utility of the technique. The dose-response model, evaluated at an unspecified value of the Bayesian parameter estimates (perhaps the median values), was compared with a modified set of parameters estimated via traditional optimization techniques and an upper limit dose-response curve corresponding to an exponential model with $r = 1$. The dose-response curve developed via Bayesian techniques fell between the other curves. However, the CI for the traditional model and the credible interval for the Bayesian model were not provided in that study, making comprehensive comparison of the models difficult. The author advocates increased use of Bayesian techniques for more complete inclusion of expert knowledge in risk assessment and because the techniques are well suited for data-scarce applications such as microbial dose-response modeling.

In their analysis of *Cryptosporidium* dose-response, Englehardt and Swartout (2004) addressed the bias introduced into dose-response models developed based on quantal dose-response experiments employing homogeneous host populations. Noting that none of the human volunteers in three *Cryptosporidium* feeding studies were immunocompromised and that the antibody positive (Ab+) population may have been over-represented, the authors conducted a parametric bootstrap analysis to generate a set of dose-response model (beta-Poisson) parameters that would better reflect risks to the general population. In their analysis, 10% of the population was assumed to be sensitive to *Cryptosporidium* infection and 20% was assumed to Ab+. Among sensitive individuals, a dose of 10 oocysts or higher was assumed to cause infection. Dose-response models from the bootstrap analysis for three isolates of *Cryptosporidium*

(analyzed separately) were not substantially different from estimates made using the original data sets—perhaps indicating that the Ab+ population offset the sensitive population in the overall population incidence of infection. Like Messner et al. (2001), Englehardt and Swartout (2004) used Bayesian methods to explore variability in response between isolates of *Cryptosporidium*. Rather than directly predicting the distribution for parameters of the beta-Poisson model, the authors determined the single pathogen probability of initiating infection, *r*, in the Bayesian meta-analysis. The likelihood function was based on the conditional probability of observing the mean single-organism probabilities of infection as determined in the bootstrap analysis described above. The prior used in the analysis is not explicitly stated.

Similar to the analysis of Teunis et al. (2005) for *C. jejuni*, Englehardt and Swartout (2008) incorporated conditional probability of illness given infection into a Bayesian analysis of *C. parvum* dose-response. Based on novel modeling and interpretation of the literature, the authors developed a dose-response model, termed the generalized beta-Poisson model, in which the proportion of infected persons becoming ill, γ, was independent of dose (one of the relationships explored in a previous study of GI illness by Teunis et al., 1999):

$$P_{ill}(d \mid \alpha,\ \beta, \gamma) = \begin{cases} \gamma\left[1-\left(\dfrac{\beta}{\beta+d}\right)^{\alpha}\right] & \beta >> 1 \text{ and } \alpha << \beta \\ \gamma\left[1-{}_1F_1(\alpha, \alpha+\beta, -d)\right] & \text{otherwise} \end{cases} \tag{24}$$

where ${}_1F_1$ denotes a confluent hypergeometric function. The modified and unmodified beta-Poisson models were both fit to aggregated (pooled) data for five isolates of *Cryptosporidium* via maximization of the log-likelihood ratio ("traditional techniques"). The improvement in fit the 3-parameter modified model provided over the 2-parameter beta-Poisson model was not significant however, and the unmodified beta-Poisson was selected over the modified version for further analyses. Parameters (α and β) for the aggregate (pooled) data set for all 5 isolates were estimated via Bayesian techniques and a joint, noninformative log-normal prior was used for the model parameters.

Chen et al. (2006) explored the impact of *Campylobacter jejuni* isolate (14 total) and origin (fresh vs. laboratory) on dose-response using Bayesian analysis. Dose-response parameters were transformed as described in Teunis et al. (2008) and priors were chosen such that their influence on the predicted parameters was deemed minimal. Significant differences in the envelope of dose-response models for the fresh and lab isolates were noted, with the ID_{50} for the fresh isolates found to be less than that for the lab isolates. The researchers also reported a greater spread (range of doses corresponding to each isolate's ID_{50}) among the fresh isolate dose-response curves. Lab isolate dose-response curves tended to have shallower slopes (higher intra-isolate variability). The authors note that Bayesian analysis allowed generation of population-level infection estimates based on a limited amount of data.

Physiologically-based biokinetic models

In their assessment of anthrax dose-response models, Gutting et al. (2008) outline the components of a hypothetical physiologically-based biokinetic (PBBK) model of infection and response to aerosols of *Bacillus anthracis*. In the model, the fate and transport of *B. anthracis* spores and vegetative cells is tracked in regions of the respiratory system, in macrophages, in the

blood and in lymph nodes. As done by Brookmeyer et al. (2005) in their development of a competing risks model for inhalation anthrax, Gutting et al. (2008) estimate model parameters for use in their biokinetic model using physiological and microbiological data not collected in quantal dose-response studies or epidemiological investigation. Details of the techniques used for parameter estimation or of the model were not provided.

An earlier mechanistic dose-response model was proposed by Coleman and Marks (2000). In that study the important events occurring in the course of a *Salmonella* illness were determined to be survival of ingested bacteria to the target, colonization, engulfment, intracellular survival, migration and multiplication, damage, and subsequent GI illness. The authors suggest use of stochastic models for each of these processes and present an alternative formulation based on a predator-prey framework. They note the existence of physiological and biological processes that do not conform to the assumptions underlying the beta-Poisson or exponential dose-response model and that can occur during infection and the progression to illness. Examples of these processes are clumping of pathogens in the ingested dose, quorum sensing, and the possibility that organisms do not exhibit independent action.

Accounting for variations between strains

Accounting for strain heterogeneity in pathogens is another cutting-edge area of QMRA dose-response research that is difficult because dose-response data for all strains may not be available and because all pathogen strains may not have been identified. The simplest means to predict response for exposure to a variety of strains is to ignore inter-strain variations by using a dose-response model based on pooled data, using a dose-response model based on the most virulent strain among the strains considered, or to select a dose-response model for a "representative strain." In some cases, models of pooled data may not exhibit goodness of fit (Coleman and Marks, 1998).

A more systematic technique for addressing strain-to-strain variation is described by Soller et al. (2007). In that study, and drawing from the previous work of Coleman and Marks (1998, 2000), Gompertz-log dose-response models (alternatively called the Weibull dose-response model) were fit to data for all strains of *Salmonella* for which data were available. As part of this effort, one of the model parameters was assumed to be a random variable drawn from a uniform distribution whose minimum and maximum values corresponded to the minimum and maximum values found in fitting the model to individual (single strain) dose-response data. This technique appears amenable to use of Bayesian techniques, since distributions other than a uniform distribution may be assessed for describing variations related to pathogen strain differences.

IV.2.2 Accounting for Susceptible Populations

Susceptibility may be defined as "a capacity characterizable by a set of intrinsic and extrinsic factors that modify the effect of a specific exposure upon the risk/severity of outcomes in an individual population" (Balbus et al., 2000). Parkin (2004) advises QMRA practitioners to define susceptibility clearly during the problem formulation phase. The author describes intrinsic factors—including age (Parkin et al., 2003), gender, prior disease, immune status (Makri et al., 2004; Parkin, 2004), pregnancy (Lanciers et al., 1999), and diabetes (Currie et al., 2000)—as well as extrinsic factors (e.g., residence, income, co-exposures, access to health care) that may result in differing susceptibility among subpopulations. Because many factors may give rise to differences in susceptibility, this suggests that, where techniques and data are available, they should be considered in all elements of a QMRA. In problem formulation,

susceptibility should be defined, susceptible groups should be identified, and susceptible populations should be included as a distinct stakeholder group (Parkin, 2004). Sampling and surveying plans should include collection of quantitative data relevant to susceptible populations—particularly because available data related to susceptible groups are, at present, limited.

Parkin et al. (2003) conducted a feasibility assessment of incorporating analyses of populations with different susceptibilities in a QMRA of the risk of GI illnesses resulting from recreational use of surface waters. In that study, qualitative and quantitative data related to differences between subpopulations were drawn from a literature search for studies describing the infection of children by enteric viruses. The authors found several studies documenting an increased risk for children (as compared with adults) for infection (including a study providing odds ratios by age group), but did not provide definitive information on the relative severity of the duration of illness associated with exposure to recreational waters. The results of this feasibility assessment led the authors to recommend that researchers should provide greater detail in reports and studies of outbreaks, distinguishing between outcomes for subpopulations, and providing more details related to risk factors for subpopulations.

To our knowledge, analysis of susceptibility is not well developed in QMRAs conducted to date. Obvious elements of QMRA analysis in which susceptibility could be addressed are dose-response modeling and exposure assessment. The dose-response models most commonly used at present (the exponential and beta-Poisson models) are derived based on an assumption that host response at a given dose is binomially-distributed. This assumption may be relaxed by assuming the exposed population may be divided into distinct groups, each of which has a uniform susceptibility. Shortley and Wilkins (1965) suggested use of such a model in dose-response and proposed a model of the form

$$P(R \mid d) = 1 - xe^{-r_1 d} - (1-x)e^{-r_2 d} \tag{25}$$

for a population comprised of two distinct subgroups. In equation 25, r_1 and r_2 are the probability that a single organism can initiate infection in subgroup 1 and subgroup 2, respectively; x is the fraction of the population belonging to subgroup 1. Shortley and Wilkins (1965) found that the model provided a good description of responses of a group of mice given interperitoneal exposure to anthrax spores. Use of dose-response models such as that of equation 25 is, however, hampered at present by (1) the lack of estimates for dose-response models for susceptible groups for most agents of concern, and (2) difficulty in estimating the proportion of the population belonging to each subgroup.

An alternative to use of models such as that described by equation 25 is to assume the responses for a population exposed to a given dose are not binomially distributed. In this regard, Haas et al. (1999) suggest the beta-binomial distribution (equation 26) as an alternative.

$$P(j \mid n) = \frac{n!}{j!(n-j)!} \frac{B(j + \frac{\pi}{\theta}, n + \frac{1-\pi}{\theta} - j)}{B(\frac{\pi}{\theta}, \frac{1-\pi}{\theta})} \tag{26}$$

In equation 26, j is the number of individuals exhibiting a response in a population of n exposed persons, π is the expected probability of response at the dose level, and θ is a parameter describing the dispersion in response. Haas and colleagues demonstrated use of the beta-

binomial host response model for an hypothetical data set that could not be fit with binomially-distributed host response models and noted that assessing the goodness of fit of models relying on beta-binomial host response is problematic.

Two studies of *Cryptosporidium parvum* infection attempted to account for sensitive sub-populations in dose-response models. Pouillot et al. (2004) used two dose-response models for *Cryptosporidium* parvum infection. For immunocompetent sub-populations, an exponential dose-response model with $r = 5.26\times10^{-3}$, 95% CI (2.88×10^{-3}, 10.9×10^{-3}) was developed using data from a human feeding study with a single isolate. For the immunocompromised sub-population, data from experiments with immunodeficient mice were used to develop an exponential dose-response model with parameter $r = 0.354$, 95% CI (0.221, 0.612). For the immunocompetent sub-population, the probability of illness given infection was assumed beta-distributed, with p = beta (9,11) based on experimental data. For the immunocompromised sub-population, a worst case scenario estimate that 100% of infected persons became ill was used. Englehardt and Swartout (2004) used a bootstrap procedure for simulating dose-response data intended to reflect responses of the population as a whole, rather than only those of healthy adult volunteers. Although the authors justified and demonstrated the effectiveness of the procedure, they found that for drinking water scenario studied, the inclusion data for sensitive and resistant sub-populations in their analysis had little effect on overall predicted risks.

To summarize, avenues for quantitative analysis of susceptibility in dose-response include the following:

- adjustment of ingestion/ventilation rates to account for variability among subpopulations;
- use of transport models of sufficient spatial resolution to estimate pathogen concentrations in different neighborhoods/vicinities;
- use of non-human dose-response data to develop alternative dose-response models for sensitive sub-populations;
- use of bootstrap techniques for development of data sets inclusive of sensitive and resistant sub-population responses, and
- integration of spatially-resolved socio-economic data into exposure models.

IV.2.3 Secondary Transmission

Secondary transmission refers to infection spreading from one infected person to another person. Secondary cases (often reported as a secondary attack rate) generally refer to cases that occur following exposure to a primary case. In some cases, direct person-to-person transmission cannot be separated from contamination of the immediate environment and subsequent transmission to another person (e.g., toddlers sharing toys versus direct physical contact during play). In most cases, it is appropriate that the definition of secondary transmission include infections that result from propagation of the specific exposure of interest, but not encompass distant transmissions (separated by time and/or space) that may be more appropriately considered to result as a function of person-to-environment-to-person transmission.

Temporal and spatial limitations should be specifically noted in the definition of secondary transmission for a given pathogen. Full discussion of the range of scenarios that qualify as secondary transmission should be included where appropriate. It is important to note that the above definition of secondary transmission is limited to avoid overlap with pathogen occurrence in the environment (person-environment-person)—even though people are part of the

environment. However, the potential for re-introduction of the pathogen into the media could also be included within the definition of secondary transmission.

Secondary transmission models may be deterministic compartmental dynamic models, stochastic compartmental models, individual-based (individual history) models, or spatially-structured models. Descriptions of these models are provided by Rothermich and Murphy (2004). Dynamic MRA models (illustrated in Figure 5) can characterize secondary cases that occur among contacts following exposure to a primary case, whereas static MRA models usually consider secondary transmission to be negligible or include it as a non-fluctuating multiplicative factor (e.g., secondary cases equal primary cases multiplied by 0.1, if 10% secondary transmission assumed). The problem formulation documentation should indicate if/how secondary transmission is included in the assessment. If it is not included, justification for this decision should be provided.

A recent study of secondary transmission for waterborne diseases by Joh et al. (2009) explored the common assumption of a threshold number of organisms is needed to initiate an infection as a component of a transmission model for diseases transmitted indirectly to humans (i.e., via environmental reservoirs whose pathogen level is linked to human and animal infection levels). The objective of employing a threshold assumption was an attempt to match the observed dynamics of diseases such as cholera with model predictions. Although the use of threshold models in QMRA dose-response is a subject of ongoing debate, the study by Joh and colleagues is significant in its treatment of environmental reservoirs and ability to predict sporadic outbreaks of disease. As noted in the preceding exposure assessment section (IV.1) of this report, modeling of the temporal variability of pathogen loading in environmental systems remains an area of research within QMRA.

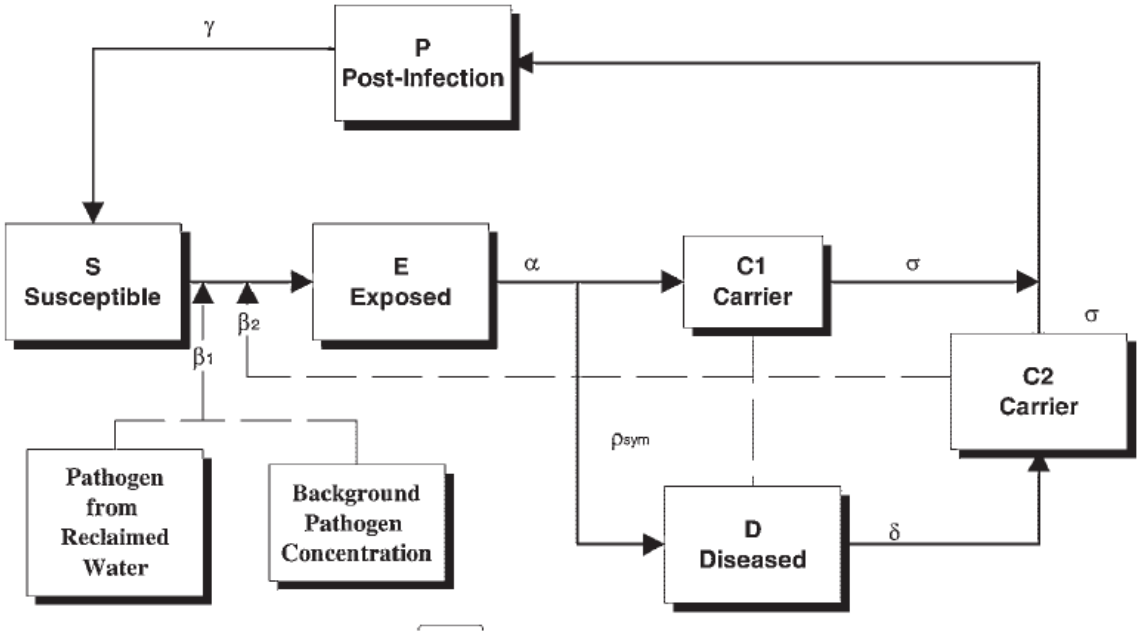

Figure 5. States and Flowpaths in a Dynamic Disease Transmission Model (SOURCE: Adapted From Soller And Eisenberg, 2008)

IV.3 RISK CHARACTERIZATION

Schaub (2004) states the major goals of risk characterization are to answer questions raised during problem formulation, describe confidence in estimates, and describe limitations of the QMRA process. The major elements that may be included in a risk characterization are listed in Table 6. Risk estimates may be expressed as individual or population estimates (ILSI, 2000) as per-event risks; as risk due to multiple exposures or as annual risk (Petterson et al., 2006); or as risk of illness, such as daily adjusted life years (DALY). Choice of the metric used for presenting risk data should be based on needs of risk managers and how estimates will answer questions from the problem formulation stage.

IV.3.1 Sensitivity Analysis

Prior studies and reports (ILSI, 2000; Mokhtari and Frey, 2005b) list the following roles for sensitivity analyses in model development and risk management:

- prioritization of potential critical control points (points, steps, or procedures at which a control can be applied in risk management);
- identification of key sources of uncertainty and variability;
- identification of data gaps; and
- model refinement, verification, and validation.

There is often a temptation to perform sensitivity analysis as a final step in a QMRA and a component of risk characterization. Delaying sensitivity analysis to this stage may result in unnecessary modeling and data collection for model components to which the solution is not particularly sensitive. Rather, the last two roles in the list above indicate that sensitivity analyses should be performed iteratively during model development and data collection (ILSI, 2000).

Sensitivity analysis techniques are chosen based on the objectives of the sensitivity analysis. These objectives might include (Mokhtari and Frey, 2005b) the following:

Table 6. Elements that May be Included in Risk Characterization (SOURCE: Adapted from ILSI, 2000)

Evaluate health consequences of exposure scenario
• Risk description (event)
• Risk estimation (magnitude, probability)
Characterize uncertainty/variability/confidence in estimates
Conduct sensitivity analysis
• Determine the most important variables and the information needs
Address items in the problem formulation
Evaluate various control measures and their effect on risk magnitude or profile
Conduct decision analysis
• Evaluate alternative risk management strategies

- ranking the importance of model inputs (e.g., critical control points);
- identifying contributions of input values that contribute to high exposure and/or risk scenarios;
- identifying and prioritizing key sources of uncertainty and variability;
- identifying critical limits; and
- evaluating the validity of the model.

Broadly, sensitivity analysis methods may be classified as mathematical, statistical, or graphical. General descriptions and specific techniques for these three types of sensitivity analyses are listed in Table 7. The choice of the QMRA model form depends on its use (screening, model selection and validation, evaluation of variability and uncertainty in risk characterization, etc.). Among complete QMRAs reviewed for this report (see Table 3), very few attempted sensitivity analyses beyond simple nominal range sensitivity analysis (NRSA) and many studies did not report sensitivity analysis results at all.

An important and cutting-edge facet of sensitivity analysis in QMRA is development of models and sensitivity analysis techniques that allow independent analysis of model sensitivity to uncertain factors and factors with variability. Several studies in which such analyses were performed are highlighted in this section as examples of QMRAs employing state of the art sensitivity analysis techniques.

Two studies (Mokhtari and Frey, 2005a; Pouillot et al., 2004) employed two-dimensional risk analysis (nested analyses in which uncertain parameters are selected from distributions and used as fixed inputs to stochastic risk models) to allow separate consideration of uncertainty and variability. Pouillot et al. (2004) addressed sensitivity through estimation of risk using pre-determined percentile estimates for model parameters. Mokhtari and Frey (2005a) addressed sensitivity directly, using and comparing an ANOVA analysis and rank correlation analyses. In their study, estimates and sensitivity rankings from two dimensional analyses (in which uncertainty and variability are addressed separately) were compared with similar sensitivity analyses for a one-dimensional model (in which uncertainty and variability were lumped). The two-dimensional model with ANOVA sensitivity analysis yielded the results best suited for use in risk management, while the correlation based methods were found to provide misleading results when used in conjunction with the two-dimensional model. Regardless of the choice of sensitivity model, the one dimensional model was insufficiently resolved to allow identification of processes over which risk managers can exert control.

Petterson et al. (2007) conducted a model sensitivity study (comparison of outputs of models developed under different assumptions) to evaluate the importance and best assumptions for including method recovery in estimates of occurrence of protozoans in drinking source waters. This study differs from those described above in that a specific uncertainty (method recovery) was addressed and in that alternative models for describing *Cryptosporidum* oocyst occurrence variability were developed. As with the above studies, however, uncertainty (related to method recovery) was separated from variability, which was characterized via distributions whose parameters and their uncertainty distributions were estimated using Bayesian techniques. These analyses indicated that the risk estimates were highly dependent on assumptions made regarding method recovery and that conservative assumptions about method recovery (low recovery rates) should be employed in future risk analyses.

Table 7. Sensitivity Analysis Methods and Techniques (SOURCE: Adapted from Frey and Patil, 2002; Frey et al., 2004)

Sensitivity Analysis Type	General Description	Techniques	Description
Mathematical	Quantification of the variation in model output with the range of variation of an input. Typically involves systematic variation of input parameters, evaluation of model, and assessment of the influence of the input parameters on the model output.	Nominal Range Sensitivity Analysis (NRSA)	Variation of individual inputs over their range while holding all other inputs at their nominal values. Sensitivity is assessed via comparison of model outputs corresponding to the range of values. When model output is probability, the difference in log odds ratio (ΔLOR) method may be preferred.
		Differential sensitivity analysis (DSA)	Variation of individual input in small range near central tendency values. Sensitivity is assessed based on variation in model output in the range around the central tendency.
		Automatic Differentiation (AD)	This method is similar to DSA, except sensitivity is assessed based on numerical partial derivatives for the variation in model output with changes in input parameters.
		Difference in log odds ratio (ΔLOR)	Similar to NRSA, except sensitivity is assessed via the ΔLOR, where $\Delta\mathrm{LOR} = \ln\left(\frac{p(\text{event} \mid \text{with changes in input})}{p(\text{Not event} \mid \text{with changes in input})}\right) - \ln\left(\frac{p(\text{event} \mid \text{w/out changes in input})}{p(\text{not event} \mid \text{w/out changs in input})}\right)$
		Worst-case determination	Similar to the ΔLOR approach, quantifies sensitivity to a factor via a factor sensitivity ratio given by $FS_k = \log\left(\frac{N_k(\text{extreme})}{N_k(\text{average})}\right)$ where *k* refers to the factor, *N* is the output (e.g., dose in the study conducted by Petterson et al., 2006) and extreme and average refer to worst-case and baseline values.
		Break-point analysis	Search for values of inputs at which decision-makers would be indifferent between two or more risk management options.

Sensitivity Analysis Type	General Description	Techniques	Description
Statistical	Inputs to models are assigned probability distributions and sensitivity is assessed via the effect of variance of the inputs on model output. Inputs may be varied using Monte Carlo simulation, Latin hypercube sampling, or other methods.	Regression techniques (sample regression or rank regression)	Linear models (either based on known relationships or analysis of scatter plots, etc.) are developed for the dependence model output on input variables. Regression is performed on a sample of data generated from the model (e.g., by Latin hypercube sampling, as demonstrated by de Vos et al., 2006). Sensitivity to input variables may be assessed via comparison of standard errors of regression coefficients or via application of stepwise regression techniques.
		Analysis of variance (ANOVA)	ANOVA is used to determine whether there is a statistical relationship between input variables and model output; in contrast to regression techniques, no functional form for the relationship is assumed and data may be qualitative or quantitative.
		Sample (Pearson) correlation or rank (Spearman) correlation	Sample correlation measures the strength of linear association between input variables and model outputs. Rank correlation is a measure of the strength of the monotonic relationship between two random variables.
		Classification and regression tree (CART)	Nonparametric technique that can select from among a large number of variables those and their interactions that are most important in determining the whether an outcome variable reaches a criterion value (Soller and Eisenberg, 2008). Output variables are divided into classes (e.g., above and below a criterion) and a tree of events leading to the output variable is developed and analyzed.
Graphical	Techniques for visualizing the change in model outputs with changes in model parameters.	Scatter plots	Plots providing information on the relationship between input variables and model outputs are constructed.
		Conditional sensitivity analysis (CSA)	Evaluating (usually graphically) the effect of changes in a subset of model inputs while other inputs are held at fixed values.

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ANNEX 2

Development of a QMRA Model to Evaluate the Relative Impacts to Human Health Risks from Animal-Impacted Recreational Waters (June 2009)

For

Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination

U.S. Environmental Protection Agency

December 2010

Development of a QMRA Model to Evaluate the Relative Impacts to Human Health Risks from Animal-Impacted Recreational Waters

Prepared by Clancy Environmental Consultants, Inc.
and Soller Environmental, LLC

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June 30, 2009

Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
Washington, DC 20460

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Executive Summary

An important goal of the Clean Water Act (CWA) is to protect and restore waters of the U.S. for swimming. A key component in the CWA framework for protecting and restoring waters for swimming is State adoption of Water Quality Standards (WQS) to protect swimmers from illnesses associated with microbes in the water. In this regard, one of EPA's key roles is to recommend recreational water quality criteria (under Section 304(a) of the CWA)) for adoption by the States.

It has been over 20 years since EPA last issued recreational criteria. The science underpinning this topic has advanced significantly during this time. EPA believes that new scientific and technical advances need to be considered, if feasible, in the development of new or revised 304(a) criteria. To this end, EPA has been conducting research and assessing relevant scientific and technical information to provide the scientific foundation for the development of new or revised criteria which are scheduled to be published in 2012. The Agency would like to be able to apply relationships from discrete epidemiology studies to the broad set of waters covered under the Clean Water Act.

The Health and Ecological Criteria Division (HECD) within the Office of Water, in conjunction with the Office of Research and Development (ORD) requested the development of a QMRA model that has the ability to mathematically encapsulate relevant scenarios for freshwater recreational waters impacted by agricultural animals (e.g., animal feeding sites and/or areas where animal manures are applied). Although specific sites of interest have been identified, EPA's goal in this respect was to identify and incorporate into a transparent and defensible model, the salient aspects of hazard fate and transport, representative exposure scenarios, and risk characterization for several important zoonotic pathogens.

This work was initiated by HECD in several phases.

- The first phase of this work (Work assignments B-01 and subsequently 1-08 Task 2) focused on model and scenario development for cattle-impacted waters. That work was developed as a key facilitating component to an EPA QMRA workshop in November 2008 at the ORD offices in Cincinnati, OH.

- The second phase of work (WA 1-08 Task 2 Amendment 3) requested an extension of the initial cattle scenario to one (or more) other agricultural animals (e.g., swine, poultry, etc.) and

- Conduct of a sensitivity analysis of the various (cattle, swine, and poultry) models and model parameters for the three exposure scenarios to determine which data and model components are the most crucial with respect to the conduct of QMRA for animal

impacted waters. The identified exposure scenarios included rainfall induced runoff transporting pathogens directly to a stream via overland flow, rainfall induced storage pond overflow, and re-suspension of pathogens stored within stream sediments.

This report summarizes the literature review, model development, QMRA simulations, and sensitivity analysis efforts that were undertaken to achieve the goals described above. During the course of this work (conducted for WA 1-08 Task 2 and subsequently Work Assignment 1-08 Task 2 Amendment 3) the following was accomplished:

- Conduct of a literature review, development, implementation and parameterization of a QMRA model in Analytica software, and development and parameterization of three exposure scenarios for cattle-impacted waters.
- Planning of and participation in an EPA QMRA workshop in November 2008 at the ORD offices in Cincinnati, OH where the cattle model was presented and demonstrated.
- Extension of the initial cattle model to two other agricultural animals (swine and poultry) for the three exposure scenarios.
- Conduct of a literature review of swine and poultry manure data to parameterize the newly extended QMRA model for *Salmonella*, *Camplyobacter jejuni*, *E. coli* O157:H7, and *Cryptosporidium parvum*.
- Conduct of a sensitivity analysis of the various models (cattle, swine, and poultry) and associated model parameters for the three exposure scenarios to identify which data and model components are the most crucial with respect to the conduct of QMRA for animal impacted waters.

The salient findings from this work includes the following:

- Onsite collection and storage of fecal material is an important barrier for preventing pathogen mobilization downstream. Operations that collect and store fecal material for land application may present short term peaks of pathogen risk, immediately following application. These peaks are estimated to be roughly equivalent to the ongoing risk associated with open grazing operations.
- When manure is to be stored and then land applied, the storage barrier is only effective for pathogen removal when a minimum storage time is ensured.
- Managing land application to avoid periods of high rainfall will reduce risk.
- Prevalence of infection in any given herd is likely to be constantly changing, and the within-herd temporal variation of can be substantial.

- Understanding the prevalence of human infectious pathogenic strains could be a critical component for not overestimating the risk associated with animal-impacted waters.
- Quantification of pathogen excretion density is a significant source of uncertainty in the overall model. In particular, the existence of super-shedders appears to drive the overall pathogen load. This aspect requires further research, particularly if identification and containment of super-shedders is possible.
- Environmental inactivation rates of pathogens are highly uncertain. Therefore, ensuring pathogen reduction via uncontrolled environmental processes is not feasible unless extended residence times can be guaranteed. Given the current state of knowledge, *Cryptosporidium* oocysts should be assumed to persist for long time periods unless site specific data indicate otherwise.

Introduction

An important goal of the Clean Water Act is to protect and restore waters for swimming. A key component in the CWA framework for protecting and restoring waters for swimming is State adoption of Water Quality Standards (WQS) to protect swimmers from illnesses associated with microbes in the water. One of EPA's key roles is to recommend recreational water quality criteria (under Section 304(a) of the CWA)) for adoption by the States. These EPA recommended criteria have been historically based on fecal matter in the water; in the 1960's the Federal government recommended a certain level of fecal coliforms as the recreational criteria. In 1986, EPA recommended certain levels of enterococci and *E. coli* as its new recreational criteria. These organisms do not cause human illness themselves (that is, they are not human pathogens); rather, they are merely indicators of fecal contamination and therefore indicators of the potential presence of human pathogenic organisms.

It has been over 20 years since EPA last issued recreational criteria. The science related to this topic has advanced significantly during this time. EPA believes that new scientific and technical advances need to be considered, if feasible, in the development of new or revised 304(a) criteria. To this end, EPA has been conducting research and assessing relevant scientific and technical information to provide the scientific foundation for the development of new or revised criteria. The enactment of the BEACH Act provided EPA with an opportunity to conduct new studies and provided additional impetus to issue new or revised criteria for coastal recreational waters (specifically, for Great Lakes and coastal marine waters) to replace or amend the 1986 EPA recommended criteria. EPA believes that the new or revised criteria must be scientifically sound, implementable for broad CWA purposes, and provide for improved public health protection over the 1986 criteria.

As one component of the work introduced above, the Agency would like to extrapolate relationships from discrete epidemiology studies to the broader set of waters covered under the Clean Water Act. Additionally, once new or revised recreational AWQC are published, the Agency would like to provide States guidance on using Quantitative Microbial Risk Assessment (QMRA) in developing WQS specific to local conditions. The Health and Ecological Criteria Division (HECD) within the Office of Water, in conjunction with the Office of Research and Development (ORD) has requested the development of a QMRA model that has the ability to encapsulate relevant scenarios for freshwater recreational waters impacted by agricultural animals (e.g., animal feeding sites and/or areas where animal manures are applied). Although the actual sites of interest have yet to be determined, EPA's goal was to identify and incorporate into a transparent and defensible model, the salient aspects of hazard fate and transport, representative exposure scenarios, and risk characterization for the zoonotic pathogens *E. coli* O157:H7 and *Cryptosporidium parvum*. The exposure scenarios of investigated were rainfall

induced runoff transporting pathogens directly to a stream via overland flow, rainfall induced storage pond overflow, and re-suspension of pathogens stored within stream sediments.

This work was initiated by HECD in several phases. The first phase of this work (Work assignments B-01 and subsequently 1-08 Task 2) focused on model and scenario development for cattle-impacted waters. That work was developed as a key facilitating component to an EPA QMRA workshop in November 2008 at the ORD offices in Cincinnati, OH. Based on the success of that work, EPA requested additional model development (Work Assignment 1-08 Task 2 Amendment 3) as follows:

- Extension of the initial cattle scenario to one (or more) other agricultural animals (e.g., swine, poultry, etc.). The extent to which the initial cattle model could be extended to other animals was to be evaluated and a justification was to be provided for extension of the initial scenario versus starting from scratch for the development of the additional scenario(s). This component of the work was to include a modest literature review of swine and poultry manure data (based on available resources) and was not intended to be an exhaustive review of all available literature.

- Conduct of a sensitivity analysis of the various (cattle, swine, and poultry) models and model parameters for the three exposure scenarios to determine which data and model components are the most crucial with respect to the conduct of QMRA for animal impacted waters.

With respect to the context of the sensitivity analysis introduced above, this effort was to be inclusive of the relationship of pathogens and indicators, including: 1) the fate and behavior of pathogens from these animal sources; 2) the fate and behavior of the indicators in these situations; and, 3) the potential divergence or uncoupling of these two groups in the environment due to the various environmental sources and sinks (i.e., regrowth, establishment, resuspension).

This report summarizes the literature review, model development, QMRA simulations, and sensitivity analysis efforts that were undertaken to achieve the goals described above.

Overview of QMRA and context for animal impacted waters

Quantitative microbial risk assessment (QMRA) (also known as MRA and pathogen risk assessment) is a process that evaluates the likelihood of adverse human health effects that can occur following exposure to pathogenic microorganisms or to a medium in which pathogens occur (1996; ILSI, 2000) To the extent possible, the QMRA process includes evaluation and consideration of quantitative information, however, qualitative information is also employed as appropriate (WHO, 1999). QMRA methodologies have been applied to evaluate and manage pathogen risks for a range of scenarios including from food (Bollaerts et al., 2009; Nauta et al., 2005; Seto et al., 2007),drinking water(Astrom et al., 2007; Medema et al., 1995; Regli et al., 1991; Soller, 2009), recycled water (Asano *et al.*, 1992; Westrell *et al.*, 2003) and recreational waters (Ashbolt and Bruno, 2003; Soller et al., 2003; Soller et al., 2006).

The principles, processes and methods for carrying out risk assessments for chemical agents were formalized in 1983 by the National Research Council (NRC) resulting in a four step process or framework (National Research Council, 1983). The steps outlined by the NRC include hazard identification, dose–response assessment, exposure assessment, and risk characterization. Many of the early MRAs employed the NRC conceptual framework to provide a structure from which the assessments could be conducted (Haas, 1983; Regli et al., 1991; Rose et al., 1991). As the field of microbial risk assessment developed, it became clear that there were some complexities associated with modelling the infectious diseases that are unique to pathogens, such as person-to-person transmission of infection and immunity. Therefore, the conceptual framework for chemicals may not always be appropriate for the assessment of risk of human infection following exposure to pathogens (ILSI, 1996).

To address this concern, the EPA Office of Water developed a conceptual framework to assess the risks of human infection associated with pathogenic microorganisms. The EPA Office of Water is also developing a framework for microbial risk assessment to support human health protection for water-based media. The EPA/ILSI framework emphasizes the iterative nature of the risk assessment process (Figure 1), and allows wide latitude for planning and conducting risk assessments in diverse situations. This framework consists of three principal components: problem formulation, analysis, and risk characterization. The analysis phase is further subdivided into the characterization of exposure and human health effects.

The problem formulation stage is used to identify: (1) the purpose of the risk assessment, (2) the critical issues to be addressed, and (3) how the results might be used to protect public health. Once identified, initial descriptions of the exposure and potential health effects are described and then a conceptual model is developed. This conceptual model is used as a starting point for the analysis phase of the risk assessment and later as an interactive tool along with components developed in the analysis phase to initiate the risk characterization.

In the analysis stage, information about both the exposure and the health effects is compiled and summarized. This compilation of quantitative and qualitative data, expert opinion, and other information results in exposure and host/pathogen profiles that explicitly identify the data to be integrated into the risk characterization and the associated assumptions and uncertainties. These two elements, while separate, must also be interactive to ensure that the results are compatible.

The final stage, risk characterization, results in a statement of the likelihood, types, and/or magnitude of effects likely to be observed in the exposed population under the expected exposure scenario, including all of the inherent assumptions and uncertainties. Often, the risk characterization phase includes data integration through parameterization of a mathematical model, numerical simulation and interpretation.

Salient aspects of each of these Framework components are briefly summarized below to provide context for the animal impacted water modelling effort undertaken in this work.

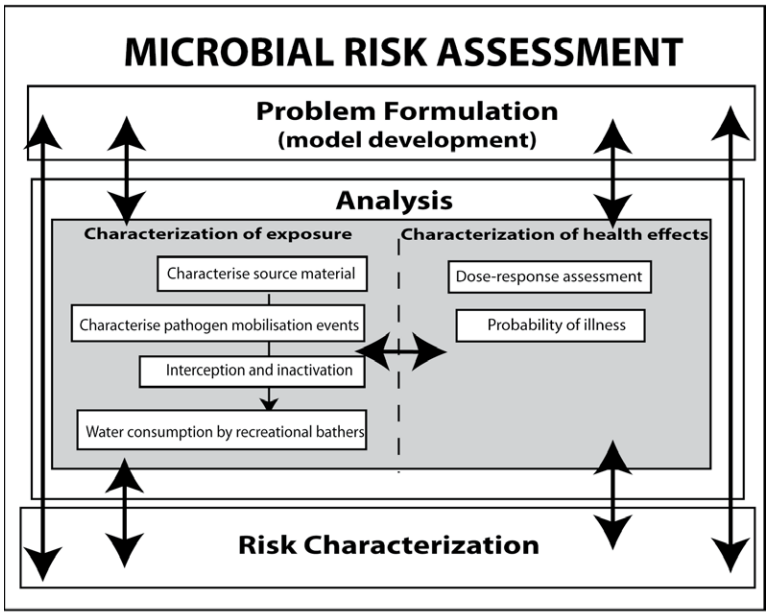

Figure 1. EPA/ILSI QMRA framework applied to animal impacted waters

Problem formulation

The objective of the model development for animal impacted waters was to facilitate the evaluation of the impact of fecal pathogens from animal feeding sites and/or areas where animal manure is applied on gastrointestinal illness among recreational bathers. The range of potential animal-impacted water sites was constrained in this work to consider dairy cattle, poultry and swine operations. Within this context, the identified hazards were enteric pathogens with the potential for zoonotic transfer from these animals to humans.

Given the scope and goals described above, a wide range of pathogens could be considered. However it is current practice within QMRA to select reference pathogens to represent the broad behaviour of the three microbial groups (bacteria, parasites, and viruses). Due to the significance of bacterial pathogens in zoonotic transfer, and the potentially different behaviour of different types of bacteria, three bacteria were selected as reference pathogens for this work: *Campylobacter jejuni*, *Salmonella* and *E. coli* O157:H7; along with one parasitic protozoa: *Cryptosporidium parvum*. While enteric viruses are thought to be primarily species specific (and hence not transmitted via zoonoses), recent evidence has demonstrated the cross-species transmission of HEV (Tei *et al.*, 2003), and concern exists regarding the potential for large scale waterborne transmission to humans (Myint and Gibbons, 2008). The evidence for this method of transmission is still building, and little environmental data is available to describe the prevalence, excretion, fate and transport of HEV. Thus, HEV was not included in this QMRA model development.

Analysis

Exposure assessment

As illustrated in Figure 1, exposure assessment for the QMRA involved modelling the occurrence of each reference pathogen in the environment from their source to the location of potential human exposure via recreational waters. The conceptual model for identifying and evaluating the hazard pathways is provided in Figure 2 which illustrates a generic animal feeding operation, where animals may either be housed within a shed, in open pens or contained in open paddocks.

Fecal material was assumed to be shed in one of these three environments. The fate of animal manure on animal feeding operations is a critical determinant of potential downstream risks. Manure handling varies significantly between operations depending on the type of facility (small, medium or large operation; breeding, feeding, laying, dairy, or finishing facility), geographic region, and intended use of manure (land application, storage, reuse for cattle feed, etc.). In general, manure is collected and stored as solids (solids comprise at least 20% of mass) or slurries (mixtures of feces, urine and potentially cleaning water, rainwater, and small quantities of feed) and the fate of the fecal material depends upon the local manure management operations. Fecal material shed on open pastures was assumed to accumulate naturally and be available for overland transport. Fecal matter from sheds and open pens is usually collected and stored and then potentially applied later to land within the same site or a nearby site.

For pathogens to present a potential health risk to recreational bathers, they must be mobilized from the farm. The sole mechanism for this mobilization was assumed to be rainfall induced runoff which could mobilize fecal material deposited on pasture land, fecal material 'spilled' during manure handling, land applied manure and potentially overflow of onsite storage facilities (pond) during large events.

Of those pathogens mobilized, some would be intercepted and some inactivated, preventing their complete transport to the recreational waters in an infectious state. In addition, some pathogens may be bound to soils (either on the soil surface or, when injected or tilled into the soil, in the vadose zone) and their transport to receiving waters may be retarded or prevented. In receiving waters, pathogens may be associated with sediments, and remain stored within the streambed to be resuspended during a future event.

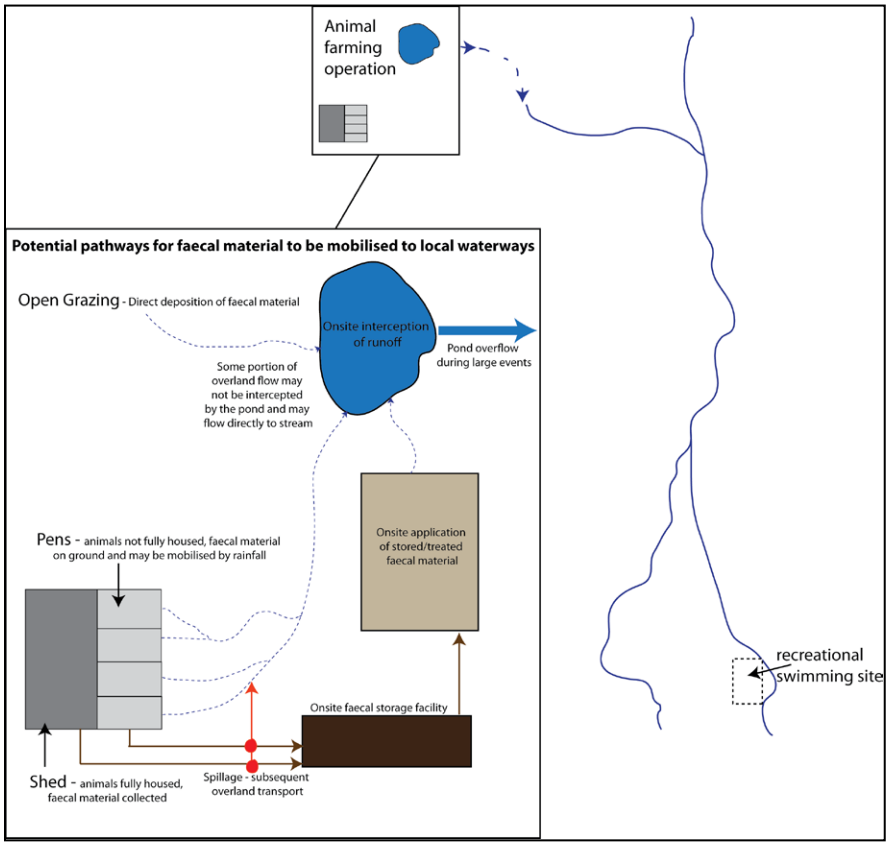

Figure 2. Conceptual model for exposure

The exposure assessment encompasses three separate pathogen mobilization events:

1. Rainfall induced runoff transporting pathogens directly to a stream via overland flow
2. Rainfall induced storage pond overflow
3. Re-suspension of pathogens associated with stream sediments

Human exposure to pathogens was assumed to occur through ingestion of ambient waters via recreational activities in waterbodies impacted by the events identified above.

Characterization of human health effects

The probability of infection and subsequent illness, given the modelled exposure was estimated based on dose response relationships published in the literature. Potentially important aspects of disease transmission such as secondary transmission (i.e. person to person or person to environment to person) and immunity were not accounted for in this work.

Risk characterization

The primary objective of constructing the QMRA model was to undertake the process of describing the system from source to exposure, review available data, to describe model inputs, and evaluate the sensitivity of the model and scenarios to the different variables. Risk characterization was conducted employing a static stochastic model.

QMRA model development

An important aspect of the model development was to translate the conceptual model for exposure (Figure 2) into a mathematical approach for quantifying the occurrence, inactivation and transport of pathogens from their source to exposure. As indicated previously, three mobilization events were considered in the model development. Those events are described below.

- Event 1: Direct overland transport to stream.
- Event 2: Overflow of the onsite storage pond. Pond overflow was assumed to occur during or as a result of a rainfall event, and therefore this event also includes the impact of direct overland transport (Event 1+ overflow of the pond).
- Event 3: Re-suspension of pathogens stored within stream sediments. An objective of the modelling was to investigate the magnitude of sediment re-suspension events on the downstream illness risk. To simulate this event, it was necessary to model the accumulation of pathogens within stream sediments prior to the hypothetical mobilisation. Of those pathogens mobilized to the stream during a runoff mobilisation event (Event 1) a portion of those were assumed to settle and remain stored within sediments.

An overview of the conceptual approach for the mathematical translation is provided in Figure 3. The pathogen load excreted each day by farm animals was calculated as the number of infected animals, multiplied by the concentration of pathogens in the feces of the infected animal and the daily fecal mass. This approach assumes that only infected animals excrete pathogens, and that infected and non-infected animals excrete the same mass of feces each day.

Fecal material was assumed to either remain on the ground or to be transferred to a storage facility. Fecal material deposited directly on the ground was assumed to inactivate over time following first order kinetics. A $\log_{10}$ basis was selected for all rate equations within the model. The total load of pathogens contained within deposited fecal material on grazing land was calculated as the sum of all previous daily loads accounting for inactivation over time since deposition. Fecal material collected for storage was assumed to inactivate over time following first order kinetics. Fecal material was assumed to be added to the facility on a daily basis, with the total load at time of application calculated as the sum of all previous daily additions, while accounting for inactivation over time in the storage facility. Conditions within the storage facility and within fecal deposits on land would be very different and different inactivation rates would be expected.

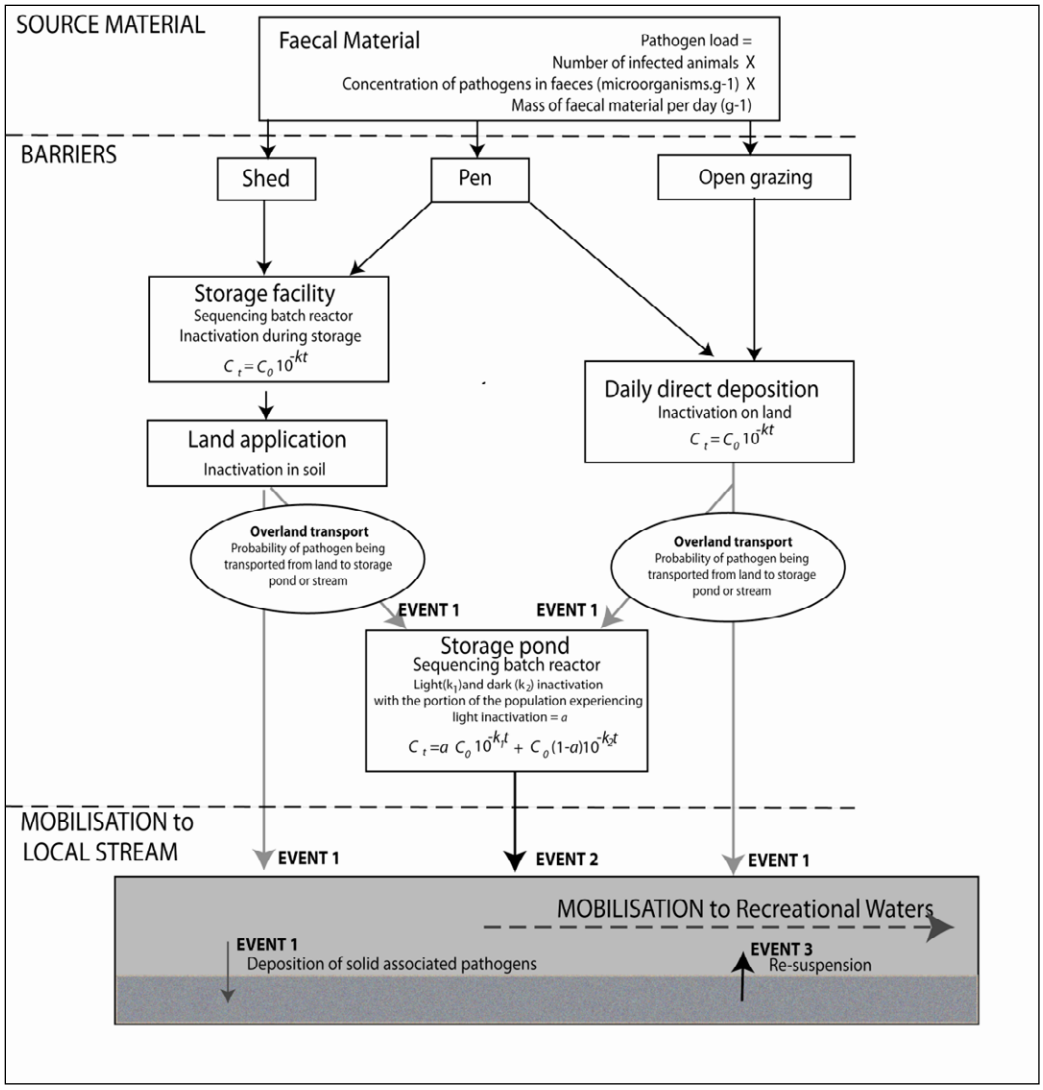

Figure 3. Conceptual approach for the mathematical translation of exposure

Overland transport of pathogens from grazing land and manure application sites is dependent upon many factors including the intensity of rainfall, vegetation cover, slope and distance to waterway. The QMRA model accounts for overland transport rates by assuming that every pathogen contained on land had a certain probability (p) of being transported to waterway during an overland flow event. This approach considers land transport as a single barrier in the same conceptual way as has been presented for drinking water treatment processes (Teunis et al., 1999a). There is the potential to describe the probability of passage (p) as a variable fraction accounting for differences in where pathogens may be deposited, vegetation cover and slope. A distribution for p could be selected based on the magnitude of the rainfall event.

Application of manure to land was assumed to occur as a single event, with the total load of pathogens in the storage facility transported to land.

The storage pond was assumed to intercept overland flow during small to moderate events. Pathogens contained within the pond were assumed to inactivate following first order kinetics, however based on reported information about the importance of sunlight in the inactivation of pathogens in water (Sinton *et al.*, 2007), light and dark inactivation phases were modelled separately. The total inactivation was assumed to be the sum of two subpopulations 1) those exposed to sunlight and 2) those protected from sunlight. The size of the total population (0-1) exposed to sunlight was described by the parameter a.

The QMRA model describes the impact of rainfall events on pathogen mobilization, and not only by a single event, but also upon the accumulation of pathogens within the storage pond and stream sediments based on a rainfall history. It was therefore necessary to include a dynamic component to the model which allowed this historical impact to be quantified (Figure 4).

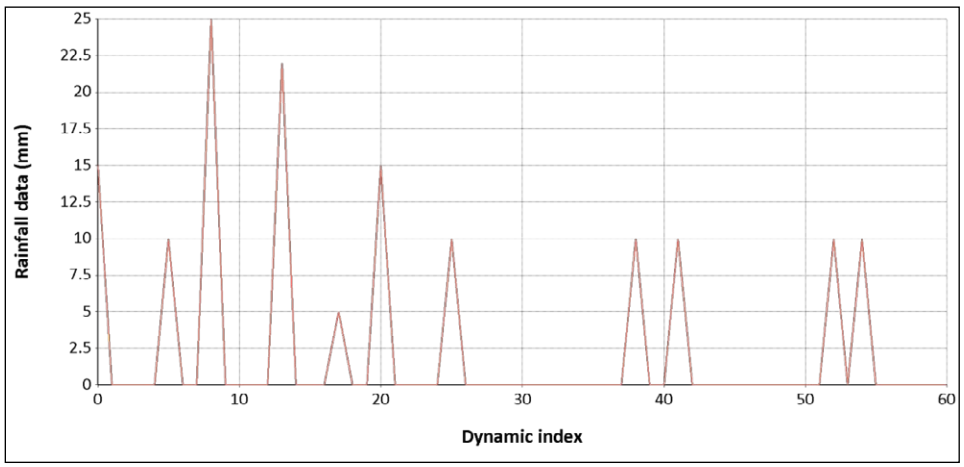

Figure 4. Hypothetical historical rainfall data used to populate the history of the QMRA model

The presented approach was then used to create the QMRA model using Analytica® Profession Version 4.1 (Lumina Decision Systems, Inc.) which allows for the construction of influence diagrams that can then be used for Monte Carlo simulation. The Analytica influence diagram and the user-interface of the model are illustrated in Figure 5 and Figure 6, respectively.

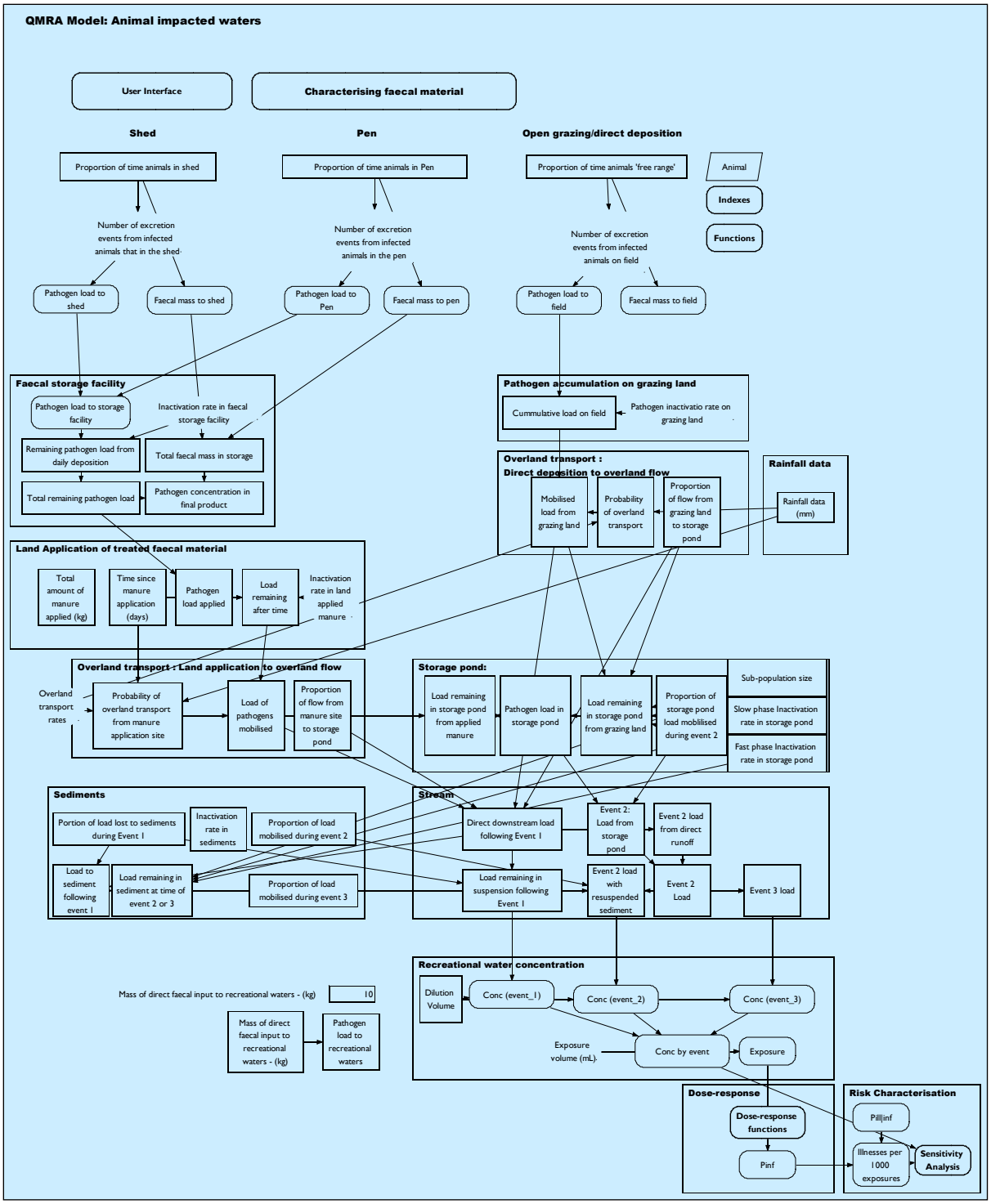

Figure 5. Influence diagram of QMRA model constructed in Analytica®

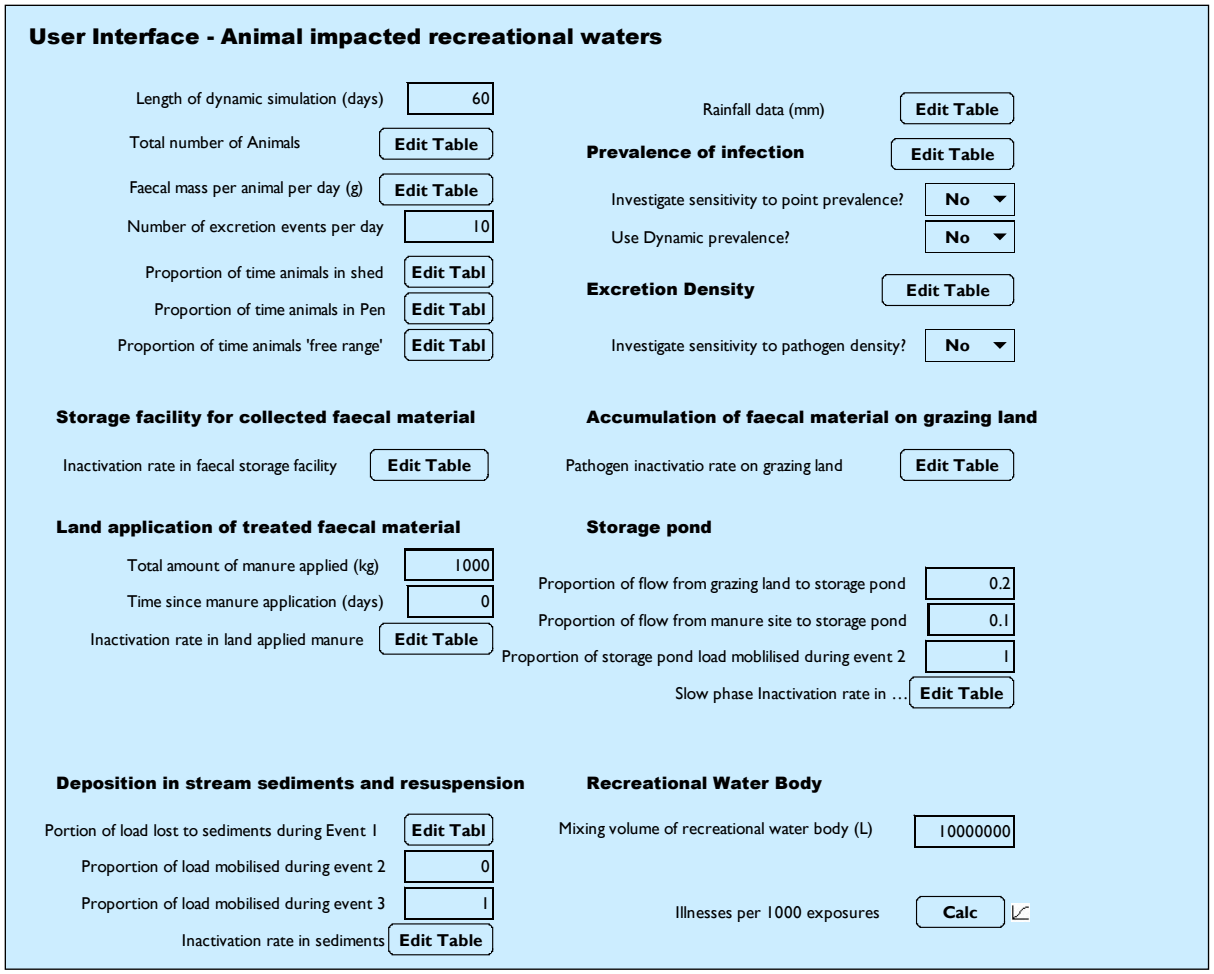

Figure 6. User Interface of the QMRA model for input of model variables in Analytica®

Quantification of model inputs through literature review

The scientific literature was reviewed with the objective of informing the selection of quantitative values for each of the model inputs (Table 1) for cattle, swine, and poultry impacted waters. A broad range of studies of relevance to the QMRA model were identified, however in many cases the studies had goals that were unrelated or tangentially related to our focus of waterborne human health risks. Special care was taken to consider the context of original datasets and the representativeness of those datasets to the input variables of interest in the QMRA model.

Table 1. Summary of inputs to the QMRA model

Input	Comments
Number of animals	The expected number of animals housed at a feeding operation for cattle, swine and poultry.
Proportion of time in shed, pen and grazing	Account for different housing conditions between each animal type
Fecal excretion rate ($kg.day^{-1}$)	The mass of feces excreted per day for each animal
Prevalence of infection	Needed to predict the number of animals infected with each reference pathogen on a farm for each animal type.
Density of pathogens in feces of infected animals ($microorganisms.g^{-1}$)	For each animal type, for each reference pathogen
Inactivation rate in fecal deposits	The rate is expected to be different for each reference pathogen and may vary for each animal due to size of deposits and composition.
Inactivation rate in stored manure	The rate is expected to be different for each reference pathogen and may vary for each animal.
Inactivation rate in land applied manure	The rate is expected to be different for each reference pathogen and may vary for each animal.
Overland transport from grazing land	Probability of passage from grazing land to surface waters expected to be different for each pathogen and animal.
Overland transport from manure amended soil	Probability of passage from manure amended soil to surface waters expected to be lower than from grazing land.
Inactivation rate in storage pond	Two population model to account for a protected sub-population
Proportion of mobilized load transported to sediments	Proportion will vary for each pathogen, and vary between runoff events.
Inactivation rate in stream sediments	Two population model to account for a protected sub-population.
Proportion of retained load mobilized during a re-suspension event	Re-suspension event will depend on individual pathogen and stream flow hydrodynamics
Dilution factor	What is the magnitude of the mixing zone of the load mobilized from agricultural land.
Exposure volume	Volume of water consumed by recreational bathers

Few of the model inputs were expected to be represented by a single value, but rather were likely to be variable, changing with time and space both on an individual farm and between farms. Variable model inputs can be described by a probability density function (PDF) which allows the variable to take one of a range of values each with defined probability of occurrence. Analytica® has the ability to describe any of the model inputs as either a point value or a distribution. The choice of whether to use a single value or a distribution was driven by two factors:

1. The nature of the input variable itself, and whether incorporating variability was deemed informative to the overall objective of the modelling exercise; and

2. Whether the available data was suitable for describing variability.

Uncertainty in quantifying model inputs from sparse data is difficult to separate from variability. However it is conceptually important to distinguish between what is considered to be actual variation in the model inputs and what is poor precision due to a lack of knowledge. In this study, uncertainty was investigated through the sensitivity analysis (described later). A summary of the data that were obtained from the literature review is provided below for the model variables summarized in Table 1.

Farm size, housing and manure handling

Cattle

About 2/3 of cattle and calves are raised on pasture and ranges, where manure collection is generally not practiced and an estimated 85% of beef cattle in the United States are fed in feedlots (Eghball and Power, 1998), primarily in the Central and Southern Great Plains. Thus, in their lifespan most beef cattle likely deposit manure both in pastures and in feedlots. Manure from grazing cattle is deposited directly on fields and pathogens and indicators in the manure are available for liberation from the manure matrix and down-slope transport. As with pigs raised on feedlots, manure from cattle on feedlots may be scraped and composted as a solid, or may be stored along with urine and other liquids as a slurry. Solids and slurries are nearly always land-applied on the farm where they are generated or on a nearby site.

Dairy cattle practices are more varied, due in part to the wider distribution of farms throughout the United States, in part to variation in practices with season, and due to the trend toward organic or other non-conventional farming practices. The two most common manure management strategies practiced on dairy farms are direct deposition on pasture lands and indoor collection and storage as solids or slurries (Hubbard and Lowrance, 1998). In both cases, manure is land-applied in the vicinity of its production. Increasing practice of organic dairy farming has produced trends toward smaller herd sizes (average of 82 compared to 153 milk cows per farm for organic and conventional farms, respectively) and increasing use of pasture for feeding cows (63% for organic dairy farms compared to 18% for conventional dairy farms) (Greene et al., 2009). Manure handling practices at dairy operations may vary with season, with

farmers housing cows indoors during the winter and collecting manure as liquid and/or solids during that period and more frequent use of pasture during other seasons.

Swine

In the US, more than 60% of swine are produced on large[1] farms with more than 5,000 head. On these large farms the majority of manure is collected as slurries and land applied; with sites for land application nearly always in the vicinity of the production facility. Small and medium sized operations are more likely to include pasture production (estimated at approximately 5%) or open feedlot production (estimated at approximately 35%). Open feedlots are scraped every two weeks, with manure stored in piles and subsequently land-applied via spreader.

Poultry

Poultry farms operate in the US for production of both eggs and broilers, with the size of operations illustrated in Figure 7 and Figure 8, respectively. Broiler operations produce only solid wastes (mixture of bedding and manure), which is almost entirely applied to land, on an annual basis following stacked storage. Laying hen operations produce both solid and liquid manure, with the liquid waste applied to land 2-3 times per year. Some solid poultry waste is used as livestock feed or in the production of biofuel, however these uses are considered to be small in comparison to land application. An increasing trend in the production of organic and free range chickens and eggs is expected, and will most likely have the greatest influence on small to medium operations.

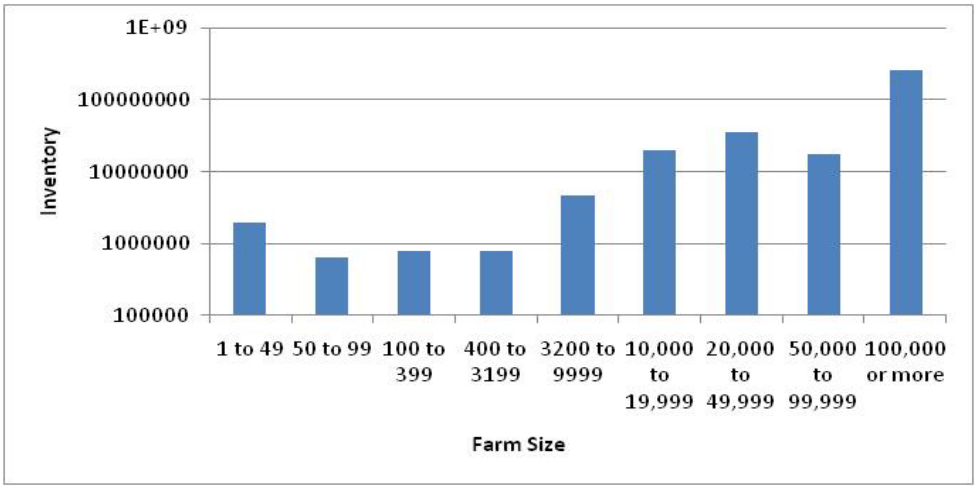

Figure 7. Egg layer farm size, 2007 (USDA NASS)

[1] The USEPA considers swine operations with at least 2500 swine weighing 55 pounds or more or 10,000 swine each weighing less than 55 pounds to be Large Concentrated Animal feeding Operations (CAFOs). Operations with 750 – 2500 swine each weighing 55 pounds or more or 3000 swine, each weighing less than 55 pounds AND a man made ditch or pipe carrying manure or wastewater or animals in contact with surface water in the confinement are considered medium CAFOs

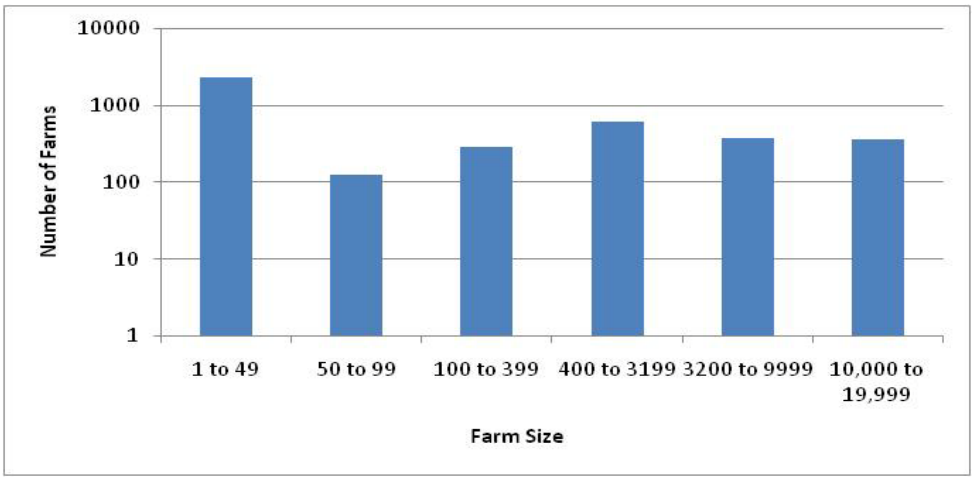

Figure 8. Broiler farm sizes, 2007 (USDA NASS)

Model input: farm size and housing conditions

While there is variability in the size of animal feeding operations across the United States, in the context of the QMRA model the farm size was considered to be a fixed point input. The model is constructed to investigate the downstream impacts of a single farming operation. The number of animals housed can be changed in the user interface of the model to compare for example the impact of a large or small farm, but it is not considered to be a random variable for the Monte Carlo simulation. A typical farm size was selected for each of the animal types (Table 2).

A similar approach was adopted for selecting the proportion of time in shed, pen and open grazing. While the review of practices across the United States indicated that there is variability between farms, any given farm was assumed to operate under one set of conditions. The most typical conditions were selected as the model input and are summarized in Table 2.

Table 2. Summary of model inputs for farm size and housing conditions

	Cattle	Poultry	Swine
Farm size	500	20,000	5,000
Proportion of time: shed	0.1 (milking)	1	1
pen	0	0	0
open grazing	0.9	0	0

Quantity of fecal material

Representative estimates of animal excretion rates from the published literature are summarized below.

Cattle

The average wet weight of feces excreted by cattle per day has been reported as 23kg (Dorner et al., 2004). The number of excretion events per day has been estimated at 12 (Larsen et al., 1994;

Thelin and Gifford, 1983) and 8.1 (Stromberg, 1997). Individual cow pats have been measured at 920g (Davies-Colley et al., 2004) and modelled assuming 1 kg per cow pat, and two cow pats per excretion event (Ferguson et al., 2007).

Poultry

Manure production estimates for poultry have been reported as 0.11 $kg.day^{-1}$ for laying hens and 0.04 $kg.d^{-1}$ for broilers (Dorner *et al.*, 2004); and 0.088 and 0.12 $kg.day^{-1}$ for layers (reviewed by Ferguson et al. (2009)).

Swine

Manure production estimates for swine included 15 $kg.day^{-1}$ for sows and gilts for breeding, 2 $kg.day^{-1}$ for nursing and weaner pigs, and 5 $kg.day^{-1}$ for growing and finisher pigs (Dorner et al. (2004)); and 5.1 and 6.2 $kg.day^{-1}$ for pigs in the US and Australia respectively (reviewed by Ferguson et al. (2009)).

Model input: mass of fecal excretion

Manure production rates vary with the health of the animal, type of feed, animal age, and potentially other factors. No data were identified that quantified the difference between excretion rates of infected and non-infected animals, and hence only average or typical rates were available for the model input. This is expected to be representative for long term endemic excretion by asymptomatic animals, but may not be representative of ill animals; a small portion of the total infected, but likely to be responsible for the bulk of the pathogen load (Chase-Topping *et al.*, 2008).

While there is variability between animals in the amount of fecal material excreted each day, the literature values were interpreted as reported averages. A point estimate was considered suitable for describing this average since adequate data were not available to describe the between animal variability. Conservative values, within the range of the reported averages, were selected and are summarised in Table 3.

Table 3. Summary of model input values for mass of fecal excretion

	Cattle	Poultry	Swine
Average fecal mass per animal per day (kg/day)	24	0.11	5

Characterization of fecal material

Point prevalence studies reviewed from the literature typically involved sampling a large number of animals, often across a number of farms, and reporting the overall proportion of those animals that tested positive for the target organism as the 'prevalence'. In the context of the current study it is worth considering the averaging effect of the adopted approaches and that localized effects can be lost. In addition, analytical methods for identifying pathogens from fecal samples are imperfect, often with poor rates of recovery. Therefore, fecal sampling may actually underestimate the true prevalence (Van Hoorebeke *et al.*, 2009).

Salmonella

Serotypes of *Salmonella enterica* differ widely in the doses required to initiate infection in humans and the severity of associated illness (Coleman and Marks, 2000; Coleman et al., 2004; Soller et al., 2007). Thus, salmonellosis risk is related to both the prevalence of *Salmonella* among animal and the serotypes prevalent in the animals. Though not conclusive, the relative risk posed by *Salmonella enterica* serotypes in animals may be inferred from comparison of the prevalent serotypes in different animal hosts and humans. The Centers for Disease Control (CDC, 2006) have identified the serotypes from human *Salmonella enterica* isolates for the period 1996-2006 and the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS, 2009) has identified the serotypes for Salmonella isolates identified in broilers, market hogs, steer and heifers, and cows and bulls for the period 1998 – 2007. The 24 most common serotypes of non-typhoid *Salmonella* from human isolates are summarized in Table 4 and Figure 9. Prevalence of serotypes for broilers, steers/heifers, cows/bulls, and market hogs are also presented in Table 4 and Figure 9. Inspection of these data indicate that the prevalence of serotypes within a given host changes significantly from year to year, though for humans, the serotypes Typhimurium (including the Copenhagen variant) and Enteriditis were consistently among the top three serotypes isolated. It is also noteworthy that there is overlap between the most common human and animal *Salmonella* serotypes (Figure 9), with all animals exhibiting relatively high prevalence of human-infecting serotypes Typhimurium, Newport, Saint-Paul, Infantis, Anatum, and Mbandaka and all of these hosts but swine are subject to infection with Montevideo. Over the period examined here, the percent of samples positive for broilers was highest (12.2%), followed by market hogs (3.3%), cows and bulls (1.3%) and steer and heifers (0.3%).

The overlap between serotypes prevalent in humans and those present in livestock can be used to develop a lower bound on the potential loading of human-infectious Salmonella from livestock. The 24 serotypes most commonly isolated from humans account for 79.5% of all positive isolates. The prevalence of the 24 most common human serotypes among livestock ranges from 52.5% to 59.8% of isolates (Table 5). Caution should be used in interpreting serotype prevalence data in risk estimation because assuming the overlap in serotype prevalence between human and livestock is an indication of relative risk disregards the role of exposure in risk. Sometimes lack of overlap between prevalent human and animal serotypes indicates no serious human health effects for those serotypes, but other times lack of overlap may indicate lack of exposure. Because *Salmonella enterica* infections are sporadic (Callaway et al., 2008) and serotype prevalence may change dramatically from year to year (USDA FSIS, 2009), there exists the possibility for an animal-associated outbreak (among humans) for a relatively uncommon or an unknown serotype.

Table 4. *Salmonella* serotype prevalence in humans, broilers, cattle, and market hogs

Serotype	Human	Broiler	Steer/ Heifer	Cow/Bull	Market Hog
Typhimurium (w. Copenhagen)	21.64	10.64	2.30	9.82	13.96
Enteriditis	17.80	6.76		0.73	
Newport	8.43		5.75	13.45	
Heidelberg	5.24	17.44	3.45	1.09	3.15
Javiana	3.46				
Montevideo	2.42	2.42	5.75	8.36	
Muenchen	2.04		1.15	1.09	0.30
Oranienburg	1.74		2.30		
Saintpaul	1.62		4.60	0.36	4.52
Infantis	1.54	0.94	2.30	3.64	7.43
Thompson	1.51	1.18			
Braenderup	1.49				
Agona	1.49		2.30		1.37
I, 4, [5], 12:i-	1.20	2.21			
Hadar	1.12	1.24		0.36	1.31
Mississippi	1.04				
Typhi	1.02				
Paratyphi B var L(+) tartrate (+)	1.02		2.30		
Poona	0.79		2.30		
Berta	0.64	0.34			
Stanley	0.58				
Anatum	0.57		4.60	5.82	9.45
Bareilly	0.52			0.36	
Mbandaka	0.52	0.75	1.15	2.55	0.36
Other or not identified	20.54	56.08	59.77	52.36	58.16

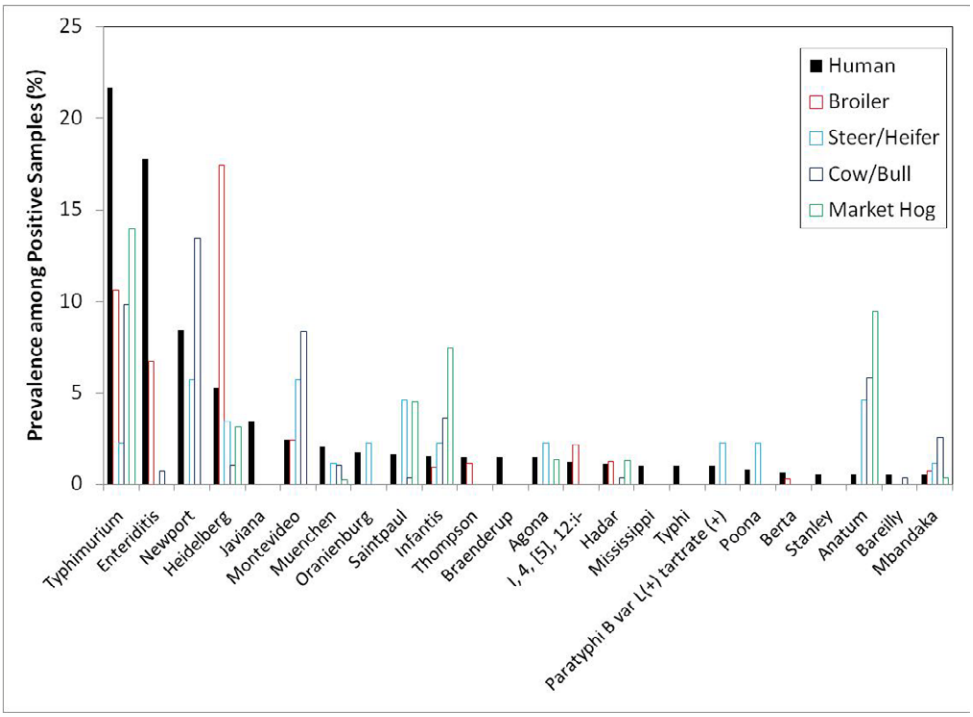

Figure 9. *Salmonella enterica* serotype prevalence in humans and livestock

Table 5. Overlap between *Salmonella enterica* serotype prevalence in humans and livestock

Host	Percent Positive Isolates with Serotype in the 24 Most Prevalent Serotypes among Human Isolates
Human	79.5
Broilers	43.9
Steers/Heifers	40.2
Cows/Bulls	47.6
Market hogs	41.8

Prior research suggested a method to account for the variation in dose-response characteristics across *Salmonella enteric* serotypes (Soller et al., 2007). In these QMRA analyses, a Gompertz dose-response model was assumed with one of the dose-response model parameters treated as a random variable. The prevalence data above suggests a potential modification to this approach, in which the selection of the dose-response model parameter is dependent on the likelihood of observing a serotype known to cause infection in humans frequently. Serotypes that appear in animals, but seldom in humans, may be assumed less able to initiate infection than those appearing regularly in both hosts.

Dairy cattle: Point prevalence studies have been undertaken by identifying the frequency of *Salmonella* positive fecal samples from cows. Reported point prevalence rates for *Salmonella spp*. in cow feces include 3.4% ((Rodriguez *et al.*, 2006); total samples n=2,496), 5.4%, (Wells *et al.*, 2001), 7.3% (Blau *et al.*, 2005), 9.96% ((Callaway *et al.*, 2005); total samples n=960), 31% (Huston *et al.*, 2002) and 0-93% ((Edrington *et al.*, 2004); total samples n=720).

The frequency of at least one positive fecal sample for each dairy (dairy prevalence) has also been reported. Those results include 21.1% (Wells *et al.*, 2001), 30.9% (Blau *et al.*, 2005), and 56% (Callaway *et al.*, 2005). Pangloli *et al.* (2008) identified seasonal variability in the prevalence of *Salmonella* in both animal and environmental samples on dairy farms. With some exceptions, prevalence increased with the seasonal temperature. Callaway *et al.*(2006) investigated the incidence of *Salmonella* in fecal samples collected from cattle feedlot pens. Within pen prevalence varied from 0 to 6.67% with the overall average prevalence at 3.75%.

Poultry: A review of studies prior to 1998 undertaken by Byrd (1998) estimated the prevalence of *Salmonella* among chicks to be 5-9%. The horizontal transmission between chicks co-housed in hatcheries was observed to be highly efficient. Garber *et al.* (2003) identified the *Salmonella enterica* serotype *enteritidis* in 7.1% of layer houses (*n* = 200, all U.S. facilities). Factors associated with higher incidence of *S. enterica* were large flock size (>100 000), young age, housing conditions (floor-reared as opposed to cage reared), and lack of cleaning and disinfection of feeders and hoppers between flocks. Hayes et al. (2000) detected *Salmonella* spp. in poultry litter and drag swabs from 48/71 (55.8%) broiler and roaster house facilities. Hutchison et al. (2004) detected *Salmonella* spp in 17.9% of fecal samples (n=67) from commercial farms.

Swine: Dorr et al. (2009) reported *Salmonella* spp. prevalence of 10.4% for 5 farms based on the detection of Salmonella spp in fecal samples. The highest and lowest observed prevalences (on individual farms) were 21.7% and 5%. *Salmonella* prevalence increased significantly with age. Hutchison et al. (2004) detected *Salmonella* in 7.9% of fecal samples (n=126) from commercial farms. Sanchez et al. (2007) undertook a systematic review and meta-analysis to identify study-level variables that could explain the variation in apparent *Salmonella* spp. prevalence estimates in swine. The median farm-level and animal-level prevalences were 59% and 17%, respectively.

E. coli O157:H7

Cattle: *E. coli* O157:H7 is commonly excreted by dairy cattle in the United States. According to Edrington *et al.* (2004) "*Research has suggested that almost all dairy farms will have cattle testing positive for E.coli O157:H7 if screened often enough*((Hancock *et al.*, 1997))". Elder and co-workers (2000) reviewed the literature regarding prevalence rates of *E. coli* O157:H7 or *E. coli* O157:nonmotile shedding in cattle and noted a widespread prevalence of E. coli O157:H7 with 63% of feedlots observing at least one positive sample. Overall cattle prevalence though appeared to be low at only 1.8% of fecal samples (Animal and Plant Inspection Service, 1995).

Method improvements employing enrichment followed by immunomagnetic separation (IMS) in the late 1990s had an important impact on analytical recovery rates, and led to an increase in reported prevalence rates. This improvement in method is demonstrated by Donkersgoed *et al.* (1999) who collected 1,247 fecal samples from 293 feedlots over 12 consecutive months; and analysed each sample by both direct culture and enrichment with IMS. Direct culture alone identified *E. coli* O157:H7 in 2.6% (33/1427) of samples while enrichment with IMS identified 7.5% (93/1247) as positive. n that study prevalence of *E. coli* O157:H7 was highest in summer (19.7%) followed by spring (4.9%), autumn (4.7%) and winter (0.7%).

Point prevalence of *E. coli* O157:H7 in fecal samples analysed with enrichment and IMS have been reported as 23% (Smith *et al.*, 2001), 0-35% (Edrington *et al.*, 2004); n=720), 5.9% (Barkocy-Gallagher *et al.*, 2003), 13% (LeJeune *et al.*, 2004); n=4790), and 13% (Fegan *et al.*, 2004). Callaway *et al.* (2006) investigated the incidence of *E. coli* O157:H7 in fecal samples collected from cattle feedlot pens. Within pen prevalence varied from 5 to 20% with the overall average prevalence at 11.6%.

Poultry: *E. coli* O157:H7 appears to have a very low prevalence in poultry. Of those studies identified in this review no detects were observed (Chapman *et al.* (1997) n=1000; Hutchison et al. (2004) n=67 fresh manure).

Swine: *E. coli* O157:H7 has been isolated from swine in ranges from 11.9 % (n = 126) by Hutchison et al. (2004) to 0.4% (n=1000) by Chapman et al. (1997). In the Chapman et al. study, *E. coli* O157 was isolated by an immunomagnetic separation technique and culture of magnetic beads on cefixime teelurite sorbitol MacConkey agar. Isolates that gave positive results were confirmed as *E. coli* by biochemical tests and as serogroup O157 or serotype O157:H7.

Campylobacter

Like *Salmonella*, the ability of *Campylobacter* isolates to infect humans varies among species and isolates and the prevalence of strains differs in animals and humans. Though other species may play smaller roles in human health effects, *Campylobacter jejuni* and *Campylobacter coli* are the most important human-disease-causing species of *Campylobacter* commensal in livestock (Wesley et al., 2000). Among all livestock hosts, the prevalence of *Campylobacter* and likely the relative prevalence of different *Campylobacter* species varies between farms and regions, with age of animal, with season, and probability with other factors (El-Shibiny et al., 2005; Weijtens et al., 1999; Wesley et al., 2000) and estimating prevalence of individual species is difficult given available data. Furthermore, the dose-response characteristics *C. jejuni* appear to differ among fresh cultures and laboratory cultures (Chen et al., 2006). Given this lack of species-specific prevalence data and the absence of a general dose-response model for human infection with *C. coli*, a reasonable approach is to assume that *Campylobacter* spp. dose-response parameter for campylobacteriosis (illness endpoint) fall within the range of related illness rates provided by Teunis et al. (2005).

Cattle: Molecular typing studies have shown that cattle and sheep are colonized with and excrete strains of *C. jejuni* which are capable of causing disease in the local community (Stanley and Jones, 2003); and outbreaks of disease from *Campylobacter* spp. have been associated with contaminated water from agricultural runoff (Hrudey and Hrudey, 2004; Vogt et al., 1982).

Poultry: Avian species are thought to be the natural hosts of thermophilic *Campylobacters* because they have a core temperature of 42°C, which is the optimum growth temperature of *C .jejuni*. According to the review undertaken by Stanley and Jones (2003), many studies have shown that once *Campylobacter* has been introduced into a shed, birds rapidly excrete high numbers in their feces and the organism spreads rapidly so that 100% of birds may be colonized within a few days.

Point prevalence of *Campylobacter* in poultry studies have been reviewed by Dorner et al. (2004)who reported a range of prevalence from 3.1% to 100% across 14 studies.

Swine: *Campylobacter coli* appears to be the predominant species found in swine. In a study of two Dutch piggeries (multiplier farms as opposed to fattening farms) with similar facilities and handling practices (Weijtens *et al.*, 1997), *Campylobacter spp.* were detected in feces of 9/10 sows (5 from each of two different farms) prior to delivery and in 10/10 sows post delivery. At 1 week after delivery *Campylobacter* was found in 29/60 piglets. At 8 weeks after delivery it was found in 56/60 piglets. Dorner *et al.* (2004) reviewed the point prevalence of *Campylobacter* in Sows and Gilts 45.9% positive (n=315); and 79.7% positive (n=59).

Cryptosporidium

Cattle: Atwill *et al.*, (2003) reviewed reported prevalence and excretion rates for *Crytosporidium* with an aim to estimate environmental loading rates and noted that "There is a wide range of reported prevalences of fecal shedding of C. parvum for adult beef and dairy cattle. Numerous investigators have reported mean prevalences of fecal shedding from ~20 to ~70% in groups of clinically healthy adult cattle (Lorenzo et al., 1993; Quilez et al., 1996; Scott et al., 1995), yet several large cross-sectional epidemiologic surveys have observed prevalences of only 2% or less in asymptomatic adult cattle populations (Atwill et al., 1999a; Hoar et al., 2001; Wade et al., 2000)."

Sources of this variation were identified as:

- Different investigators using diagnostic assays of differing sensitivity and specificity (Faubert and Litvinsky, 2000; Fayer et al., 2000; Pereira et al., 1999)
- Different populations (beef versus dairy), age distributions and management practices. For example, they found in two different studies that calving duration for beef herds was associated with a three- to sixfold difference in the proportion of cattle shedding *C. parvum* (Atwill et al., 1999b; Hoar et al., 2001), making interstudy comparisons of the shedding prevalence for beef cattle potentially confounded if not adjusted for calving duration.

Atwill and co-workers (2003) reported the overall apparent prevalence of adult beef cattle testing positive for *C. parvum* was 7.1% (17 of 240), with 8.3 and 5.8% of cattle shedding oocysts during the pre- and postcalving periods, respectively. Starkey and co-workers (2005) investigated the prevalence of *Crytosporidium* oocysts in the feces of dairy cows from a New York watershed. Of the 9914 fecal samples collected, 747 (7.5%) were found to contain *C. parvum*.

Fayer *et al.* (2006) investigated the prevalence of *Cryptosporidium* species in 571 1–2-year-old heifers on 14 dairy farms in seven states on the East Coast of the United States and reported 11.9% of 571 heifers were infected with *Cryptosporidium*, 0.7% with *Cryptosporidium parvum*. Of 68 PCR-positive specimens 1, 4, 10, 24, and 29 calves were infected with *Cryptosporidium suis*, *Cryptosporidium parvum*, *Cryptosporidium* deer-like genotype, *Cryptosporidium bovis*, and *Cryptosporidium andersoni*, respectively. These findings demonstrate a lower prevalence of infection in 1–2-year-old dairy cattle than in younger cattle as well as an increase in the diversity of species present. Consequently, the risk of humans acquiring infection with *C. parvum* from exposure to feces from yearling and older cattle appears much lower than from exposure to pre-weaned calves.

Poultry: Ferguson *et al.* (2009) reviewed two studies that reported prevalence rates for *Cryptosporidium* in poultry: Ley *et al.* (1988) reported prevalence rates in the US at 6% (n=17) and 27% (n=33) in broiler and layer chickens; Medema et al. (2001) reported prevalence of 27% (n=16) in The Netherlands.

Swine: Ferguson *et al.* (2009) reviewed 6 studies that investigated the prevalence of *Cryptosporidium* in swine, rates ranged from 0 - 100%.

Dynamic prevalence

As indicated by the summaries presented above, the reported rate of prevalence was highly variable for all reference pathogens. Several detailed prevalence investigations have been conducted which evaluated the dynamics of herd prevalence. Chapagain *et al.* (2008) modelled the dynamics of *Salmonella Cerro* infection in a US dairy herd. The data collected for that study included tracking an outbreak of *Salmonella Cerro* in a single milking herd. In March, 2004 only one (n=102) cow was shedding *Salmonella* at this time and the isolates from this sample were identified as *S. Enteric Typhimurium* (var. Copenhagen). Six months later, 43.5% of the herd was reported to be infected with *Salmonella enterica Cerro*. Within 6 weeks, the fecal prevalence rate of *S. Cerro* dramatically increased to 75% and persisted at or near this level for ~6 months. By August, 2005 the number of cows shedding *Salmonella* had dropped to 9% and the results of a subsequent sampling in September indicated that 29% of the cows were shedding this organism.

Van Kessel *et al.* (2007a) described the course of a *Salmonella* outbreak and subsequent endemic infection on a dairy farm in Pennsylvania. Shedding of *Salmonella* Cerro was reported to be

greater than 60% of the cows in the herd throughout the fall of 2004 and the spring of 2005. With this high level of persistent shedding, most of the environmental samples tested positive for *Salmonella* during this time frame. The animal level prevalence of *Salmonella* fell dramatically between March (67%) and August 2005 (8%) before rising to 88% by December 2005.

The dynamic nature of within herd prevalence observed for *Salmonella*, at least in part appears to explain the variability in reported point prevalence rates. It is logical to assume that other organisms may exhibit similar behaviour within a herd - with differences driven by the relative virulence and infectivity of the different organisms in the host.

Model input: prevalence of infection

Given the high level of variability in the reported results, selection of prevalence rates for each pathogen and animal was difficult, and most likely not representative of the all realistic situations. While a large number of studies have been reviewed, it was not considered reasonable to simply fit a distribution to these data for determining a variable input for the QMRA. Investigating the significance of a changing the prevalence rate on the model output is considered to be a more important attribute of the QMRA model, with a view to modelling dynamic prevalence rates within a single herd in the future. As a starting point for the simulations, point estimates were selected that reflected the general patterns reported in the literature. The values employed in the modelling effort are summarised in Table 6.

Table 6. Summary of model input values for infection prevalence (%)

	Cattle	Poultry	Swine
Salmonella	10	10	10
E. coli O157	20	0	15
Campylobacter	40	80	40
Cryptosporidium	30	10	10

Excretion density

The vast majority of studies aimed at quantifying the prevalence of infection have sought to identify the presence/absence of the target organism in fecal material. Not only the presence of pathogens however, but the magnitude of their concentration is essential for quantitative risk assessment calculations. Far fewer studies have been undertaken with the aim of quantifying pathogen *concentration* in feces of infected animals.

Salmonella

Cattle: Very limited data were identified describing the density of *Salmonella* in the feces of infected cattle. In their modelling study Chapagain *et al.* (2008) assumed the excretion density of infected cows to be 10^9 (units not specified).

Poultry: Byrd (1998) reported on challenging day-old chicks with 10^2, 10^4 or 10^6 *Salmonella typhimurium* by gavage. The concentration of *Salmonella* in litter ranged from $10^{2.05}$ to $10^{4.55}$

cfu/g litter (*n = 10*), with the highest concentrations resulting from chicks inoculated with 10^4 and 10^6 cfu. Hutchison *et al.* (2004) reported the mean and maximum concentration of *Salmonella* in fresh chicken manure to be $10^{2.34}$ and $10^{4.34}$ cfu/g (*n* = 12).

Swine: Hutchison *et al.* (2004) reported the geometric mean and maximum concentration of *Salmonella* in fresh swine manure to be $10^{2.78}$ and $10^{4.89}$ cfu/g (*n* = 10).

E. coli O157:H7

Cattle: A great deal more information was available for *E. coli* O157:H7 in comparison to *Salmonella*. Reported concentrations of *E. coli* O157:H7 in cow feces include: $2x10^2$ to $8.7x10^5$ cfu/g (Shere *et al.*, 2002); 4 to $>1.1x10^6$ per 10 g of feces (Widiasih et al., 2004); and <100 to > $36x10^6$cfu/g (Chase-Topping *et al.*, 2007). Providing insight into the nature of the variability, Fegan *et al.* (2004) reported the concentration of *E. coli* O157:H7 in cattle feces to vary from undetectable <3 MPN to 10^5 MPN/g with 67% of samples < 10 MPN/g and 8% in the range of 10^3–10^5 MPN/g. Matthews *et al.* (2006) undertook bacterial counts on 440 fecal samples positive for *E. coli* O157. Approximately one quarter of samples had concentrations >100 cfu/g reaching a maximum of around 10^6 cfu/g. The authors reported an outlier of $3.6x10^7$ cfu/g that had been excluded from the statistical analysis.

The most comprehensive dataset identified regarding the variability in shedding was undertaken by Robinson *et al.* (2004) in which two groups of naturally infected calves were intensively sampled for periods of 5 and 15 days, respectively. In that study the reported median level of shedding was 10^3 up to a maximum up 10^6 cfu/g (Robinson *et al.*, 2004). Recovery of the method was not taken into account with quantitative estimates, however the limit of detection was estimated at 100 cfu/g feces. The authors argued that the highly variable excretion densities may have been be due to actual variability in shedding, clumping of organisms within the fecal material, or variability in method recovery.

Poultry: No studies were identified that described the excretion density of *E. coli* O157:H7 in infected birds. Prevalence of *E. coli* O157:H7 in poultry appears to be very low.

Swine: Cornick and Helgerson (2004) challenged three month old pigs with graded doses of *E. coli* O157:H7. Pigs were housed indoors on concrete floors or decks in Iowa. Shortly after inoculation fecal *E. coli* O157:H7 density ranged between 10^3 and 10^7 cfu/g. After two weeks and then two months, the shedding density ranged from 50 to 1000 cfu/g, and non-detect to 10^4 cfu/g, respectively. Hutchison *et al.* (2004) reported the mean and maximum concentrations of *E. coli* O157:H7 in fresh swine manure at $10^{3.59}$ and $10^{5.88}$ cfu/g respectively (n = 15).

Campylobacter

Cattle: Stanley *et al.* (1998) reviewed the excretion patterns of *Campylobacter* in sheep and cattle and concluded that young cattle are exposed to *Campylobacter* infection within the first few days of life, and calves can excrete very high numbers (10^8 per g feces); while adult cattle

shed *Campylobacter* intermittently throughout their lives at excretion densities of around 10^2–10^3 per g feces.

Poultry: Cox *et al.* (2002) reported concentrations of *Campylobacter* in breeders of $10^{2.8}$ – $10^{3.9}$ cfu/g feces and broilers of $10^{3.5}$–$10^{6.5}$ cfu/g feces. Studies reviewed by El-Shibiny et al. (2005) reported a range of 10^6– 10^9 cfu/g feces.

Swine: Average density of *Campylobacter* in sow feces at two separate farms one week before delivery were $10^{5.0\pm1.1}$ and $10^{3.6\pm0.4}$ cfu/g feces (Weijtens *et al.*, 1997).

Cryptosporidium

Cattle: For adult cattle, the total load has been has been estimated to be 3900 – 9200 oocysts per adult animal per day (Atwill *et al.*, 2003). In contrast Davies and co-workers (2005a) reported that the mean number of oocysts excreted by apparently healthy grazing adult cattle was 331 per g feces (dw), which relates to a total load of around 10^7 oocysts per animal per day. The prevalence and shedding intensity of *Cryptosporidium* oocysts among animals in the Sydney drinking water catchment has also been investigated by Cox *et al.* (2005). In that study, the concentration of *Cryptosporidium* across cows, sheep and horses ranged from 0 to >6897 oocysts per g feces (ww). This expected mean excretion density on the order of hundreds of oocysts per g feces has also been reported in Canada (Heitman *et al.*, 2002) and Uganda (Nizeyi *et al.*, 2004).

Calves however, may excrete higher numbers of oocysts during infection in comparison to older cows with reported averages of 1.3×10^5 (Starkey *et al.*, 2005) up to 10^7 oocysts per gram of feces (Blewett 1989). Nydam *et al.* (2001) investigated the shedding patterns of calves naturally infected with *Cryptosporidium* and reported that an infected 6 day old calf would produce 3.89×10^{10} oocysts until 12 days old. This is a much higher loading, particularly given the high expected prevalence of symptomatic *Cryptosporidiosis* infection among calves. In addition, the results of genotyping studies suggest that the risk of human infection from oocysts excreted by pre-weaned calves may be considerably higher than from yearling and older cattle(Santín *et al.*, 2004).

Poultry: Among the two *Cryptosporidium* species most often isolated from poultry, *C. meleagridis* and *C. bayeli* (de Graaf et al., 1999; Thompson et al., 2008), only *C. meleagridis* is known to infect humans (Ramirez et al., 2004). Tůmová et al. (2002) found that shedding rates for broilers experimentally infected with *C. meleagridis* ranged from 0 oocysts to approximately 20,000 oocysts per mL of liquid chicken feces (data were presented graphically). Shedding began approximately 2 days after experimental infection, peaked after about 7 days and lasted for more than 15 days post infection. Ferguson et al. (2009) note a *Cryptosporidium* spp. shedding rate of 2100 oocysts/g feces for layers in the Netherlands.

Swine: Natural *Cryptosporidium parvum* infection by bovine and porcine genotypes of *C. parvum* has been observed in pigs (Guselle et al., 2003). This porcine genotype has been identified in a human infection (Xiao et al., 2002). Reported shedding rates of *Cryptosporidium parvum* from pigs ranged from 0 oocysts at 18 days post-weaning to a maximum geometric mean for 33 pigs (94% infection rate) of 1596 oocysts/g feces at 53 days post-weaning. Dorner et al. (2004) fit the same data with a gamma distributions to describe shedding intensities observed during the course of the study. In their review of pathogen loads from animal sources, Ferguson et al. (2009) note *Cryptosporidium* spp. shedding rates from prior studies include 14.3 oocysts/g for pigs in an Australian study and 472 oocysts/g for 6 – 8 week old pigs.

Excretion patterns and super-shedders

All studies that investigated the concentration of pathogens in the fecal material of infected animals reported a wide variability in the measured concentration. In a review by Chase-Topping *et al.* (2008) the authors argue that for *E. coli* O157 excretion density could not be described by a single distribution as most (75%) fecal samples were positive for bacterium containing $<10^2$ cfu/g of feces, while some animals excreted up to $>10^7$ cfu/g of feces. The implications could be substantial, since in one study the high shedders (defined as $>10^4$ cfu/g feces) made up 9% of a sample of slaughter cattle but were responsible for >96% of all *E. coli* O157 bacteria shed.

Chase-Topping et al. (2008) recommend the following definition of a 'super-shedder': "An individual who for a period yields many more infectious organisms of a particular type than most other individuals of the same host species. Typically, many more infectious units are released from a super-shedder. The term is most useful when there is a clear biological basis for the distinction between super -shedders and non-super-shedders (such as host genetic differences, host immune suppression, type differences in the infectious organism, or the presence or absence of co-infections)."

Based on the brief review presented in this paper, the pattern of heterogeneity in excretion density appears to exist for all reference pathogens.

Model input: excretion density

Two separate excretion densities were selected for each pathogen to represent endemic excretion rates and 'super-shedder' excretion rates (Table 7). The initial simulations were undertaken with zero super-shedders in the catchment (i.e. only endemic excretion), but then additional simulations were undertaken including 1 to 10 super-shedders on top of the baseline endemic level. The literature consistently reported variability in shedding rates within each group, and therefore a variable shedding density was assumed. Sufficient data were not available to define the shape of the distribution, therefore, the triangular distribution was used to describe the estimated range of likely values. Distinguishing between different animal types was not possible.

Table 7. Summary of model input values for excretion density (Log_{10}. microorganisms/g)

	Endemic	Super-shedder
Salmonella	Triangular(2, 3, 4)	Triangular (5, 6, 7)
E. coli O157	Triangular (1, 2, 3)	Triangular (5, 6, 7)
Campylobacter	Triangular (2, 3, 4)	Triangular (6, 7, 8)
Cryptosporidium	Triangular (1, 2, 3)	Triangular (5, 6, 7)

Persistence in fecal deposits, manure and soils

Bacteria (*E. coli* O157:H7, *Salmonella and Campylobacter*)

Muirhead *et al.*(2005) reported that there was an initial increase in the number of bacteria in defecated cow feces for the first 2 weeks, and by the 5th week the bacteria were back to their initial levels. A study undertaken by Van Kessel *et al.* (2007b) concurred with these results reporting that cowpats may remain a substantial source of *E. coli* for at least 30 days after deposition and maybe much longer, mostly because of a substantial growth in *E. coli* (up to 1.5 log_{10}) during the first 4–8 days. Temperature appeared to be the leading factor affecting the magnitude of the initial growth of the *E. coli* population in freshly deposited bovine feces. The range of temperatures between 20 and 35°C appeared to be the most favourable for the post-deposit growth.

Regrowth of bacteria including *Salmonella* has been previously reported (Gibbs *et al.*, 1997; Zaleski *et al.*, 2005) and has been associated with increasing soil moisture after rainfall events (Pepper *et al.*, 1993). Gagliardia and Karns (2000) examined the impact of intermittent wetting and drying on *E. coli* O157:H7 survival and transport through soil columns. The researchers expected the level of *E. coli* O157:H7 in leachate to significantly decrease during dry periods, however no decrease was observed indicating that only limited soil moisture was required to support bacterial survival and possible growth.

E. coli O157:H7 has been shown to survive in sewage sludge for greater than 2 months (Avery *et al.*, 2005). *Campylobacter* spp. were thought to survive poorly in digested sludge applied to land (Jones *et al.*, 1990), but subsequent work identified that 8% of swab samples taken from sludge put to land were positive for *C. jejuni* (Jones, 2001), identifying the need for further work to evaluate the persistence of *Campylobacter* in sludge amended soil.

Rosen (2000) reported that pathogenic organisms including *Salmonella typhimurium* were observed to survive less than half as long in aerated manure than in non-aerated manure, largely because temperatures in aerated manure were significantly higher than those observed in the non-aerated manure .

Microcosm studies (Åström *et al.*, 2006) indicate that *Campylobacter* from piggery effluent is much less persistent than either *Salmonellae* or indicator bacteria. *Campylobacter coli*

inoculated into microcosms with high soil moisture, moss, and lichen covers was not detected within 3 hours post inoculation, even in microcosms kept in the dark and at relatively low temperature (conditions believed optimal for *Campylobacter* survival). The authors note that other studies conducted in wastewaters and surface waters have shown greater *Campylobacter* persistence than observed in their study. Guan and Holley (2003) reviewed the persistence of *Campylobacter* in a range of matrices, and reported persistence in water for 8-120 days at 4–8°C but <2 days at 20 – 30°C; in soil for 20 days (4-6 °C) and 10 days (20-30 °C); in human feces for 12-21 days (4 -6 °C); in cattle manure for 3 days (20-37 °C); and in cattle manure slurry for 3 days (4-37 °C).

Dieoff constants for E. coli and fecal coliform reported by Van Kessel et al. (2007b) are summarized in
Table **8**. The average air temperature during the observation period was 25.8°C. Rate constants in laboratory incubated samples were substantially smaller than the rate constants in field incubated samples, however the difference between open and shade groups from the field was not significant.

Table 8. Exponential die-off constants for E. coli and fecal coliform
Data from Van Kessel et al. (2007b)

	Field		**Laboratory**		
	Open	Shade	21.1°C	26.7°C	32.2°C
E. coli	0.205±0.070	0.230 ±0.012	0.08 ±0.02	0.125 ±0.044	0.166 ±0.028
Fecal coliform	0.225±0.017	0.169 ±0.030	0.071 ±0.018	0.103 ±0.039	0.125 ±0.016

Cattle Manure: Guan and Holley (2003) reviewed *Salmonella* survival and reported persistence for 27-60 days (temperature range 4–37°C) in liquid manure (slurry); for 48 days in solid manure at 4–5°C and 48 days in solid manure at 20–37°C. Rosen (2000) reported a 0.246–0.986 cfu/d reduction in *Salmonella* in cattle manure slurry. Inactivation rates for *E. coli* O157 and Salmonella in fresh cow manure and slurry reported by Himathongkham et al., (1999) are summarized in Table 9.

Table 9. Inactivation rates of *Salmonella* and *E. coli* O157 in fresh cow manure and slurry Data from Himathongkham et al. (1999)

		Inactivation rate k Log_{10} inactivation/day			
		Fresh manure		Slurry	
Temp	Location in Pile	*Salmonella*	*E. coli* O157	*Salmonella*	*E. coli O157*
4 °C	Middle and bottom	0.049	0.054	0.061	0.04
	Top	0.079	0.111		
20 °C	Middle and bottom	0.107	0.074	0.079	0.068
	Top	0.040	0.046		
37°C	Middle and bottom	0.578	0.279	0.422	0.315
	Top	0.120	0.112		

Poultry Manure: Hutchison et al. (2005a) reported *Salmonella* inactivation rates of k = 0.565 $\log_{10}$ cfu/d in farmyard manure; *E. coli* inactivation rates of k = 0.725 $\log_{10}$ cfu/d; and *Campylobacter* of k = 0.395 cfu/d. Inactivation rates for *E. coli* O157 and Salmonella in fresh poultry manure and slurry reported by Himathongkham et al., (1999) are summarized in Table 10.

Swine Manure: Hutchison *et al.* (2005a) reported *Salmonella* inactivation rates of k = 0.552 $\log_{10}$ cfu/d in farmyard manure; and E. coli inactivation rates of k = 0.55 $\log_{10}$ cfu/d.

Table 10. Inactivation rates of *Salmonella* and *E. coli* O157 in fresh poultry manure and slurry Data from Himathongkham et al. (2000).

		Inactivation rate k Log10 inactivation.day-1			
Temp	**Location in Pile**	**Fresh manure**		**Slurry**	
		Salmonella	*E. coli* O157	*Salmonella*	*E. coli O157*
4 °C	Middle and bottom	0.086	0.061	0.022	0.0064
	Top	0.117	0.070		
20 °C	Middle and bottom	0.627	0.689	0.151	0.145
	Top	0.631	0.688		
37°C	Middle and bottom	1.634	1.683	0.571	1.527
	Top	1.634	1.683		

Cryptosporidium

For *Cryptosporidium*, the mechanism(s) of inactivation in fecal material and soils are not well understood. However temperature has been shown to be an important factor determining the

inactivation rate of oocysts, with a rapid increase in inactivation as temperatures exceeded 35°C (Davies et al., 2005b; Jenkins et al., 1999). Other factors considered important included soil/biotic effects since much higher rates of inactivation were observed in fecal material in comparison to water (Olson *et al.*, 1999). By implication, pathogens may be expected to be inactivated more quickly in microbially rich matrices such as cow pats. Other factors including ammonia (microbial production of ammonia in stored animal wastes) can also contribute to inactivation of oocysts in stored fecal material (Jenkins *et al.*, 1998).

Davies *et al.* (2005b) investigated *Cryptosporidium* oocysts persistence in closed soil microcosms over time, using fluorescence *in situ* hybridization (FISH) as an estimate of oocyst 'viability'. Time for one $\log_{10}$ inactiviation (calculated from reported inactivation rates) ranged from 13-24 days at 35°C (Log_{10} k=0.077 to 0.042 day^{-1}) to 45–75 days at 20°C (Log_{10} k= 0.022 to 0.013 day^{-1}) depending on soil type.

Model input: persistence of reference pathogens in feces and soil

Manure handling has a profound influence on pathogen persistence, with greater persistence observed, in general, in liquid and slurry manure storage than in solid manure storage. Input assumptions for the model are summarised in the following sections and in Table 11.

Cow pats: The inactivation rate for bacterial pathogens in cow pats was assumed to be zero for the first 4 days, and then follow open field conditions of 0.2 day^{-1} exponential decay rate (0.087 day^{-1} on a Log_{10} scale). Inactivation rate for *Cryptosporidium* oocysts was assumed to be similar to inactivation rates at 20°C, and hence a triangular distribution of (0.05, 0.04, 0.03) was selected. This equates to T(0.02,0.015,0.013) on a Log_{10} scale. Higher rates can be expected at higher temperatures, and hence this assumption is likely to be conservative.

Cattle manure: The inactivation rate for bacterial pathogens in cow manure was based on results reported by Himathongkham et al. (1999) for 20 °C, in the middle or bottom of the pile, rounded down. No additional data were available for *Cryptosporidium*, so inactivation was assumed to be the same as in cow pats.

Poultry manure: The inactivation rate for bacterial pathogens in poultry manure was based on results reported by Himathongkham et al. (2000) for 20 °C, in the middle or bottom of the pile, rounded down. *Campylobacter* was assumed to inactivate at a rate equal to the value reported by Hutchison et al. (2005a) . No additional data were available for *Cryptosporidium*, so inactivation was assumed to be the same as in cow pats.

Poultry slurry: The inactivation rate for bacterial pathogens in poultry slurry was based on results reported by Himathongkham et al. (2000) for 20 °C, in slurry, rounded down. *Campylobacter* was assumed to inactivate at a rate equal to the least persistent of *E. coli* O157 and *Salmonella*. No additional data was available for *Cryptosporidium*, so inactivation was assumed to be the same as in cow pats.

Swine manure slurry: The inactivation rate for bacterial pathogens in swine manure was based on the only available values reported by Hutchison *et al.* (2005a) of 0.5 Log_{10} inactivation.day^{-1}. No additional data were available for *Cryptosporidium*, so inactivation was assumed to be the same as in cow pats.

Manure amended soil: In the absence of any additional data, inactivation rates in manure amended soil were assumed to be the same as in the manure alone.

Table 11. Model inputs for inactivation rates of reference pathogens in fecal deposits and manure

	Inactivation rate k Log10 inactivation per day			
	Salmonella	*E. coli* O157:H7	*Campylobacter*	*Cryptosporidium*
Cow pats	zero inactivation for first 4 days then 0.087 day-1	zero inactivation for first 4 days then 0.087 day-1	zero inactivation for first 4 days then 0.087 day-1	T(0.02,0.015,0.013)
Cattle manure	0.10	0.07	0.10	
Poultry manure	0.60	0.60	0.395	
Poultry slurry	0.15	0.14	0.15	
Swine manure slurry	0.50	0.50	0.50	

Inactivation in the storage pond

Bacteria (*E. coli* O157:H7, *Salmonella and Campylobacter*)

Sinton et al., 2007 investigated the inactivation rates of *E. coli*, *Salmonella enterica* and *Campylobacter jejuni* under light and dark conditions. Temperature was maintained at 14°C. Inactivation was consistently slower in 'dark' conditions in comparison to sunlight. The reported inactivation rates are included in Table 12.

Table 12. Inactivation rates and times for *E. coli*, *Salmonella enterica* and *Campylobacter jejuni*
Data from Sinton et al. (2007)

Indicator/Pathogen	**Dark**		**Sunlight**			
	T_{90} (hours)	k (Log 10 decay rate/d)	Winter (T_{90} hours)	k (Log 10 decay rate/d)	Summer (T_{90} hours)	k (Log 10 decay rate/d)
E. coli	548	0.04	17.3	1.4	3.85	6.3
Salmonella enterica	67.4	0.36	26.8	0.9	4.81	5.0
Campylobacter jejuni	82.6	0.30	1.58	15	0.8	30

Cryptosporidium

Ives *et al.* (2007) undertook bench-scale survival studies with *Cryptosporidium parvum*. *C. parvum* inactivation rates ranged from 0.0088 Log_{10}/day at 5°C to 0.20 Log_{10}/day at 30°C.

Temperature, surface water or groundwater type, and the interaction of these factors had statistically significant effects on the survival of *C. parvum*.

Model input: persistence of reference pathogens in the storage pond

Given the results and relevance of the work undertaken by Sinton *et al.* (2007), the QMRA model was constructed to allow for differentiating between light and dark inactivation. The inactivation rates as reported by Sinton *et al.* (2007) were used in the model, using winter sunlight values to be conservative and a sub-population size of 10% (chosen arbitrarily) to differentiate between those organisms protected from sunlight at depth in the pond (Table 13).

Cryptosporidium is less sensitive to inactivation by sunlight in comparison to bacterial pathogens (Mendez-Hermida *et al.*, 2005); however whatever the key mechanism of inactivation is for *Cryptosporidium* a similar sub-population phenomena was considered likely, with a small portion of organisms finding protection. Using the results of Ives *et al.* (2007) that highlight the importance of temperature on *Cryptosporidium* survival, the slow phase was assumed represented by the low temperature inactivation rate and fast phase for high temperature inactivation. The sub-population was assumed to be 10% (chosen arbitrarily). The inactivation rate for 30 °C of 0.2 Log_{10} per day appears to be high, and including the two population model allows for a more conservative consideration of persistence. These values should be tested and refined in the future.

Table 13. Model inputs for inactivation rates of reference pathogens in storage pond

	Inactivation rate k Log10 inactivation per day			
	Salmonella	*E. coli* O157:H7	*Campylobacter*	*Cryptosporidium*
Fast (Light) inactivation	0.9	1.4	15	0.2
Slow (dark) inactivation	0.36	0.04	0.3	0.0088
Persistent sub-population size (%)	10	10	10	10

Pathogen mobilization

Overland flow across agricultural land occurs when rainfall intensity exceeds the infiltration capacity, or when the soil becomes saturated. Once overland flow occurs, there is potential for mobilization of pathogens to surface waters. The ease with which contaminants are mobilized is influenced by their partitioning status; free unattached cells are more easily incorporated into mass flow in comparison to those microorganisms attached to soil or manure particles. For the contaminant to reach surface waters, these forces of entrainment must be maintained for the overland distance to the waterway.

Grazing land

For all animals, the mobilization of pathogens from fresh fecal deposits depends on numerous factors including nearness to the waterway. For this work, it was assumed that for any specific pathogen, the animal source did not impact the relative mobilization. Davies-Colley *et al.* (2004) documented the water quality impact of a herd of 246 dairy cows crossing a stream. The cows defecated approximately 50 times more per metre of stream crossing than elsewhere on the raceway. In this example, access to the stream combined with the increased frequency of defecation would lead to a high probability of mobilization.

Muirhead and coworkers have undertaken several studies investigating the mobilization of *E. coli* from fresh cowpats with the following conclusions:

- The number of *E. coli* in the cowpat runoff was highly variable and was strongly correlated with the number of *E. coli* in the cowpat (Muirhead et al., 2006c);
- *E. coli* mobilized from cowpats were transported as single cells, and only a small percentage (approximately 8%) were attached to particles; implying that particle transport is not necessary for pathogen mobilization (Muirhead *et al.*, 2005); and
- These results implied that in runoff generated by saturation-excess conditions, bacteria are rapidly transported across the surface and have little opportunity to interact with the soil matrix. The removal of *E. coli* from overland flow under saturation excess conditions was also investigated, and reported to be limited (< 50%). Instead, most bacteria remained entrained within the overland flow down the length of the plots (5m long) (Muirhead et al., 2006b).

On soil blocks from Sydney's catchment, Davies *et al.* (2004) investigated the dispersion and initial transport of *Cryptosporidium* oocyst from fecal pats under simulated rainfall events. The oocyst load in runoff was significantly affected by the vegetation status, the slope of the soil, and the event characteristics in terms of rainfall intensity. These same factors significantly affected the concentrations of oocysts retarded on the surface soil a short distance (10 or 30cm) downhill. Devegetated or heavily grazed soils represented a higher risk than vegetated soils. The freshly crusted cow pats containing 10^7 oocysts transported from $10^{0.2}$ oocysts on vegetated loam soil to $10^{4.5}$ on unvegetated loam soil over a distance of 1m. In addition, the bovine manure matrix has been reported to enhance the attachment of *Cryptosporidium* oocysts to soil (Kuczynska et al., 2005b).

Atwill *et al.* (2002) suggested that vegetated buffers constructed with sandy loam or soils with higher bulk densities were less effective at removing oocysts (1-2 $\log_{10}$ reduction per m) than buffers constructed with silty clay, loam, or solids with lower bulk densities (2 to 3 $\log_{10}$ reduction per m). Their study suggested that on slopes of <20% a length of 3m should function to remove 3 $\log_{10}$ (99.9%) of *C. parvum* oocysts from agricultural runoff generated during events involving mild and moderate precipitation.

Ferguson *et al.* (2007) studied the dispersion and transport of *Cryptosporidium* parvum oocysts, Escherichia coli and PRD1 bacteriophage seeded into artificial bovine fecal pats during simulated rainfall events. Transportation efficiency increased with decreasing size of the microorganism studied; *Cryptosporidium* oocysts were the least mobile followed by *E. coli* and then PRD1 phage. Rainfall events mobilized 0.5 to 0.9% of the *Cryptosporidium* oocysts, 1.3–1.4% of *E. coli* bacteria, and 0.03–0.6% of PRD1bacteriophages from the fresh fecal pats and transported them a distance of 10m across the bare soil sub-plots.

Model input: probability of overland transport from grazing land

Several studies were identified that investigated the mobilisation of pathogens from direct fecal deposits on agricultural land. While trends have been identified, quantification of the probability of passage of the reference pathogens from the grazing land to surface waters is still not possible, and realistically is going to be site specific. To consider what could be quantified however, animal access to waterways can reasonably lead to an assumption of p=1, and vegetated buffers may allow the probability to be estimated at $p < 0.001$ (3 Log_{10}).

As a starting point in the model, the probability of transport to waterway was based on results by Ferguson *et al.* 2007, with a uniform distribution selected to represent the reported ranges. Assuming no direct access of animals with the stream or pond, this is likely to be a conservative estimate since they are estimates for a distance of 10m (Table 14).

Table 14. Model inputs for probability of passage from grazing land to surface waters

	Salmonella	***E. coli* O157:H7**	***Campylobacter***	***Cryptosporidium***
Probability of passage (p)	Uniform (0.013,0.014)	Uniform (0.013,0.014)	Uniform (0.013,0.014)	Uniform (0.005,0.009)

Manure amended soil

In comparison to the mobilisation of pathogens from grazing land, similar principals apply for the conditions that lead to overland transport form manure attenuated soil. Importantly however, the pathogen transport rates would be expected to be lower from manure amended soil. Amending soil leads to higher infiltration, increased surface retention as demonstrated for human biosolids application (Joshua *et al.*, 1998; Moffet *et al.*, 2005) and increased vegetative cover. In addition, sites selected for manure application tend to be of lower relief.

Model input: probability of overland transport from manure amended soil

Because the objective for this study was a screening-level analysis of animal-derived pathogen risk in recreational waters, a coarse, conservative model (with a tendency to overpredict pathogen loading) was implemented for the transport of pathogens from manure amended soils; models with higher resolution were not implemented because manure management practices are diverse and give rise to widely differing pathogen loading rates. As a conservative starting point, the same probability of transport rates applied for grazing land were applied to describe transport

from manure amended land (Table 15). This is certainly an overestimate of pathogen transport rates given the known reduction in pathogen runoff when best practices for land application are used (Goss and Richards, 2008; Vinten et al., 2004).

Table 15. Model inputs for probability of passage from manure amended land to surface waters

	Salmonella	***E. coli* O157:H7**	***Campylobacter***	***Cryptosporidium***
Probability of passage (p)	Uniform (0.013,0.014)	Uniform (0.013,0.014)	Uniform (0.013,0.014)	Uniform (0.005,0.009)

Streambed sedimentation

During low flow conditions bacterial and protozoan pathogens can settle to a stream or river bed and be retained. Searcy *et al.* (2006) investigated the transfer of *Cryptosporidium* oocysts from surface water to the sediment beds of streams and rivers using controlled laboratory flume experiments. The association of *C. parvum* with other suspended sediments increased both the oocysts' effective settling velocity and the rate at which oocysts were transferred to the sediment bed.

Model input: proportion of mobilized load retained in stream sediments

In the absence of real quantitative estimates of sediment transport rates, and given the results of Searcy et al. (2006), the proportion of organisms associated with solids was used as a first pass to estimate that portion that may be retained (Table 16). For bacteria, this proportion was assumed to be 8% (given 8% solids associated (Muirhead et al., 2006a)) and for Cryptosporidium representative values were not found for attachment of Cryptosporidium to stream sediments. In experiments with soils and manure amended soils (Kuczynska et al., 2005a), Cryptosporidium was observed to adsorb readily to sandy loam and clay loam soils (72% and 93% attachment, respectively) when introduced to the soils in a suspension in dilution water and even more readily (97.4% and 97.7 %) when oocysts were introduced to the soils in a dilute suspension of bovine manure. In a study of urban stormwater Clostridium perfringens partitioning following high rainfall events ranged from 20 to 60% (Characklis et al., 2005). Based on the very high tendency of Cryptosporidium to attach to soils, Clostridium perfringens is expected to be a conservative surrogate for Cryptosporidium mobilization from stream sediments in this case. Therefore 20% was selected as a reasonable conservative estimate (here, conservative indicates C. perfringens is expected to over-predict Cryptosporidium mobilization to the water column) for Cryptosporidium.

Table 16. Model input for the proportion of load transported to stream sediments

	Salmonella	***E. coli* O157:H7**	***Campylobacter***	***Cryptosporidium***
Proportion of load	0.08	0.08	0.08	0.2

Inactivation in stream sediments

No studies were identified that specifically addressed the inactivation rate of pathogens within stream sediments. The studies identified to address the persistence of pathogens in the storage pond were considered equally relevant to the freshwater stream context.

Model input: persistence of reference pathogens in stream sediments

The same values as applied to estimate persistence in the storage pond, were applied to the persistence in stream sediments (Table 17).

Table 17. Model inputs for inactivation rates of reference pathogens in stream sediments

	Inactivation rate k Log10 inactivation per day			
	Salmonella	*E. coli* O157:H7	*Campylobacter*	*Cryptosporidium*
Fast (Light) inactivation	0.9	1.4	15	0.2
Slow (dark) inactivation	0.36	0.04	0.3	0.0088
Persistent sub- population size (%)	10	10	10	10

Resuspension

Re-suspension of microbes from stream sediments can be a source of increased pathogen concentrations during high flow events. While research has been undertaken to quantify re-suspension (Jamieson et al., 2005; Muirhead et al., 2004); no real data relevant to the current modelling exercise was identified. Jamieson *et al.* (2005) reported that bacterial re-suspension was primarily limited to the rising limb of storm hydrographs implying that a finite supply of sediment-associated bacteria are available for re-suspension during individual storm events.

Model input: proportion of retained load mobilized during a re-suspension event

In the absence of any quantitative data from the literature, and given the results of Jamieson *et al.* (2005), the impact of resuspending the total sediment load was investigated in a conservative approach to evaluate the potential impacts of reasonable worst case conditions (Table 18).

Table 18. Model input for the proportion of retained load that is re-suspended

	Salmonella	***E. coli* O157:H7**	***Campylobacter***	***Cryptosporidium***
Proportion of load	1	1	1	1

Consumption by recreational bathers

In a study undertaken by Dufour et al. (2006), fifty-three recreational swimmers participated using a community swimming pool disinfected with cyanuric acid stabilized chlorine. The swimmers were asked to actively swim for at least 45 minutes and to collect their urine for the next 24 hours. Results of the study indicated that non-adults ingest slightly more water than

adults do during swimming activity (Figure 10). The predicted median volume of water swallowed by all participants combined was approximately 19mL.

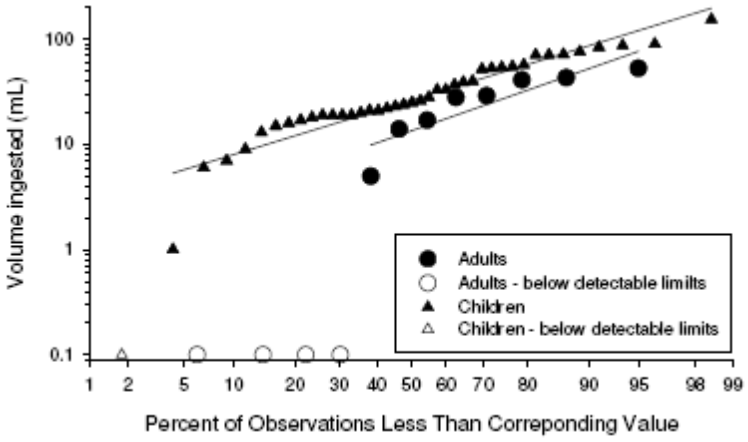

Figure 10. Ingestion volumes during recreational activities
Data from Dufour et al. (2006)

Model input: Consumption of water by recreational bathers

A normal distribution was fitted to the natural log transformed combined adult and child dataset: Ln mean of 2.92 with Ln sd of 1.43 this yields a mean of 18.6 ml.

Human health effects: Dose response relationships

During dose-response analysis, data from human clinical studies, epidemiological studies, animal studies, and/or outbreaks are used to develop a mathematical relationship between the intensity of exposure or amount of intake and the subsequent occurrence of disease or infection. Dose-response models are mathematical functions that take as input the dose to which individuals or populations are exposed and yield a probability (bounded by 0 and 1) of the particular adverse health effect (Haas et al., 1999).

Dose-response models are generally derived using statistical estimation techniques, and the form of the relationship between exposure and response is determined by (1) assumptions related to the biological processes leading to infection, and (2) the "shape" of the relationship found in the data between exposure and the health outcome of interest. The mathematical form of the dose-response model may vary with pathogen or strain, route of administration, distribution of host statuses, and other factors. For this study, dose response models were obtained from the scientific literature and applied. The dose-Response models applied in the QMRA model are summarised in Table 19.

Table 19. Summary of dose response relationships used in QMRA model

	Dose Response Model	Parameter values	Reference	Probability of illness given infection	Reference
Salmonella	*Gompertz function for Bareilly strain*	*log(a) = 11.68; b=0.82*	*(Coleman and Marks, 1998, 2000; Coleman et al., 2004; Soller et al., 2007)*	*1.0 (Note that dose response is for illness not infection)*	
E. coli O157:H7	*Hypergeometric beta-Poisson model**	*Median value of Markov chain Monte Carlo sample alpha=0.37 and beta =37.6*	*(Teunis et al., 2008)*	*0.825*	*(Havelaar et al., 2003)*
Campylobacter	*Beta-Poisson approximation*	*alpha=0.145* *beta=7.59*	*(Haas et al., 1999; Medema et al., 1996; Teunis et al., 1996)*	*0.2*	*Point estimate from results presented by Teunis et al. (1999b)*
Cryptosporidium	*Exponential*	*r = uniform distribution (0.04-0.16)*	*(U.S. EPA, 2006)*	*0.71*	*(Havelaar and Melse, 2003)*

* Beta-Poisson approximation at high doses and at low dose (<0.1) with the exponential model r=a/a+b

Risk Characterization

The QMRA model was run with the described inputs using a Monte Carlo simulation approach for 10,000 iterations to construct the cumulative density function (CDF) for downstream illness risk following Event 1, Event 2, and Event 3. As indicated in the previous sections of the report, all of the inputs to the QMRA were highly uncertain. The variability in the input values extracted from the literature was considerable. In addition, uncertainty exists regarding whether the relatively simple model structure was a reasonable representation of types of realistic exposure scenarios that were investigated. Therefore, the output from these simulations is best used to compare and understand the relative risks associated with the various scenarios and to prioritize the types of data that could be collected in the future to most significantly enhance interpretation of future analyses.

Despite limited predictive capabilities, the simplified model structure was more than adequate for investigating the interactions of model parameters and to identifying the most important sources of uncertainty - or rather the sources of uncertainty that have the largest impact upon the illness predictions. This approach to model exploration is termed sensitivity analysis and is the study of how the uncertainty in the output of a model can be apportioned to different sources of uncertainty in the model input.

Model simulation results

The model output represented in terms of CDFs of predicted illnesses per 1000 exposures for each reference pathogen and animal type are illustrated in Figure 11 (cattle), Figure 12 (swine) and Figure 13 (poultry). In all simulations the predicted rates of gastrointestinal illness were consistently higher for events 1 and 2 compared to event 3. Thus, mobilization of fresh fecal material by overland flow was the primary driver of downstream risk. Intercepted and stored fecal material was less of a concern due to the inactivation of pathogens, even for *Cryptosporidium* which was modelled with a persistent inactivation rate. Event 2 risks were slightly higher than Event 1. The difference between event 1 and 2 was the impact of the storage pond overflow (in addition to direct overland flow alone) on the overall downstream risk, which was surprisingly small. The difference was greater for *Cryptosporidium* in comparison to the bacterial pathogens due to the persistence of oocysts, however the high order of magnitude of daily pathogen excretion still overshadowed the build up of pathogens in the storage pond.

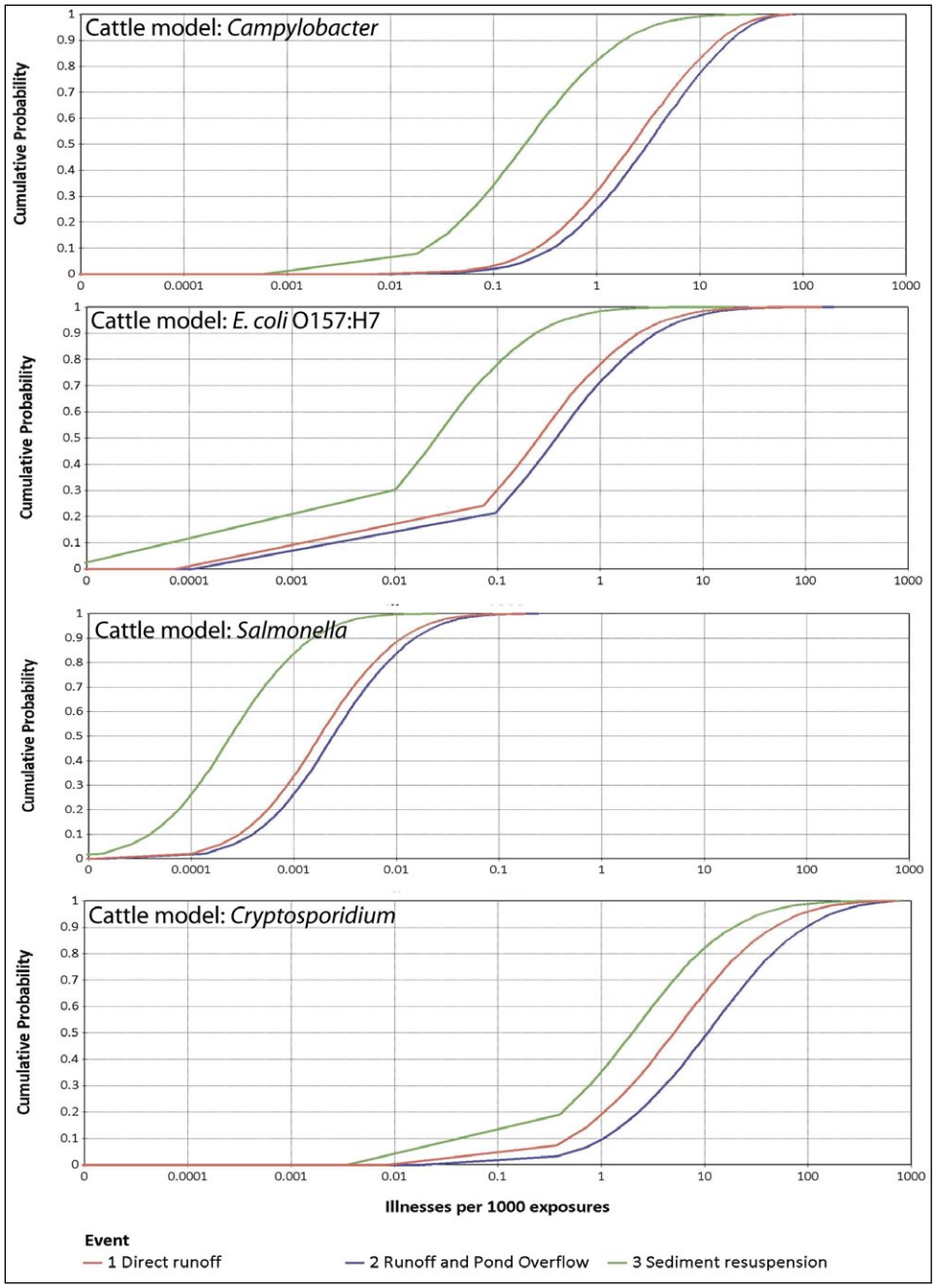

Figure 11. Downstream illness risks from the Cattle model for each reference pathogen

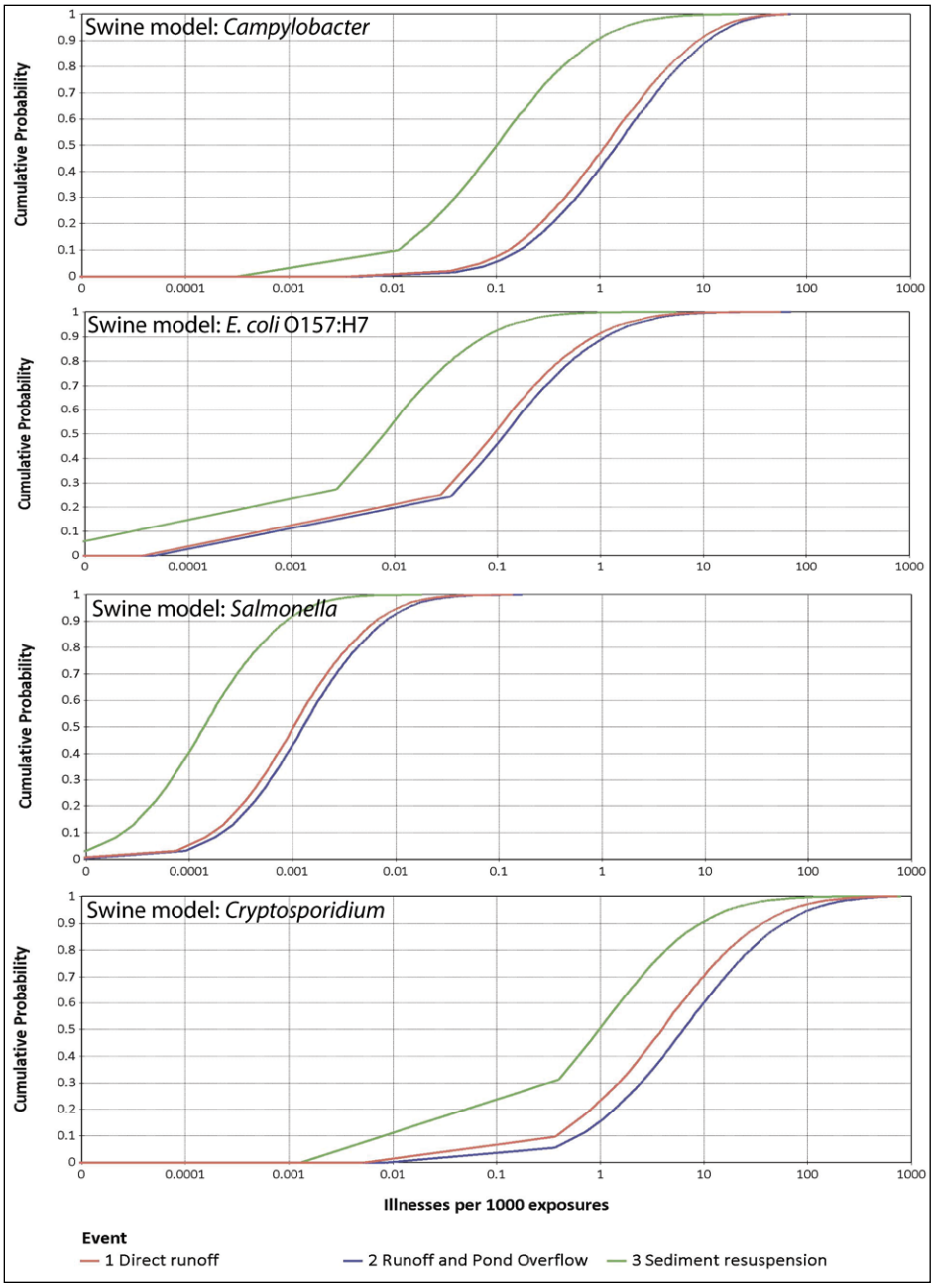

Figure 12. Downstream illness risks from the Swine model for each reference pathogen

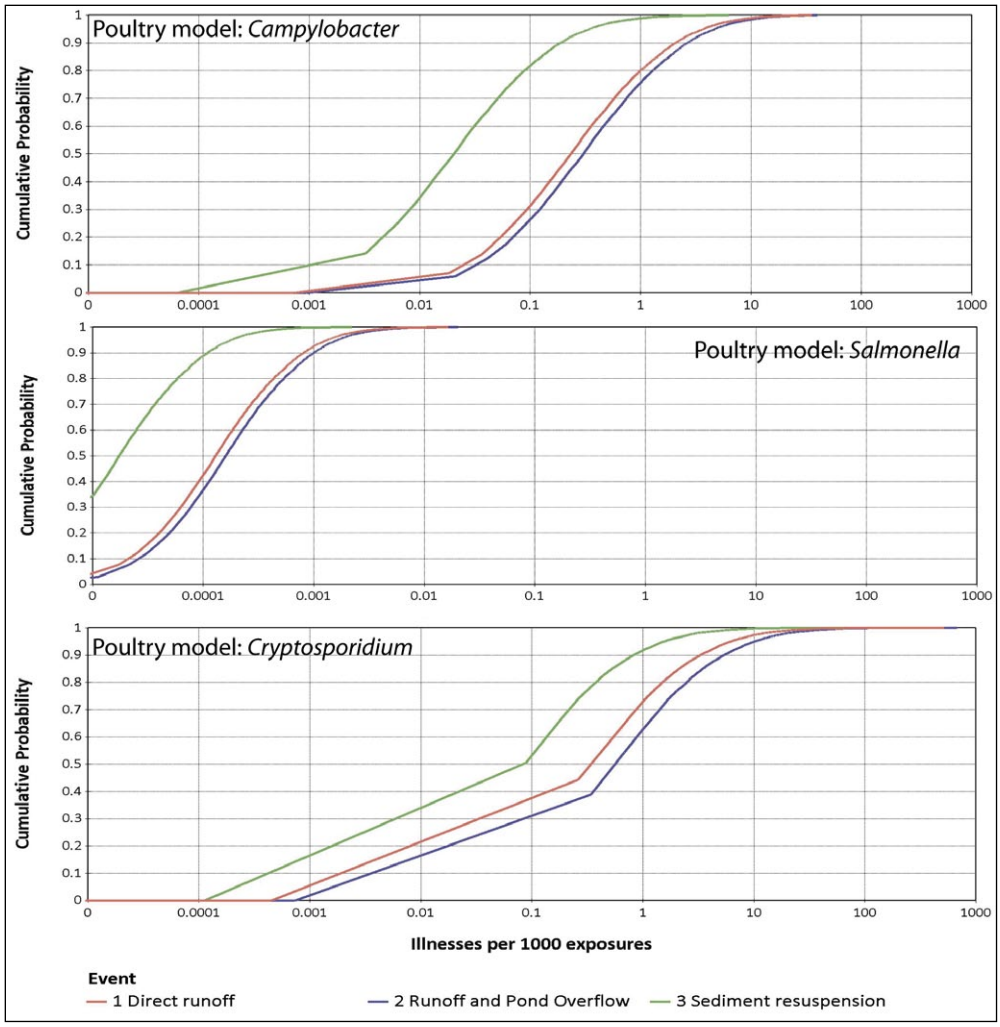

Figure 13. Downstream illness risks from the Poultry model for each reference pathogen
Note for Figure 13: no results for E. coli O157:H7 as the prevalence was assumed to be 0.

Comparison between *Campylobacter* and *Cryptosporidium* illness risks for poultry (Figure 13) indicates that the risk from *Cryptosporidium* was predicted to be higher than for *Campylobacter*. This was due to the relatively high prevalence of *Cryptosporidium* (~25%) in the few reported studies, and the high persistence in the environment. These results however highlight the importance of accounting for the human infectious component of the animal infections. While poultry do have C*ryptosporidium* infections, the majority appear to be *C. meleagridis* which is unlikely to cause infection in healthy humans. In contrast, poultry are frequently infected with *Campylobacter jejuni* and *Campylobacter coli* which are most frequently responsible for *Campylobacter* infection in humans. Failure to consider which strains of each organisms are most likely to cause infection in humans can lead to an overestimation of risk, and unnecessarily conservative illness estimates. In this case, despite the relatively high prediction for *Cryptosporidium* risk from poultry, epidemiologic evidence allows this to be discounted with a greater focus on *Campylobacter.*

Comparison of the predicted risks for *Campylobacter* and *Cryptosporidium* between the swine and cattle operations show that they are of a similar magnitude. This was surprising due to the significant differences in the onsite management of fecal material on each type of feeding operation. While cattle were assumed to openly graze 90% of the time, swine operations were assumed to be entirely housed with fecal material collected and stored before managed agricultural application. A comparison between the modelled load of pathogens to grazing land and storage is illustrated in Figure 14.

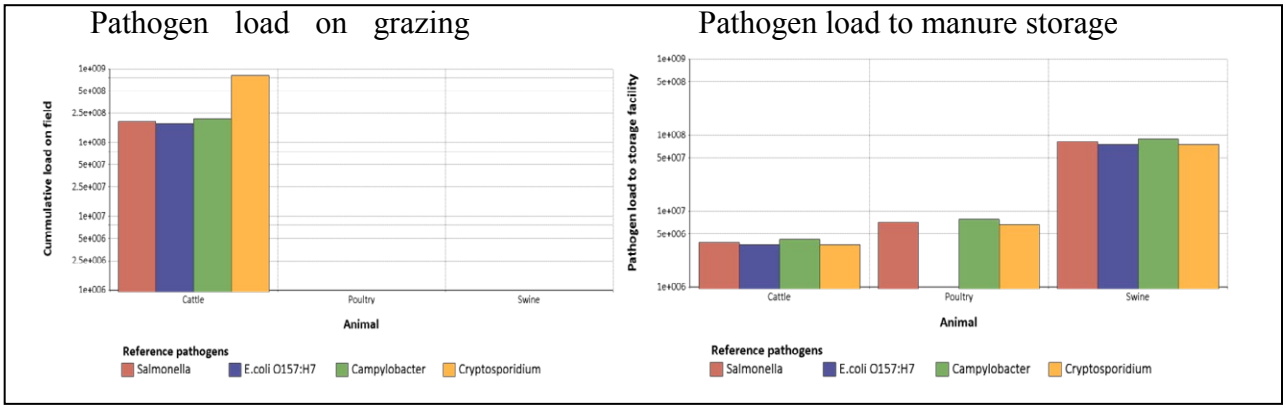

Figure 14. Modeled pathogen loads to grazing land and manure storage

The fact that the risks associated with each of these operations were predicted to be roughly equivalent does not seem reasonable. There are two factors that contribute to this result: the importance of the minimum storage time for manure prior to application; and the impact of time between application and the rainfall mobilising event.

Minimum storage time

While the manure was stored prior to application, the design of the storage facility assumed that fecal material was added every day. At the time of application, all manure from the storage facility was assumed to be applied to land. While the mean storage time may have been some months, the modelled load of pathogens in the manure was still high - leading to high predicted downstream risk. For the same reasons as mentioned previously, the magnitude of the daily input of pathogens to the storage facility overshadowed the impact of the inactivation on the final load. Therefore the pathogen load to agricultural land with manure application was driven by the *minimum* storage time, rather than the overall mean storage time (Figure 15).

Storage time has a profound influence on pathogen loads in land-applied manures (Himathongkham et al., 1999; Himathongkham et al., 2000; Hutchison et al., 2005b; Hutchison et al., 2005d; Kasorndorkbua et al., 2005; Kelley et al., 1994; Meals and Braun, 2006; Nicholson et al., 2005), regardless of the manure origin, type (liquid v. solid), and storage system. Yet, while pathogen reduction is a consideration in farmer choice of timing and rate of manure application and of storage facility volume, other considerations including nutrient requirements

of soils, generation of noxious odours, rainfall, availability of labour, construction cost, and available land also play roles in design and operation of manure application schemes. Some of these considerations may be at odds with long storage times for pathogen reduction, as in the case of odour, which tends to be lowest for fresh manure (Dougherty et al., 1998). Because storage systems must meet multiple objectives, minimum storage times and timing of land application are generally best management practices (BMPs) though it is likely that for some individual NPDES permits numerical limits on minimum storage times may have been stipulated.

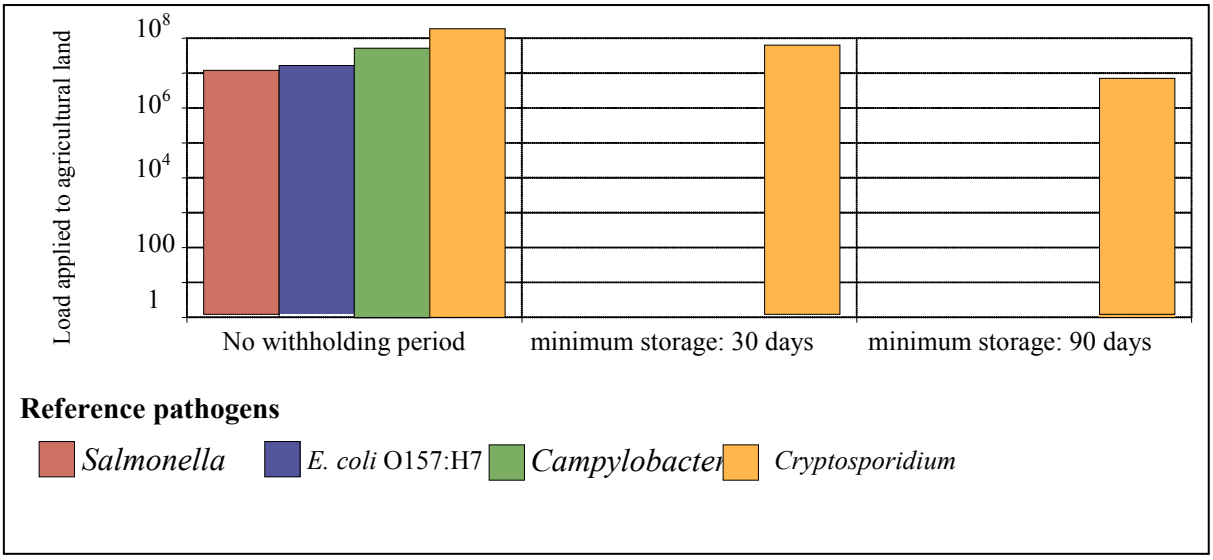

Figure 15. Impact of minimum storage time on load applied via manure application

Time between application and rainfall event

Unlike the continuous contribution of grazing animals openly defecating on land, manure application is an infrequent occurrence; perhaps 2 - 3 times per year. The pathogen load available for transport to surface waters can be expected to decline over time following application due to environmental inactivation. For the purposes of conservatively estimating the downstream risks associated with land application, the rainfall mobilizing event was assumed to occur immediately following application. Figure 16 illustrates the modelled load of pathogens mobilized with a rainfall event versus time since manure was applied. In comparing the risks associated with swine and cattle operations it is necessary to consider the duration of the potential risk. While open grazing poses a potential risk throughout the year, risk associated with manure application exhibits short term peaks that follow the time of application. The magnitude of these peaks appear to be, at worst, equivalent to the ongoing risk associated with open grazing.

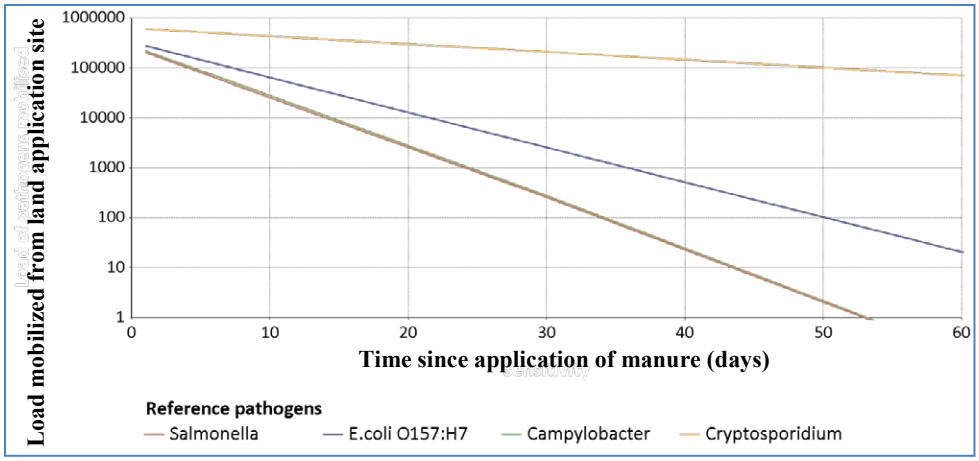

Figure 16. Mobilized pathogen loads from cattle versus time between application and rainfall event

Sensitivity analysis

Sensitivity to prevalence

Pathogens are only assumed excreted by infected animals and therefore the prevalence of animal infection with human infectious pathogens was the basis for the QMRA model. A broad range of prevalence rates were identified in the literature ranging from 1% to 100% of animals on a given farm. The sensitivity of the predicted illness risk to the prevalence rate was explored and the results for *E. coli* O157:H7 illness is illustrated in Figure 17. The broad range of prevalence rates had a relatively limited impact on the predicted illness rates (2 Log_{10}) largely due to the high order of magnitude in excretion rate that overshadowed the calculations. In contrast however, the number of supershedders in the catchment had a strong influence on the predicted illness rates (Illustrated in Figure 18 for the swine model). Once a single super shedder was assumed present, this high level excretion of pathogens dominated the risk model, and the risks rapidly approached their maximum. Increasing the number of supershedders above 1 had less of an impact.

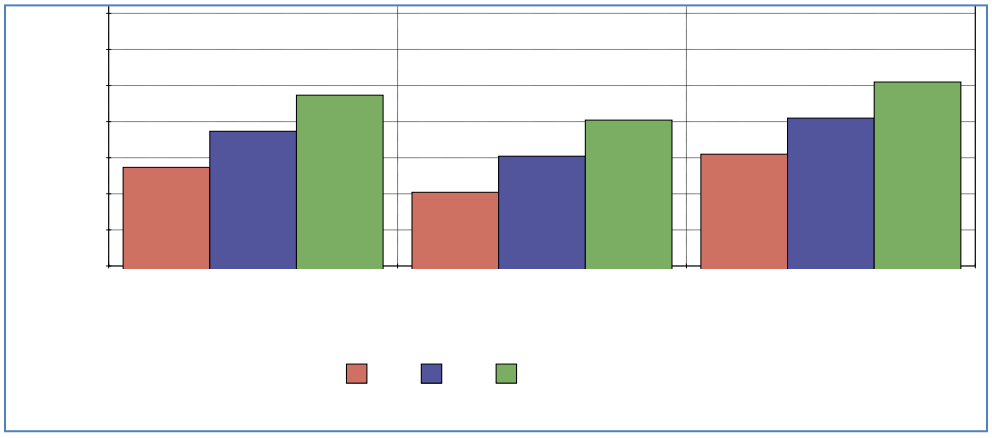

Figure 17. Sensitivity of predicted illness rates to the prevalence of infection in farm animals

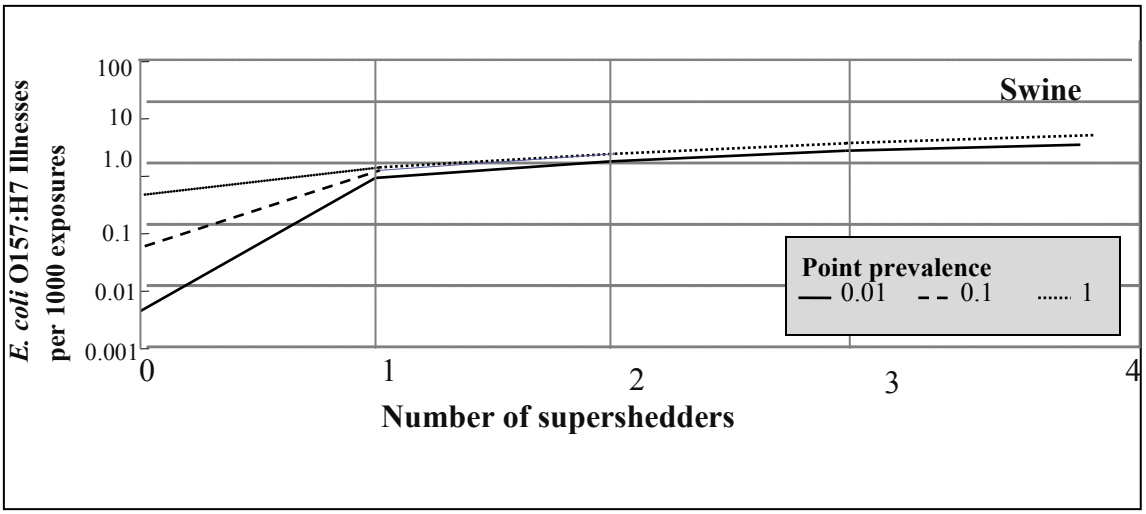

Figure 18. Influence on the predicted illness rates of the number of supershedders among swine

Sensitivity to excretion rate

The sensitivity of the model to the assumed endemic excretion rate for cattle is illustrated in Figure 19. Reported excretion rates from the literature cover several orders of magnitude (<10-10^9 organisms per g), indicating that uncertainty in this input is an important driver in uncertainty in the predicted illness risk.

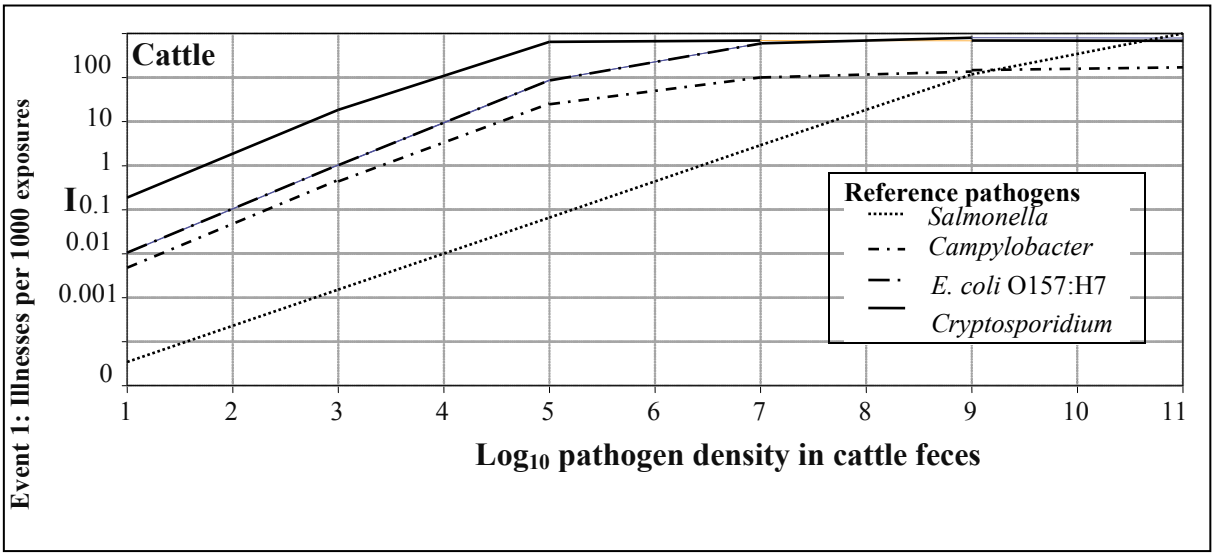

Figure 19. Sensitivity of the risk model to cattle excretion rate for event 1 illness risks

Sensitivity to Inactivation rate

Pathogen inactivation under uncontrolled environmental conditions is a key barrier between animal feeding operations and humans engaging in recreational activities. In the QMRA model, inactivation of pathogens is predicted on open grazing land (within cow pats); in manure piles; in

manure applied to land; in the storage pond; and in stream sediments. Despite a large body of research investigating the kinetics of pathogen inactivation in a variety of media, and under laboratory and field conditions, these inputs are still extremely uncertain. While general patterns can be described (such as pathogen inactivation increases with temperature), the actual inactivation rates are not well known. The inactivation rates in the model are at best a starting point to explore model sensitivity and to further motivate research into better defining these quantitative relationships.

To illustrate the impact of the uncertainty in the inactivation rates, the inactivation of *Campylobacter* and *Cryptosporidium* in manure applied to land is illustrated in Figure 20 and Figure 21, respectively. Both figures illustrate that the QMRA model is very sensitive to the assumed inactivation rate. Some references report *Campylobacter* to be inactivated readily in the environment. When this upper value is used, the load on manure applied land is reduced quickly and as soon as 5-7 days following application, the concentration of *Campylobacter* is predicted to be negligible. Some references however have indicated that under some circumstances *Campylobacter* can persist in the environment, represented by the 'low' inactivation rate. Under this assumption, even after 3 months in the field, a reasonable concentration of *Campylobacter* remains. While good reduction of bacterial pathogens could be assumed in the environment, this uncertainty should be reduced by identifying conditions that support bacterial persistence before this reduction can be relied upon as a critical barrier for the protection of public health.

Results of the sensitivity analysis for the inactivation of *Cryptosporidium* oocysts indicate that even the faster reported inactivation rates support the assumption that oocysts persistence in the environment, and that environmental inactivation is a poor barrier for protection of human health unless extended residence times can be assured.

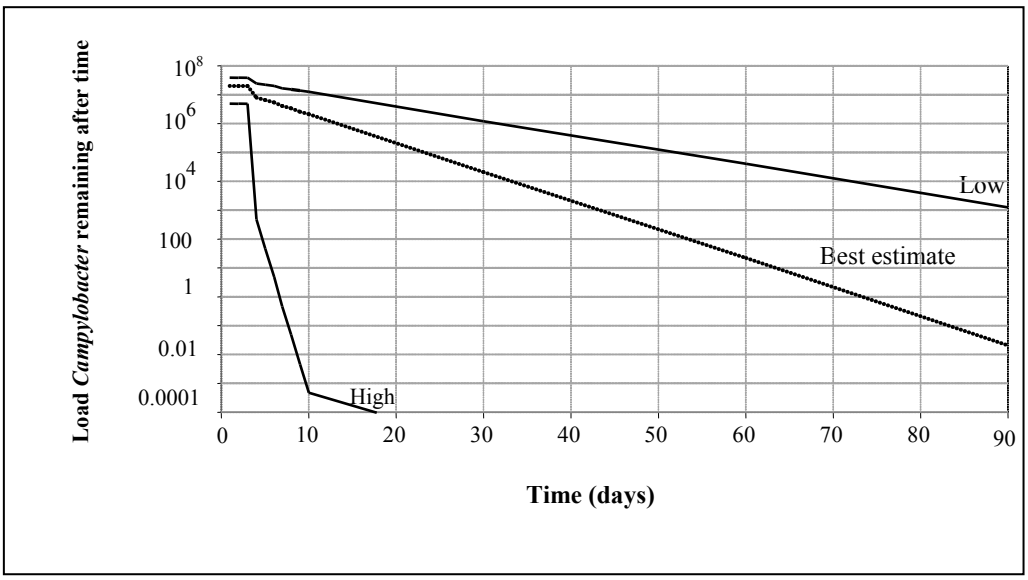

Figure 20. Estimated *Campylobacter* load remaining on manure applied ground over time

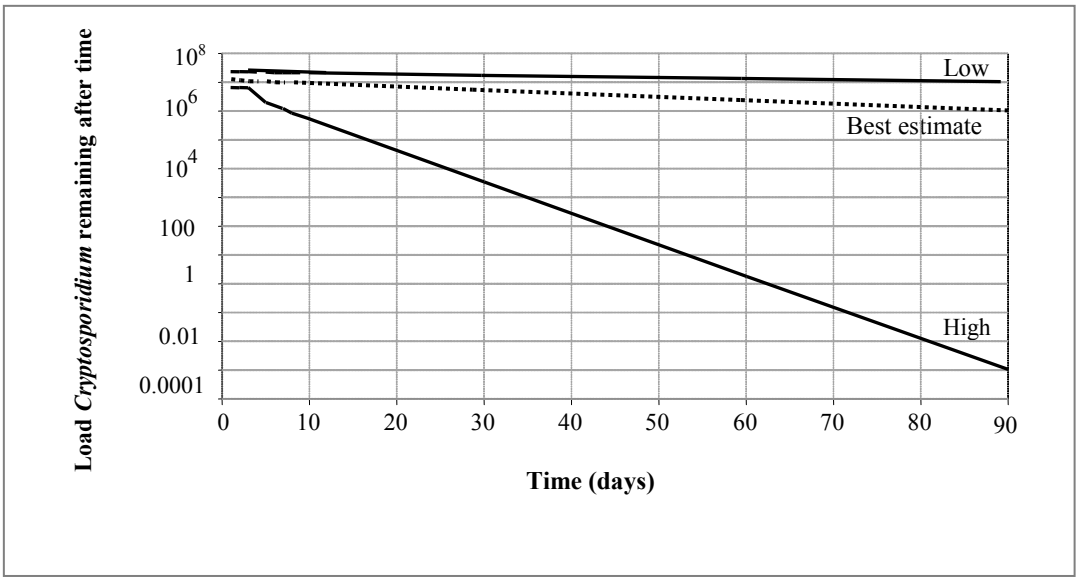

Figure 21. Estimated *Cryptosporidium* load remaining on manure applied ground over time

Sensitivity to storage pond catchment size

Onsite interception of runoff can be used to mitigate the downstream risks following rainfall events. The sensitivity of the predicted illness rates following an overland flow event (Event 1) to the assumed storage pond catchment size is illustrated for the cattle model in Figure 22. The results indicate that the downstream risk from Event 1 is eliminated when 100% of the catchment is intercepted by the storage pond. However, when 90% of the catchment was assumed to be intercepted (only 10% outside the storage pond catchment) by the storage pond, the downstream risks were predicted to be similar (within 1 Log_{10}) to when only 10% or 0.1% interception was assumed. These results indicate that for the storage pond to effectively reduce downstream risks, it must intercept all the runoff, as even a small portion of flow can contain a significant load of pathogens.

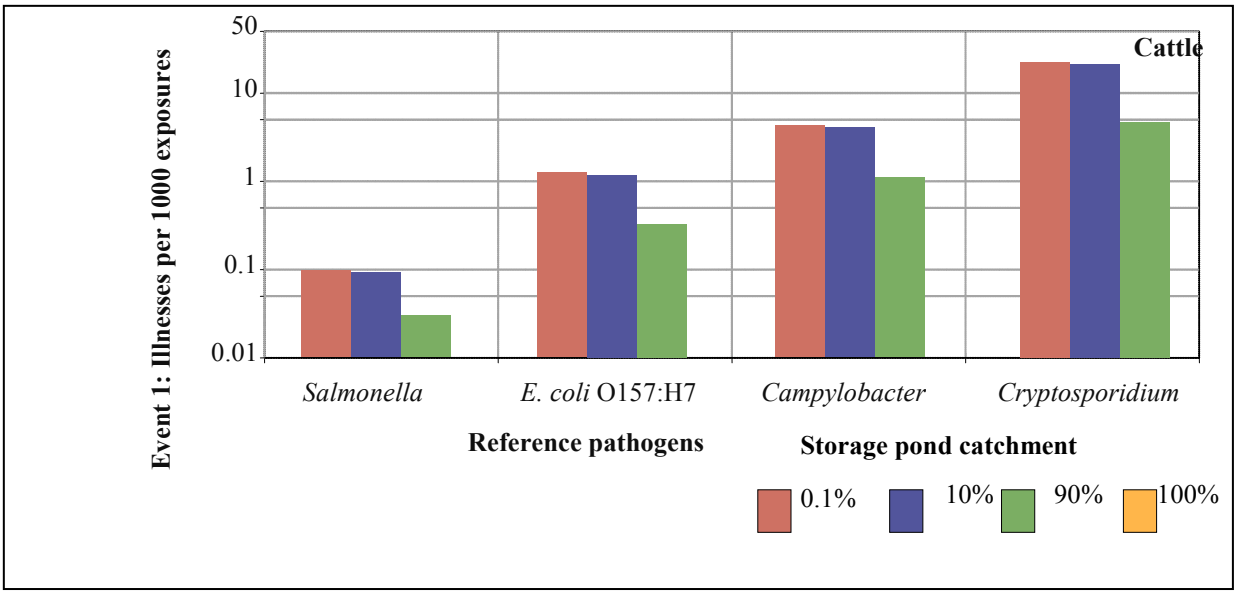

Figure 22. Sensitivity of the Event 1 illness risks to storage pond catchment size for the cattle model

Conclusions

During the course of the work that was conducted for WA 1-08 Task 2 (including the first phase of this work conducted under 1-08 Task 2 and subsequently Work Assignment 1-08 Task 2 Amendment 3) the following was accomplished:

- Conduct of a literature review, development, implementation and parameterization of a QMRA model in Analytica software, and development and parameterization of three exposure scenarios for cattle-impacted waters.
- Planning of and participation in an EPA QMRA workshop in November 2008 at the ORD offices in Cincinnati, OH where the cattle model was presented and demonstrated.
- Extension of the initial cattle model to two other agricultural animals (swine and poultry) for the three exposure scenarios.
- Conduct of a literature review of swine and poultry manure data to parameterize the newly extended QMRA model for *Salmonella*, *Camplyobacter jejuni*, *E. coli* O157:H7, and *Cryptosporidium parvum*.
- Conduct of a sensitivity analysis of the various models (cattle, swine, and poultry) and associated model parameters for the three exposure scenarios to identify which data and model components are the most crucial with respect to the conduct of QMRA for animal impacted waters.

The construction, parameterization, and evaluation of the QMRA model to describe the potential impacts of an animal feeding site and/or areas where animal manures are applied on gastrointestinal illness risk among recreational water users has allowed for the important environmental variables to be evaluated and numerous issues to be identified and discussed. The salient findings from this work includes the following:

- Onsite collection and storage of fecal material is an important barrier for preventing pathogen mobilization downstream. Operations that collect and store fecal material for land application may present short term peaks of pathogen risk, immediately following application. These peaks are estimated to be roughly equivalent to the ongoing risk associated with open grazing operations.
- When manure is to be stored and then land applied, the storage barrier is only effective for pathogen removal when a minimum storage time is ensured.
- Managing land application to avoid periods of high rainfall will reduce risk.

- Prevalence of infection in any given herd is likely to be constantly changing, and the within-herd temporal variation of can be substantial.
- Understanding the prevalence of human infectious pathogenic strains could be a critical component for not overestimating the risk associated with animal-impacted waters.
- Quantifying pathogen excretion density is a significant source of uncertainty in the overall model. In particular, the existence of super-shedders appears to drive the overall pathogen load. This aspect requires further research, particularly if identification and containment of super-shedders is possible.
- Environmental inactivation rates of pathogens are highly uncertain. Therefore, ensuring pathogen reduction via uncontrolled environmental processes is not feasible unless extended residence times can be guaranteed. Given the current state of knowledge, *Cryptosporidium* oocysts should be assumed to persist for long time periods unless site specific data indicate otherwise.

References

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ANNEX 3

Distribution and Prevalence of Selected Zoonotic Pathogens in U.S. Domestic Livestock

For

Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination

U.S. Environmental Protection Agency

December 2010

DISTRIBUTION AND PREVALENCE OF SELECTED ZOONOTIC PATHOGENS IN U.S. DOMESTIC LIVESTOCK

U.S. Environmental Protection Agency
Office of Water
Health and Ecological Criteria Division

August 2010

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Acronyms

AIAO	all-in-all-out management
APHIS	Animal and Plant Health Inspection Service
AWQC	ambient water quality criteria
BEACH Act	Beaches Environmental Assessment and Coastal Health Act
BMP	best management practice
CFU	colony forming unit
CPSP	Critical Path Science Plan
CWA	Clean Water Act
EPA	U.S. Environmental Protection Agency
GI	gastrointestinal
MMS	manure management systems
MPN	most probable number
NAAS	National Agricultural Statistics Service
PCR	polymerase chain reaction
POTW	publicly owned (wastewater/sewage) treatment works
QMRA	quantitative microbial risk assessment
QPCR	quantitative polymerase chain reaction
SE	*Salmonella* Enteriditis
STEC	Shiga toxin producing *Escherichia coli*
U.K.	United Kingdom
U.S.	United States
USDA	U.S. Department of Agriculture
WHO	World Health Organization (United Nations)
WQS	water quality standard(s)

Executive Summary

This report describes the distribution, prevalence,[1] and abundance[2] of key waterborne zoonotic pathogens in domestic cattle, swine, and poultry (livestock) in the United States. It evaluates if the prevalence and/or abundance of these pathogens from livestock varies systematically due to geography, farm management, or manure handling practices. The methods employed are summarized in the text box to the right.

Methods

This work was conducted into four phases, (1) selection and justification of key zoonotic pathogens whose net risk may be used to represent risks posed by livestock wastes, (2) review of literature to develop a datasets of distribution and prevalence of the key zoonotic pathogens in the United States, (3) review of peer-reviewed literature on the impact of farm management practices on key pathogen prevalence and persistence, and (4) evaluation of the datasets.

The literature review includes collection of national-scale geospatial data, studies on the prevalence of pathogens in regions of the United States, and studies exploring factors that can affect pathogen distribution and prevalence, including best management practices (BMPs) and manure handling practices.

The major finding is that U.S. farm management conditions are more important determinants of pathogen occurrence and abundance in livestock than location within a specific region. The conditions with the greatest influence are farm size, whether the farm is a feedlot, seasonality, and the age of the animals on the farm.

As depicted in Exhibit 1, exposure to zoonotic pathogens during recreational activities depends upon the occurrence of the pathogens in manure and subsequent manure and farm management practices. Risks to users of recreational waters can be reduced significantly via management of surface water runoff from stored or land-applied manures and through treatment of manures prior to land application.

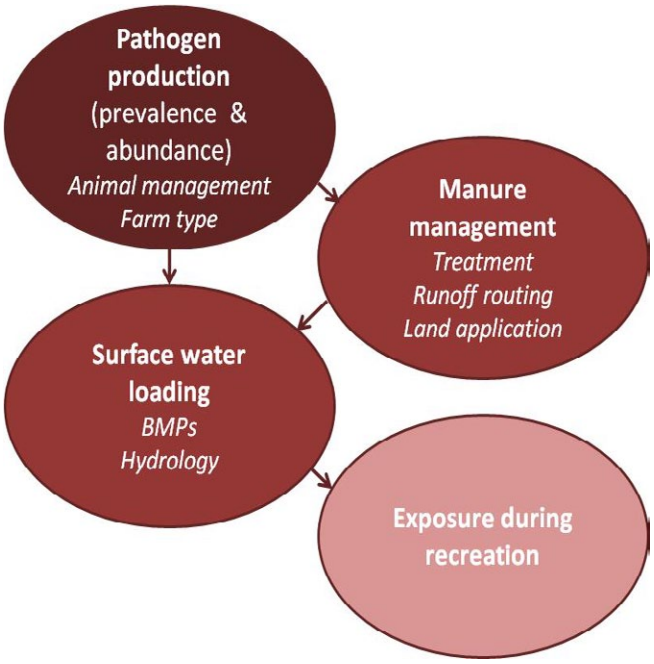

Exhibit 1. Pathogen Flow from Farms to Recreation Sites

The zoonotic pathogens[3] evaluated are the bacterial pathogens *Salmonella*, *Campylobacter*, and *E. coli* O157:H7, and the protozoan parasites *Cryptosporidium* and *Giardia*. All of

[1] Defined as the population proportion (%) of animals shedding a particular pathogen at a specific point in time.
[2] Defined as the density of organisms in manures of shedding individuals (# of organisms per g wet weight of solid feces or per L of manure slurry).
[3] From "Review of Zoonotic Pathogens in Ambient Waters" (EPA 822-R-09-002).

Zoonotic Pathogen Species and Human Infections

In cattle, all of the key zoonotic pathogens discussed in this report occur frequently. The species and serotypes found in cattle feces are similar to those posing hazards to humans. Swine *Salmonella*, *Campylobacter*, *Giardia*, and *E. coli* O157:H7 tend to be the same species/serotypes as the most commonly implicated species/serotypes in human infections. Most swine Cryptosporidia appear to be host-adapted and pose a reduced hazard to humans. For chickens, the only key zoonotic pathogens that occur with frequency and are similar to the pathogens implicated in human infections are *Salmonella* and *Campylobacter*. Both are highly prevalent among U.S. flocks.

these pathogens are found in the feces of cattle, swine, and chicken, except *Giardia* that does not occur in chicken feces.

The particular pathogen species and types prevalent in livestock feces are not necessarily the species and types posing the greatest risk of human infection and subsequent illness (see text box above). The species and types of the key pathogens in cattle are the most similar to those infecting humans. For chickens, *Salmonella* and *Campylobacter* are the only key pathogens occurring as human-infectious species.

Risks to users of recreational waters impacted by animal wastes depend on the following:

1) whether human-infectious pathogens are present in animal waste,
2) the abundance of the pathogens in those wastes,
3) the survival of those pathogens during manure management and overland transport (to a stream), and
4) the in-stream transport of those pathogens to a recreation site.

This report focuses on the first three of these factors with particular emphasis on identifying specific geographical or farm management conditions that influence the relative prevalence or abundance of the key zoonotic pathogens.

Pathogen Occurrence in Livestock

Prevalence and abundance of zoonotic pathogens

Representative ranges for the prevalence and abundance of the key zoonotic pathogens are summarized in Exhibit 2 below. These ranges were chosen from values reported in the literature because they are from studies with large scales and long durations and because the authors believe they reflect typical conditions in the United States. Despite high variability in pathogen abundance in all livestock wastes, the following general conclusions can be made:

- *E. coli* O157:H7 abundance is greatest in cattle.
- *Campylobacter* abundance is similar in all livestock evaluated and nearly ubiquitous in chickens and swine.
- *Salmonella* and *Cryptosporidium* abundance are greatest in cattle and swine.
- *Giardia* abundance is greatest in swine.

Exhibit 2. Pathogen occurrence and abundance ranges for cattle, swine, and chicken manures

Fecal Source		*E. coli* O157:H7	*Campylobacter*	*Salmonella*	*Cryptosporidium*	*Giardia*
Prevalence	Cattle	9.7–28	5–38	5–18	0.6–23	0.2–37
	Swine	0.1–12	46–98	7.9–15	0–45	3.3–18
	Chickens	NA	57–69	0–95	NA	NA
Abundance	Cattle	$10^{3.1}$–$0^{8.4}$	$10^{1.2}$–$10^{7.3}$	10^{3}–$0^{5.8}$	$10^{2.3}$–$10^{3.9}$	10^{0}–$10^{4.9}$
	Swine	ND–10^{7}	$10^{2.0}$–$10^{5.7}$	$10^{2.8}$–$10^{4.9}$	$10^{1.7}$–$10^{3.6}$	10^{0}–$10^{6.8}$
	Chickens	NA*	$10^{2.8}$–$10^{6.5}$	$10^{-1.0}$–$10^{4.5}$	NA	NA

Conditions and Factors that can Influence the Prevalence or Abundance of the Key Zoonotic Pathogens

Geography and pathogen occurrence

Studies of national scope indicate some U.S. regional differences in the occurrence of pathogens in animal wastes. In some cases, regional differences may be the result of regional differences in agricultural practice (e.g., tendency toward different herd size or feed type) and not intrinsic differences in the occurrence or survival of pathogens. General findings on regional differences include the following:

- There is a higher prevalence of *Salmonella* in layers (chickens) and dairy cattle in the Midwest (including Great Lakes states) than in other U.S. regions.
- Regional clustering of *Salmonella*-positive feedlot cattle was not observed. However, cattle on feed longer appear associated with higher *Salmonella* shedding prevalence.
- *Campylobacter* are uniformly distributed among dairy cattle in the United States.
- *E. coli* O157 occurs at low levels in dairy manure across the United States. One study noted prevalence is highest in the southwestern states (7.6% of fecal samples) and lowest the northeast (1.6% in the northeast). Dairies in the southwestern United States typically have larger herds than other U.S. regions.
- Samples from pens of feedlot cattle receiving barley were 2.7× more likely to have positive samples for *E. coli* O157 than for pens without cattle fed barley. Because feeding barley to cattle is effectively a regional practice, this finding might partially explain high regional prevalence of *E. coli* O157 in the southwest.
- In a 13-month study of feedlot beef cattle, *E. coli* O157 occurs at low levels in beef cattle manure across the United States, but prevalence is highest in the southwestern states (13% of fecal samples from California, New Mexico, and Texas). Only the top 11 feedlot cattle states were included in that study.

- In both dairy and feedlot beef cattle operations, prevalence was consistently higher during summer months and for larger herd sizes.
- Beef feedlot cattle with the shortest time on feed had the highest *E. coli* O157 prevalence. This may be the result of stress during travel and greater susceptibility of animals newly arrived at feedlots.

Farm management conditions

Numerous farm management conditions can influence the relative prevalence or abundance of the key zoonotic pathogens. Important findings include the following:

- The prevalence of most of the key zoonotic pathogens increases with larger farm size (number of animals) for cattle and chicken operations.
- *Campylobacter* and *Salmonella* prevalences are higher among feedlot cattle than among pasture or range cattle. This difference is likely (primarily) due to diet and housing.
- Cattle *Cryptosporidium* infection is more related to animal age than housing or feed; very young calves shed *Cryptosporidium* with higher prevalence and abundance than older cattle.
- *E. coli* O157:H7 shedding in cattle is highly variable. However, the prevalence is higher in spring/summer than fall/winter.
- Chicken *Campylobacter* prevalence is higher in the summertime than other seasons, and may be the result of pathogen aerosol transport via ventilation systems.
- Differences in pathogen prevalence between conventional and organic farming operations can be related to intrinsic differences in these farming practices—such as farm size (organic farms tend to be smaller), animal housing, and age of the animals on the operation. For example, *Campylobacter* prevalence increases with chicken age and organic chickens are typically older when slaughtered.

Manure management practices

- Pathogen loads to streams may be substantially reduced via manure management and use of BMPs.
- Effective BMPs include routing stormwater away from manures and farm areas with high potential for pathogens, and use of filter strips and other means for slowing overland flow.
- Treatment alternatives may be used to reduce manure pathogen density by as much as 5-logs, with higher reductions associated with higher costs and maintenance requirements.

1. Introduction

1.1. Purpose

This report summarizes available information on the prevalence, distribution, and abundance of select waterborne zoonotic pathogens in domestic livestock cattle, swine, and chicken in the United States. It is, in large part, a continuation of the U.S. Environmental Protection Agency (hereafter EPA or the Agency) report *Review of Zoonotic Pathogens in Ambient Waters* (USEPA, 2009). In that report, the following six key ("reference") waterborne zoonotic pathogens were identified based on their relevance in the United States and their potential to be associated with outbreaks in ambient recreational waters and/or drinking water: pathogenic *E. coli*, *Campylobacter*, *Salmonella*, *Leptospira*, *Cryptosporidium*, and *Giardia.* The first four microorganisms are bacteria while the last two are parasitic protozoa. With the exception of *Leptospira* (see Text Box 1), the remaining five zoonotic waterborne pathogens are discussed throughout this report.

1.2. Background

A central goal of the Clean Water Act (CWA) is to protect and restore waters of the United States for swimming and other recreational activities. A key component in the CWA framework for protecting and restoring recreational waters is for EPA to recommend ambient water quality criteria (AWQC) to provide public health protection from illnesses—historically gastroenteritis or gastrointestinal (GI) illness—associated with exposure to fecal contamination during recreational water contact and for subsequent adoption by the States as water quality standards (WQS). Water quality criteria, WQS, guidelines, or their equivalent, as they relate to microbial waterborne illness, are generally specified throughout the world in terms of densities of fecal indicator organisms because fecal matter can be a major source of pathogens in ambient water and because it is not practical or feasible to monitor for the full spectrum of all pathogens that may occur in water (NRC, 2004). For decades, these fecal indicator organisms have served as surrogates for potential pathogens and subsequent health risks in recreational and drinking waters.

The EPA currently recommends recreational AWQC under CWA Section 304(a) and utilizes the fecal indicator bacteria enterococci and/or *E. coli* (USEPA, 1986), which are non-pathogenic and present in both human and animal feces. This approach effectively assumes that animal fecal material is as hazardous as human fecal material and does not allow the exclusion or "discounting" of disease risk associated with animal fecal contamination.

According to the World Health Organization (WHO, 2004), zoonoses are "those diseases and infections which are naturally transmitted between vertebrate animals and man." Examples of zoonotic infections have been recognized among all the major groups of infectious agents: bacteria, protozoa, viruses, helminths, and prions—though the latter three groups are not discussed in this paper. Some zoonoses may infect only one type of animal other than humans, while others may infect several types of animals as well as humans (Moe, 2004). Fenton and Pederson (2005) reported that most pathogens can infect several host species; for example, >60% of human pathogens and >90% of domesticated animal pathogens infect multiple hosts.

Text Box 1
Selection of Key Waterborne Zoonotic Pathogens

There are many zoonotic pathogens and many waterborne pathogens; however, there is a much more limited subset of pathogens that are both. In the EPA report, *Review of Zoonotic Pathogens in Ambient Waters* (USEPA, 2009), a total of 70 pathogens from warm-blooded animals were evaluated for their potential to be both waterborne and zoonotic using the following four criteria (partially adapted from Bolin et al., 2004):

1. *The pathogen must spend part of its lifecycle within one or more warm-blooded animal species.*
2. *Within the lifecycle of the pathogen, it is probable or conceivable that some life stage will enter water.*
3. *Transmission of the pathogen from animal source to human must be through a water related route.* There are zoonotic pathogens for which waterborne exposure has not been detected as a significant route of cross-species transmission. This does not exclude the possibility that these zoonotic pathogens could be transmitted via water.
4. *The pathogen must cause infection or illness in humans.* There are animal pathogens that have waterborne transmission between animals, yet are not known to cause illness in humans.

Of the 70 pathogens evaluated, 20 met the above criteria. Notably, several well-known waterborne pathogens were excluded from analysis because they are not zoonotic. See Appendix A, Table A-1 of USEPA (2009) for a summary of waterborne pathogens that meet the above criteria and selected pathogens that meet some, but not all, of the criteria. Six of the 20 waterborne, zoonotic pathogens from warm-blooded animals were selected for further discussion based on their relevance in the United States. Five (*E. coli*, *Campylobacter*, *Leptospira*, *Cryptosporidium*, and *Giardia*) were selected based on their potential for outbreaks in ambient (untreated) recreational water and one (*Salmonella*) was included based on outbreaks in drinking water. Notably, this list correlates very well with the top five waterborne pathogens for recreational and drinking waters identified previously by Craun et al. (2004). Of the 6 key pathogens, *Leptospira* is excluded from further discussion in this report. This is because the source of infection in humans is usually either direct or indirect contact with the urine (not feces) of an infected animal (USEPA, 2009), and because there are no peer-reviewed, livestock-associated dose-response data for this pathogen. Notably, other studies reviewing risks associated with zoonotic organisms in livestock feces also do not include *Leptospira* among the main pathogens of concern (Bicudo and Goyal, 2003; Goss and Richards, 2008; Venglovsky et al., 2009). The key pathogens selected for analysis in this study are the same as those suggested for emphasis in research and regulation in another review of zoonotic pathogens (USEPA, 2005). On the basis of surveillance of water and foodborne outbreaks in the United States, the authors suggested that priority for standard methods and recreational and drinking water guidelines should be given to *Salmonella* spp., *Campylobacter jejuni*, *E. coli* O157:H7, *Cryptosporidium*, *Giardia*, and selected viral agents indicative of viral contamination.

Interested readers may refer to USEPA (2009) for detailed descriptions of strain variation and zoonotic potential, routes of exposure, health implications, incidence, and interactions and survival in the aquatic environment, for each of these key pathogens.

The presence of fecal indicator organisms in recreational waters generally indicate point- and/or nonpoint sources of human and/or animal fecal wastes from agricultural animals (e.g., cows, pigs, and chickens), domestic animals (e.g., dogs and cats), and/or wildlife (NRC, 2004). Excreted feces and other animal waste products (e.g., urine) are the predominant sources of waterborne zoonoses (WHO, 2004). Zoonotic pathogens in the feces of an animal or human reservoir can be transported to a particular waterbody where their stability in that environment will ultimately influence their infectivity and disease risk to exposed humans. There is evidence

that zoonotic pathogens may change in infectivity, virulence, and the severity of health outcomes they cause in humans depending on their previous host environment. There is also evidence that some of these host-factor changes can influence subsequent infection cycles in exposed hosts. The key mechanisms of phenotypic change in pathogens are genetic diversity (coinfection and quasispecies), cryptic genes, mutators, and epigenetic effects, and are reviewed in USEPA (2009).

Understanding which pathogens could be present depending on the source of fecal contamination might allow the Agency to better estimate human health risks from identified sources of fecal matter. To this end, the information in this report could be used to support quantitative microbial risk assessment (QMRA) in two ways, (1) to estimate potential risks from warm-blooded animal feces in ambient (untreated) recreational waters; and (2) as a complement to recreational water epidemiological studies in support of the development of new or revised recreational water quality criteria and/or implementation. Risks of illness from recreation in waters receiving livestock fecal pollution arise from the presence of human-infectious pathogens in livestock wastes, the abundance of the pathogens in the fecal wastes, the survival of pathogens during on-farm treatment of the wastes or during transport to streams, and the transport of pathogens from their points of entry (often diffuse) to surface waters or recreation sites. This report focuses on the on-farm portion of this risk scenario—particular emphasis is placed on the impact of the animal source and farm operations on the prevalence and abundance of key zoonotic pathogens in manures of swine, cattle, and chicken.

EPA has conducted a significant amount of research since the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 was enacted and plans to issue new or revised criteria for coastal recreational waters (under the CWA, defined as Great Lakes and coastal marine waters) by 2012. EPA believes that the new or revised criteria must be scientifically sound, implementable for broad CWA purposes, and provide for improved public health protection over the current (1986) criteria.

1.3. Approach

Preparation of this report was divided into (1) a literature review to develop a dataset of available studies on the U.S. distribution and prevalence of five key waterborne zoonotic pathogens; and (2) evaluation of the dataset. The literature search included the identification, collection, and summarization of available data and studies (e.g., peer-reviewed literature, EPA or other State agency reports) on the distribution and prevalence—and the factors affecting distribution and BMPs or manure handling practices—of these key zoonotic pathogens in U.S. domesticated livestock cattle, swine, and chicken. The review and analyses focused on ambient water transmission, to include animal feeding operations (AFOs), concentrated feeding operations (CAFOs), and small farms. The report also identifies and discusses seasonal and regional variability of pathogen occurrence and abundance. These data and information were used along with available national-scale geospatial data to support extensive mapping and analysis of the spatial distribution of zoonotic pathogen sources and their potential impacts on water quality in the United States.

Following this introduction, Section 2 provides an overview of the distribution of livestock in the United States. Section 3 summarizes information on pathogen occurrence in U.S. livestock, while Section 4 discusses important factors affecting pathogen occurrence. Section 5 includes all references cited within this report. Appendix A summarizes the approach and results of the literature review. Appendices B and C summarize individual studies of zoonotic pathogen occurrence and abundance, while Appendix D summarizes studies of farm factors and their impact on zoonotic pathogen occurrence and abundance.

2. Distribution of Livestock in the United States

In this section, the distribution of livestock cattle, swine, and chicken in the conterminous United States is presented. Distribution, together with data on pathogen prevalence and abundance in livestock manures (discussed in Sections 3 and 4), suggests regions of the United States where pathogen occurrence in recreational waters differ from "typical" values and where the risks associated with fecal indicator densities differ from those associated with waters impacted by human fecal pollution. Different livestock species are associated with different suites of pathogens. Those pathogens may differ in their ability to infect humans and also differ in the abundances in which they occur in manures. Thus, while livestock distribution alone does not allow estimation of risks associated with swimming in recreational waters receiving wastes from livestock operations, it contributes to the comparison of U.S. regions with respect to their risks.

2.1. Methods

Livestock data were acquired from the 2007 Agricultural Census (NASS, 2010). This data set provides data on all livestock species of interest to this report. It is a single and consistent data set for the entire conterminous United States and contains data available at a county level scale for visualization of regional differences in livestock. The Agricultural Census (the census) provides the number of animals for each livestock type as well as related data not used in this report.

Cattle data are presented as all cattle (adults and calves) and are also subdivided into milk cows and cattle on feed. Cattle on feed are those being fed some ration of grain, silage, hay and/or protein supplement in preparation for slaughter. As described in Sections 3 and 4, these distinctions are important because cattle on different operations have different prevalence and shedding rates of the key pathogens.

Swine data available from the census includes data for hogs (swine weighing over 120 pounds) and pigs (swine weighing under 120 pounds), and also hogs/pigs used for breeding. Because the data do not indicate a significant difference between these two groups, the combined hog and pig data were extracted from the census and used in this section, while the data for breeding hogs/pigs was not used.

For chicken, the census provides layer and broiler data separately. Broilers are raised for meat while layers are raised for egg production. The data for broilers and layers were combined for this report because no significant difference between prevalence of pathogens is apparent between both types of operations.

To allow meaningful comparison of livestock numbers between counties and regions, the number of animals for each livestock type was normalized by county area, and livestock numbers were presented as densities (number of animals/mi^2). Data from numerous counties was withheld from the livestock census to prevent disclosure of data on individual operations that are identified in the maps shown below. Because the proportion of counties with suppressed data was relatively minimal, this did not preclude interpretation of maps and identification of

trends in livestock density for the conterminous United States. However, the absence of these counties does preclude state-level or finer spatial scale analyses of livestock density.

Maps illustrating density by county for each of the livestock types (all cattle, milk cows, cattle on feed, all swine, and all chickens) were developed using ArcGIS 9.2. The maps are graduated in color (light to dark representing low to high density), categorized by quartile, and the counties withholding data are shown in yellow. Alternative schemes for presenting data were explored, however categorization by quartiles most suitably affords easy discrimination of regions of high and low density on maps of the entire United States.

2.2. Cattle Distribution

2.2.1. All cattle

The distribution of all cattle (including milk, beef and cattle on feed) in the conterminous United States is shown in Figure 1. Several large regions of the United States show relatively high densities. These include the central United States (from North Dakota through Texas); the Great Lakes states; less urbanized portions of Pennsylvania, New York, and Vermont; portions of Virginia, Maryland, Tennessee, and Kentucky; and counties in California and Florida. A majority of these regions lie within temperate climate zones and drain to inland waters, with a large portion of the high-density counties located within the Mississippi River basin. The potential for runoff impacting coastal waters is relatively high for Wisconsin and western Michigan coastal streams draining to Lake Michigan, for Florida and East Texas streams draining to the Gulf of Mexico, and for California streams draining to the Pacific Ocean.

2.2.2. Milk cows

Figure 2 shows milk cow density in the conterminous United States. The distribution of U.S. milk cows differs substantially from that of all cattle, with clear high-density regions extending from the Great Lakes through the Northeast states, through the mid South, in a cluster in southwestern Missouri, and along the Pacific coast. The regions with highest milk cow densities drain to the Great Lakes and to inland waterways including the waters of the Chesapeake Bay system, the Ohio and Tennessee Rivers, inland waters in the Pacific Northwest and California, and the upper Mississippi River.

2.2.3. Cattle on feed

The density of cattle on feed is shown in Figure 3. The distribution of feedlot cattle differs substantially from overall cattle distribution and from distribution of milk cows. There is a clear high-density region of cattle on feed in the upper Midwest (a majority of Nebraska, most of Iowa, southern Minnesota and Wisconsin, eastern South Dakota, much of Kansas, and eastern Colorado), and there are regions of medium density from the Great Lakes states through the mid-Atlantic states. Data was withheld by the census for many counties in Texas, Virginia, and Tennessee. It can be assumed that Texas also has high feedlot cattle density. The majority of high-density regions drain to the Mississippi River basin, while many of the medium-density counties drain to the Great Lakes.

2.3. Swine Distribution

Because few data were available to differentiate between swine operations, all swine density data are presented in a single map (Figure 4). There are clear regional trends in swine density, with high-density regions in the Midwest (the highest densities seen in Iowa), the Great Lakes States (particularly in Illinois, Indiana, southern Michigan, and western Ohio), eastern North Carolina, and in south-central Pennsylvania. These regions drain primarily to the Mississippi River basin, the Ohio River, the Great Lakes, and mid-Atlantic coastal streams.

2.4. Chicken Distribution

Among the different livestock species, chicken density (shown in Figure 5) appears the most uniform. Although the census provided bird counts for layers and broilers, these data were combined because insufficient evidence was found that would differentiate these types of operations with respect to their anticipated pathogen productions. Chicken production is common across the entire eastern United States, with particularly high bird densities in the Southeast (excluding Florida) and Arkansas, the upper Midwest (Wisconsin, Minnesota, and Iowa), and inland counties of the Pacific states (California, Oregon, Washington). The high-density regions drain to coastal (Great Lakes and Atlantic) waters, and many U.S. inland waters.

2.5. Summary

Cattle and swine tend to be clustered in identifiable regions of the United States, whereas chickens are more disperse. Regions of particularly high milk cow density are the Great Lakes, Northeast, and inland counties of California and Washington. Cattle on feed have different prevalences of many of the key pathogens than milk cows. High densities of cattle on feed are noted in the Midwest (Texas to Minnesota). Swine are the most heterogeneously distributed of these livestock types, with high-density regions in the upper Midwest and North Carolina. Chicken are more evenly distributed than other livestock types, with production occurring throughout the eastern United States and inland counties of the Pacific states. These livestock distributions may be used, along with pathogen prevalence data, to identify regions where risks from recreation may differ from those either in waters primarily impacted by publicly-owned treatment works (POTW) discharge (wastewater/sewage) or waters impacted by mixed sources of fecal pollution.

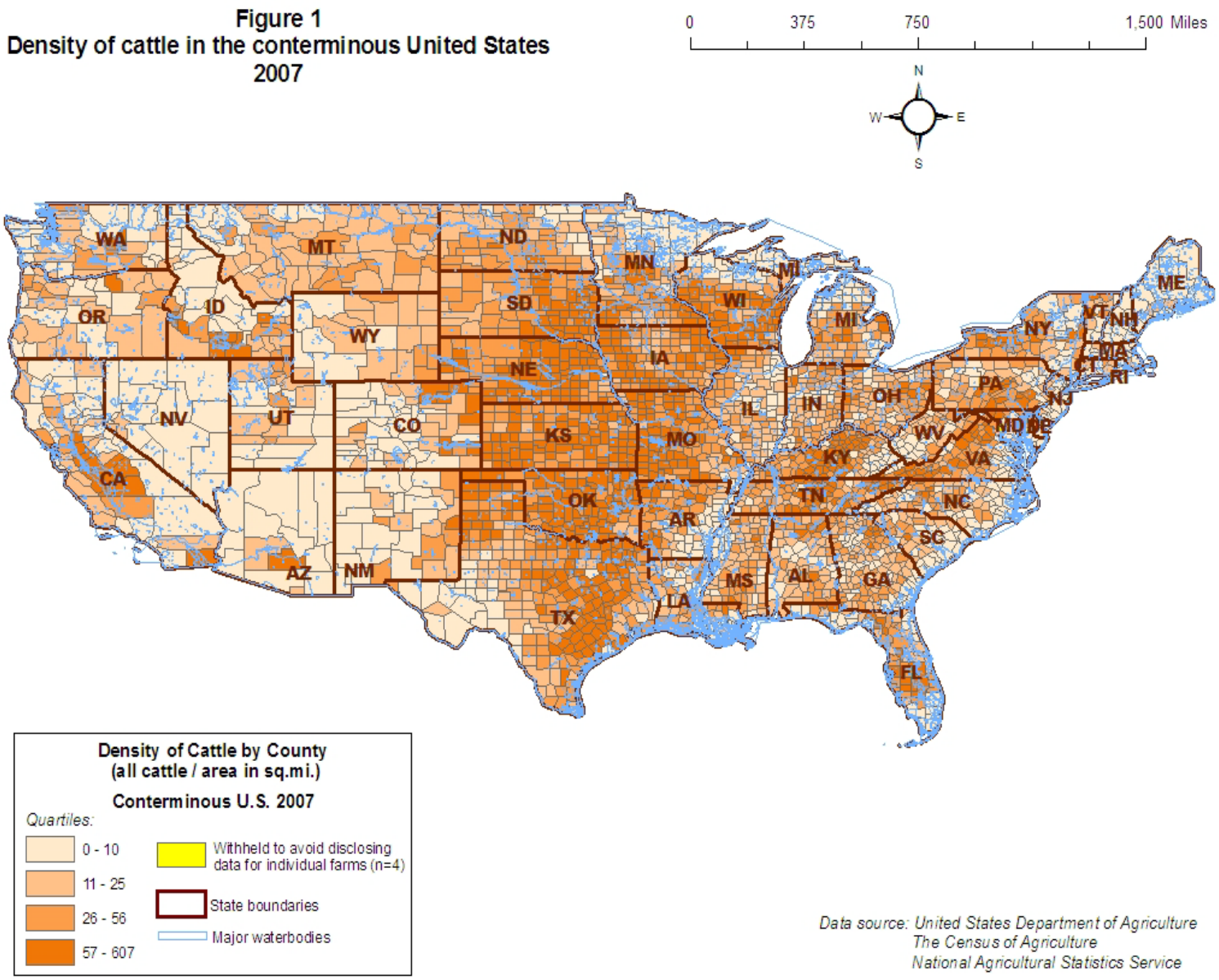

Figure 1. Cattle density in the conterminous United States, 2007

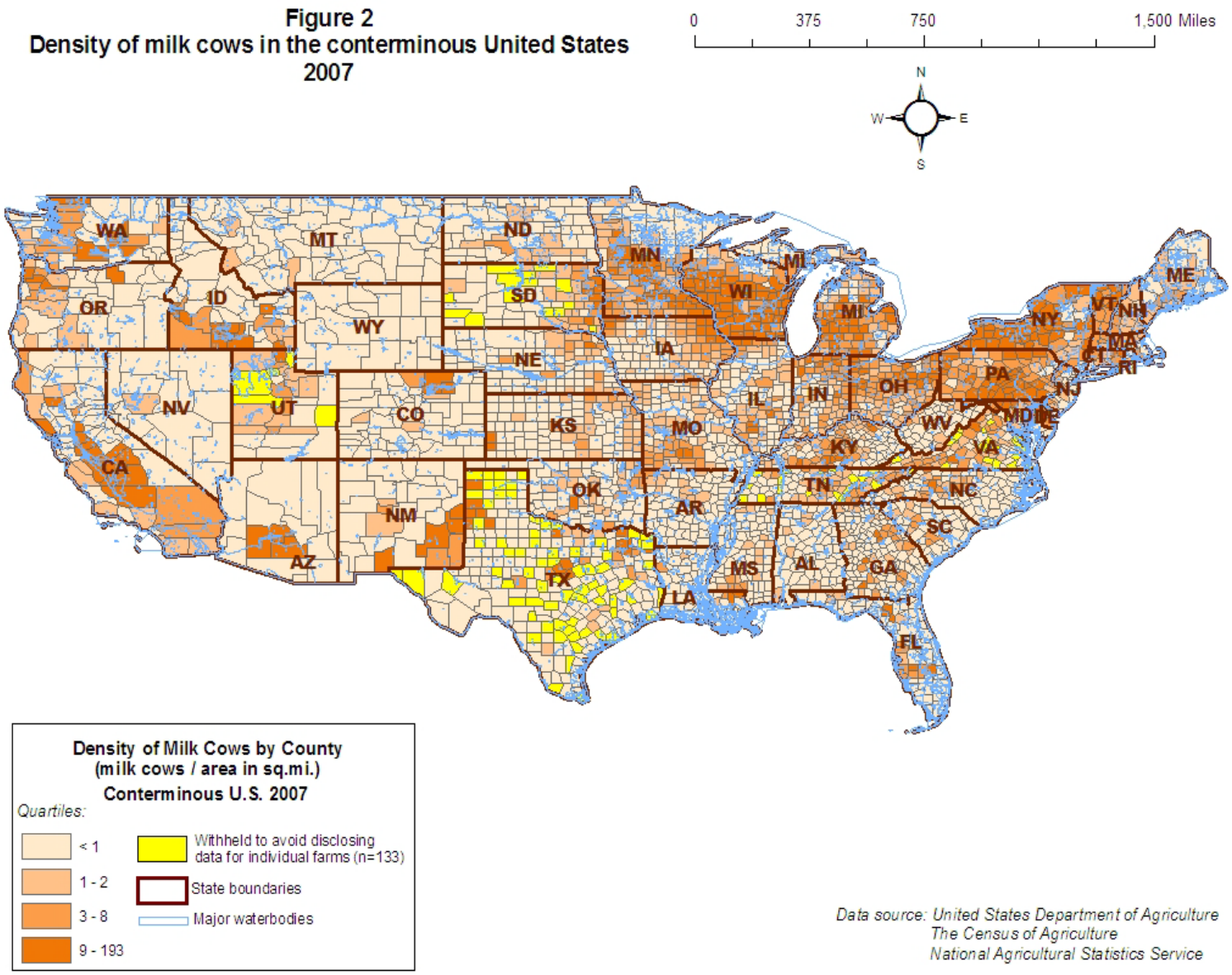

Figure 2. Milk cow density in the United States, 2007

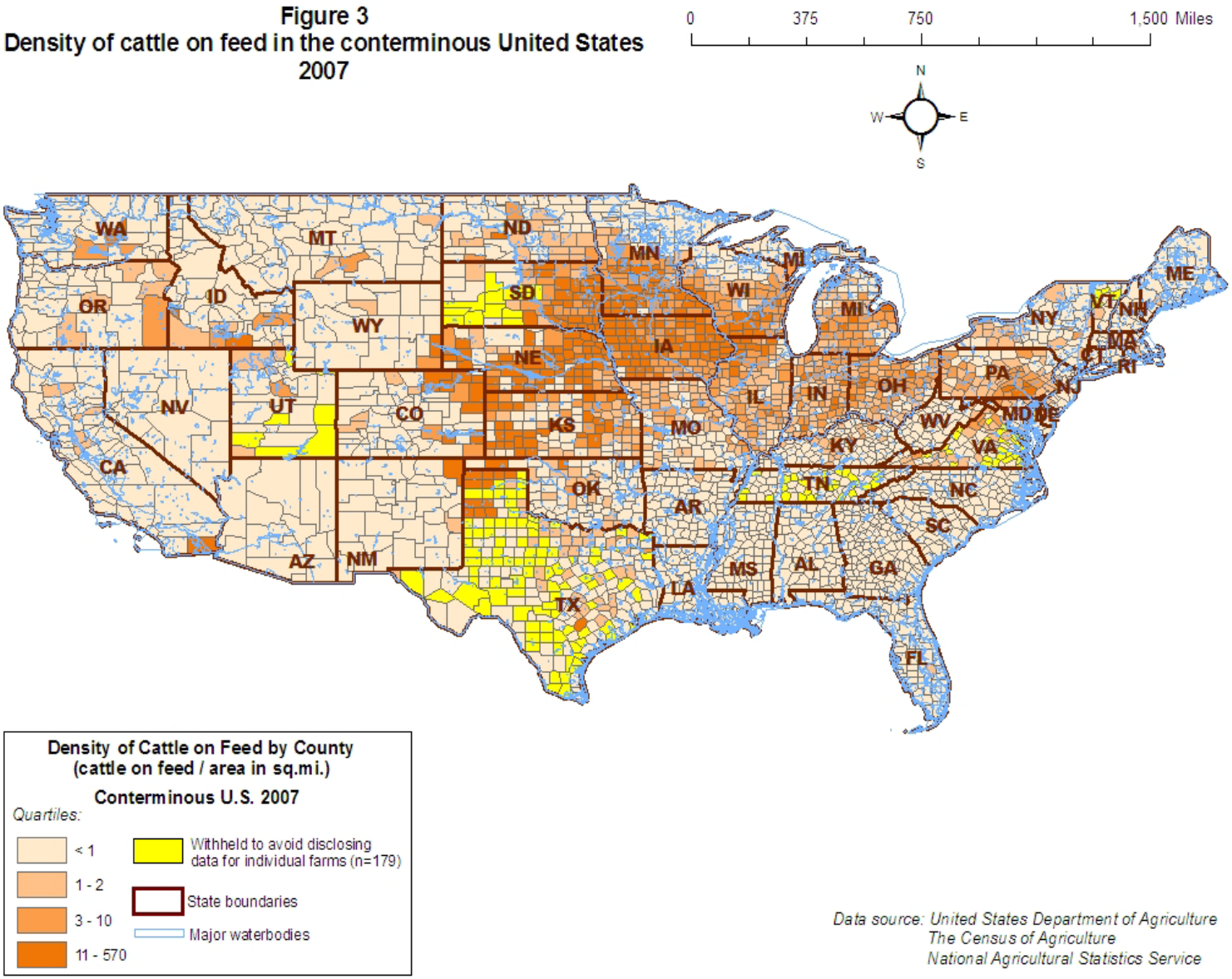

Figure 3. Density of cattle on feed in the United States, 2007

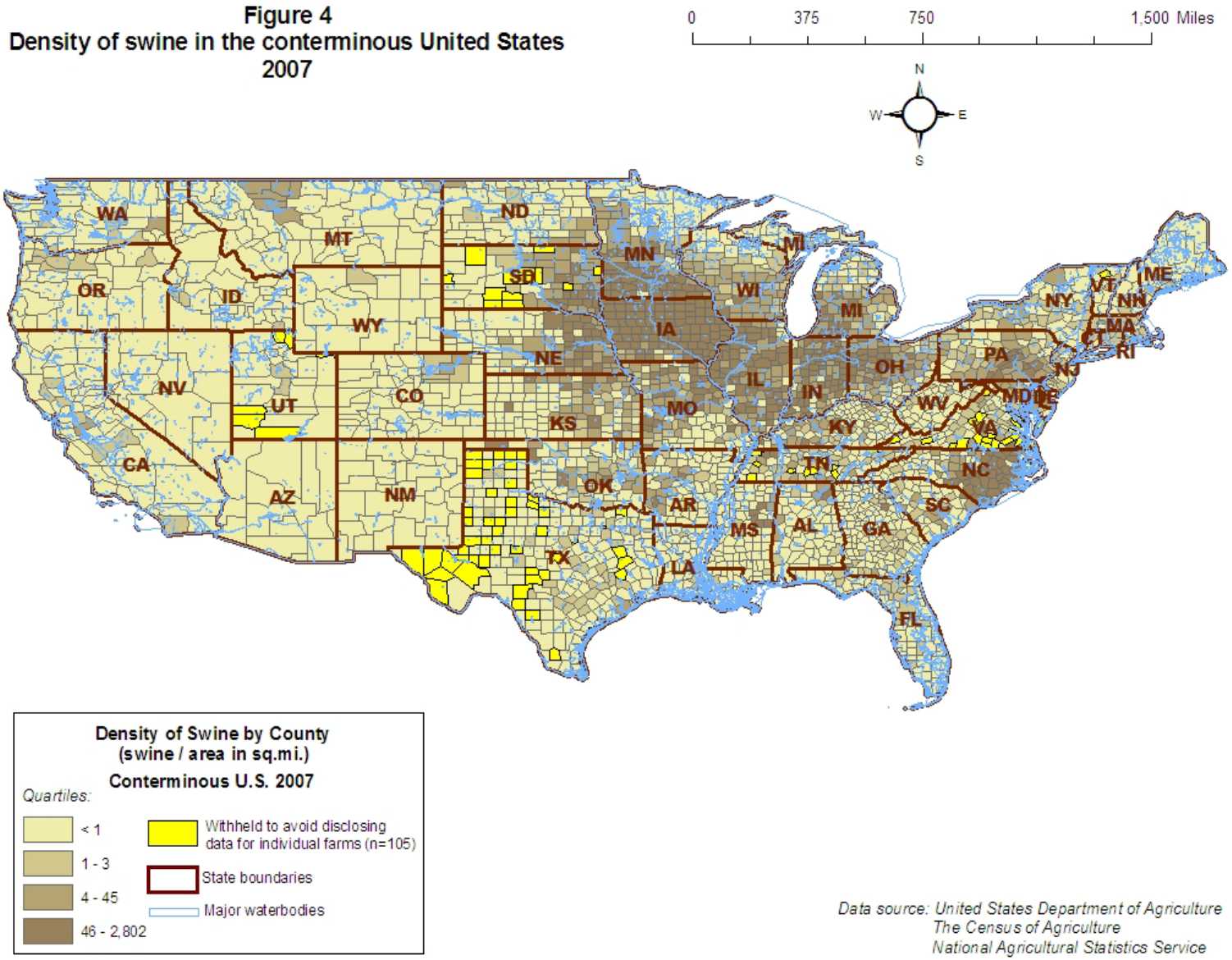

Figure 4. Swine density in the United States, 2007

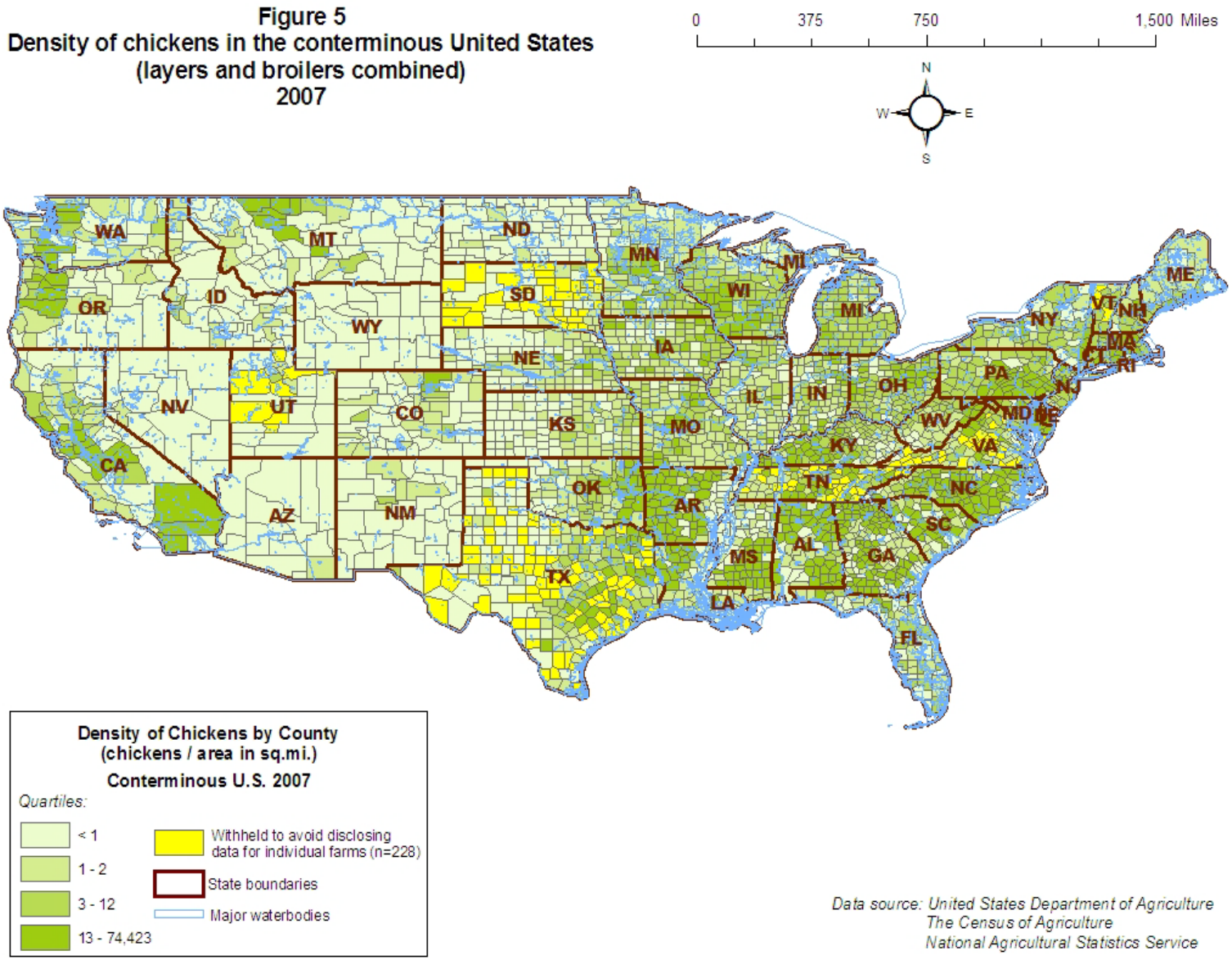

Figure 5. Chicken density in the United States (layers and broilers combined), 2007

3. Pathogen Occurrence in Livestock

The livestock distribution data in Section 2 provide an indication of where gross pathogen loads may occur in the United States, but additional data are required to develop a more nuanced and defined understanding of the distribution of specific pathogens. This section presents and describes those additional data and, where sufficient, the data are then related to the regional distribution of pathogens. It is important to emphasize that the risks associated with particular livestock species are specific for each host-pathogen combination. Further, pathogen occurrence and shedding rates, host-specificity, and manure handling practices all play roles in the generation and transport of pathogens with the potential to infect humans. These factors and processes differ among host-pathogen combinations.

To characterize the occurrence and distribution of pathogens among livestock wastes, relevant studies were collected and data extracted characterize the occurrence of the five key zoonotic pathogens listed in Section 1. Numerous study types and data sets contributing to these characterizations were identified and grouped as follows:

- review papers describing the hosts for the individual pathogens and the extent to which host adaption may have occurred;
- studies reporting prevalence (either herd- or animal-level) of the pathogens in manures of cattle, swine, and chickens;
- studies reporting the abundance (number of organisms per mass of feces);
- studies reporting the national distribution of the reference pathogens; and
- studies relating farm factors to the occurrence of pathogens.

The following sections provide summaries of each of these groupings and refer to expansive tables in the appendices (B, C, and D) in which all studies identified during the literature review are presented.

In these reviewed studies, the authors use the terms pathogen prevalence and pathogen abundance, albeit with different meanings. In general, pathogen prevalence is the fraction of manure samples, herds, individual animals, or other measurable quantities associated with pathogens. The three measures of prevalence with the greatest relevance and that are the most frequently used in this report are (1) herd-level prevalence (the percentage of herds having at least one positive sample for a given pathogen); (2) sample-level prevalence (the percentage of fecal samples positive for a given pathogen); or (3) individual animal prevalence (the percentage of tested animals positive, either based on samples of fresh feces or other measures of infection). Note that throughout this report, the terms prevalence and occurrence are used interchangeably.

Abundance refers to the count of pathogens or indicator in a known mass or volume of a given environmental medium. Because it is the most useful measure of pathogen density, this report focuses on fecal abundance of pathogens (units of organisms/g for solid feces or organisms/L for slurries). In most cases, studies presented pathogen densities as number of organisms per wet weight of feces, though several studies presented fecal abundance data as organisms per dry weight of feces.

3.1. Key Zoonotic Hosts

The five reference zoonotic pathogens occur in different hosts, with the hosts frequently associated with specific species, serotypes, or genotypes of the pathogens. This section associates the species and genotypes of the reference pathogens with hosts—both to identify the most important pathogen species/serotypes/genotypes in human infections and to identify the hosts likely to produce human-infectious pathogens. For all of these pathogens, and for *Salmonella* in particular, the prevalence of species or serotypes among specific host populations appears to shift over time, so the data presented in this section must be interpreted cautiously.

Among the more than 16 species of *Cryptosporidium* identified to date, *C. parvum* and *C. hominis* are believed to cause the majority of human infections among immunocompetent hosts. As noted in Table 1 below, other animals are considered major hosts for both species. Humans are considered minor hosts for other *Cryptosporidium* species, including *C. muris*, *C. meleagridis*, *C. felis*, and *C. canis*. Among livestock species, cattle prevalence of *Cryptosporidium* species aligns closely with species infecting humans, whereas swine cryptosporidia are more seldom isolated in human infections (Xiao et al., 2006). Chicken cryptosporidia do not appear to overlap with species causing human infections, with the exception of *C. meleagridis* that has been implicated in infections of immunocompromised persons (Hunter and Nichols, 2002).

Giardia taxonomy remains unsettled and the species of *Giardia* causing the majority of human illnesses is called *G. lamblia*, *G. duodenalis*, and *G. intestinalis* by different researchers (Adam, 2001; Thompson, 2004). Thompson (2004) notes that *Giardi*a isolates from humans fall into one of two major genotype assemblages and that some *Giardia* genotypic groupings are confined to specific animal hosts. Table 2 (Adam, 2001) presents the most important *Giardia* species and genotypes and their associated hosts. Based on this listing, cattle and pigs appear to have the potential for shedding human-infectious *Giardia*, though chicken are not a significant source of human-infectious *Giardia* cysts.

Table 1. *Cryptosporidium* species and associated major and minor hosts (SOURCE: adapted from Xiao et al., 2004, 2006)

Reference Pathogen	Species or Serotype	Major Host	Minor Host
Cryptosporidium	*C. muris*	Rodents, Bactrian camels	Humans, rock hyrax, mountain goats
	C. andersoni	Cattle, Bactrian camels	Sheep
	C. parvum	Cattle, sheep, goats, humans	Deer, mice, pigs
	C. hominis	Humans, monkeys	Dugongs, sheep
	C. felis	Cats	Humans, cattle
	C. canis	Dogs	Humans
	C. meleagridis	Turkeys	Parrots, humans
	C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches, duck
	C. galli	Finches, chicken, capercalles, grosbeaks	—
	C. bovis	Cattle	—
	C. suis	Pigs	—

Table 2. Significant *Giardia* species and genotypes and associated hosts (SOURCE: adapted from Adam, 2001)

Reference Pathogen	Species	Genotype	Hosts
Giardia	*G. agilis*		Amphibians
	G. muris		Rodents
	G. lamblia	Genotype A-1	Human, cat, beaver, lemur, sheep, calf, dog, chinchilla, alpaca, horse, pig, cow
		Genotype A-2	Human, beaver
		Genotype B	Human, beaver, guinea pig, dog, monkey, cow, sheep, alpaca, goat, pig, rat
	G. ardae		Herons
	G. psittaci		Psittacine birds
	G. microti		Voles and muskrats

Campylobacter species may be grouped as those playing a major role in human infection, those playing a minor role, and those unlikely to cause human infection. Ketley (1997) designated *C. jejuni* and *C. coli* as the species playing a major role in human infections (80% to 90% of *Campylobacter* infections) and notes that other species have the potential for initiating human infections. *Campylobacter* species in humans, livestock, and other hosts are summarized in Table 3. *C. jejuni* and *C. coli* are prevalent among cattle, pigs, and chickens. The non-human reservoirs of *C. upsaliensis* are not fully known, though dogs are known to shed that species and have been implicated in transmission to humans (Bourke et al., 1998). *C. hyointestinalis* has been identified in pig and cattle feces and hamster intestines (Gebhart et al., 1985) and is a suspected cause of human enteric disease (Edmonds et al., 1987).

Salmonella serotypes prevalent among different hosts appear to be fluid; over the past 20 years the predominant serotype for both swine and chickens has changed (Foley et al., 2008). Nonetheless, examining the predominant serotypes for different hosts provides insight into the potential for animal species to shed zoonotic Salmonellae. Bäumler et al. (1998) associated humans, livestock, and selected other host species with *Salmonella* serotypes frequently encountered (Table 4) and noted the age groups that are most susceptible to infection and illness. Given the fluidity in serotype frequency for each of the hosts, the authors believe that the associations presented in Table 4 demonstrated in that all three livestock species of interest (cattle, swine, and chicken) have the potential to shed human-infectious Salmonellae. Callaway et al. (2008) caution against making inferences on the zoonotic potential of Salmonellae based only on serotype prevalence.

As discussed in Section 3.2 below, *E. coli* O157:H7 are frequently detected in cattle feces, less prevalent in swine feces, and seldom reported in chicken feces. Assessing the potential for cattle and other wildlife to generate virulent *E. coli* O157 is difficult given the apparent ability of Shiga-toxin-negative *E. coli* O157 to acquire the *stx* virulence gene in a variety of hosts and settings (Wetzel and LeJeune, 2007), and the potential for differences in virulence between isolates from humans and other sources, though these differences were not observed in a recent study by (Lenahan et al., 2009). Given the variability of *E. coli* O157:H7, even among those isolates originating from the same source, we adopt a conservative approach and assume that *E. coli* O157:H7 from any source pose the same hazard to humans.

Table 3. Significant *Campylobacter* species and genotypes and associated hosts

Reference Pathogen	Role in Human Infections	Species	Non-Human Hosts
Campylobacter	Major	*C. jejuni*	Cattle, pigs, chickens
		C. coli	Cattle, pigs, chickens
	Minor	*C. upsaliensis*	Dogs
		C. hyointestinalis	Pigs, cattle, hamsters
		C. lari	Sea gulls, chickens
		C. pylori	Not known
	Unlikely	*C. fetus*	Cattle, sheep

Table 4. Frequently-encountered Salmonella serotypes for select hosts (SOURCE: Bäumler et al., 1998)

Reference Pathogen	Host Species	Disease	*S. Enterica* Subspecies/ Serotypes most Frequently Encountered	Most Susceptible Age Groups
Salmonella	Humans	Salmonella enteritis	Typhimurium, Enteritidis	Children (<4 years)
		Typhoid fever	Typhi	Children and adults
		Paratyphoid fever	Sendai; Paratyphi A, B and C	Children and adults
	Cattle	Salmonellosis	Typhimurium, Dublin	Calves and adults
	Chicken	Pullorum disease	Pullorum	Newly-hatched birds
		Fowl typhoid	Gallinarum	Growing stock & adults
		Avian paratyphoid	Enteriditis, Typhimurium	Newly-hatched birds
	Pigs	Pig paratyphoid	Cholerasuis	Weaned and adult pigs
		Salmonellosis	Typhimurium	Weaned pigs (>4 months)
		Chronic paratyphoid	Typhisuis	Not specified
	Sheep	Salmonellosis	Abortusovis	Lambs, adult sheep
			Typhimurium	Lambs
	Horses	Salmonellosis	Abortusequi	Foals and adults
			Typhimurium	Foals
	Wild rodents	Murine typhoid	Typhimurium, Enteritidis	Not specified

3.2. Occurrence and Abundance of Reference Pathogens in U.S. Cattle, Swine, and Chicken

The major determinants of whether swimmers are exposed to pathogens of livestock origin include the following:

- whether livestock are shedding the pathogen (prevalence/occurrence),
- the rate of shedding (abundance), and
- the attenuation of pathogens between their introduction in the watershed and their arrival at the swimmer.

This section summarizes occurrence and abundance data for each of the livestock species for each of the reference pathogens. The data characterizes host-pathogen occurrence and abundance and includes data collected as a component of preparing the report "State-of-the-Science Review of Quantitative Microbial Risk Assessment: Estimating Risk of Illness in

Recreational Waters" (August 2010) and data identified during the literature review (see Appendix A). For all host-pathogen combinations, both occurrence and abundance are widely variable. Occurrence varies between herds/flocks and within herds/flocks. For some host-pathogen combinations infection is typically chronic (e.g., *Campylobacter* in chickens) whereas for others infection is epidemic or more prevalent within a particular age cohort (e.g., *Cryptosporidium* in calves). Likewise, shedding (abundance) typically varies during the course of infection for an individual animal, with some animals shedding asymptomatically, some animals shedding at mean rates, and some animals acting as "super-shedders" (animals shedding at above-average rates).

The occurrence of the reference pathogens in livestock manure (% of samples positive) is summarized in Table 5 and a full listing of occurrence data extracted from the literature is presented in Appendix C. In Table 6, the maximum and minimum reported prevalences of pathogens in feces are presented for each of the livestock animals and pathogens. Notable important findings from Table 5 are

- *Campylobacter* is quite prevalent (typically >50%) in both pig and chicken manure and is detected often, but less frequently in cattle manure;
- although *E. coli* O157:H7 shedding was observed in pigs, the most important source of *E. coli* O157:H7 is cattle;
- *Salmonella* occurs in all of the livestock species and is highly variable among chickens; and
- chickens are not significant sources for *Cryptosporidium* and *Giardia* (*Cryptosporidium* species typical of chicken infection pose a low risk of human infection and *Giardia* have not been observed in chicken feces).

Reported abundances of pathogens in feces of livestock are summarized in Table 6 and all data are provided in Appendix D. The studies reviewed employed various sampling strategies and where possible, the authors attempted to provide consistent abundance data in Table 6. The basis refers to the weight basis for the density ([W]et vs. [D]ry). The sample type is either composite (C) or direct (D), and the chicken manure type is either fresh (F) or litter (L). Notable observations from Table 6 include the following:

- fecal shedding abundances exhibit wide variability, with extreme variability observed for cattle shedding of *E. coli* O157:H7 and *Campylobacter*, for pig shedding of *Giardia*, and for chicken shedding of *Salmonella*;
- cattle have the highest manure production rate among the livestock species and therefore may produce very high pathogen loads (number of pathogens excreted by an animal over a period of time); and
- no data were available for estimating *E. coli* O157:H7 shedding rates in chickens.

Table 5. Prevalence (occurrence) of human infectious species of pathogens in livestock manures

	Cattle (Beef & Dairy)			Pigs			Chicken		
Pathogen	Minimum observed prevalence (%)	Maximum observed prevalence (%)	Reference(s)	Minimum observed prevalence (%)	Maximum observed prevalence (%)	Reference(s)	Minimum observed prevalence (%)	Maximum observed prevalence (%)	Reference(s)
E. coli O157:H7	3.3	28	Berry et al. (2007)	0.1	12	Cornick and Helgerson (2004); Hutchison et al. (2004);	0	0	Chapman et al. (1997); USDA (2001)
Campylobacter spp.	5	38	Wesley et al. (2000); Hoar et al. (2001)	46	98	Dorner et al. (2004)	57.1	68.5	Cox et al. (2002) and El-Shibiny (2005)
Salmonella enterica	5	18	Hutchison et al. (2004); Fossler et al. (2005)	7.9	15	Hutchison et al. (2004); Dorr et al. (2009)	0	95	Byrd (1998); Martin et al. (1998)
Cryptosporidium spp.	0.6	23	Sturdee et al. (2003); Atwill et al. (2006);	0	45	Heitman et al. (2002); Xiao et al. (2006)	6	27	Ley et al. (1988)
Giardia spp.	0.2	46	USDA (1994); Fayer et al. (2000); Wade et al. (2000)	3.3	18	Heitman et al. (2002); Xiao et al. (2006)	None reported		

3.3. Large-Scale Studies of Pathogen Occurrence in U.S. Livestock

Relatively few national-scale studies of U.S. livestock pathogen prevalence are available in the open literature. Among the studies identified, most were either of *Salmonella*, *Campylobacter*, or *E. coli* O157:H7 occurrence. A few focused on *Cryptosporidium* or *Giardia* occurrence. Because these studies provide the most direct evidence of regional differences in pathogen occurrence and properties, they were reviewed and are summarized in Table 7 (for *Salmonella*), Table 8 (for *Campylobacter*), Table 9 (for *E. coli* O157:H7), Table 10 (for *Cryptosporidium*), and Table 11 (for *Giardia*).

Table 6. Abundance of human infectious pathogens in livestock manures

Pathogen	Cattle[e]					Pigs[e]				Chicken					
	Log_{10} range[a]		Basis[b]	Sample type[c]	Reference	Log_{10} range		Sample type	Referernce	Log_{10} range		Basis	Sample type	Manure type[d]	Reference
	a	b				a	b			a	b				
E. coli O157:H7	3.1[i]	8.4	W	C	Hutchison et al. (2004)	ND[g]	7	D	Cornick and Helgerson (2004)	NR[h]					
Campylobacter	1.2	7.3	W	D	Moriarty et al. (2008)	2.0	5.7	D	Weitjens et al. (1999)	2.8	6.5	W	D	F	Cox et al. (2002)
Salmonella	3[i]	5.8	W	C	Hutchison et al. (2004)	2.8[i]	4.9	C	Hutchison et al. (2004)	-1.0	4.5	D	D[j]	F	Kraft et al. (1969)
Cryptosporidium	2.3	3.9	W	C	Atwill et al. (2006)	1.7[i]	3.6	C	Hutchison et al. (2004)	NA[f]					
Giardia	0.0	4.9	W	D	Wade et al. (2000)	0	6.8[k]	D	Maddox-Hyttel et al. (2006)	NA					

Notes:
[a] Units are $\log_{10}$(cfu L^{-1} or oocysts L^{-1} or cysts L^{-1}). "a" denotes the minimum observed value and "b" denotes the maximum observed value
[b] Basis refers to weight basis for manure. D denotes dry weight and W denotes wet weight
[c] Sample type is either composite (C) or direct (D)
[d] Poultry manure type is litter (L) or fresh (F)
[e] All cattle and swine fecal abundances reported are for solid, fresh fecal samples (not slurries or treated manure)
[f] Not applicable
[g] Not detected
[h] None reported
[i] Geometric mean among samples (minimum abundance among positive samples not provided in the original study)
[j] Samples taken at random from the top of the litter pile; because the droppings were fresh, it is presumed they were derived from a single bird
[k] Estimated from data presented graphically

Table 7. Review of large-scale studies of *Salmonella* prevalence on livestock operations

Study	Livestock Species	Regional Variations	Serotype Prevalence Trends
Ebel et al. (1992)	Spent laying hens	Prevalences of *Salmonella enteriditis* (SE) in positive layer houses Northern U.S.: 45% Southeastern U.S.: 3% Central/Western U.S.: 17%	No significant regional differences in occurrence of *Salmonella* reported; serotype prevalence among positive flocks not assessed
Foley et al. (2008)	Swine and chickens	Not considered	Chickens: Prior to 2000, serotype *S. heidelberg* replaced serotype SE as the predominant serotype among U.S. chickens. The decline in SE prevalence may be the result of targeted programs or in development of natural resistance among chicken flocks. *Salmonella* (all serotypes) prevalence is highly variable, varying with time and with operation/chicken type. Swine: From 1986 to 1995, *S. cholerasuis* was the most common serovar among U.S. swine isolates. In 1995, *Derby* was identified as the most common serotype, and since 1996, *Typhimurium* has been most common. As with chickens, prevalence of *Salmonella* infection is highly variable from year-to-year and herd-to-herd; estimates of overall prevalence were 1.4–3.1% and 3.4–33%.
Fossler et al. (2005a)	Dairy cows	*Salmonella* shedding was more prevalent in midwestern states (MI, MN, WI) than in NY	Not considered
Garber et al. (2003)	Layers	Overall, SE was isolated from 7.1% of U.S. layer houses, regional prevalence estimates were: Southeast: 0%; Central region: 9.0% (standard error = 7.2); West: 4.4% (standard error = 2.5); and Great Lakes region: 17.2% (standard error = 13.7)	Only SE studied; regional differences in prevalence may differ for other serotypes
Kabagambe et al. (2000)	Dairy cows	Herds from the south (defined as study states CA, NM, TX, FL, TN) had 5.7× odds of *Salmonella* shedding (from at least one animal) than herds from the north (study states OR, WA, ID, MN, IN, IO, MI, WI, NO, OH, NY, VT, and PA). Prevalences (% of herds with at least one shedder) were: South: 66.7% North: 15.7%	Not considered

<table>
<tr><th>Study</th><th>Livestock Species</th><th>Regional Variations</th><th>Serotype Prevalence Trends</th></tr>
<tr><td>USDA (2000)</td><td>Layer houses</td><td>Environmental samples of manure, egg belts, elevators, and walkways in layer houses used to estimate the prevalence of SE. Regional prevalences (based on at least one positive sample per layer house studied) were:
Great Lakes: 17.2%
Southeast: 0%
Central: 9.0%
West: 4.4%
All farms: 7.1%</td><td>Only SE was studied; non-detects of SE in the south may reflect a difference in serotype prevalence from other regions rather than a complete absence of Salmonella</td></tr>
<tr><td>USDA (2009a)</td><td>Swine operations</td><td>Regional differences in swine Salmonella prevalence were not reported; overall prevalence was 7.2% of fecal samples positive and 52.6% of sites with at least one fecal sample positive</td><td>Top four serotypes from Salmonella isolates:
Derby: 29.6% of isolates and 23.0% of sites
Typhimurium (Copenhagen): 22.6% of isolates and 15.6% of sites
Agona: 10.8% of isolates and 9.6% of sites
Anatum: 7.5% of isolates and 5.2% of sites

The isolate prevalence among swine has not changed since 1995</td></tr>
<tr><td>USDA (2009b)</td><td>Dairy cows</td><td>Overall percentage of Salmonella-positive dairy operations has increased with each APHIS study since 1996, from 21.1% in 1996 to 39.7% in 2007.

Percentage of Salmonella-positive cows increased from 5.4% in 1996 to 13.7% in 2007; percentage of operations with any Salmonella-positive samples by herd size:

<table><tr><th rowspan="2">Region</th><th colspan="2">Herd size</th></tr><tr><th>1 to 499</th><th>500 or more</th></tr><tr><td>West</td><td>30.0</td><td>36.4</td></tr><tr><td>East</td><td>42.9</td><td>79.5</td></tr><tr><td>All operations</td><td>41.5</td><td>61.0</td></tr></table></td><td>From 1996 to 2007, the most common serotypes from dairy cattle isolates shifted from S. montevideo and Meleagridis to Cerro and Kentucky</td></tr>
<tr><td>USDA (2009c)</td><td>Beef cow-calf operations</td><td>Number of Salmonella-positive cow-calf operations did not vary by region or farm size. Number of positive operations declined from 11.2% in 1997 to 9.2% in 2007/8. Number of positive sampled cows declined from 1.4% in 1997 to 0.5% in 2007/8.</td><td>Serotype Montevideo identified most among isolates (17.6%), followed by I 6, 7:k:- (8.8%) and Braenderup, Meleagridis, Newport and I 3,10:-:1,w (all 5.9%)</td></tr>
</table>

Study	Livestock Species	Regional Variations	Serotype Prevalence Trends
Wells et al. (2001)	Dairy cows	Regional herd-level prevalences (a positive herd had at least one positive fecal sample): Northwest: 20.0% Midwest: 15.4% Northeast: 9.5% South: 45% Authors noted farm-size differences in shedding rates that may manifest as regional differences if sizes of farms are different in different regions.	48 different serotypes were identified in the studied herds, with the top 5 most prevalent being *Montevideo* (21.5% of isolates), *Cerro* (13.3%), *Kentucky* (8.5%), *Menhaden* 7.7%), and *Meleagridis* (6.1%). Distribution significantly different from prior reported national distributions of serotypes in which serotype *Typhimurium* was the most commonly identified serotype. Regional differences in the abundance of individual serotypes not reported.

Table 8. Large-scale studies of *Campylobacte*r prevalence among U.S. livestock operations

Study	Livestock Species	Regional Variations	Species Prevalence Trends
Harvey et al. (2004)	Dairy cattle	Prevalences of *Campylobacter* relatively low and regional differences not significant, regional prevalences were: Northeast: 2.9% Desert southwest: 5.2% Pacific west: 5.0%	70% of *Campylobacter* determined to be *C. jejuni*; other studies report a range of species prevalence among dairy cattle isolates
USDA (2009b)	Dairy cattle	*Campylobacter* found to be present on most dairy operations; in 2007, at least one healthy cow in 92.6% of sampled operations (n = 121) was shedding *Campylobacter*. Percentage of cows positive for *Campylobacter* decreased from 51.3% in 2002 to 33.7% in 2007.	In 2007, number of *C. coli* isolates was very small compared with the number of *C. jejuni* isolates
Wesley et al. (2000)	Dairy cattle	No significant differences in herd-level and animal-level prevalence of *Campylobacter* for the north (ID, IL, IO, MI, MN, NY, OR, PA) and south (CA, FL, TN, TX) regions of the United States	80.6% of herds positive for *C. jejuni* and 19.4% of herds positive for *C. coli.* 30.3% of cows positive for *C. jejuni* and 2.5% positive for *C. coli*.

Table 9. Review of large-scale studies of *E. coli* O157 prevalence on livestock operations

Study	Livestock Species	Overall Prevalence and Regional Trends	Observations and Notes
USDA (2003)	Dairy cows	Overall: Prevalence for culture-positive *E. coli* O157 was 4.3%; 38.5% of operations had one or more positive cows. Prevalence highest in summer (June – 8.2%) and lowest in spring (April – 1.5%). Regional: Highest prevalence of positive cows found in West region (7.6%), Midwest (3.5%), Southeast (3.1%), and Northeast (1.6%). Large operations (>500 cows) were more likely to have positive samples than medium operations (100-499 cows) or small operations (<100 cows). The majority of large dairies are in the West region.	Samples collected from March to September 2002; total of 3,733 samples for culture and ID of *E. coli* O157, *stx 1*, *stx 2*, and antigens. Samples collected from 5 operations from each of the 21 participating states: • West region: CA, CO, ID, NM, TX, WA • Midwest region: IL, IN, IA, MI, MN, MO, OH, WI • Northeast region: NY, PA, VT • Southeast region: FL, KY, TN, VA
USDA (2001)	Beef cattle (feedlot)	Overall: Ranged from 3.3 % in samples taken in the winter (Feb) to 19.9% in fall samples (Sept). No geographic trends for STEC prevalence. All feedlots had at least one positive sample during the study. Regional: Prevalence of culture-positive samples per region: 8.4% in Middle Region (CO, KS, and OK), 11.5% in Northern Region (ID, IA, NE, SD, WA), 13% in Southern Region (CA, NM, TX)	73 feedlots/422 pens in 11 leading cattle feeding states sampled for STEC from Oct '99 to Sept '00. Total of 10,415 samples. Samples from pens for cattle that had been on feed the shortest (13.9%) were more likely to be positive than samples from pens for cattle that had been on feed the longest (8.6%).
USDA (1998)	Dairy cows	Overall: 24.2% of operations and 30.9% of markets had at least one culture-positive *E. coli* O157 sample. Prevalence on farm – 0.9% of samples positive. Prevalence for cows to be culled with 7 days – 2.8% of samples positive. Prevalence for culled dairy cows at markets – 1.8% of samples positive. Regional: Authors did not comment on regional differences.	Fecal samples collected from 91 dairy operations and 97 cull dairy cow markets in 19 states during a one-time sampling event. Samples collected from Feb-July 1996. Seasonal pattern of shedding was observed, samples more likely to be positive after May 1 than before May 1. No significant differences found between cows on farm and cows going to slaughter. Prevalence was higher for herds with 100 or more cows (39.1% of herds had at least one positive sample) than for herds with fewer cows (8.9 % of herds had at least one positive sample), however seasonality may have been a factor.

USDA (1995)	Beef cattle (feedlot)	Overall: Prevalence 1.61% of collected samples. Regional: Prevalence of positive feedlots per region: 59.4% in Middle Region (CO, NE, KS, OK), 58.3% in Northern Region (ID, IA, IL, MN, SD, WA), 71.9% in Southern Region (CA, AZ, TX)	Pens in feedlots from 13 leading cattle feeding states sampled for E. coli O157:H7 in fall of 1994. Total of 11,881 samples. Samples from pens for cattle that had been on feed the shortest (47.1%) were more likely to be positive than samples from pens for cattle that had been on feed the longest (16.8%).

Table 10. Review of large-scale studies of *Cryptosporidium* prevalence on livestock operations

Study	Livestock Species	Overall Prevalence and Regional Trends	Observations and Notes
USDA (1994)	Beef calves from beef cow/calf operations (Fresh fecal samples included both diarrheic calves <3 months and nondiarrheic calves <6 months)	Overall: Prevalence of positive calves was 20.1% for diarrheic calves and 11.2% for nondiarrheic calves. Prevalence of positive operations submitting samples from diarrheic calves was 39.1%. Prevalence of positive operations submitting samples from nondiarrheic calves was 41.8%. Prevalence was related to and decreased with age of calves (23.1% for 1-30 days old; 9.2% for ≥121 days old). Regional: No discussion regional differences in prevalence.	Study included 391 samples from diarrheic calves from 69 operations and 1,053 samples from nondiarrheic calves from 141 operations. Average age of diarrheic calves testing positive was 41.1 days. Average age of nondiarrheic calves testing positive was 75.8 days. Shedding was common in calves of beef herds whether the calves had diarrhea or not.
USDA (1993)	Dairy calves (preweaned)	Overall: Prevalence across U.S. 22% of calves and >90% of farms. Prevalence increased slightly with herd size but still high prevalence on (about 80%) on smaller farms (<100 cows). Prevalence higher in summer months than in other months. Prevalence was highest in heifers 1-3 weeks old (>50%). Prevalence drops to <15% for calves over 5 weeks old. Regional: Prevalence higher in western herds; authors note that these are also the largest operations.	Study included 1,103 farms in 28 states, with 7,369 samples collected. States included: • West: WA, OR, CA, ID, CO • Midwest: NE, IA, MN, WI, MI, IL, IN, OH • Northeast: ME, VT, NH, NY, PA, CT, MA, RI • Southeast: VA, NC, TN, GA, AL, FL, MD

Table 11. Review of a large-scale study of *Giardia* prevalence on livestock operations

Study	Livestock Species	Overall Prevalence and Regional Trends	Observations and Notes
USDA (1994)	Beef calves from beef cow/calf operations (Fresh fecal samples included both diarrheic calves <3 months and nondiarrheic calves <6 months)	Overall: Prevalence of positive calves was 26.9% for diarrheic calves and 45.9% for nondiarrheic calves. Prevalence of positive operations submitting samples from diarrheic calves was 63.8%. Prevalence of positive operations submitting samples from nondiarrheic calves was 90.8%. Prevalence peaked in calves 61-90 days old (59.6%) and decreased with age of calves (29.9% for calves ≥121 days old). Regional: No discussion of regional differences in prevalence.	Study included 391 samples from diarrheic calves from 69 operations and 1,053 samples from nondiarrheic calves from 141 operations. Average age of diarrheic calves testing positive was 47.1 days. Average age of nondiarrheic calves testing positive was 79.1 days. Shedding common in calves, especially older calves, of beef herds whether the calves had diarrhea or not.

These large-scale studies were "snapshots" of prevalence of the pathogens. Prevalence was either as herd prevalence (the fraction of herds for which one or more samples were positive for the pathogen of interest) or individual level (the number of positive samples at a farm or among all samples in a study). Because large-scale studies were snapshots, data from these studies must be interpreted in light of known seasonal and non-seasonal (e.g., epidemic) variations in pathogen prevalence and prevalence of specific pathogen species, serotypes, (etc.), and in light of market and other conditions that differentiate the regions from each other. For example, farm size is known to influence prevalence of both *Salmonella* and *Campylobacter* in most livestock operations. Regions with a greater proportion of large operations may have an attendant high prevalence of shedding of these pathogens. In those cases, care should be taken to associate at least some of the cause for shedding prevalence difference among regions with farm size rather than other regional factors such as climate.

3.3.1. Large-scale studies of *Salmonella* prevalence

More large-scale studies have focused on *Salmonella* prevalence than any of the other reference pathogens (Table 7). Studies of layers and dairy cows indicate a higher prevalence of *Salmonella* shedding in the Midwest and very low levels of shedding in the Southeast. Notably, at least two large-scale studies considered only *Salmonella enteriditis* serotype enteriditis and so may have missed the presence of unexpected *Salmonella* serotypes among animals in the Southeast. A single study (Kabagambe et al., 2000) observed higher shedding in states broadly classified as "south" than those classified as north, though Florida was the only southeastern state included in that study. Regional differences in shedding prevalences were not observed for swine of beef calf-cow operations.

No studies evaluated regional differences in the prevalence of *Salmonella* isolate serotypes. However, multiple studies noted significant shifting in the dominant serotypes among layer operations, dairy operations, and beef calf-cow operations for the period between 1996/7 and 2007/8.

3.3.2. Large-scale studies of *Campylobacter* prevalence

Large scale studies of *Campylobacter* prevalence were conducted only for dairy cattle. These study results (Table 8) are difficult to interpret. All studies indicate a relatively uniform prevalence of *Campylobacter* across the conterminous United States and a preponderance of *C. jejuni* isolates. However, one study indicates low prevalence (Harvey et al., 2004) whereas other studies (Wesley et al., 2000; USDA, 2009b) indicate high prevalence among operations and among individual animals. It is not possible to generalize the findings of these studies, except to note that they do not indicate regional differences in either *Campylobacter* prevalence or the prevalence of individual *Campylobacter* species.

3.3.3. Large-scale studies of *E. coli* O157:H7 prevalence

Large-scale studies of *E. coli* O157:H7 prevalence were conducted for dairy and beef cattle. These study results (Table 9) indicate consistently low albeit widespread prevalence of *E. coli* O157:H7 across the conterminous United States for dairy operations and for cattle on feed. The results of these studies indicate slightly higher prevalences for feedlots in southwestern states (i.e., California, Texas, Arizona, New Mexico). Authors of a study relating these national results to farm factors (USDA-APHIS-VS, 1997) believe the presence of barley in the diet of cattle on feed in these states may be a region-based contributing factor. Additionally, in feedlots, prevalence was higher in cattle that had been on the feedlot a short time compared to cattle that had been on the feedlot for a longer time. In one study of dairy operations (USDA, 2003), again there was a slightly higher prevalence in the western states (California, Colorado, Idaho, New Mexico, Texas, Washington). This finding may be related to the size of the operation; the largest dairy operations are in this region. In all of these large-scale studies, higher prevalence occurred during the summer/fall months. And, when evaluated, prevalence was higher for larger herds than it was for smaller herds (USDA, 1997, 1998).

3.3.4. Large-scale studies of *Cryptosporidium* and *Giardia* prevalence

The USDA conducted a large-scale study of *Cryptosporidium* in dairy calves (USDA, 1993) and a large-scale study of *Cryptosporidium* and *Giardia* in beef calves (USDA, 1994). These study results (Tables 10 and 11) indicate widespread prevalence of both *Cryptosporidium* and *Giardia* in U.S. dairy and beef calves. The results of these studies indicate a higher prevalence of *Cryptosporidium* in dairy operations in western states (i.e., California, Oregon, Washington, Idaho, and Colorado). This finding may be related to the size of the operation; the largest dairy operations are in this region. Prevalence of *Cryptosporidium* peaked in very young calves (<30 days old) while *Giardia* prevalence peaked in slightly older calves (31-60 days old). In the study of dairy calves, higher prevalence occurred in the summer months. In both studies, parasites were found in calves with and without diarrhea, indicating that many producers may be unaware of the extent of infection in their herds. Large-scale studies of *Cryptosporidium* and *Giardia* among beef and dairy cow/calf operations in the United States indicate that prevalence of these parasites is widespread, and that there may be slight regional differences in prevalence with the west having the highest *Cryptosporidium* prevalence of the regions studied.

3.4. Summary

With the exception of the absence of *Giardia* in chickens, all of the key zoonotic pathogens evaluated in this report (*Salmonella*, *Campylobacter*, *E. coli* O157:H7, and *Cryptosporidium*) are all found in cattle, swine, and chicken—though the particular species and types prevalent in the livestock are not necessarily the species and types posing the greatest risk of human infection and subsequent illness. All of the key zoonotic pathogens occur frequently among cattle and the species and serotypes found in cattle feces are generally similar to those posing hazards to humans. Swine *Salmonella*, *Campylobacter*, *Giardia*, and *E. coli* O157:H7 tend to be the same species/serotypes as those most commonly implicated in human infections, whereas most swine *Cryptosporidia* appear to be host-adapted and pose a reduced hazard to humans. Among chickens, the only key zoonotic pathogens that occur with some frequency and are similar to the pathogens implicated in human infections are *Salmonella* and *Campylobacter*—both of which are highly prevalent among U.S. flocks.

Relatively few studies reporting regional differences in pathogen prevalence for specific host-pathogen combinations were identified. Large-scale studies of both layer and dairy cattle operations indicate higher prevalence of *Salmonella* among Midwest (including Great Lakes States) operations than in other U.S. regions. In particular, the serotype SE was frequently encountered in Midwest layer operations but has not been reported in operations in the south. However, these observations must be interpreted cautiously. Differences among regions detected in these studies may relate less to intrinsic (climatic) differences among regions than to farm management decisions and practices, such as the types of units used for housing animals and the typical farm sizes in different regions. Additionally, studies based only on SE (rather than all *Salmonella* serotypes) may not accurately indicate the presence of human-infectious *Salmonella*. Large-scale studies of *Campylobacter* among dairy herds indicate *Campylobacter* are widespread in the United States (no regional differences) and the dominant species among positive manure samples is *C. jejuni*.

Large-scale studies of *E. coli* O157 among the largest beef and dairy operations in the United States indicate that a low prevalence of *E. coli* O157 is widespread, and that there may be slight regional differences in prevalence with the west/southwest having the highest prevalence of the regions studied.

4. Farm Factors and the Occurrence of Pathogens in Livestock Manures

Livestock distributions and the relatively few large-scale studies on pathogen occurrence are insufficient to allow an assessment of how livestock pathogens might differ between U.S. regions. However, additional data related to farm factors (farm type, animal management, farm size, etc.) can be used to evaluate whether pathogen occurrence and abundance from a particular operation could be expected to fall at the top, bottom, or middle of the ranges reported in Section 3.2. This section divides farm factors into those with potential regional implications and those without regional implications. An example of a farm factor with regional implications is farm size. Farm sizes differ by region, with large farms often concentrated in specific regions (e.g., swine operations in North Carolina) and small farms in other regions. Farm factors without regional implications are generally animal rearing and manure management choices implemented on farms. Individual farmers choose how to raise animals and manage manure based on operation of their farms. Although the latter choices play a role in the pathogen occurrence and discharges from the individual farms, there appears to be no systematic or consistent reason (e.g., besides "tradition") for these choices to be clustered or have regional differences. However, because there are no data to allow an evaluation of these types of factors, they are not discussed in this report.

This section presents synopses of studies relating farm factors to the prevalence and abundance of the key pathogens in manures. First addressed are farm factors <u>with</u> regional implications. We identify those factors as farm type (e.g., cattle feed lot vs. dairy operation vs. pasture operation) and farm size. Next discussed are results from longitudinal studies comparing prevalence of infection among different age cohorts of animals or among animals before and after changes in animal management. Also discussed are studies on seasonality of pathogen prevalence. This is followed by an evaluation of farm factors <u>without</u> regional implications and is limited to those farm factors for which we could obtain data for multiple pathogens and livestock species. The section concludes with a brief review of manure management practices. The discussion of manure management differs from the other portions of this section because it relates to the probability that manure-borne pathogens reach receiving waters—not the probability that the pathogens are excreted in manures.

It is important to note that the literature review for this section identified many studies comparing organic and conventional farming practices. Most of the reported differences in pathogen prevalences among conventional and organic farms can be explained based on intrinsic differences between these farming practices, such as farm size (organic farms tend to be smaller), animal housing, and age of the animals on the operation. For example, *Campylobacter* prevalence increases with chicken age and organic chickens are typically older when slaughtered. Therefore, in reviewing studies reporting pathogen prevalence differences among conventional and organic operations, we sought to identify the underlying cause rather than ascribing the observed pathogen prevalence to a generic organic vs. conventional difference. The results presented cannot be used for comparing risks and benefits of organic farming. Such a comparison must include many factors not relevant for discussion in this report. A total of 21 articles were reviewed to compare and describe the *Salmonella*, *E. coli* O157:H7, and *Campylobacter* prevalence and variability reported for organic and conventional farming

practices for cattle, swine, and chicken. Detailed reviews of those studies are provided in Appendix D.

Differences in pathogen prevalence and abundance between CAFOs and non-CAFO operations can be attributed to multiple factors such as herd size, animal density, selection of feed, and others. These factors are likely more important than the distinction of whether or not a farm is a CAFO. As noted in the USEPA (USEPA, 2005), "The U.S. Environmental Protection Agency defines a concentrated animal feeding operation (CAFO) as an animal feeding facility that houses more than 1,000 animal units (AU), has 300 to 1000 AU but meets certain conditions, or is designated a CAFO by the state. The number of animal units is based on an equivalent number of beef cattle. Therefore, 1,000 AU equals 1,000 beef cattle, 700 mature dairy cattle, 2,500 swine, 5,000 ducks, 10,000 sheep, 55,000 turkeys, or between 30,000 and 100,000 laying hens or broilers depending on the animal waste management system employed." This definition is a useful distinction with respect to the volume of manure produced by the operation and the manure management techniques likely employed. However, for the purposes of this report, designation as a CAFO is used only to provide information on whether a farm is above a certain size threshold.

4.1. Farm Factors with Regional Implications

Regional differences in pathogen prevalence may relate to intrinsic differences between the regions (precipitation, temperature, solar radiation, microbial ecology, and other features related to the survival of pathogens in regions) or may relate to practices that differ by region. In addition to regional pathogen prevalence and animal density data, the authors identified studies from the literature that relate pathogen prevalence (either herd-level or animal level prevalence) to farm practices and features. Some of the farm factors and features may be different among U.S. regions and have the potential to cause different pathogens prevalences in different regions. Examples of the factors and features that could give rise to regional differences in pathogen prevalence are the following:

- operation type;
    - cattle (dairy, beef cattle pasture, beef cattle feedlot)
    - chicken (broilers, layers, broiler-breeder)
    - swine (farrow to finisher operations or birth to market; farrow to feeder operations or birth to about 15 days; feeder to finisher operations)
    - or for any livestock, use of organic or conventional farming practices (as self-defined in studies); and
- farm size.

Because different operations house animals of different ages and because, in many cases, prevalence of pathogens varies with animal age, this section includes a discussion on longitudinal studies of pathogen prevalence in livestock cohorts. The results of these studies provide additional data for understanding and interpreting pathogen prevalence differences reported for operations of different types. Likewise, seasonality provides insights into some findings of the farm factors studies.

4.1.1. Farm size

Regional difference in the proportion of large farms might give rise to regional differences in pathogen prevalences in livestock manures. As described below, there is a general association between larger farms and increased prevalence of pathogens among herds (herds with at least one positive sample) and within herds (prevalence from sample to sample). However, this association is not universal and studies reporting farm-size related differences cannot directly identify the mechanisms by which larger farms are prone to higher pathogen prevalences.

Cattle

An association between large dairy herd sizes and *Salmonella* prevalence in feces has been observed in numerous studies (Kabagambe et al., 2000; Wells et al., 2001; Huston et al., 2002; Warnick et al., 2003; Cummings et al., 2009). Although the mechanism(s) underlying the association of *Salmonella* infection with large herd sizes is unclear, it may relate to intensive management practices, introduction of more cows, and stress due to crowding, transportation, and animal mixing (Huston et al., 2002). Huston and colleagues studied dairy cattle from 105 Ohio dairy farms and found that the odds of a herd being infected by *Salmonella* increased by about 5% per each 25 additional cows on the farm. In a large study of *Salmonella* at dairy operations, Wells et al. (2001) observed prevalence of *Salmonella* in fresh feces for farms with fewer than 100 head to be 0.7% during late winter (February to April) and 0% during early summer (May to July). For farms with more than 100 head, the prevalences were 3.3% and 14.0%, respectively. Fossler and colleagues conducted the largest study of *Salmonella* shedding in dairy cows (Fossler et al., 2005a) and the only one evaluating herd level characteristics. *Salmonella* shedding was more likely on farms with at least 100 cows. A single study (Fossler et al., 2005b) was identified that did not report an association between dairy herd size and prevalence of *Salmonella* in feces. We find that the weight of evidence points toward increasing prevalence of *Salmonella* shedding with increased cattle herd size for both dairy and beef operations.

Several studies associating cattle herd sizes with *Campylobacter* shedding at both the herd and animal level were identified. Hoar et al. (2001) found an association between herd size, measured as number of females on the farm, and *Campylobacter* fecal shedding prevalence on beef cattle farms. Ellis-Iversen et al. (2009) observed that larger cattle herd size was associated with increased fecal shedding of *Campylobacter* on dairy and beef cattle operations in England and Wales. Sato et al. (2004) found that *Campylobacter* prevalence was significantly higher in smaller farms than in large farms. Wesley et al. (2000) could not associate increased dairy farm *Campylobacter* shedding prevalence with herd size at the herd level, but did find that increased fecal shedding at the animal level was associated with larger farms. Together, these studies point to increased *Campylobacter* shedding prevalence with increasing farm size.

Two large-scale studies of *E. coli* O157 occurrence among the largest U.S. dairy operations found that prevalence was related to farm/herd size. One study (USDA, 2003) found that large operations (>500 cows) were more likely to have positive *E. coli* O157 samples than medium operations (100 to 499 cows) or small operations (<100 cows). Another similar study (USDA, 1998) found that prevalence was higher for herds with 100 or more cows (39.1% of herds had at

least one positive sample) than for herds with fewer cows (8.9 % of herds had at least one positive sample). When comparing prevalence of Shiga toxin producing *E. coli* (STEC) at conventional and organic dairy farms, Cho et al. (2006) found significantly higher prevalence in organic farms when data from all farms were combined, but found no difference in prevalence between organic and conventional farms when only farms with 100 or fewer head were included in the analysis. Ellis-Iversen et al. (2009) associated increased cattle *E. coli* O157 prevalence with farm size.

Hoar et al. (2001) did not find an association between herd size (measured as number of females on the farm) and *Giardia* fecal shedding prevalence on beef cattle farms. However, in a nationwide study of dairy calves, the USDA (USDA, 1993) found that almost all dairy farms with over 100 milks cows tested positive for *Cryptosporidium*, while farms with <100 cows had slightly lower prevalence (~80%).

Swine

A single study was identified assessing the influence of swine herd size on shedding prevalence. Hurd et al. (2002) conducted a longitudinal study of pigs before and after transport to abattoirs (slaughter houses) to determine the role transportation and housing conditions at the abattoir play on acquisition of *Salmonella* infections among pigs. All farms selected for the study were small to moderate sized operations (approximately 193 sows) located in Midwestern states. Although linear regression of infection rates demonstrated no effect of herd size on infection rates, the statistical power of this finding was low because of small sample size.

Chicken

Ebel et al. (1992) describe a general trend of greater SE prevalence at larger farms, citing differences in prevalence among Canadian and northern U.S. farms as evidence of this trend. In a nationwide study of layers (Garber et al., 2003), approximately 4% of houses with fewer than 100,000 layers were environmentally positive for SE, whereas 16.5% of houses with 100,000 or more layers were environmentally positive for SE. Young et al. (2009) summarized the prevalence of zoonotic and potentially zoonotic bacteria in organic and conventional chicken using systematic review and meta-analysis methodology. Those results indicate that the prevalence of *Salmonella* spp. was higher in conventional laying hen flocks than in organic flocks. This finding could however be confounded by larger flock sizes.

A limited number of studies (Berndtson et al., 1996; Newell and Fearnley, 2003) indicate that chicken *Campylobacter* prevalence increases with farm size. Mechanisms responsible for this finding may be greater numbers of animals feeding and watering from common sources.

4.1.2. Operation type

Operation types differ from each other with respect to typical herd/flock sizes, animal densities, feed, hygienic conditions, manure handling, and other features. As shown in Section 2, the geographic distributions of different types of operations are uneven in the United States. In particular, feedlot cattle are far more prevalent in the Midwest states than in other regions. The

following section compares operations where different types of animals are kept, different age classes of animals are kept, or animals are housed and/or fed in a specific manner.

Cattle

Cattle operations include beef cattle calving operations, dairy cattle calving operations, dairy operations, beef cattle fed on pasture, and beef cattle in feedlots. There are also differences between organic and conventional operations. With respect to shedding prevalence of pathogens, the primary differences in these operations are the following:

- the presence or absence of calves;
- the feed provided to cattle;
- whether cattle are on feedlots (in pens), on pasture, or in irrigated pasture;
- manure handling practices;
- use of antibiotics; and
- animal density.

In a study of the impact of transport on shedding of *Salmonella* and *Campylobacter*, Beach et al. (2002) sampled rectums of feedlot cattle and adult pasture cattle before and after transport to facilities for slaughter. There were marked differences in shedding prevalence among feedlot and pasture cattle: 64% (n = 100) of feedlot samples and 6.3% (n = 96) of pasture cattle samples were positive for *Campylobacter*. Similarly, Riley et al. (2008) determined that feedlot steers had greater odds of *Campylobacter* detection (odds ratio 8.5; 95% confidence interval [3.7, 19.5]).

Midwestern states were more likely to have cattle shedding *Salmonella* than cattle from New York. Cattle that had been treated with antibiotics within 14 days were less likely to be shedding *Salmonella* (Fossler et al., 2005a,b,c; Wilhelm et al., 2009). In the United States, there was a significantly higher ($p = 0.0001$) seroprevalence of *Salmonella* from anti-microbial free herds (54%) than from conventional indoor reared herds (39%), although there were some geographical variations in *Salmonella*. Wisconsin had the highest prevalence at 59%, followed by North Carolina at 34%, and Ohio at 34% (Gebreyes et al., 2008).

Bae et al. (2005) studied the prevalence of *Campylobacter* in cattle at different farm types. Prevalence of *C. jejuni* and *C. coli* excretion differed by farm type. The highest *C. jejuni* prevalence was observed at beef cow-calf operations (47.1%) and the lowest at calf rearing operations (23.8%). The highest *C. coli* prevalence was at calf rearing operations (20.0%) and the lowest was at beef calf-cow operations (0.6%). Comparing feedlot and pasture beef cattle, Krueger et al. (Krueger et al., 2008) found higher *Campylobacter* shedding prevalence among pasture cattle than cattle fed concentrate. Among cattle shedding *Campylobacter*, abundance in feces were a factor of 10× less for animals fed concentrate than pasture-fed animals. Riley et al. (2008) determined shedding prevalences of steer fed on pasture or in feedlots. Feedlot cattle had greater odds (odds ratio 8.5; confidence interval [3.7, 19.5]) of shedding *Campylobacter* than steers grazing wheat. In a study that evaluated conventional and organic dairy herds, the prevalence of *Campylobacter spp*. in organic and conventional farms was 26.7% and 29.1%, and the prevalence was not statistically different between the two types of farms (Sato et al., 2004).

Different feedlot operations use different feed mixes. In a large U.S. study of farm factors affecting the prevalence of *E. coli* O157 on beef cattle feedlots (USDA, 1997), samples from cattle pens receiving barley in the ration were 2.75× more likely to be positive for *E. coli* O157 than samples from cattle pens receiving no barley. The prevalence of *E. coli* O157:H7 was reported as 14.8% for organically raised cattle, 14.2% for naturally raised[1] cattle, and 11.2% for conventionally raised cattle (Reinstein et al., 2009). There were no statistically significant differences in herd-level prevalence of STEC in the United States but researchers found greater individual prevalence on organic dairy farms (Cho et al., 2006). No differences in STEC prevalence were found between organic and conventional farms in Switzerland (Kuhnert et al., 2005) or the Netherlands (Franz et al., 2007).

Swine

Davies et al. (1997) compared *Salmonella* prevalence in swine operation feces for multiple-site production (different phases of production raised on separate sites) and all-in-all-out (AIAO) management (all animals are removed from a location before introducing a new group) of both nursery and finisher phases of production to prevalence in traditional operations at which multiple age groups were on the farm simultaneously. Contrary to expectations, Davies and colleagues found that *Salmonella* prevalence was not lower in multiple-site production systems using all-in-all-out management of finishing pigs compared with conventional farrow-to-finish systems. As with many other studies, very high variability in both the *Salmonella* prevalence and serotype prevalence among positive samples was noted. Conflicting results were reported in studies that examined the prevalence of *Salmonella* spp. in swine on farms and at slaughter in the United States, Denmark and Germany. Studies conducted in the United States showed higher *Salmonella* prevalence in organic farms (Gebreyes et al., 2008) whereas international studies showed contrary results.

Chicken

Campylobacter in chicken has received relatively more attention on the topic of organic vs. conventional farming practices than most of the other animal/pathogen combinations. Heuer et al. (2001) evaluated three rearing systems (organic, conventional, and extensive indoor) and reported that *Campylobacter* spp. were isolated from 100% of organic broiler flocks, 36.7% of conventional broiler flocks, and 49.2% of extensive indoor broiler flocks. The proportion of *Campylobacter* positive flocks was significantly higher for organic flocks compared with the others. Furthermore, they found that no single factor related to organic broiler production can be pointed out as the sole determinant of high *Campylobacter* prevalence. Rather, the prevalence results reported reflect the combined effect exerted by factors that are inextricably related to each broiler rearing system.

The above results are similar to those reported by (1) Luangtongkum et al. (2006) who indicate that the prevalence of *Campylobacter* on conventional broiler farms was slightly lower (44 to 80%) than organic farms (70 to 100%) and the prevalence on conventional turkey farms was

[1] The study does not explicitly define "naturally raised cattle." In general, natural rearing programs disallow use of antimicrobials, ionophores, and hormones, which necessitates special animal management, including handling of sick animals (SDES, 2007).

similar (63-98%) to organic farms (6-100%); (2) Young et al. (2009) who report in a review article that the prevalence of *Campylobacter* was higher in organic broiler chickens than those raised conventionally; and (3) Van Overbeke (2006) who found that *Campylobacter* infections were significantly higher in organic flocks (however, organic flocks were slaughtered at 12 weeks, compared to 6 weeks for conventional flocks).

4.1.3. Longitudinal (life stage) studies

For each animal-pathogen combination, there may be variation in the within-herd prevalence and abundance of the pathogen in feces as animals age, are moved between facilities, are provided different diets, or are in contact with other animals of the same or different species. Numerous studies have explored longitudinal variation in infection rates from birth to slaughter and, as described below, noted consistent trends in pathogen shedding prevalence consistent with age groups. In this section, studies that have identified trends in shedding prevalence for specific livestock host-pathogen combinations are summarized.

<u>Cattle</u>

Besser et al. (2005) conducted a longitudinal study of *Campylobacter jejuni* prevalence in fresh feces at U.S. cattle feedlots. In that study, prevalence of *C. jejuni* increased markedly from cattle arrival. In samples from pens with newly-arrived cattle *C. jejuni* was detected in 1.6% of fecal samples. In samples from pens with animals within two weeks of slaughter prevalence was 62.2%. *Campylobacter* appears to be persistent in at least a portion of feedlot cattle (Inglis et al., 2004). Among those cattle, species prevalence of *Campylobacter* varied between lots and from season to season. In their 4-month controlled study of *Campylobacter* in feedlot cattle, Inglis et al. (2004) observed *C. lanienae* to be the most prevalent species, followed by *C. jejuni*. No *C. coli* were detected. In a study comparing conventional and organic dairy herds in Wisconsin, Sato et al. (2004) found that *Campylobacter* prevalence was significantly higher in calves than in cows.

E. coli O157:H7 appears to differ between calves and adult cattle and between cattle before and after their arrival on feedlots. *E. coli* O157:H7 infection peaks in young cattle between 3 and 18 months of age, and declines thereafter (Ellis-Iversen et al., 2009a). In a large study of feedlot beef cattle, LeJeune et al. (2004) observed a general trend of increasing prevalence of *E. coli* O157:H7 among animals with their duration in the feedlot. However, during periods of highest *E. coli* O157:H7 detection, prevalence in individual pens was sporadic. A reduced risk for the presence of STEC was found for older than younger cows (Kuhnert et al., 2005).

Calves (<6 months) and particularly young calves (<2 months) are especially prone to *Cryptosporidium* infection and high rates of fecal shedding. Wade et al. (2000) found *Cryptosporidium* shedding prevalence was strongly dependent on cattle age, with much higher *C. parvum* prevalence among calves than older cattle (Table 12). Age-related differences in *Cryptosporidium* prevalence were confirmed in a study of runoff water from cattle operations. Miller et al. (2008) measured *Cryptosporidium* densities in runoff from various locations on dairy farms and estimated *Cryptosporidium* loading from areas housing cattle in different age classes. Runoff from areas housing calves <2 months of age had much higher runoff water

Table 12. *Cryptosporidium* shedding prevalence among cattle of different age groups (SOURCE: Wade et al., 2000)

Parasite	Prevalence (%)			
	All ages	<6 months	6-24 months	>24 months
C. parvum	0.9	2.4	0	0
C. muris	1.1	0.5	1.7	1.5

density of oocysts and overall loading than that from areas housing cattle 3 to 6 months of age. Runoff from areas housing dry, milking, and calving cows was even lower than from areas housing calves 3 to6 months of age.

Swine

Dorr et al. (2009) observed significant increases of *Salmonella* prevalence in pig feces with age. At late nursery age, prevalence of fecal shedding of Salmonellae ranged from 5.0 to 21.7%, whereas at slaughter infection rate varied from 25 to 63%. The authors note that other studies have not reported monotonically-increasing shedding with age and suggest that environmental contamination, particularly during transport, may account for increasing prevalence with age.

Alter et al. (2005) evaluated how *Campylobacter* varied over rearing time and found that no *Campylobacter* were detectable in the feces of piglets at the day of birth. The *Campylobacter* incidence rose within days to 32.8%. After transfer to the nursery unit the prevalence increased to 56.6%. Approximately two-thirds of the pigs remained *C. coli* shedders in the fattening unit. The detection rate before transportation was 79.1%.

Chicken

Stern et al. (2001) studied 32 flocks belonging to 4 major U.S. chicken producers. Low flock- and sample-level prevalences of *Campylobacter* were observed for young flocks and high flock- and sample-level prevalences were observed for flocks pre-slaughter. In most infected flocks, increasing prevalence among samples in successive sampling events indicated rapid spread of infection among the chickens. For many infected flocks 100% of fecal samples were positive for *Campylobacter* within a short period after the initiation of infection.

In a study of *Campylobacter* changes with broiler chicken age, Bull et al. (2006) examined fecal droppings from 10 houses in the U.K. over a production cycle for meat chickens.

Flock-level prevalence of *Campylobacter* increased from 10% (n = 10) at 18 days to 40% between 28 and 33 days and to 60% at depletion. Once colonized with *Campylobacter*, flocks' fecal droppings tended to have consistent and high densities of *Campylobacter*. Five flocks were colonized by *C. jejuni* exclusively, one with *C. coli* exclusively, and one with both species. Similar increases in flock-level prevalence of *Campylobacter* in broiler chickens were also

observed by Arsenault et al. (2007) in a study of broiler houses in Quebec, Canada, and by Gregory et al. (1997) in a study of broiler flocks in northeastern Georgia. Other investigators have reported that the risk of flock infection increased with the age of the broilers (Berndtson et al., 1996; Evans and Sayers, 2000). Newell and Fearnley (2003) found that the prevalence of flock positivity is dependent on flock size and the type of production system. Flock positivity is generally higher in organic and free-range flocks compared to intensively reared flocks presumably due to the level of environmental exposure as well as the increased age of the birds at slaughter.

4.1.4. Seasonality

Seasonal effects include seasonal life-cycle effects (e.g., calving periods), seasonal temperature fluctuations (with related fluctuations in survival of microorganisms and changes in microbial ecology), and changes in precipitation (particularly in climates with distinct dry and wet seasons). As described below, seasonal variations in occurrence are frequently associated with the lifecycles of the livestock. Occurrence of pathogens may differ among different livestock age cohorts (e.g., high *Cryptosporidium* shedding among calves) or may have a trend with animal age (e.g., increasing prevalence of *Campylobacter* with chicken age). These differences are not intrinsically regional—a farm in the northern United States experiences the same life-cycle-driven seasonality as a farm in the South. Other seasonal factors are less important than life-cycle seasonality, but could cause regional differences in pathogen shedding. Among these, the seasonal changes most directly related to potential regional difference in pathogen occurrence are temperature fluctuations.

In the reviews of studies addressing seasonality below, attempts were made to ascertain the underlying cause (temperature, precipitation, life-cycle, etc.) of the changes in prevalence, though in many cases researchers did not explore or could not determine the underlying causes.

<u>Cattle</u>

Several studies report seasonal variations in *Salmonella* prevalence within cattle herds, with fecal shedding higher in the summer months than winter and early spring (Wells et al., 2001; Edrington et al., 2004; Fossler et al., 2005b). Edrington et al. (2004) also noted that the most prevalent serotypes of *Salmonella* changed with season, though the changes may have been related to epidemiological factors rather than consistent seasonal differences. In contrast to these studies, Huston et al. (2002) did not find a seasonal pattern in shedding rates among cattle on a large number of Ohio dairy farms, and Kunze et al. (2008) did not find seasonal variations in *Salmonella* prevalence in feces from feedlots in the U.S. southern high plains.

Prevalence of *E. coli* O157 in cattle appears to be seasonal. In two large studies of *E. coli* O157 shedding in dairy operations, the USDA found a higher prevalence of *E. coli* O157 in the summer months (USDA, 1998, 2003). Another large USDA study of beef cattle feedlots found the highest prevalence of *E. coli* O157 during September (USDA, 2001). Edrington et al. (2004) observed a generally high prevalence of *E. coli* O157:H7 shedding among dairy cows on large commercial dairy operations in the southwest United States as well as seasonality in prevalence of shedding. In that study of relatively large farms, no positive samples for *E. coli* O157:H7

were collected during winter sampling events. Summer *E. coli* O157:H7 was higher than winter prevalence, but highly variable. Similarly, in a 26-month study of feedlot cattle, Berry et al. (2007) observed higher summer and fall (22.7% in fall) prevalence of *E. coli* O157:H7 in manure samples from feedlot pens than in winter samples (9.7% of samples), while Chapman et al. (1997) observed higher spring and summer *E. coli* O157:H7 prevalences than winter prevalences for U.K. cattle herds.

Miller et al. (2008) noted seasonality in *Cryptosporidium* oocyst runoff from coastal California dairy operations, which was attributed to rainfall patterns. Oocyst runoff was high during the wet season and was highest early in the wet season and during early portions of storms. In these conditions, fecal matter is easily mobilized and density of oocysts in fecal material is relatively high. Trotz-Williams et al. (2007) observed higher fecal shedding prevalences of *C. parvum* for calves born and raised in summer months than in winter. A nationwide USDA study (USDA, 1993) found prevalence of *Cryptosporidium* in dairy calves to be higher during the summer months than in other months. In a U.K. study of cattle, Hutchison et al. (2005) observed significantly higher *Cryptosporidium* prevalence in June and December than in other months, though overall seasonal trends were not observed. In contrast, Wade et al. (2000) did not find seasonality in *Cryptosporidium* oocyst shedding from dairy cattle, but did find a distinct pattern of decreasing *C. parvum* shedding prevalence with increasing animal age.

Sato et al. (2004) observed significantly higher cattle prevalence of *Campylobacter* shedding in March than September. This observation was consistent with Wesley et al. (2000) who noted a seasonal trend in dairy cattle shedding of *Campylobacter*. In their review of both dairy and beef cattle studies, Stanley and Jones (2003) note that in both the northern and southern hemispheres there is a bimodal, seasonal trend in *Campylobacter* shedding prevalence among cattle, with northern hemisphere peaks in the spring and autumn and southern hemisphere peaks in prevalence during climatic periods similar to the summer and autumn. Although these observations point toward a likely climatic component to the seasonality, no clear explanation of the role of temperature or climate was offered.

Chicken

Campylobacter prevalence in chickens appears to be seasonal (Stern et al., 2001; Newell and Fearnley, 2003), with summer rates generally higher than winter rates, and with peak rates varying with latitude. *Campylobacter* prevalence in chicken (layer) feces was observed to vary significantly with season (Doyle, 1984). In a 42-week study, the authors observed two peak periods of high prevalence in layer feces—September/October and April/May. During low prevalence months, prevalence was found to be generally less than 8% of fecal samples, whereas in high prevalence months, rate of infection was as high as 25%. However, the authors could not relate the seasonal variation in *Campylobacter* prevalence to any particular factor or phenomenon. Newell and Fearnley (2003) suggest use of ventilation during summer months as a possible cause of higher summertime infection rates. Studies by Berndtson et al. (1996) and Evans and Sayers (200) found that if a broiler flock is *Campylobacter* infected, a large proportion of the birds within the flock is infected; and that there is a seasonal variation in the prevalence of *Campylobacter* positive broiler flocks (i.e., significantly higher in the period from May to October than the period from November to April)

4.2. Farm Factors without Regional Implications

Farm features and practices beyond those listed in Section 4.1 are important determinants of pathogen prevalences, but their use cannot be related to regions or states. The authors of the studies we reviewed have attempted to relate many of these non-regional farm factors to manure pathogen prevalence. Note that measures for reducing the carriage of pathogens in livestock are different from those designed to limit the transport of pathogens occurring in livestock to surface waters (Sobsey et al., 2006).

Farm practices may be modified to reduce prevalence of pathogens in livestock. Although these practices cannot be related to spatial distribution of pathogens (no data were found on the prevalence of farm practices in the United States), farm practices and their relationship with pathogen prevalence are reviewed below. This section provides a survey of the relevant literature but is not exhaustive. Doyle and Erickson (2006) and Sobsey et al. (2006) suggest the following farm practices for reducing the carriage of pathogens in livestock:

- mono-species farms;
- genetic selection of animals resistant to colonization;
- breeding treatments (e.g., antibiotic treatment of semen, antimicrobial egg dips);
- sanitation and hygiene for farm and transportation environments;
- choice of bedding material;
- maintenance of dry litter;
- housing design;
- elimination of pathogens from water;
- elimination of pathogens from feed;
- feed withdrawal (prior to shipping and during molting);
- animal diet modifications;
- feed and water additives;
- vaccination; and
- prophylactic antibiotic treatment.

A relatively small list of these types of farming practices is reviewed in this report. The following factors were selected because they are considered the most important and because sufficient data were found in the literature to adequately comment on their association with manure pathogen prevalence:

- watering practices (type of watering, use of disinfected water)
- practice of mixed farming; and
- use of BMPs for manure management.

Among these factors, manure management is clearly the most important. However, a comprehensive review of the relationship between manure management options and pathogen occurrence is beyond the scope of this report, which primarily deals with the occurrence of key pathogens in livestock waste—not the treatment, fate, and transport of these zoonotic pathogens.

Nonetheless, this report provides reviews of several studies illustrating the relationship between manure management BMPs and pathogen loading of receiving waters.

4.2.1. Water disinfection and hygiene

Water source and use of disinfected water do not provide consistent reductions in pathogen prevalence. Besser et al. (2005) did not observe a significant difference in feedlot cattle *Campylobacter jejuni* prevalence among cattle provided chlorinated water and cattle provided unchlorinated water. Wesley et al. (2000) determined that chlorination of drinking water was not a protective factor for *Campylobacter jejuni* infection in dairy herds. However, Ellis-Iversen et al. (2009) found an association between emptying water troughs at cattle operations and prevalence of *Campylobacter* excretion. Note that this study did not explore the benefits of disinfection.

Arsenault et al. (2007) did not observe a difference in prevalence in *Salmonella* infection between chicken flocks using disinfected and undisinfected water, while LeJeune et al. (2004) did not find a difference in fecal prevalence of *E. coli* O157:H7 between cattle provided chlorinated water in their troughs and those provided unchlorinated water.

The manner in which water is offered to animals also appears to play a role in the prevalence of pathogens in the animals. Bahnson et al. (2006) observed that pigs from herds with at least some bowl drinkers had 8× the odds of testing positive for *Salmonella* than did pigs from herds with only nipple drinkers. Berndtson et al. (1996) did not find an association between chicken drinking facilities (bell, cup, or trough) and *Campylobacter* prevalence on Swedish broiler chicken operations. Moreover, Newell and Fearnley (2003) indicate that the water source is a low-risk factor for *Campylobacter* infection and water contamination usually follows rather than precedes colonization of a flock suggesting that this is a consequence of the tracking up through the water lines of organisms excreted from the birds.

Together, these studies show that drinking water is just one of many exposure routes for livestock to acquire infections and the reduction in transmission of infectious agents via drinking water might not produce a measurable reduction in prevalence of infection among herds and flocks.

4.2.2. Mixed production

Mixed production provides a route for infection of one livestock species by another, either via direct contact, aerosol transmission, transmission on equipment, boots, and other materials used across operations, and possibly other routes. Mixed production may also entail use of practices different from those used on farms where single species are raised. Despite these potential transmission routes, the studies on mixed production did not demonstrate higher pathogen prevalences on mixed production farms than on farms with single species and, in fact, one study reported lower pathogen prevalence on a mixed production farm than on comparable operations with single species.

Boes et al. (2005) did not find a significant herd-level or animal level difference in prevalence of *Campylobacter jejuni* or *C. coli* in swine herds that were on farms with or without mixed production (commercial cattle or chicken production). Further, the ratio of species of *Campylobacter* present in swine herds (high proportion of *C. coli*) was consistent across herds with and without mixed production. This finding, along with high prevalence of *C. jejuni* infection rates in both chickens and cattle, led the authors to suggest transmission among mixed livestock populations is not an important factor in swine infection.

Wright et al. (2008) studied *Campylobacter* prevalence, overall and by species, in turkey and swine farms in eastern North Carolina. Although high *Campylobacter* prevalence was observed among swine and turkey populations, the prevalence of *C. coli* (by far the most prevalent *Campylobacter* species among the swine herds studied) in swine was not a good predictor of *Campylobacter* infection prevalence among turkeys on the same farm. Further, adult turkeys were far more likely to be colonized by *C. jejuni* than *C. coli*. These findings led the authors to hypothesize that even though turkeys and swine grown in proximity to each other may both be colonized by thermophilic campylobacters, the *C. jejuni* and *C. coli* in the animals are likely host-associated. Arsenault et al. (2007) indicate that risk factors for *Campylobacter* spp. include vertical transmission; contamination from previous flock; and exposure to potential sources of the bacterium, such as other animals on the farm, insects, rodents, environment, litter, and drinking water. Odds of *Salmonella* colonization were 2.6× greater for chicken flocks without permanently locked houses.

Ellis-Iversen et al. (2009) observed lower prevalence of *E. coli* O157:H7 infection among cattle on farms where chicken were also present. They speculated that this lower prevalence among cattle may be related to the adoption of practices for chicken rearing that had a mutually beneficial effect on cattle health.

4.2.3. BMPs and manure management

Manure management (the choice of manure treatment, storage, and ultimate disposition) is a critically important farm factor, both because it determines the pathogen loads ultimately reaching waters used for recreation, and because it is a class of activities over which farmers can maintain control. While it is outside the scope of this report to provide detailed quantitative BMP and manure treatment system performance data, a brief review is provided of the role of manure management in determining the risks of livestock fecal pollution as well as a list of commonly used manure management techniques with their anticipated ranges in pathogen reduction. The data and descriptions presented below may be used as a starting point for collection of data on pathogen reduction in treatment and BMPs or for developing or refining a QMRA framework for risks from livestock pathogens. Manure management and use of BMPs for attenuation of pathogens in runoff to receiving waters do not relate to regional differences in pathogen loads to streams. Although manure management and installation of BMPs are expected to have profound effects on manure loads to receiving waters, agriculture decision making usually takes place at the farm level (Garcia et al., 2008) and is unrelated to differences among regions of the United States.

A model showing pathways by which zoonotic pathogens may reach either surface waters or groundwater is presented in Figure 6 (Goss and Richards, 2008). The sources may be in barns, yards, or grazed fields. As described above, the prevalence and abundance of shedding depends on the animal and farm features. Pathogens shed in barns, yards, and grazed fields may be transported directly to receiving waters via rainwater, may be given some degree of treatment, and/or may be applied to arable fields. Manure management determines the proportion of pathogens present in manure that reaches streams and groundwater. The presence of tile drains can dramatically change the connection between farm and receiving water and result in high indicator and pathogen loads to receiving waters even for relatively small operations (USEPA, 2005).

Manure management systems are primarily designed to match nutrient content of wastes to nutrient needs of crops (Eghball and Power, 1994; Van Horn et al., 1994; Moore et al., 1995; Garcia et al., 2008). Nonetheless, substantial changes occur in the pathogen and fecal indicator densities of wastes during storage and treatment. Wastes may be added intermittently to treatment processes (e.g., compost heaps or lagoons), resulting in an accumulation of wastes with a distribution of residence times, indicator levels, and pathogen levels.

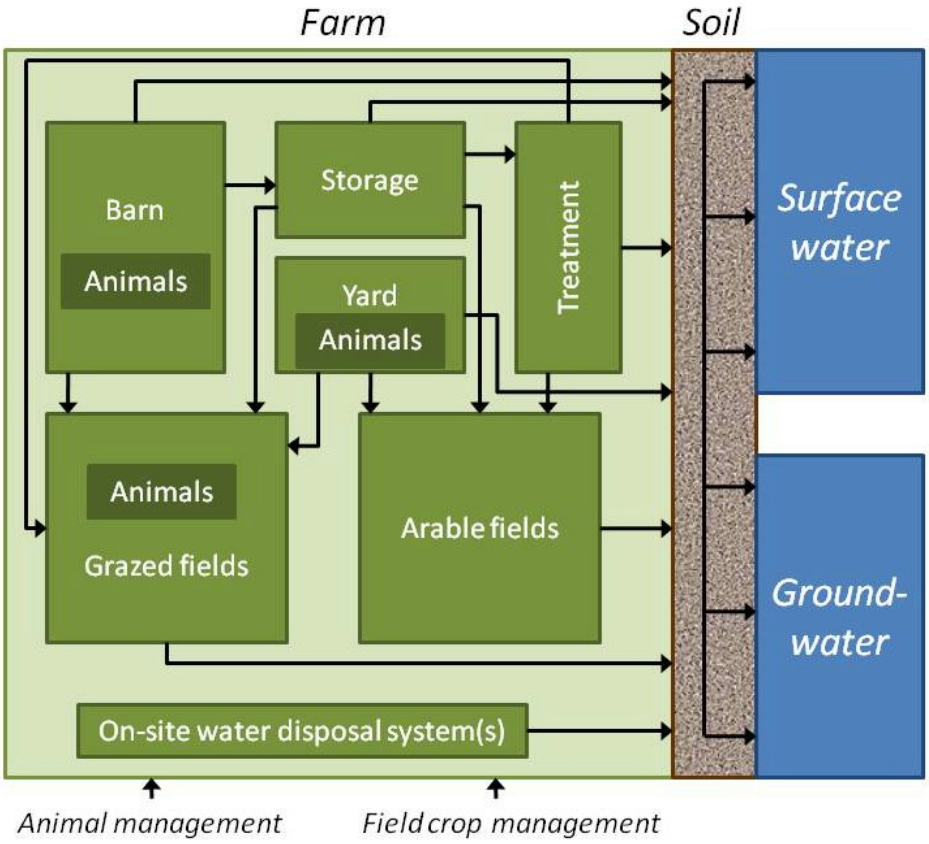

Figure 6. Farm sources of zoonotic pathogens and pathways to receiving waters (SOURCE: adapted from Goss and Richards, 2008)

Overview of manure treatment systems

Bicudo and Goyal (2003) reviewed studies of manure management systems and divided the manure management process into components that determine the number of microorganisms in manures (e.g., choice of feed) and components relating to the ultimate discharge of those microorganisms into surface or subsurface waters. The processes and farm factors they described differ from the others listed in Section 4. Specifically, the other factors in Section 4 relate to the occurrence of pathogens in the fresh manures of livestock whereas the factors and processes explored by Bicudo and Goyal (2003) relate to the pathogen loads in manures intended for land application or other processes (after some level of treatment). The pathogen and indicator reductions for the options they identified and assessed (Table 13) are variable and differ between implementations at different farms and with season and other factors at a given farm. In general, high, rapid reductions in microorganisms have attendant high-energy requirements or use of chemicals that may be expensive or whose handling may be difficult. The adoption of manure management strategies is likely related to the labor and capital costs associated with the processes. Manure management strategies may be classified as passive or active on the basis of the operational requirements of the system (USEPA, 2005). Lagoons, storage prior to disposal,

Table 13. Effect of manure management options on the number of microorganisms contained in manure (SOURCE: Bicudo and Goyal, 2003)

Type of Option	Management Option	Effect	Notes
Animal housing and feed	Diet changes	Reduction of acid-resistant *E. coli*	Hay diet seems to have a significant effect on the reduction in *E. coli* numbers in cattle manure
		Reduction in *Salmonella* numbers	Conflicting results from swine nutrition experiments
	Production systems	Reduction in *Salmonella* numbers in swine manure	Prevalence of *Salmonella* was lower on slotted floors compared with all other floor types
Physical treatment	Vegetative filter strips	Reduction of bacterial indicator and *Cryptosporidium* oocyst numbers	Wide variations; reductions from 20 to 90% have been reported
	Thermal treatment	Reduction of viruses	Temperatures between 60°C and 70°C appear to be high enough to inactivate several viruses in pig slurry
	Electrolytic treatment	Reduction of bacterial indicators	Energy costs may be a concern, as the system consumes 26 kW per m^3 of pig slurry
Chemical treatment	Chemical addition	Reduction of bacterial indicators	Does not attract much interest because of high concentrations needed and toxic nature of chemicals
	Ozonation	Reduction of bacterial indicators	High doses (up to 3 g/L) required to achieve a 99.9% reduction in *E. coli*
Biological treatment	Anaerobic lagoon	Reduction of bacterial and viral indicators	Reduction of enteric microbes and coliphages varies between 90 and 99%
	Anaerobic digester	Reduction of bacteria and viruses	Rapid inactivation of pathogens is achieved under thermophilic conditions
	Aeration	Reduction of bacteria and viruses	Temperature and time of treatment play a significant role
	Thermophilic aerobic stabilization	Reduction of bacteria and protozoa	Most microorganisms are inactivated within 24 hours when the temperature reaches 50°C
	Aerobic sequencing batch reactor	Reduction of bacterial indicators	Reductions between 90 and 99.9% can be achieved with treatment times ranging from 5 to 10 days

vegetated buffer strips, constructed wetlands, separation of different ages of animals, and land application are examples of passive systems and composting, anaerobic digesters, aerobic digesters, and actively operated lagoons are examples of active systems.

Goss and Richards (2008) provide general ranges of bacteria reductions that may be expected in manure treatment processes and the stresses (inactivation or removal mechanism) for each of the processes (Table 14). The ranges proposed by Goss and Richards are not directly comparable to those presented by Bicudo and Goyal (2003) because the classification of treatment processes differs for the two studies. Further, given the between- and within-operation variabilities in the treatment processes, the ranges presented in those two studies are expert judgments and, in the case of Goss and Richards' estimates, are not specific to classes of pathogens. Table 15 (from Sobsey et al., 2006) summarizes typical viral pathogen reductions for various physical, chemical, and biological treatment processes. Though we are not considering manure-borne viruses to be as significant a risk as bacterial and protozoan pathogens in this report, these data provide a

Table 14. Typical reductions of pathogens during manure treatment processes (SOURCE: Goss and Richards, 2008)

Treatment		Log Reduction	Stress
Lagoon		1–3	Time
Constructed wetland		2–3	Time, filtration
Deep stack (composting)		1–?	NH_3, heat
Digestion	mesophilic	1–2	Time, heat
	thermophilic	5	
Composting		1–5	Heat, time
Air drying		1–2	Desiccation
Heat drying		4–5	Heat, desiccation
Pasteurization		5	Time, heat
Alkaline processes		3–5	Heat, NH_3

Table 15. Typical reductions of viruses during animal waste treatment processes (SOURCE: Sobsey et al., 2006)

Treatment Process	Estimated Virus Reduction (log_{10})
Physical processes	
Heat/thermal processes	
Mesophilic	Typically 1–2
Thermophilic	Typically >4
Freezing	Variable; depends on temperature, type of waste and pathogen
Drying or desiccation	Typically >4 at <1% moisture; typically <1 at >5% moisture
Gamma irradiation	Typically >3
Chemical processes	
High pH (> 11)	Inactivation at high pH (e.g., alkaline/lime stabilization; typically >3)
Low pH (< 2 to < 5)	Inactivation at low pH; acidification; typically <2
Ammonia	Inactivation at higher pH (> 8.5) where NH_3 predominates
Biological processes	
Aerobic, mesophilic	Typically 1–2
Aerobic, thermophilic (composting)	Typically >4
Silage treatment (mesophilic)	Variable
Land application	Highly variable and largely unknown; potentially high

means for evaluating that assumption and are of relevance to other sources of fecal pollution including land-applied biosolids.

Composting is a relatively simple and flexible means of manure treatment and reduction of pathogen densities in solid manures, manures mixed with bedding or other materials, or litter. Composting may be conducted in heaps or reactors (Heinonen-Tanski et al., 2006) and the degree of pathogen inactivation depends upon the temperature maintained in the compost and the availability of oxygen in the heap. Heinonen-Tanski and colleagues report large variations in measured reductions of indicator bacteria in composted manures for composting conducted over different durations and with different techniques. Larney and Hao (2007) compared straw-bedded and wood-bedded windrow composting of feedlot cattle manures. Both types of compost performed similarly and produced declines in *E. coli* of >99.95% during the first 7 days of composting, despite relatively low temperatures in the compost (34 to 42°C). High inactivation despite low temperatures lead Larney and Hao to conclude that desiccation played a significant role in *E. coli* reduction.

Based on a review of the literature, Larney and Hao noted that *Giardia* and *Cryptosporidium* reductions in composts are more gradual than those observed for *E. coli* and also more dependent on achieving a high temperature in the compost heap. The authors suggest that composting for 15 days at temperatures > 55°C is adequate for inactivation of *Giardia* and *Cryptosporidium* in feedlot wastes. Shepherd et al. (2007) explored within-stack differences in inactivation of non *stx* E. coli O157:H7 inoculated into dairy manure composts comprised of dairy manure, feed waste, sawdust, calf feces and fresh hay. Heaps were kept on a concrete slab during the experiments and were not turned. Commensal *E. coli* populations in the heaps declined ~4.6 to 4.9 logs during the first 3 days of composting and were not detectable via direct plating after seven days, though they were detectable via enrichment for up to 14 days. A 6-log reduction in *E. coli* O157:H7 was observed in the center and at the top of the stack after 3 days and a 4-log reduction was observed at the bottom of the stack. After the third day surface samples from the heap had consistently higher *E. coli* O157:H7 abundance than samples from elsewhere in the stack. Inactivation of *E. coli* O157:H7 and commensal *E. coli* were correlated.

The following generalizations may be drawn concerning composting:

- Removal of pathogens and indicators is highly variable and dependent on
    - duration,
    - temperature achieved in the heap, and
    - mixing of the heap.
- Composting heaps may be comprised of different materials. The make-up of the compost heap may be less important than management of the heap to maintain high temperature and mixing.
- Inactivation is variable within heaps, with the highest temperature and removal achieved at the heap interior.
- Vegetative bacterial removals in composting are similar and greater than those of *Giardia* and *Cryptosporidium.*

Land application and passive treatment systems

With respect to land-applied manure, Bicudo and Goyal (2003) identified that the application technique and timing (with respect to rainfall) influenced microbial water quality in both surface runoff and in subsurface drainage. Management options in land application of manures include (Midwest Plan Service, 1993)

- timing of land application;
- land application technique;
- degree to which land-applied manures are tilled/incorporated into soil;
- selection of ground cover for lands receiving manures;
- choice of land application site; and
- mass or volume applied per land area.

Wastes must be stored prior to land application and waste holding facilities are designed to meet storage requirements dictated either by regulations or by practical considerations like the staging of land-application with other activities. The combination of storage time prior to land application, timing and nature of land application determine the risks land-applied manures pose to recreational waters.

Lewis et al. (2005) measured storm-flow fecal coliform densities in key locations on dairy farms to determine the routes by which fecal indicators reach streams and to identify the farm management practices with the greatest potential for reducing loads. Although between storms loads from various portions of the dairy facilities were highly variable, the relative loading by the sources, averaged over all storms, may be assumed mode consistent. Relative contributions from various components of the dairy operations' manure management systems (MMS) > lots > stockpiles > drains > runoff > pastures > gutters and the relative average fecal coliform density in waters from these locations were MMS > stockpiles > lots > drains > runoff > pasture > gutters. These results indicate that the greatest potential for reducing loads to streams lies in management of runoff from manure management systems and stockpiles, whereas pastures and gutter flows present less opportunity for transport of fecal bacteria loads to streams.

Miller et al. (2008) estimated *Cryptosporidium* oocyst loading to surface waters from dairy farms similar to those studied by Lewis et al. (2005). Attempts were made to relate loading to both infrastructure and non-infrastructure factors including age class of cattle and implementation of specific BMPs. In this study the BMPs were vegetative buffer strips (grassy channels for directing and slowing lot runoff) and the use of straw mulch. Vegetative buffer strips and use of straw mulch both were associated with significant reductions in *Cryptosporidium* loads from dairy operations. Each additional 10% of straw mulch coverage placed on dairy cattle high-use areas was associated with a reduction in stormwater oocyst load by a factor of 0.76 and each meter increase in vegetative buffer length was associated with a reduction in the stormwater oocyst load by a factor of 0.98. Age class of cattle in a given farm area was a strong determinant of the oocysts load in runoff from the area. Mean oocyst density and load from areas housing calves under two months of age were 2000 oocysts/L; mean oocyst density from areas housing older cattle was around 6 oocysts/L (Table 16). Collectively, the findings of Miller et al. (2008)

Table 16. Dairy farm oocyst stormwater density and loading by age class (SOURCE: Miller et al., 2008)

	Cryptosporidium		***Giardia***	
Age Class	**Mean Runoff Density**	**Mean Loading Rate**	**Mean Runoff Density**	**Mean Loading Rate**
<2 months	2000 oocysts/L	1400 oocysts/s	989 cysts/L	7908 cysts/s
3–6 months	50 oocysts/L	320 oocysts/s	38.9 cysts/L	109 cysts/s
Dry, milking, and calving cows	~ 6 oocysts/L	1–20 oocysts/s	86.9 cysts/L	450 cysts/s

support the targeted use of infrastructure BMPs on farm areas with greater potential for oocyst stormwater loading (areas housing calves <2 months of age).

Miller et al. (2007) also studied the efficacy of BMPs in reducing *Giardia* cyst loads to receiving waters. In addition to BMPs related to isolation of manures from young animals, the authors examined structural BMPs, including use of vegetative buffer strips, application of straw mulch, seed application, scraping of manure, and cattle exclusion. Among these BMPs, only vegetative buffer strips were found to reduce *Giardia* load to receiving streams and the density of *Giardia* in receiving streams. Other farm factors found to influence *Giardia* loads coming from pens were animal age and cumulative annual precipitation. An association between event rainfall and cyst loading was also observed. Cyst loading increased with prior 24-hours' precipitation up to a net rainfall of 30mm. Above 30mm cyst loading was observed to decline with increasing rainfall.

Meals and Braun (2006) compared the impacts of manure storage, timing of land application ground cover, and tilling of manures into soils to determine which BMP reduced runoff from land-applied manures most. Runoff density of *E. coli* from land-applied aged manure declined significantly with manure age—runoff from land-applied 90-day-old manure was 99.6% less than that from non-stored manures. Delay to rainfall (from the time of land application) also reduced E. coli in runoff. Manures applied 1 day before rain resulted in twice the E. coli density in runoff water than manures applied 3 days before a rain event. Neither vegetation height nor incorporation of manures into soils alone produced a significant reduction in runoff *E. coli* density, though incorporation accompanied with either storage or 3-day lag between application and rain resulted in significant reductions in runoff *E. coli* density. This study indicates that manure storage results in the greatest reduction in pathogen density compared with timing of land application, height of vegetation, or tilling the applied manure into the soil. These results are expected to be general to all bacterial pathogens.

4.3. Summary

Animal management factors with the potential to influence the regional and local prevalence of the key pathogens include operation type, farm size, and whether the operation uses practices typical of organic operations. A general association was found between increased prevalence and larger farm size (number of animals) for cattle and chicken operations—though for at least one host-pathogen combination (*Giardia* in beef cattle), this trend was not observed. Only one study attempted to relate swine operation size to prevalence of *Salmonella*. In that study, no association was found; however, the sample size was small and the results may not be

representative of general trends. Studies indicate little difference in pathogen prevalence among chicken operation types (layer vs. broiler) and swine operation types (all-in-all-out multisite systems vs. conventional systems) and significant differences among cattle on feedlots and other cattle. Prevalence of both *Campylobacter* and *Salmonella* were markedly higher among feedlot cattle than among animals prior to their arrival at feedlots. *Cryptosporidium* infection among cattle appears to be more related to animal age than housing and feed, and no studies were found associating *Cryptosporidium* prevalence with cattle operation type. Differences in pathogen prevalences between farms using organic and conventional practices could typically be explained based on differences such as farm size (organic farms tend to be smaller), animal housing, and age range of animals on the operation (organic chickens are typically older when slaughtered).

Although numerous animal management factors that are individual farmer choices were identified and evaluated, they are not believed to contribute to regional differences in zoonotic pathogen occurrence. However, these farm practices do relate to local pathogen prevalences and points for controlling zoonotic pathogen sources. Examples of animal management practices with the potential to influence the prevalence of zoonotic pathogens in manures include the following: mixed-production practices; genetic selection of animals resistant to colonization; breeding treatments (antibiotic treatment of semen, antimicrobial egg dips); sanitation for farm and transportation environments; choice of bedding material; maintenance of dry litter; elimination of pathogens from water; elimination of pathogens from feed; feed withdrawal (prior to shipping and during molting); feed and water additives; and vaccination. A brief survey of studies related to these animal management factors indicates that mixed production facilities are not generally associated with higher prevalence of pathogens (via cross-infection), and that drinking water chlorination may be of limited value for limiting infections among herds (though the vessel used for delivering drinking water may be important).

The exposure of humans to zoonotic pathogens during surface water recreation relates both to the source of the pathogens (as quantified by the occurrence and prevalence of the pathogens in fresh livestock manures) and to the manure handling practiced on individual farms. Manure handling entails collection, storage, treatment, and eventual use of collected manures. Each of these steps affords opportunities for reducing pathogen densities in source materials or preventing transport of the pathogens to receiving waters. Best management practices, including infrastructure for isolating runoff waters from manure stocks or for promoting the removal of pathogens from runoff waters (e.g., vegetative buffers), are effective for reducing pathogen loading to streams. High reductions in pathogen loads may be achieved via treatment of the animal wastes. Treatment systems may be physical, chemical, or biological, and the level of treatment varies widely among alternative systems. In general, higher pathogen removal rates are accompanied by higher costs (energy, chemical, or complexity of systems). Regardless of the treatment process, removal varies widely between systems (whose designs and operations may vary) and for systems over time. Land application of manures is typically designed based on nutrient considerations, though how and when manures are land-applied can have profound impacts on the runoff of manure indicators and pathogens to groundwater and streams.

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Appendix A. Literature Search Strategy and Results

Literature Search Overview

Literature searches were conducted to obtain data on livestock and pathogen geographic distribution, data on the range of pathogen prevalences and abundances in livestock manures, data on the relationship between farm factors and pathogen prevalence and abundance, and manure management impacts on pathogen loads to streams. All searches were conducted using the following databases:

- Highwire (full text searchable)
- Pubmed (full text searchable)
- Web of Science (keywords and titles searchable)
- American Society of Agricultural and Biological Engineers archives (keywords, titles and abstracts searchable) and
- USDA APHIS online resources

Additional papers were acquired based on reviews of references cited in articles obtained from database searches. Approximately one-third of papers used in the study were identified in references obtained from studies from the database searches.

Geospatial data were searched both in databases and from online sources. Initially, state agency (environmental protection agencies, departments of natural resources or state geospatial data clearinghouses) databases were searched individually, but this strategy was abandoned in favor of seeking a single source for consistent livestock data for the United States. The sole source providing a consistent data set was the USDA agriculture survey.

Search Terms Used in Database Searches

A relatively simple search strategy was used to obtain studies related to occurrence and abundance of pathogens in manures. Searches were conducted with manure as the primary search term, cattle, swine or (poultry or chicken*) as a secondary search term and each of the key pathogens or "pathogen*" as a tertiary search term. These searches typically returned fewer than 500 studies whose titles and abstracts were scanned to determine the relevant studies. Highwire and Pubmed tended to return more studies since full text searches were used in those databases. Studies related to microbial source tracking, fate of pathogens, and microbial methods were not considered relevant. Abstracts of relevant studies were reviewed and highly relevant studies were selected from the lists for inclusion in the report. In many cases studies were not selected for inclusion in the report because they described farms not in the United States (though select non-US studies were used to fill data gaps), because the emphasis was molecular biology, or because the emphasis was human health epidemiology.

Specific searches were conducted for several types of studies, including the following:

- Studies documenting the impact of herd or flock size on pathogen occurrence,
- Studies comparing pathogen occurrence in conventional and organic operations; and

- factors relating manure management to pathogen runoff.

Search terms used for herd and flock size studies were livestock type (primary search term) "herd/flock size" OR "farm size" (secondary search term) and "pathogen*" (tertiary search term). Searches for data on conventional and organic operations used the primary search term "organic" the secondary search term "livestock type" and the tertiary search term "manure." The search terms used to identify manure management factors were primary search term "manure management" and secondary search term either pathogen* or [key pathogen name].

Summary of Literature Survey Results

The literature survey resulted in collection of 176 highly-relevant studies, of which over 120 are cited in this document.

Appendix B. Occurrence Data

Table B-1. Summary of studies reporting prevalence of *Cryptosporidium* in cattle, swine, poultry, other domestic animals, wildlife, and environmental samples

Study	Animal/Source	Media	Species	Prevalence	Observations and Notes
Moriarty et al. (2008)	Dairy cattle	Freshly-collected manure	spp.	Overall prevalence among 4 farms 5.2% (n=155); oocysts detected at 2 farms	
Atwill et al. (2006)	Beef cattle	Feedlot manure samples	*C. parvum*	Overall prevalence of *C. parvum* in samples 0.2% (n=5274); highest point prevalence (one farm, one sampling event) of 1.7% (n=239)	An alternate estimate of 0.99–1.08% point prevalence was developed based on statistical analyses and consideration of false negative rate.
Xiao et al. (2006)	Swine	Slurry	*C. suis*, pig genotype II, *C. muris*	Oocysts detected in 45% (n=56) of pig slurries collected from 33 farms in Ireland. *C. suis*, pig genotype II and *C. muris* were detected in 62%, 42%, and 2% of positive samples, respectively.	*Cryptosporidium* spp. and individual species prevalences varied with type of slurry sample (liquid vs. solid).
Hutchinson et al. (2005)	Cattle	Manure collected as composite samples from fresh and stored stocks	*C. parvum*	Prevalence among samples of fresh manure 5.4%; prevalence among samples of stored manure 2.8%	*Cryptosporidium* prevalence was significantly higher in June and December than in other months, though overall seasonal trends not observed.
Hutchinson et al. (2005)	Swine	Manure collected as composite samples from fresh and stored stocks	*C. parvum*	Prevalence among samples of fresh manure 13.5%; prevalence among samples of stored manure 5.2%	
Atwill et al. (2003)	Beef cows, pre-parturition and post-parturition	Manure	*C. parvum*	Overall prevalence for three herds 7.1%. Prevalence by herd ranged from 6.25% to 8.75%. Prevalence among pre-parturient cows and post-parturient cows 8.3% and 5.8%, respectively.	Data do not indicate a significant difference in shedding during the pre- and post-parturition periods. This finding indicates the potential for inter-herd transmission, particularly to calves. Calves potentially shed very high numbers of *Cryptosporidium* oocysts.

<table>
<tr><th>Study</th><th>Animal/Source</th><th>Media</th><th>Species</th><th>Prevalence</th><th>Observations and Notes</th></tr>
<tr><td>Sturdee et al. (2003)</td><td>Cattle</td><td>Manure collected as rectal samples or recently deposited feces</td><td>C. parvum</td><td><table><tr><th>Description</th><th>Prevalence over 6 years</th><th>Highest annual prevalence</th></tr><tr><td>Bull beef</td><td>3.6%</td><td>8.7%</td></tr><tr><td>Dairy cow</td><td>3.5%</td><td>8.8%</td></tr><tr><td>Home-bred calf</td><td>52%</td><td>66.7%</td></tr><tr><td>Bought-in calf</td><td>23.2%</td><td>48%</td></tr></table></td><td>C. muris not detected in any livestock samples; occurrence highest in autumn and lowest in spring.</td></tr>
<tr><td>Heitman, (2002)</td><td>Dairy cattle, both young and adult</td><td>Manure (from pasture)</td><td>spp.</td><td>19.6% on two farms (n=92); prevalences on the two farms were 8% and 28%, respectively</td><td>Cryptosporidium detected most frequently in the spring and summer.</td></tr>
<tr><td>Heitman et al. (2002)</td><td>Hogs</td><td>Manure (collected from a single hog operation from pasture)</td><td>C. parvum, C. muris</td><td>0% (n=40)</td><td></td></tr>
<tr><td>Fayer et al. (2000)</td><td>Dairy cattle, post-weaned and adult</td><td>Feces (rectal samples)</td><td>C. parvum, C. andersoni</td><td>On a single farm, C. andersoni detected in 12.5% of fecal samples (n=24)
On a second farm, C. parvum detected in 9.5% of samples (n=42). Prevalence among cows was 10.5% and prevalence among heifers was 9.0%.</td><td>Recovery of oocysts from spiked samples was very low in this study.</td></tr>
<tr><td>Sischo et al. 2000</td><td>Dairy cattle and calves</td><td>Fecal manure (rectal sampling)

Water samples</td><td>C. parvum</td><td>91% of the dairy farms; 15% of calves 0–3 weeks of age; 90% of stream samples</td><td>Eleven dairy farms sampled over a 6-month period, in these farms manure slurry and calves (feces) were sampled; calves sampled from three age groups (0–3, 4–8, and 9–12 weeks of age)</td></tr>
<tr><td>Wade et al. (2000)</td><td>Dairy cattle, all ages</td><td>Manure</td><td>C. parvum, C. muris</td><td><table><tr><th rowspan="2">Parasite</th><th colspan="4">Prevalence (%)</th></tr><tr><th>All ages</th><th><6 mo</th><th>6–24 mo.</th><th>>24 mo.</th></tr><tr><td>C. pavum</td><td>0.9</td><td>2.4</td><td>0</td><td>0</td></tr><tr><td>C. muri</td><td>1.1</td><td>0.5</td><td>1.7</td><td>1.5</td></tr></table></td><td>No significant seasonal patterns observed; C. parvum recovered only from calves less than 30 days of age; C. muris was detected from animals with a wide age range</td></tr>
</table>

Study	Animal/Source	Media	Species	Prevalence	Observations and Notes
Atwill et al. (1997)	Feral (wild) pigs	Fecal samples	*C. parvum*	5.4% (12/221)	No association found between oocyst shedding and sex of pig, body condition, and presence of cattle in the area. However, there was an association between oocyst shedding and pig age and density population: younger pigs (≤8 months) and high-density area (>2 feral pigs/km^2) significantly more likely to shed oocysts.
USDA (1994)	Beef calves from beef cow/calf operations	Fresh fecal samples included both diarrheic calves <3 months and nondiarrheic calves <6 months	spp.	Prevalence of positive calves was 20.1% for diarrheic calves and 11.2% for nondiarrheic calves. Prevalence of positive operations submitting samples from diarrheic calves was 39.1%. Prevalence of positive operations submitting samples from nondiarrheic calves was 41.8%. Prevalence was related to and decreased with age of calves (23.1% for 1-30 days old; 9.2% for ≥121 days old).	Study included 391 samples from diarrheic calves from 69 operations and 1,053 samples from nondiarrheic calves from 141 operations. Average age of diarrheic calves testing positive was 41.1 days. Average age of nondiarrheic calves testing positive was 75.8 days. Shedding was common in calves of beef herds whether the calves had diarrhea or not.
USDA (1993)	Dairy calves (preweaned)	Fresh fecal samples	spp.	Overall prevalence across U.S. was 22% of calves and >90% of farms. Prevalence increased slightly with herd size but still high prevalence on (about 80%) on smaller farms (<100 cows). Prevalence higher in western herds; these are also the largest operations. Prevalence higher in summer months than in other months. Prevalence was highest in heifers 1-3 weeks old (>50%). Prevalence drops to <15% for calves over 5 weeks old.	Study included 1,103 farms in 28 states, with 7,369 samples collected. States included: • West: WA, OR, CA, ID, CO • Midwest: NE, IA, MN, WI, MI, IL, IN, OH • Northeast: ME, VT, NH, NY, PA, CT, MA, RI • Southeast: VA, NC, TN, GA, AL, FL, MD

Table B-2. Summary of studies reporting prevalence of *Giardia* in cattle, swine, chicken, other domestic animals, wildlife, and environmental samples

Study	Animal/Source	Media	Species	Prevalence	Observations and Notes
Hutchison et al. (2004)	Cattle (assumed to include samples from both dairy and beef cattle operations)	Feces (farm yard manures) and slurries	*intestinalis*	3.6%	Samples taken from operations throughout U.K.
Hutchison et al. (2004)	Swine	Feces (farm yard manures) and slurries	*intestinalis*	2.4%	Samples taken from operations throughout U.K.
Ralston et al. (2003)	Range beef calves and their dams	Feces	spp.	100% of calves shed Giardia cysts at some point during the study Cow shedding prevalences 10% prior to calving, up to 15% one week post calving, and to 0% within 23 weeks after calving	
Heitman et al. (2002)	Dairy cattle	Feces	spp.	10–20%	Study of Canadian dairy farms
Heitman et al. (2002)	Beef cattle	Feces	spp.	15%	Study of Canadian beef cattle farms
Fayer et al. (2000)	Beef cattle 7–9 months old	Feces	*duodenalis*	37.3%	Study conducted in three Maryland cattle farms (one beef and two dairy operations).
Fayer et al. (2000)	Dairy cattle	Feces	*duodenalis*	0–17.3%	Study conducted in three Maryland cattle farms (one beef and two dairy operations); dairy cattle include replacement heifers and milk cows
Wade et al. (2000)	Dairy cattle	Feces	spp.	Giardia prevalence varied among animals with different age groups. Age group: Prevalence All ages: 8.9% < 6 months: 20.1 % 6 – 24 months: 3.5% > 24 months: 0.2%	No seasonal variation in *Giardia* prevalence was observed.
Olson et al. (1997)	Beef cattle	Feces	spp.	Giardia prevalence varied with age group. Among animals < 6 months prevalence was 30% and among animals > 6 months of age the prevalence was 11%	

Study	Animal/Source	Media	Species	Prevalence	Observations and Notes
USDA (1994)	Beef calves from beef cow/calf operations	Fresh fecal samples included both diarrheic calves <3 months and nondiarrheic calves <6 months	spp.	Prevalence of positive calves was 26.9% for diarrheic calves and 45.9% for nondiarrheic calves. Prevalence of positive operations submitting samples from diarrheic calves was 63.8%. Prevalence of positive operations submitting samples from nondiarrheic calves was 90.8%. Prevalence peaked in calves 61-90 days old (59.6%) and decreased with age of calves (29.9% for calves ≥121 days old).	Study included 391 samples from diarrheic calves from 69 operations and 1,053 samples from nondiarrheic calves from 141 operations. Average age of diarrheic calves testing positive was 47.1 days. Average age of nondiarrheic calves testing positive was 79.1 days. Shedding was common in calves, especially older calves, of beef herds whether the calves had diarrhea or not.
Xiao et al. (1994)	Swine	Feces (via rectal swab samples)	spp.	On two farms, 0–17% of litters of pigs positive for *Giardia* and 3–25% of weanlings positive for *Giardia*	Two Ohio farms with different animal housing types studied.

Table B-3. Summary of studies reporting prevalence of *Campylobacter* in cattle, swine, chicken, other domestic animals, and environmental samples

Study	Animal/Source	Species	Media	Prevalence	Observations and Notes
Hakkinen and Hänninen (2009)	Dairy cattle	*C. jejuni*	Fecal samples, water samples from troughs, and milk samples	*C. jejuni* detected in 169 out of 340 fecal samples, 1 out of 3 farm drinking water supplies (one sampling event), and in no milk samples. *C. coli* detected in 3.2% of fecal samples and *C. hyointestinalis* detected in 15.3% of fecal samples.	Finland; infection prevalence differed significantly between farms and, for farms with relatively low prevalence, by season. For two farms, the lowest *C. jejuni* prevalence (% of herd infected) coincided with indoor housing of cattle; higher *C. jejuni* prevalence coincided with grazing periods.
McLaughlin et al. (2009)	Swine (sows, nursery swine, and finishers)	spp.	Lagoon waters	100% of sow slurry in lagoons (n = 7 farms, 102 samples); 100% of nursery slurries (n = 10 lagoons and 60 samples); and 100% of finisher slurry in lagoons (n = 10 lagoons and 60 samples) positive for *Campylobacter*	Farms located in mid-south United States
Kwan et al. (2008)	Dairy cattle	*C. jejuni*	Manure	Overall prevalence 35.9% (5 farms); ranged from 26–50.8% for low and high months; *C. jejuni* prevalence among isolates 68%	Seasonal variation in *Campylobacter* spp. prevalence differed among the five U.K. dairy farms studied, though overall prevalence (annual average) among farms was similar; genotype diversity for *C. jejuni* isolates differed significantly between farms
Moriarty et al. (2008)	Dairy cattle	*C. jejuni, C. coli*	Freshly-excreted feces	96% of *Campylobacter* spp. positive samples positive for *C. jejuni*. 10% of *Campylobacter* spp. positive samples positive for *C. coli*. 7% of *Campylobacter* spp. positive samples positive for C. *jejuni* and *C. coli*	New Zealand farms, 4 regions
Berry et al. (2007)	Beef cattle (feedlot)	spp.	Manure (composite samples)	Ranged from 2.2 % in samples taken in the spring to 14.9% of samples in the summer.	Samples collected every four weeks for 26 months; manure samples were composite samples collected from feedlots

<table>
<tr><th>Study</th><th>Animal/Source</th><th>Species</th><th>Media</th><th>Prevalence</th><th>Observations and Notes</th></tr>
<tr><td>Englen et al. (2007)</td><td>Dairy cattle from 96 operations in 21 states</td><td>C. jejuni,
C. coli</td><td>Feces samples</td><td>97.9% of operations and 51.2% of samples positive for Campylobacter</td><td>Study also assessed antimicrobial resistance of isolates</td></tr>
<tr><td>Bull et al. (2006)</td><td>Broiler chickens at U.K. farms</td><td>C. jejuni,
C. coli</td><td>Droppings (feces) taken from floors</td><td>Flock level prevalence of Campylobacter increased from 10% of flocks (n = 10) at 18 days to 40% of flocks between 28 and 33 days and to 60% at depletion</td><td>Among flocks colonized at depletion, 71% colonized exclusively by C. jejuni, 14% colonized by C. coli exclusively, and 14% were colonized by both</td></tr>
<tr><td>Luangtongkum et al. (2006)</td><td>Broiler chickens from conventional and organic operations</td><td>spp.</td><td>Intestinal tracts of birds a slaughter</td><td>Prevalence on conventional broiler farms slightly lower (44–80%) than organic farms (70–100%)</td><td>Higher prevalence in organic operations can be explained, in part, by the higher age at which organic chickens are typically slaughtered</td></tr>
<tr><td>Bae et al. (2005)</td><td>Dairy cattle, beef cattle, feedlot cattle, cattle in calf-cow operations</td><td>C. jejuni,
C. coli,
C. fetus,
C. hyointestinalis</td><td>Fecal samples (rectal or recently deposited)</td><td>C. jejuni and C. coli prevalence differed with operation type; prevalences for each farm type and species were
<table>
<tr><th rowspan="2">Operation type</th><th colspan="2">% positive</th></tr>
<tr><th>C. jejuni</th><th>C. coli</th></tr>
<tr><td>Beef cow-calf</td><td>47.1</td><td>0.6</td></tr>
<tr><td>Calf rearer</td><td>23.8</td><td>20.0</td></tr>
<tr><td>Dairy</td><td>31.2</td><td>5.8</td></tr>
<tr><td>Feedlot</td><td>31.6</td><td>13.3</td></tr>
<tr><td>Total</td><td>34.1</td><td>7.7</td></tr>
</table></td><td>Samples collected from 15 farms in Washington state; findings based on a relatively large (n = 686) sample size</td></tr>
<tr><td>Besser et al. (2005)</td><td>Beef cattle (feedlot)</td><td>C. jejuni</td><td>Manure from feedlot (10 different fresh samples per sampling date, 10 sampling dates)</td><td>For newly-arrived animals, prevalence was 1.6% (n=10); after final sampling (2 weeks prior to slaughter) prevalence was 62.2% (n=10)</td><td>Study conducted in a large commercial feedlot (>50,000 head)</td></tr>
<tr><td>Devane et al. (2005)</td><td>Dairy cattle</td><td>spp.,
C. coli,
C. jejuni</td><td>Feces</td><td>spp.: 98% (n = 91)

C. jejuni: 100% of Campylobacter spp. positive samples.

C. coli: 10% of Campylobacter spp. positive samples.</td><td>Some serotypes prevalent in dairy cattle feces also prevalent in human feces in samples collected in the same region</td></tr>
</table>

Study	Animal/Source	Species	Media	Prevalence	Observations and Notes
Devane et al. (2005)	Beef cattle feces	spp., *C. coli,* *C. jejuni*	Feces	spp.: 84% (n =8 7) *C. jejuni*: 100% of *Campylobacter* spp. positive samples *C. coli*: 19% of *Campylobacter* spp. positive samples	Some serotypes prevalent in beef cattle feces also prevalent in human feces in samples collected in the same region
Devane et al. (2005)	Surface waters, stream source	spp., *C. coli,* *C. jejuni*	Water	spp.: 55% (n = 293) (result reflects less than 100% recovery/sensitivity) *C. jejuni*: 100% of *Campylobacter* spp. positive samples *C. coli*: 7.4% of *Campylobacter* spp. positive samples	High prevalence of *Campylobacter* spp. positive samples in surface waters consistent with expectations
El-Shibiny et al. (2005b)	Chickens (free-range and organic)	*C. jejuni* and *C. coli*	Animals (rate of infection)	Free-range birds: *Campylobacter* isolated from 68.5% (n=54) of birds during the rearing cycle; first incidence of *Campylobacter* colonization observed at an age of 31 days Organic birds: *Campylobacter* was isolated from 90% (n=42) of birds during the rearing cycle; first incidence of *Campylobacter* colonization observed at 8 days	Studies performed on organic and free range chicken farms in the U.K. Practices on these farms differ significantly from those on more conventional farms. *C. jejuni* more prevalent in chicks between 0 and 5 weeks of age. After 5 weeks of age *C. coli* detected more frequently. An estimated 80% of U.K. chicken meat believed to be contaminated with *Campylobacter* (27% *C. coli* and 73% *C. jejuni*)
Dorner et al. (2004)	Sows or gilts	spp.	Animals (i.e., infected proportion)	45.9% positive (n = 315); and 79.7% positive (n = 59)	Authors suggest representing prevalence with a beta distribution. For the two studies, beta distribution parameters were (α, β) = (146,172) and (α, β) = (48,13)).
Dorner et al. (2004)	Growers or finishing pigs	spp.	Animals (i.e., infected proportion)	91.9% positive (n = 595); 100% positive (n = 24); and 98.1% positive (n=160)	For the three studies, beta distribution parameters were (α, β) = (548,49) and (α, β) = (25,1) and (α, β) = (158,4)
Dorner et al. (2004)	Nursing or weaner pigs	spp.	Animals (i.e., infected proportion)	63.6% positive (n = 93) and 79.3% positive (n = 294)	For the two studies, the beta distribution parameters were (α, β) = (60,35) and (α, β) = (234,62)

Study	Animal/Source	Species	Media	Prevalence	Observations and Notes
Dorner et al. (2004)	Chicken, broilers	spp.	Animals (infected proportion)	8 studies were reported; the three with the highest number of observations were 3.1% positive (n=19,700,000), 42.5% positive (n=89,110), and 27% positive (n=12,233)	For the three studies with the largest number of observations, beta distribution parameters (α, β) = (606 001, 19 094 001), (α, β) = (37 873, 51 240), and (α, β) = (3305, 8930)
Dorner et al. (2004)	Chicken, layers	spp.	Animals (infected proportion)	66.1% positive (n=280) and 42.9% positive (n=105)	For the two studies, the beta distribution parameters were (α, β) = (186, 96), (α, β) = (46, 61)
Inglis et al. (2004)	Feedlot cattle	*C. jejuni*, *C. lanienae*, *C. hyointestinalis*, *C. coli*	Abundance in fresh feces from feedlot cattle housed individually; longitudinal study duration of 4 months	*Campylobacter jejuni* detected 13.4% of fecal samples (range 8.2–16.7% over 4 sampling events); *C. laneinae* detected in 55.5% of samples (range 46.7– 63.3%); *C. coli* not detected in any sample	
Minihan et al. (2004)	Beef cattle (heifers)	*C. jejuni* and *C. coli*	Rectal samples, fecal samples, water trough samples, feed samples, and samples from carcasses	On arrival at the feeding lot, rectal fecal samples from cattle indicated 12% infection rate; after four months at the feedlot, the infection rate was 76%. Among rectal fecal samples positive for *Campylobacter* spp., *C. jejuni* accounted for 68.5% of isolates, *C. coli* accounted for 29.9% of isolates and *C. lari* accounted for 1.6% of isolates.	Irish feedlots; infection prevalence differed between pens at a feeding lot and with residence of cattle at the feeding lot; prevalence increased from 12% at the introduction of cattle to the feeding lot to 76% after cattle had been at the feeding lot for 4 months. *Campylobacter* spp. more prevalence in rectal swabs than from feces on the pen floors, trough water, feed, or dust. Environmental occurrence of *Campylobacter* increased with the duration of cattle at the feedlot. *C. coli* were more prevalent than *C. jejuni* among environmental samples.
Cox et al. (2002)	Chicken (breeders and broilers)	spp.	Feces	Overall: 57.1% of fecal samples Within flock low: 0% of fecal samples Within flock high: 100% of fecal samples	Based on a total of 350 observations for 14 different flocks located in diverse U.S. locations; transmission from breeder to offspring through the egg noted in the study discussion

Study	Animal/Source	Species	Media	Prevalence	Observations and Notes
Hoar et al. (2001)	Beef cattle	spp., *C. jejuni, C.coli*	Fecal samples, 17 herds	*Campylobacter* spp. detected in 20 out of 401 fecal samples (5%) *C. jejuni* detected in15 out of 20 samples culture positive for *Campylobacter* spp. (75%) *C. coli* detected in 3 of 20 samples culture positive for *Campylobacter* spp. (15%)	Proportion of female animals was positively associated with occurrence of *Campylobacter* spp. in individual herds.
Whyte et al. (2001)	Chicken, broilers	spp.	Animals (infected proportion, based on fecal samples)	69% positive (n=70); 10 farms tested with n=7 fecal samples per farm. At 7 farms there were either 6 or 7 positive samples out of 7. At three farms *Campylobacter* was not detected in any fecal samples.	Farms tended to be 100% infected or not infected at all; transport and holding in processing facilities appears to have limited or no influence on occurrence
Wesley et al. (2000)	Dairy cattle	*C. jejuni, C. coli*	Fecal samples (assumed to be fresh) from 13 farms in 23 U.S. states	*C. jejuni* isolated from 37.7% (n = 2085) of fecal samples *C. coli* detected in 1.8% (n=2085) of fecal samples	*C. jejuni* prevalence higher among lactating cows than non-lactating cows (46.9% vs. 39.8%) and on farms with more than 100 head than smaller farms (45.2% vs. 37.7%)
Donnison and Ross (1999)	Sentinel freshwater mussel (*Hydridella menziesi*)	*C. jejuni*, *C. coli*	Mussel slurries; river samples; wastewater samples	<u>Recoveries from mussels by site</u>: MP = *C. jejuni* 67% (2/3) DF = *C. jejuni* 75% (3/4) SW1 = *C. jejuni* and *C. coli* 75% (3/4) SW2 = *C.jejuni* 33% (1/3) <u>Recoveries from untreated wastewater</u>: Sheep = *C. jejuni* and *C. coli* 80% (8/10) Cattle = *C. jejuni* and *C. coli* 91% (10/11) Sewage/human = *C. coli* 86% (6/7)	Analyzed mussels recovered from river/stream systems with inputs of fecal pollution from known sources (MP=meat-processing wastewater; DF=non-point inputs from dairy farms; SW1 and 2=two treated sewage plants)

Study	Animal/Source	Species	Media	Prevalence	Observations and Notes
Weijtens et al. (1997)	Swine (sows and piglets)	*C. coli*	Feces	All *Campylobacter* isolated were *C. coli* Sows: 9/10 infected before delivery, 10/10 infected after delivery Piglets: 8/10 infected at 1 week post-delivery; 10/10 infected at 4 and 8 weeks post delivery	Conducted on two Dutch farms; between 1 and 4 weeks post-delivery, piglets rapidly acquired infections and began shedding. Fecal samples from more than half of the piglets positive for *Campylobacter* at the first fecal sample (1 week post-delivery).
Humphrey and Becket (1987)	Dairy cattle	*C. jejuni*	Fecal samples, rectal swabs, 12 herds	Overall, 24.5% (n = 668) of cows had positive *C. jejuni* fecal samples; herd prevalence ranged from 0% to 36%	Prevalence did not appear significantly different for cows whose water source was chlorinated vs. unchlorinated

Table B-4. Summary of studies reporting prevalence of *Salmonella* in cattle, swine, chicken, other domestic animals, and environmental samples

Study	Animal/Source	Media	Prevalence	Observations and Notes
Cummings et al. (2009)	Dairy cattle	Rectal fecal samples	Herd-level prevalence 11%; prevalence among all individual samples 22.5%	Large study in geographic extent (NE United States) and number of samples analyzed (n = 2565 dairy cattle)
Dorr et al. (2009)	Pigs (nursery)	Fecal samples	10.4% positive for *Salmonella* (5 farms). Highest and lowest observed prevalences (on individual farms) were 21.7% and 5%. Serotype prevalences were *Typhimurium* (48%); *Derby* (33%); *Muenchen* (7.3%); London (4.1%); and *Mbandaka* (3.1%).	*Salmonella* prevalence increased significantly with age
Dorr et al. (2009)	Pigs (finisher)	Fecal samples	15.4% positive (based on fecal samples) for 9 farms. Highest and lowest observed prevalences (on individual farms) were non-detect (0) and 33.9%.	*Salmonella* prevalence increased significantly with age
Haley et al. (2009)	Various	Receiving waters (streams)	*Salmonella* spp. were found in 79.2% of environmental waters collected (n=72). Studies were conducted in north central Georgia. Among positive samples 13 serotypes were identified. The prevalence of subspecies and serotypes was: *S. enterica* subsp. *arizonae* 41%; *Muenchen* 14%; *Rubislaw* 13%; *Mikawasima* 6%; *Braenderup* 6%; *Saint Paul* 5%; other serotypes were *Bareilly*, *Liverpool*, I 4,[5]:b, *Gaminara*, *Montevideo*, *Anatum*, I47:z4z23, not typed.	The strongest determinants of *Salmonella* occurrence were antecedent rainfall and temperature (highest *Salmonella* abundance in August). Occurrence of serotypes highly variable.
Kunze et al. (2008)	Feedlot beef cattle	Feces from feedlots	*Salmonella enterica* recovered from 30.3% of fecal samples (n=182)	No apparent seasonal variation in prevalence reported
Callaway et al. (2005)	Dairy cattle	Dairy cattle feces	*Salmonella* found in 56% of herds studied; overall prevalence (among all fecal samples) of 9.07%	Study conducted on herds from 4 states, with state selection weighted toward western states
Fossler et al. (2005a)	Dairy cattle	Dairy cattle and calf feces	Prevalence of *Salmonella* spp. for all data (collected in 5 states) ranged from 2.7% (n=5220) in the winter to 6.4% (n = 6417) in the summer. Prevalence 5.2% on organic farms and 4.8% on conventional farms; the difference between prevalence by farm type was not statistically significant.	Factors associated with increased prevalence of *Salmonella* were: • season • health status of cattle • Midwest farm location • herd size >100 head

Study	Animal/Source	Media	Prevalence	Observations and Notes
Edrington et al. (2004)	Dairy cattle	Fecal samples from large dairy operations	*Salmonella* spp. detected in 0–93% of samples taken at each sampling event (4 farms, two summer samples and one winter sample)	While a general seasonal trend in *Salmonella* shedding was reported (higher summer shedding than winter shedding), variability was extremely high. Although farms sampled were relatively close in proximity, serotype prevalence was very different from farm to farm.
Hutchison et al. (2004)	Pig feces	Commercial farms	*Salmonella* spp. detected in 7.9% of fecal samples (n = 126)	Wastes taken from farms throughout Great Britain and results believed representative of overall prevalence in the region
Hutchison et al. (2004)	Chicken feces	Fecal samples from commercial farms	*Salmonella* spp. detected in 17.9% of fecal samples (n = 67)	Wastes taken from farms throughout Great Britain and results are believed representative of overall prevalence in the region
Garber et al. (2003)	Chickens	Hen houses (layers)	*Salmonella enterica* serotype *enteritidis* found in 7.1% of layer houses (n=200, all U.S. facilities)	Factors associated with higher incidence of *S. enterica* serotype *enteritidis* were the following: • flock size >100,000 • flocks 0–16 weeks post-molting • young flocks • floor-reared (rather than cage reared) • location in Great Lakes region • no cleaning and disinfection of feeders and hoppers between flocks
Johnson et al. (2003)	Various	Streams	*Salmonella* detected in 14/468 (3%) of water samples taken over two years	The highest prevalence was in storm drain waters; the lowest prevalence was in samples taken in an urbanized area
USDA (2003)	Dairy cows	Rectal swabs	Overall prevalence for culture-positive E. coli O157 was 4.3%; 38.5% of operations had one or more positive cows. Prevalence highest in summer (June – 8.2%) and lowest in spring (April – 1.5%). Highest prevalence of positive cows found in West region (7.6%), Midwest (3.5%), Southeast (3.1%), and Northeast (1.6%). Large operations (>500 cows) more likely to have positive samples than medium operations (100-499 cows) or small operations (<100 cows). The majority of large dairies are in the West region.	Samples collected from March to September 2002; total of 3,733 samples for culture and ID of *E. coli* O157, *stx 1*, *stx 2*, and antigens. Samples collected from 5 operations from each of the 21 participating states: • West region: CA, CO, ID, NM, TX, WA • Midwest region: IL, IN, IA, MI, MN, MO, OH, WI • Northeast region: NY, PA, VT • Southeast region: FL, KY, TN, VA
Warnick et al. (2003)	Dairy cattle	Fresh feces	Within-herd prevalence highly-variable and ranged from 0% to 100% of animals; overall, *Salmonella* isolated from 9.3% of 4049 fecal samples	Large, multi-state study; over the course of the study, *Salmonella* isolated from at least one fecal sample from every farm sampled

Study	Animal/Source	Media	Prevalence	Observations and Notes
Huston et al. (2002)	Dairy cattle	Feces	Overall herd-level prevalence 31.4% and individual cow prevalence 17.7% among cows from infected herds	The only factors associated with higher shedding prevalences were farm size; larger farms were associated with higher *Salmonella* prevalences.
USDA (2001)	Beef cattle (feedlot)	Fresh manure from feedlot pens	Ranged from 3.3 % in samples taken in the winter (Feb) to 19.9% in fall samples (Sept). No geographic trends for STEC prevalence. All feedlots had at least one positive sample during the study. Prevalence of culture-positive samples per region: 8.4% in Middle Region (CO, KS, OK); 11.5% in Northern Region (ID, IA, NE, SD, WA); 13% in Southern Region (CA, NM, TX)	73 feedlots/422 pens in 11 leading cattle feeding states sampled for STEC from Oct '99 to Sept '00. Total of 10,415 samples. Samples from pens for cattle that had been on feed the shortest (13.9%) were more likely to be positive than samples from pens for cattle that had been on feed the longest (8.6%).
Wells et al. (2001)	Dairy cattle	Fresh feces	Overall prevalence of *Salmonella* 10% (n = 6595)	Prevalence varied with season (higher shedding prevalence in summer than winter) and with herd size (higher shedding prevalence in herds with more than 100 cows than in herds with fewer than 100 cows.
Hayes et al. (2000)	Chicken litter and drag swabs	Broiler and roaster houses	*Salmonella* spp. detected in 48/71 (55.8%) of facilities	Litter water content found to be a poor predictor of *Salmonella* occurrence.
Donnison and Ross (1999)	Freshwater mussel (*Hydridella menziesi*)	Mussel slurries; river samples; wastewater samples	Recoveries from mussels by site: MP = 67% (2/3) *S. typhimurium* DF = 0% (0/4) SW1 = 0% (0/4) SW2 = 0% (0/3) Recoveries from untreated wastewater: Sheep = 50% (7/10) Beef = 50% (11/22) Sewage (human) = 44% (4/9)	Analyzed mussels recovered from river/stream systems with inputs of fecal pollution from known sources (MP = meat-processing wastewater; DF = non-point inputs from dairy farms; SW1 and 2 = two treated sewage plants).
Byrd (1998)	Chicks	Chickens	Incidence of *Salmonella* infection among chicks leaving hatcheries was estimated at 5–9%. Within three weeks of entering growing houses, prevalence rose to between 72 and 95%.	Based on studies conducted prior to 1998. Horizontal transmission between chicks co-housed in hatcheries was shown to be highly efficient. Fecal shedding (and transmission) was dependent on doses ingested by chicks when infection was acquired.

Study	Animal/Source	Media	Prevalence	Observations and Notes
USDA (1998)	Dairy cows	Rectal swabs of dairy cows, cows to be culled within 7 days, and cows at cull dairy cow markets	24.2% of operations and 30.9% of markets had at least one culture-positive *E. coli* O157 sample. Prevalence on farm – 0.9% of samples positive. Prevalence for cows to be culled with 7 days – 2.8% of samples positive. Prevalence for culled dairy cows at markets – 1.8% of samples positive.	Fecal samples collected from 91 dairy operations and 97 cull dairy cow markets in 19 states during a one-time sampling event. Samples collected from Feb-July 1996. Seasonal pattern of shedding was observed, samples more likely to be positive after May 1 than before May 1. No significant differences found between cows on farm and cows going to slaughter. Prevalence was higher for herds with 100 or more cows (39.1% of herds had at least one positive sample) than for herds with fewer cows (8.9 % of herds had at least one positive sample), however seasonality may have been a factor.
USDA (1995)	Beef cattle (feedlot)	Fresh manure from feedlot pens	Overall prevalence was 1.61% of collected samples. Prevalence of positive feedlots per region: 59.4% in Middle Region (CO, NE, KS, OK); 58.3% in Northern Region (ID, IA, IL, MN, SD, WA); 71.9% in Southern Region (CA, AZ, TX)	Pens in feedlots from 13 leading cattle feeding states sampled for E. coli O157:H7 in fall of 1994. Total of 11,881 samples. Samples from pens for cattle that had been on feed the shortest (47.1%) more likely to be positive than samples from pens for cattle that had been on feed the longest (16.8%).
Ebel et al. (1992)	Spent layer hens	Cecal contents	Overall prevalence (% of layer houses) of *Salmonella* was 24% and overall prevalence of *S. enteriditis* was 3%; no differences in Salmonella prevalence associated with U.S. regions. *S. enteriditis* more prevalence in the northern U.S. (45%) than in the southeastern or western/central U.S.	Authors speculate that high *S. enteriditis* prevalence in the northern U.S. may relate to the tendency toward larger flocks in that region

Table B-5. Summary of studies reporting prevalence of *E. coli* O157:H7 in cattle, swine, chicken, other domestic animals, wildlife, and environmental samples

Study	Animal/Source	Media	Prevalence	Observations and Notes
Berry et al. (2007)	Beef cattle (feedlot)	Manure (composite samples)	Ranged from 9.7 % in samples taken in the winter to 22.6% of samples in the fall.	Samples collected every four weeks for 26 months. Manure samples were composite samples collected from feedlots.
Cornick and Helgerson (2004)	Swine	Animals (based on multiple studies)	0.1–6% of animals	Range based on studies performed in Europe and North America.
Cornick and Helgerson (2004)	Swine	Feces	Shortly after inoculation fecal *E. coli* density ranged between 10^3 and 10^7 CFU/g. Two weeks after inoculation fecal *E. coli* O157:H7 density ranged from 50 to 1000 CFU/g. Two months after inoculation fecal *E. coli* density ranged from non-detect to 10^4 CFU/g.	Three month old pigs challenged with graded doses of *E. coli* O157:H7. Pigs housed indoors on concrete floors or decks. Experiments conducted in Iowa.
Edrington et al. (2004)	Dairy cattle	Feces	Variable prevalence observed from farm to farm and season to season. Prevalence ranged from 0–35% (of fecal samples collected from a given farm on a sampling event). No positive samples were found on any farm among winter samples.	Prevalences from the same farms on successive summers were highly variable.
Hutchison et al. (2004)	Swine	Samples of fresh manure	11.9 % (n = 126) of samples were positive for *E. coli* O157	Wastes taken from farms throughout Great Britain and results are believed representative of overall prevalence in the region.
LeJeune et al. (2004)	Feedlot cattle	Fresh manure from feedlot pens	13.3% (n = 4790)	There was no apparent influence of trough water chlorination on fecal shedding prevalence.
Feder et al. (2003)	Swine	Pig colon	2% (6 out of 305)	No inferences could be made of *E. coli* O157:H7 isolation rates with respect to the season, or swine or herd prevalence. PCR confirmed two genotypes: isolates harboring the *eaeA*, *stx1*, and *stx2* genes and isolates harboring the *eaeA*, *stx1*, and *hly933* genes; ribotyping did not discriminate among isolates within the *E. coli* O157:H7 serotype.
Johnson et al. (2003)	Various	Streams in a mixed use watershed	*E. coli* O157:H7 detected in 13 out of 1483 (0.9%) of water samples taken from 10 locations over 2 years	Highest prevalence (9.1% of samples) observed for a stream reach within an urbanized area; *E. coli* O157:H7 not detected at many sites, including those draining high-intensity livestock regions and in storm drains
Booher et al. (2002)	Swine	Fecal samples	High dose experiments: recovery after 2 weeks for STEC strains varied from 75 (6/8) to 100% (8/8) and for all other three strains at 12.5% (1/8); however,	High (inoculation) and low (feeding) dose experiments using 3-month old pigs and a mixture of five *E. coli* strains, including two STEC O157:H7 strains, two enterotoxigenic *E. coli* (ETEC) strains,

Study	Animal/Source	Media	Prevalence	Observations and Notes
			at 2 months, most of the recovered strains were STEC that varied from 37.5 (3/8) to 50% (4/8). Low dose experiments: recoveries varied from 50% (all strains but one STEC) to 67%. No STEC strain was recovered at 2 months. Although one STEC strain was not recovered from the low dose group at 2 months, it was recovered from the cecum (but not elsewhere in the alimentary tract) of 2/6 pigs at necropsy.	and one enteropathogenic *E. coli* (EPEC) strain. High dose: 3-month old pigs inoculated with the mixture at 10^{10} CFU per strain). Low dose: 3-month old pigs fed both STEC O157:H7 strains at a dose of 10^{7} CFU per strain and other strains at a dose of 10^{10} CFU per strain. STEC strains persisted in the alimentary tracts of some pigs at 2 months post-inoculation for high and low dose mixtures; when all strains were given at 10^{10} CFU (high dose), STEC strains persisted in greater numbers and in more pigs than did the other *E. coli* strains.
Donnison and Ross (1999)	Sentinel freshwater mussel (*Hydridella menziesi*)	Mussel slurries; river samples; wastewater samples	Mussel slurries (shucked and homogenized for analysis) and river samples collected	3×10^{2}–5×10^{5} CFU/100g slurry (mussel); 90–2×10^{3} CFU/mL (water samples)
Chapman et al. (1997)	Chicken	Fecal samples, taken immediately after slaughter	0/1000 (0%) chickens	Studies conducted in the U.K.
Chapman et al. (1997)	Swine	Fecal samples, taken immediately after slaughter	4/1000 (0.4%) of pigs	Studies conducted in the U.K.
Chapman et al. (1997)	Dairy cattle	Fecal samples, taken immediately after slaughter	16.1% (n=1661) of culled dairy cattle	Studies conducted in the U.K.; highest prevalence observed in late spring and early summer.
Chapman et al. (1997)	Beef cattle	Fecal samples, taken immediately after slaughter	13.4% (n=1840) of beef cattle	Studies conducted in the U.K.; highest prevalence observed in late spring and early summer.
Rice et al. (1995)	Cattle/deer	Fresh fecal samples collected from cattle and deer	1.85% (2/108) deer and 2.6% (5/191) cow samples tested positive	Fecal samples measured for *E. coli* O157

Study	Animal/Source	Media	Prevalence	Observations and Notes
Schoeni and Doyle (1994)	Chickens	Fresh fecal samples collected after 1 hour of defecation	83–100% of chicks administered 2.6×10^{1} to 2.6×10^{5} *E. coli* O157:H7 colonized at some time during the 12 weeks of examination; *E. coli* recovered from cecal tissue of two of six chickens (33%) *E. coli* also isolated from the shells of eggs but not from the yolks and whites at a rate of 13.9% (14/101)	*E. coli* inoculated orally: 2.6×10^{1} to 2.6×10^{5} per chick; *E. coli* colonization persisted up to 4 months when inoculated up to 10^{5} CFU/chicken and up to 10–11 months when inoculated with 10^{8} CFU/chicken

Appendix C. Abundance Data

Table C-1. Summary of studies reporting abundance (concentrations) of *Cryptosporidium* in feces and related media of domestic animals

Study	Source/Media	Species	Description	Abundance	Notes
Moriarty et al. (2008a)	Dairy cattle manure	spp.	Samples taken from freshly-deposited manure	Among positive samples, *Cryptosporidium* density ranged from 1–25 oocysts/g feces	Prevalence low in the herds studied
Berry et al. (2007a)	Beef cattle feces from feedlot	spp.	Manure from beef feedlots sampled (composite samples) each 4 weeks during a 26 month study	Average: 14 oocysts/g Range: 0.5 oocysts/g manure to 1510 oocysts/g manure	*Cryptosporidum* identified in 58% of composite manure samples collected over a 26-month study
Atwill et al. (2006b)	Beef cattle feces from feedlot	*C. parvum*	Manure from 22 feedlots in 7 western and central states sampled from August 2000 to January 2002	Among samples positive for *C. parvum*, the geometric mean was 447 oocysts/g manure (range 203–7702 oocysts/g)	*C. parvum* detected in only 0.2% of samples; abundance data fit with a negative binomial distribution
Hutchison et al. (2005b)	Fresh and stored pig manure	*C. parvum*	Composite samples from fresh and stored manure collected between April 2000 and December 2002	Geometric mean densities 58 for fresh manure and 33 for stored manure	
Hutchinson et al. (2004)	Cattle manure	*C. parvum*	Manure samples collected from throughout U.K.	For fresh manure, geometric mean density was 19 oocysts/g (n = 44); maximum density 3500 For stored manure, geometric mean density was 10 oocysts/g (n=12); maximum density 480	
Hutchison et al. (2004)	Fresh and stored chicken manure	*C. parvum*	Composite samples from fresh and stored manure collected between April 2000 and December 2002	No *C. parvum* were identified in any chicken manure samples	
Atwill et al. (2003)	Beef cow (> 24 months) feces	*C. parvum*	Manure samples from preparturient and postparturient beef cows on three California farms were sampled and *C. parvum* was enumerated via a sensitive method.	For samples positive for *C. parvum*, the arithmetic mean oocyst density was 3.38 oocysts/g feces and the standard deviation was 2.64 oocysts/g feces	No significant difference in prevalence or shedding of *C. parvum* between pre-parturient and post-parturient cows

Study	Source/Media	Species	Description	Abundance	Notes
Sturdee et al. (2003)	Cattle feces	*C. parvum*	Rectal and recently-deposited fecal samples collected at a farm with beef and dairy cattle and calf rearing operations	Description: Mean of positive samples (oocysts/g) Bull beef: 1371 Dairy cow: 1778 Calf, Home-bred: 107,025 Calf, brought-in: 24,448	Highest observed density was 280,000 oocysts/g feces for a home-bred calf
Heitman et al. (2002)	Manure from dairy cattle	*C. parvum*	Manure from two dairy operations collected from pasture	Mean densities in manure from the two farms were 18.8 and 490 oocysts/g (considering only positive samples)	*C. muris* not detected in any fecal samples
Wade et al. (2000)	Dairy cattle feces	*C. muris*	Fecal samples collected rectally from dairy cattle at 109 farms in southeastern New York; data stratified by cattle age	Mean: 24,413 oocysts/g feces Range: 1–100,000 oocysts/g feces	*C. muris* recovered from animals with a wide range of ages
Wade et al. (2000)	Dairy cattle feces	*C. parvum*	Fecal samples collected rectally from dairy cattle at 109 farms in southeastern New York; data stratified by cattle age	Mean: 21,090 oocysts/g feces Range: 1–79,040 oocysts/g feces	*C. parvum* recovered only from calves less than 30 days of age

Table C-2. Summary of studies reporting abundance (concentrations) of *Giardia* in feces and related media of domestic animals

Study	Source/Media	Species	Description	Abundance	Notes
Hutchison et al. (2004)	Cattle farmyard manures and slurries	*G. intestinalis*	Results are for samples collected throughout Great Britain	Geometric mean and maximum cyst densities 10 and 5000 cysts/g, respectively	
Hutchison et al. (2004)	Swine farmyard manures and slurries	*G. intestinalis*	Results are for samples collected throughout Great Britain	Geometric mean and maximum cyst densities 68 and 160,000 cysts/g, respectively	
Ralston et al. (2003)	Range beef calf and dam manures	spp.	Fecal samples were collected from calves and dams from range operations in Canada	*Giardia* abundance in feces varied with animal age group. Density ranged from 0 at 1 week of age to a maximum of 2230 cysts/g (range 0–574,933 cysts/g of feces) of feces at 5 weeks of age. The geometric mean decreased after week 5 to a low of 2 cysts/g at 25–27 weeks of age	
Heitman et al. (2002)	Dairy cattle manure	spp.	Fecal samples collected from farms in Canada	Mean cyst range 1.5–29.9 cysts/g	
Heitman et al. (2002)	Pig manure	spp.	Fecal samples collected from farms in Canada	Mean cyst density 16.1 cysts/g	
Wade et al. (2000)	Dairy cattle manure	spp.	Fecal samples collected from 212 farms in southeastern New York	1–85,217 cysts, mean of 3039 cysts/g feces	

Table C-3. Summary of studies reporting abundance (concentrations) of *Campylobacter* in feces and related media of domestic animals

Study	Source/Media	Species	Description	Abundance	Notes
McLaughlin et al. (2009)	Swine lagoons	spp.	Samples taken from swine lagoons receiving wastes from sows, nursery pigs, or finishing pigs.	Sows lagoon: mean density of 5100 CFU/100 mL Nursery lagoon: mean density of 3500 CFU/100 mL Finisher lagoon: mean density of 1900 CFU/100 mL	For each type of waste, 10 lagoons were sampled; all farms located in the mid-south U.S.
Moriarty et al. (2008)	Dairy cattle	spp.	Samples taken from 4 farms considered to span conditions in New Zealand	For all seasons: median 430 CFU/g, range 15–1.8×10^7 CFU/g	Prevalence of *C. jejuni* and *C. coli* reported, but not related to abundance in manure; *Campylobacter* abundance bi-modally distributed among samples
Bull et al. (2006)	Chicken breeder and broiler flocks	spp. (study included speciation)	Samples taken from environmental areas (puddles, air) and from fresh fecal deposits on house floors	Densities varied within a relatively small range among flocks (for flocks colonized by *Campylobacters*) and did not change significantly once a flock was colonized; the range of observed densities was 4.0×10^4–5.0×10^6 organisms/g feces	Species prevalence among samples positive for *Campylobacter* differed between flocks; 5 flocks colonized by *C. jejuni* exclusively, 1 flock with *C. coli* exclusively, and 1 flock with both
El-Shibiny et al. (2005b)	Chicken (range of values from published studies)	spp.	Estimates based on multiple published studies	10^6–10^9 CFU/g excreta	
Hutchison et al. (2005)	Cattle	spp.	Composite samples of manure from pens collected	320 CFU/g for fresh feces 530 CFU/g for stored feces	
Hutchison et al. (2005)	Swine	spp.	Composite samples of manure from pens collected	310 CFU/g for fresh feces 1600 CFU/g for stored feces	
Hutchison et al. (2005)	Chicken	spp.	Composite samples of manure from pens were collected	260 CFU/g for fresh feces 590 CFU/g for stored feces	
Dorner et al. (2004)	Chicken (broiler) feces	spp.	Abundance data from multiple studies were pooled and fit to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β) = (27.78, 0.2558)	
Dorner et al. (2004)	Nursing or weaner pigs	spp.	Abundance data from a single study (Weijtens et al., 1999) fitted to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β) = (4.419, 0.6319)	

Study	Source/Media	Species	Description	Abundance	Notes
Dorner et al. (2004)	Sows and gilts	spp.	Abundance data from two studies (Weijtens et al., 1997; Weijtens et al., 1999) fitted to a gamma distribution	Gamma-distributed abundance, distribution parameters (α, β)= (4.207, 0.8859)	
Inglis et al. (2004)	Feedlot cattle feces	*C. jejuni*, *C. lanienae*, *C. hyointestinalis, C. coli*	Abundance in fresh feces from feedlot cattle housed individually	*C. jejuni* density range 0.01–1.03 $\log_{10}$ cells/g (via RT-PCR); *C. lanienae* density ranged from 1.47–4.74 $\log_{10}$ cells/g; *C. coli* not detected in any sample; *C. jejuni* detected 1.4% of fecal samples (range 8.2–16.7%)	Longitudinal study duration of 4 months
Cox et al. (2002)	Chicken feces	spp.	Results from composite of samples taken from 35 commercial broiler farms; results segregated by age of chicken (breeders vs. broilers)	Breeders: 2.8–3.9 $\log_{10}$ CFU/g feces Broilers: 3.5–6.5 $\log_{10}$ CFU/g feces	*Campylobacter* less prevalent in broilers (offspring) than breeders, but shedding (colonization) was higher in broilers than breeders
Whyte et al. (2001)	Chicken feces	spp.	Fecal samples from sacrificed chickens from 10 Irish farms were enumerated for *Campylobacter*. Although samples analyzed before, during, and after transport to a processing facility, the only values quoted here are for before transport. Studies conducted in Ireland.	6.11±0.37 $\log_{10}$ CFU/g feces for 5 farms 6.61±0.38 $\log_{10}$ CFU/g feces for 5 additional farms	

Study	Source/Media	Species	Description	Abundance	Notes
Weijtens et al. (1999)	Fattening pig feces from 10 weeks of age to 25 weeks	spp.	For each sampling event, 6 feces samples collected per pig. Pigs monitored from birth and housed with 16 pigs each on an experimental farm.	At 13 weeks: mean fecal *Campylobacter* density 4.1±0.7 $\log_{10}$ CFU/g (*n* = 8 pigs, average of 6 fecal samples per sampling event per pig) At 19 weeks: mean fecal *Campylobacter* density 3.3±1.0 $\log_{10}$ CFU/g (*n* = 8 pigs, average of 6 fecal samples per sampling event per pig) At 25 weeks: mean fecal *Campylobacter* density 2.0±0.1 $\log_{10}$ CFU/g (*n* = 8 pigs, average of 6 fecal samples per sampling event per pig)	The abundance (and prevalence) of *Campylobacter* varied weekly and between fecal samples on a given sampling event. Several pigs had periods of non-detectable fecal *Campylobacter* between periods of high fecal *Campylobacter* abundance. Abundance highest shortly after colonization and generally decreased with age.
Stanley et al. (1998)	Beef cattle feces	spp.	Fresh beef cattle sampled at slaughter	610 MPN/g feces	
Stanley et al. (1998)	Dairy cattle feces	spp.	Fresh dairy cattle manure samples collected in pens of 4 dairy herds in the United Kingdom	Adult cows: 69.9 MPN/g feces (SD 3) Calves: 33,000 MPN/g (SD 170)	Two peak periods (seasonal) of shedding were noted
Weijtens et al. (1997)	Sow feces at one week prior to delivery	spp.	Sow feces were sampled and bacteria were enumerated 1 week prior to delivery	5.0±1.1 $\log_{10}$ CFU/g (farm 1, n=5) and 3.6±0.4 $\log_{10}$ CFU/g (farm 2, n=5)	Prevalence data for sows and piglets were also collected at 1 week, 4 weeks and 8 weeks post-delivery

Table C-4. Summary of studies reporting abundance (concentrations) of *Salmonella* in feces and related media of domestic animals

Study	Source/Media	Serotype	Description	Abundance	Notes
Haley et al. (2009)	Stream waters	spp.	Water samples from a mixed use (livestock, on-site septic system, small community) watershed were sampled for *Salmonella*	Geometric mean of *Salmonella* in waters did not vary greatly among sampled sites. The highest and lowest mean densities were 0.746 MPN/100 mL and 0.496 MPN/100 mL	
McLaughlin et al. (2009)	Anaerobic primary lagoon effluent	spp.	37 lagoons sampled; lagoons received waste from sow, nursery, or finisher operations	Mean densities for sow, nursery and finisher operations were 28, 34, and 6.2 CFU/100mL, respectively	All lagoons sampled were located in the mid-south of U.S.
Kunze et al. (2008)	Feedlot cattle feces	All	Fecal samples taken from feedlots	Among samples positive for *S. enterica*, mean fecal density was 0.75 $\log_{10}$ / g	
Boes et al. (2005a)	Swine manure slurry from 62 herds	*Typhimurium*	Samples were drawn from swine manure slurry and from soil after application of swine manure slurry	*Salmonella*e detected in all slurry samples. Average *Salmonella Typhimurium* density was 0.2 CFU/g (note: not $\log_{10}$ CFU); maximum density was estimated to be 2500 CFU/g for a sub-clinically-infected herd; observed abundance among 112 slurry samples was 33% of samples with < 0.1 MPN, 13% of samples between 0.1 and 1 MPN, 28% between 1 and 10 MPN, 12% between 10 and 110 MPN, and 14% > 100 MPN.	Danish farms; authors proposed a polynomial survival model for *Salmonella* in soil
Hutchison et al. (2004)	Fresh pig manure	spp.	Multiple commercial farms	Geometric mean of 600 CFU/g (n = 10) Maximum observation of 78,000 CFU/g	Wastes taken from farms throughout Great Britain and results believed to be representative of overall prevalence in the region
Hutchison et al. (2004)	Fresh chicken manure	spp.	Multiple commercial farms	Geometric mean of 220 CFU/g (n = 12) Maximum observation of 22,000 CFU/g	Wastes taken from farms throughout Great Britain and results believed to be representative of overall prevalence in the region

Study	Source/Media	Serotype	Description	Abundance	Notes
Hutchison et al. (2004)	Fresh cattle manure	spp.	Multiple U.K. commercial farms	Geometric mean of 2100 CFU/g (n=62) Maximum observation of 580,000 CFU/g	Wastes taken from farms throughout Great Britain and results believed to be representative of overall prevalence in the region; *Salmonella* density higher in stored manure than fresh manure
Byrd (1998)	Cecal material and chicken litter from hatcheries	*Typhimurium*	Day-old chicks were challenged with 100, 10^4, or 10^6 *Salmonella typhimurium* by gavage. Litter and cecal contents were monitored for 17 days.	Pens containing chicks inoculated with 100 *Salmonella*e: 2.05 to 3.03 $\log_{10}$ CFU/g litter (n=*10*) Pens containing chicks inoculated with 10^4 *Salmonella*: 2.39 to 4.55 $\log_{10}$ CFU/g litter (n=*10*) Pens containing chicks inoculated with 10^6 *Salmonella*: 3.65 to 4.42 $\log_{10}$ CFU/g litter (n=*10*)	Cecal colonization rate and *Salmonella* count in cecal contents varied according to challenge dose. The number of chicks inoculated (5%, 10%, 25% and 50% of chicks in a pen) did not influence the overall incidence of infection in the pen.

Table C-5. Summary of studies reporting abundance (concentrations) of *E. coli* O157:H7 in feces and related media of domestic animals

Study	Animal/Source	Media	Description	Abundance	Notes
Hutchison et al. (2004)	Swine	Manure	Samples collected from multiple commercial farms in the U.K.	Geometric mean of 3900 CFU *E. coli* O157/g (n=15); highest observed density 750,000 CFU *E. coli* O157/g	
Booher et al. (2002)	Swine	Fecal samples	Fecal samples taken on 3 sucessive days during each of the following three post-inoculation periods: days 2–4; days 14–16; days 58-60	High dose experiments: 5 to 10^3 CFU/g feces Low dose experiments: 5 to 10^4 CFU/g feces (Note: values are approximate because they were obtained from a visual inspection of figures in the paper)	High dose: pigs inoculated with a mixture of 5 *E. coli* strains at 10^{10} CFU per strain Low dose: pigs fed 2 STEC O157:H7 strains at a dose of 10^7 CFU per strain and the other 3 strains at a dose of 10^{10} CFU per strain (low dose)
Kudva et al. (1998)	Sheep	Manure pit	Composite samples (from manure pits receiving waste from multiple animals) collected and enumerated for *E. coli* O157:H7. Experiments conducted in Idaho	1.15×10^8 CFU/g feces from a composite sample	Prior to shedding, sheep experimentally inoculated with *E. coli* O157:H7; some of the animals contributing to the manure pit were not infected
Kudva et al. (1998)	Cattle	Manure	Composite samples (from manure pits receiving waste from multiple animals) collected and enumerated for *E. coli* O157:H7; experiments conducted in Idaho	Two samples yielded 2.04×10^7 CFU/g feces and 4.35×10^8 CFU/g feces	Prior to shedding, cattle experimentally infected with *E. coli* O157:H7; some of the animals contributing to the manure pit were not infected
Kudva et al. (1998)	Cattle	Manure slurry	Untreated slurries and treated slurries (the retentate post-storage and separation) sampled and enumerated for *E. coli* O157:H7; experiments conducted in Idaho	Two samples of untreated slurry yielded 1.02×10^6 CFU/mL and 2.36×10^6 CFU/mL. A single sample of treated slurry yielded 2.35×10^6 CFU/mL	Prior to shedding, cattle were experimentally infected with *E. coli* O157:H7; some of the animals contributing to the manure pit were not infected
Schoeni and Doyle (1994)	Chickens	Fresh fecal samples	Chickens inoculated orally with *E. coli* varying from 2.6×10^1–2.6×10^5 CFU/chicken	Short-term experiment: highest level of inoculation (2.6×10^5) *E. coli* detected in feces averaging 4.6×10^2 CFU/gram of feces Long-term experiment: chickens inoculated with 1.3×10^8 CFU/chicken average recovery picked at 4 months at 3.2×10^6 CFU	

Appendix D. Farm Factors Data

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Alter et al. (2005)	*Campylobacter* (all isolates identified as *C. coli*)	Swine farms including slaughter pig producing operations (farrow to finishing) operations for finishing only A single "ecological" farm with free range access	Factors evaluated with respect to their association with *Campylobacter* in feces from: • individual animals • animal age (new-born/weaner, nursery pig, fattening, at slaughter) • organic vs. conventional farming	No *Campylobacter* detectable in feces of piglets at the day of birth. *Campylobacter* incidence rose within days to 32.8%; after transfer to the nursery unit prevalence increased to 56.6%. Approximately two-thirds of pigs remained *C. coli* shedders in the fattening unit; detection rate before transportation was 79.1%. Based on a single organic operation, infection with and shedding of *C. coli* at the organic operation appeared to occur at an earlier age than in conventional operations. On conventional farms, the best predictor of prevalence in a given growth stage is infection prevalence in prior growth stage. Greatest increase in prevalence occurred during piglet weaning. Prevalence of infection in piglets was not related to prevalence of infection in mothers (sows). A single farm had a much lower infection rate at slaughter than all other farms (5.3%); this difference could not be attributed to difference in rearing system or hygienic conditions.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Arsenault et al (2007)	*Salmonella* spp. *Campylobacter* spp.	Broiler chicken and turkey farms in Quebec, Canada	Factors evaluated with respect to presence of *Campylobacter* and *Salmonella* in pooled cecal contents of approximately 30 birds per flock evaluated The most important factors were: • age at slaughter • # birds in chicken house • cleaning practices • vermin control • distance to nearest manure heap • permanent locking of chicken house	Prevalence of *Salmonella*-positive flocks was 50% for chickens and 54% for turkeys Odds of *Salmonella* colonization were 2.6× greater for chicken flocks that failed to lock the chicken house permanently. No other factors were associated with a significant change in odds of *Salmonella* in chicken houses. In turkeys, odds of *Salmonella* colonization were 4.8–7.7× times greater for flocks that failed to be raised by <2 producers with no other visitors allowed onto the premises, or origin from a hatchery. Prevalence of *Campylobacter*-positive flocks was 35% for chickens and 46% for turkeys Odds of colonization were 4.1× higher for chicken flocks raised on farms with professional rodent control and 5.2× higher for flocks with manure heap >200 m from the chicken house, and also increased with the number of birds raised per year on the farm and with the age at slaughter. For turkeys, odds of *Campylobacter* flock colonization were 3.2× times greater in flocks having a manure heap at >200 m from chicken house and 4.2× greater in flocks drinking unchlorinated water.
Bae et al. (2005)	*Campylobacter* (all isolates speciated)	Cattle farms, including calf rearing, dairy, beef, and feed lot operations	Factors evaluated with respect to their association with the prevalence of *Campylobacters* in fresh feces; including: • farm type • calf rearing • dairy • beef • feedlot	Prevalence of *C. jejuni* and *C. coli* excretion differed by farm type. Highest *C. jejuni* prevalence was observed at beef cow-calf operations (47.1%) and the lowest at calf rearer operations (23.8%). Highest *C. coli* prevalence was at calf rearer operations (20.0%) and the lowest was at beef calf-cow operations (0.6%).

<table>
<tr><th>Study</th><th>Organism(s)</th><th>Farm Type(s)</th><th>Factor(s) and Notes</th><th>Major Findings</th></tr>
<tr><td>Barwick et al. (2003)</td><td>Cryptosporidium spp. and Giardia spp.</td><td>Dairy farms</td><td>Factors evaluated with respect to occurrence of Cryptosporidium or Giardia in dairy farm soils; evaluations made via multivariate logistic regression

The most important factors studied were:
• land use in the immediate vicinity of sample site
• soil pH
• herd prevalence of Cryptosporidium or Giardia</td><td><u>Cryptosporidium</u>
Risk factors associated with occurrence of Cryptosporidium in individual soil samples were:
• land use at the soil sample site (farming fields were 4× times more likely to have oocysts in soil than non-farming areas)
• soil pH (acidic soils were associated with a higher likelihood of Cryptosporidium detection)

<u>Giardia</u>
Risk factors positively associated with Giardia detection in soil were
• cattle access to soils
• herd-level prevalence of Giardia infection
• presence of grass cover and soil moisture (linear association with Giardia detection frequency, with higher prevalence in soils with higher moisture)</td></tr>
<tr><td>Beach et al. (2002)</td><td>Campylobacter spp. and Salmonella spp.</td><td>U.S. cattle feedlots and pasture operations</td><td>Factors evaluated with respect to their association with presence of Campylobacter and Salmonella in rectal samples of individual cattle.

Factors related to pre- and post-transport (to slaughter) infection rates were evaluated. The only factor related to pre-transport prevalence was animal origin
farm type (feedlot vs. pasture).</td><td>Campylobacter and Salmonella prevalences in pre-transport cattle are provided in the table below

<table><tr><th></th><th colspan="2">Pre-transport prevalence</th></tr><tr><th>Pathogen</th><th>Feedlot</th><th>Pasture</th></tr><tr><td>Campylobacter</td><td>64%</td><td>6.3%</td></tr><tr><td>Salmonella</td><td>3%</td><td>1%</td></tr></table></td></tr>
<tr><td>Besser et al. (2005)</td><td>C. jejuni</td><td>U.S. cattle feedlots</td><td>Factors evaluated with respect to association with C. jejuni in fresh feces taken from feedlots
duration of animal at feedlot
water chlorination</td><td>Prevalence of C. jejuni increased markedly from cattle arrival; in samples from pens with newly-arrived cattle, C. jejuni detected in 1.6% of fecal samples; in samples from pens with animals within two weeks of slaughter prevalence was 62.2%.

Water chlorination did not result in a significant difference in the prevalence of C. jejuni in fecal samples.</td></tr>
</table>

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Boes et al. (2005)	*C. jejuni*, *C. coli*	Danish swine herds from farms with and without mixed livestock production	Factors evaluated with respect to their association with *C. jejuni* and *C. coli* in individual fecal samples, and mixed livestock on farm	No significant herd-level or animal level difference in prevalence of *C. jejuni* or *C. coli* in herds on farms with or without mixed production. The ratio of *C. coli to C. jejuni* in swine feces was consistent across herds with and without mixed production.
Bull et al. (2006)	*C. jejuni*, *C. coli*	Housed broiler chicken operations in the U.K.	Factors evaluated with respect to their association with occurrence of *C. jejuni* and *C. coli* in floor fecal droppings. Factors evaluated were: • time (bird age and time since arrival in house) • environmental conditions (presence of *Campylobacter* in the broiler house environment)	Once colonized with *Campylobacter*, flocks' fecal droppings tended to have consistent and high densities of *Campylobacte*r. Flock level prevalence of *Campylobacter* increased from 10% of flocks (n=10) at 18 days to 40% of flocks between 28 and 33 days and to 60% at depletion. Among colonized flocks, five colonized by *C. jejuni* exclusively, one colonized with *C. coli* exclusively, and one colonized with both species.
Cho et al. (2006)	STEC*i*	Dairy cattle, farm environment and county fairs in Minnesota	Organic and conventional farms, calf pens, county fairs Note that organic agriculture is a production system that seeks to promote and enhance the health of agroecosystems by using few inputs, avoiding synthetic substances and promoting animal welfare (Codex Alimentarius Commission, 1999).	Shiga toxic bacteria (STB) prevalence greater in organic farms compared to conventional farms especially at the individual sample level, but was not statistically significant when restricting the analysis to only herds with <100 cows or in a herd-level analysis. In samples collected from conventional farms, 2.3% of fecal samples were STB-positive and 65% of farms had at least one positive animal; 6.6% of fecal samples from organic farms were STB-positive and 87.5% of farms had at least one positive animal. STB detected from 17.4% of samples and 58.3% of manure piles at county fairs. Organic farms smaller than conventional herds with a mean of 37 and 132 milking cows per herd on organic and conventional farms, respectively ($p<0.01$). The percent of STB-positive samples for each farm (within-herd prevalence for each farm) ranged from 0 to 26% (median 5.4%) on organic farms and from 0 to 13.9% (median 1.1%) on conventional farms.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Cummings et al. (2009)	*Salmonella*	Dairy cattle from herds in the NE United States	Factors evaluated with respect to their association with prevalence of *Salmonella* in rectal fecal samples; factors evaluated were: • herd size • housing type • vaccination status • history of *Salmonella* infection	Herd size only significant predictor of *Salmonella* prevalence
Ebel et al. (1992)	*Salmonella* spp. and *S. enteriditis*	Spent hens from farms throughout the United States	Regional differences in pooled cecal contents for layers were evaluated	*Salmonella* recovered from 24% of pooled samples; *S. enteriditis* was recovered from % of pooled samples. Layer house prevalences of *S. enteriditis* among northern, southeastern, and western/central layer houses were 45%, 3% and 17%, respectively.
El-Shibiny el al. (2005)	*C. coli* and *C. jejuni*	Free range and organic chicken farms in the U.K.	Rearing cycle of free range (56 days) and organic chickens (73 days) evaluated for *C. coli* and *C. jejuni*	*Campylobacter* isolated from 68.5% of the organic birds and 90% of the free-range birds over the rearing cycles. Following initial colonization campylobacters were detected throughout the rearing period in both flocks, with the exception of a single bird at day 31 from the free-range flock. Organic flock was colonized by campylobacters susceptible to the majority of the antibiotics tested. Authors noted that the most intensively reared broilers are killed at approximately 35 days old, which is the time at which we have observed the succession of *C. jejuni* by *C. coli* as the dominant species in the free-range and organic flocks.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Edrington et al. (2004)	*Salmonella* and *E. coli* O157:H7	Dairy farms in the Midwest and NE United States	Factors evaluated with respect to their association with fecal shedding of pathogens, including: • season • year-to-year variation	*Salmonella* prevalence varied widely within farms, from year-to-year and from season to season. Although there was a general trend toward higher summertime *Salmonella* prevalences, this trend is small in the context of the overall variability in prevalence. *E. coli* O157:H7 prevalence was also highly variable, though seasonal effects were more easily observed; no *E. coli* O157:H7 positive samples were observed on any farm during any winter sampling event.
Ellis-Iversen et al. (2009)	*C. jejuni*, *C. coli*, *E. coli* O157	25 dairy and 10 beef cattle operations distributed through England, Wales, and Scotland	Farm factors evaluated were: • contact with other herds • housing • herd size • visit • number of suckling calves on farm • water trough hygiene • operation type • presence of ringworm-infected animals	*Campylobacter* Only herd size, water trough hygiene and number of suckling calves related to *Campylobacter* prevalence. Higher herd size associated with increased prevalence of *Campylobacter*, whereas more frequent water trough emptying and presence of suckling calves associated with decreased *Campylobacter* prevalence. *E. coli* O157 Larger herds associated with increased *E. coli* O157 prevalence and presence of chicken and suckling calves associated with reduced *E. coli* O157 prevalence.
Fossler et al. (2005a)	*Salmonella*	Multiple cattle and farm types evaluated, including organic and conventional	Factors evaluated were: • size • season • state • treatment with antibiotics • cattle type (sick, periparturient, to be culled, preweaned calf, healthy) • organic vs. conventional farm	Season, state, farm size and cattle status associated with *Salmonella* shedding . Farm type (organic vs. conventional) not associated with shedding. Midwestern states more likely to have cattle shedding *Salmonella* than cattle from NY *Salmonella* shedding more likely on farms with at least 100 cows. Cattle that had been treated with antibiotics were within 14 days less likely to shed *Salmonella.* There were too few large organic farms to evaluate the role of large herd sizes on *Salmonella* shedding in organic herds.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Fossler et al. (2005b)	*Salmonella*	Multiple cattle and farm types evaluated, including organic and conventional	Paper discusses herd level factors that modify the *Salmonella* shedding factors described in Fossler 2005a (above); many herd level characteristics were considered. Examples include presence of chickens, turkeys, pigs, and wild geese; maternity pens present; type of bedding; protein feeds stored in enclosed buildings (refer to Appendix A of that report for complete listing)	Herd size, season, and state forced into the models; note that this is the largest study of *Salmonella* shedding in dairy cows and the only study evaluating herd level characteristics. Herd levels factors in the model were: • lack of use of tiestall or stanchion facilities to house lactating cows • not storing all purchased concentrate or protein feeds in an enclosed building • not using monensin in weaned calf or bred heifer diets • access of lactating or dry cows to surface water • disposal of manure in liquid on owned or rented land • cows eating or grazing of roughage from fields where manure was applied in solid or liquid form and not plowed under during the same growing season Herd size not associated with *Salmonella* shedding. Season and State (location) associated with *Salmonella* shedding. Farm type not associated with *Salmonella* shedding (authors noted a lack of information in the literature on this topic).

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Fossler et al. (2005c)	*Salmonella*	Multiple cattle and farm types evaluated, including organic and conventional	Paper reviews herd level factors for *Salmonella* shedding in calves; many herd level characteristics considered (refer to Appendix A of that paper for complete listing)	Herd size, season, and state were forced into the models; however, herd size not associated with *Salmonella* shedding Management practices and characteristics that <u>were not</u> associated with *Salmonella* shedding in calves at $p<0.20$ after adjustment for effects of herd size, season, state of origin and the multiple sampling occasions per herd included: • farm type (organic vs. conventional) • percent of cows born off the farm • type of maternity facility • use of a chlorinated water source for dairy cattle • type of coccidiostats used • amount of colostrums fed to calves • washing of calf milk buckets between feedings • placement of sick cattle in a pen separate from other lactating cows • average herd milk somatic cell count The following <u>were associated</u> with an increased odds of *Salmonella* shedding in calves: • presence of *Salmonella* positive cow on the operation • lack of routine feeding of milk replacer containing antibiotics to preweaned calves • use of maternity housing as a hospital area for sick cows more than once a month
Franz et al. (2007)	STEC and *E. coli* O157:H7 genes	Organic (ORG) and low- input conventional (LIC) dairy farms in the Netherlands	Organic and low- input conventional (LIC) dairy farms; note, the majority of the management practices such as feeding regimen and housing conditions remains unclear and under debate	Prevalence of a gene specific for O157 was 52% overall, and was higher at organic farms (61%) than at LIC farms (36%), but the difference was not significan. Relatively more LIC farms were positive for all STEC virulence genes. The four manures that best supported *E. coli* O157:H7 (all organic) were derived from farms with exclusively Frisian Holstein cows, while two of the four farms from which the manure supported the worst survival of *E. coli* O157:H7 (two ORG and two LIC) harbored another breed (both ORG) next to Frisian Holsteins.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings

Garber et al. (2003)	SE	Layer operations–environmental samples	Factors explored with respect to their association with the presence of SE on environmental surfaces of layer operations included:
			• geographic region
			  ○ Southeast
			  ○ Central
			  ○ West
			  ○ Great Lakes
			• molting/age
			• floor rearing (vs. cage rearing)
			• rodent presence (measured via trapping)
			• cleaning and disinfecting between flocks
			• manure handling (flush vs. high; rise vs. deep pit)
			• presence of SE in feed
			• age of layer house
			• floor area per bird
				Overall, SE was isolated from 7.1% of layer houses; regional prevalence estimates were:
				• 0% in the southeast
				• 9.0% (standard error = 7.2) in the central region
				• 4.4% (standard error = 2.5) in the west, and 17.2% (standard error = 13.7) in the Great Lakes region
				
				Approximately 4% of houses with fewer than 100,000 layers were environmentally positive for SE, whereas 16.5% of houses with 100,000 or more layers were environmentally positive for SE.
				Molted flocks were more likely to be associated with SE than unmolted flocks of the same age.
				No association with SE was found for presence of SE in feed age of layer house floor area per bird.

Gebreyes et al. (2008)	*Salmonella*	Anti-microbial free and conventional swine systems	Locations (WI, NC, OH) Anti-microbial free and conventional production systems Note: swine raised in outdoor production units have full or partial outdoor access on dirt with open access to soil, vegetation and wild fauna.	Significantly higher (p=0.0001) seroprevalence of *Salmonella* from anti-microbial free herds (54%) than conventional herds (39%). *Salmonella* more common in anti-microbial free, outdoor niche market than conventional indoor reared herds, although there was some geographical variation in *Salmonella* (WI highest at 59%, followed by NC at 34%, and Ohio at 34%).
Harvey et al. (2004)	*C. jejuni*, *C. coli*	Dairy	Prevalence in fecal samples evaluated with respect to association with region of the U.S. (northeast, southwest, or Pacific west) predominant *Campylobacter* species	Low prevalence of *Campylobacter* observed overall (5.2% for the desert southwest, 2.9% for the northeast, and 5.0% for the Pacific west) and on-farm prevalence ranged from 0–10%. No difference in *Campylobacter* prevalence observed between regions.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Heuer et al. (2001)	*Campylobacter* spp.	Conventional and organic chicken (broiler) flocks	3 rearing systems evaluated: • organic • conventional • extensive indoor production farms	Campylobacters isolated from 100% of organic broiler flocks, from 36.7% of conventional broiler flocks and from 49.2% of extensive indoor broiler flocks. Proportion of *Campylobacter*-positive flocks significantly higher for organic flocks compared with conventional flocks ($p<0.001$) and extensive indoor flocks ($p<0.001$). Organic broiler flocks constitute a strong potential for introduction of *Campylobacter* to the processing line upon arrival at slaughter No single factor related to organic broiler production can be pointed out as the sole determinant of high *Campylobacter* prevalence; rather, prevalence results reported reflect the combined effect exerted by factors that are inextricably related to each broiler rearing system.
Hoar et al. (2001)	*Campylobacter*, *Giardia*, *Cryptosporidium parvum*	Beef cattle from 18 counties in CA	Factors evaluated were: • herd size (as number of females on the farm) • increased weaning age • scouring calves • purchase of replacement calves	*Campylobacter* Only herd size (number of females in herd) was associated with increasing prevalence of *Campylobacter.* *Giardia* No factors associated with an increased prevalence of *Giardia.* *Cryptosporidium* Prevalence associated with length and timing of the calving season, scouring calves, and mean herd proportion of cows.
Huston et al. (2002)	*Salmonella* spp.	Conventional dairy farms in OH	Factors evaluated with respect to their association with fecal shedding of *Salmonellae*; the most significant factors evaluated were: • herd size • season • housing • use of straw bedding	*Salmonella* prevalence significantly associated with herd size, use of free stalls for lactating and non-lactating cows, and use of straw bedding for non-lactating cows. No seasonal shedding pattern observed.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Kabagambe et al. (2000)	*Salmonella* spp.	Dairy farms, nationwide	Factors evaluated with respect to herd-level prevalence of *Salmonella* in feces; the most significant factors evaluated were: • herd size • region of the country (U.S.) • manure disposal method • manure management • drinking water hygiene and disinfection • feeding brewers yeast products	Herd size and region have a significant impact on *Salmonella* prevalence; cows from large herds (> 100 cows) had 5.8× greater odds of shedding *Salmonella* than cows from smaller farms. Cows from the south (defined as a large region including CA, and NM) had 5.7× greater odds of shedding *Salmonella* than cows from the north (defined as a large region including Oregon and Washington).
Kuhnert et al (2005)	STEC	Conventional and organic dairy farms in Switzerland	250 risk factor parameters evaluated including • management data (farm size, hosing condition, etc) • current milk production, others specified in Roesch et al. (2004) Note, several parameters known to be different between these two types of farming, ranging from feeding, therapy, animal husbandry, and to processing of the meat.	In general, no significant differences between the two farm types concerning prevalence or risk for carrying STEC or O157:H7 observed (cows tested 30 days postpartum). Overall prevalence level based on PCR was 58%. STEC detected in all farms and O157:H7 were present in 25% of organic farms and 17% of conventional farms. STEC detected in 58% and O157:H7 were evidenced in 4.6% of individual feces Risk-factors mainly related to the potential of cross-contamination of feeds and cross-infection of cows, and age of the animals. A reduced risk for the presence of STEC found for older than younger cows Increased risk for carrying STEC was associated with elevated milk concentrations of lactose or urea, with farms that had an Unifeed trailor (used for mixing feed) or a paddock. For O157, chain or lateral fixation versus bar fixation, and final milking manually or automatically were factors associated with an increased risk for the presence of O157:H7 Note, previous studies produced conflicting results regarding impact of diet on EC, some showed hay diet resulted in reduction of EC, others showed exact opposite results.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Le Jeune et al. (2004)	*E. coli* O157:H7	Beef cattle feedlots	Factors explored with respect to their association with prevalence (% positive samples) in feedlot pen manure, including cattle duration in feedlot supply of chlorinated water for troughs	There was a general trend toward increasing *E. coli* O157:H7 prevalence with duration of cattle in feedlot; however, periods of high prevalence were sporadic and unrelated to season or length of time cattle were in the feedlot. No significant difference in prevalence among cattle provided chlorinated drinking water and those not provided chlorinated drinking water.
Luangtongkum et al. (2006)	*Campylobacter*	Conventional and organic chicken farms	Conventional and organic broiler and turkey farms; antimicrobial resistance to widely used antibiotics evluated	*Campylobacter* species highly prevalent in both the conventional and organic chicken operations. Broiler rates for *Campylobacter* prevalence 65% (conventional) and 89% (organic) (significantly different); turkey rates 83% (conventional) and 87% (organic) Prevalence on conventional broiler farms slightly lower (44–80%) than organic farms (70–100%). Prevalence on conventional turkey farms similar (63–98%) to organic farms (6–100%) The high prevalence of Campylobacter strains in organically raised broilers in part seems to be associated with the increased age of the birds at slaughter. Study also indicates the influence of conventional and organic chicken production practices on antimicrobial resistance of *Campylobacter* on chicken farms.
Miller et al. (2008)	*Cryptosporidium*	Dairy farms in coastal CA	Factors evaluated with respect to their impact on density of oocysts in runoff water and oocyst loading from various farm locations, including: • use of structural BMPs (vegetative buffer strips, straw mulch) • animal age class	Both straw mulch and vegetative buffer strips provided significant reductions in oocysts loads. For vegetative buffer strips, load reduction found to be a function of the vegetative buffer strip length. For straw mulch, reductions related to the areal extent of mulch cover and the age class of cattle in lots where mulch was applied.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Miller et al. (2007)	*Giardia duodenalis*	Dairy lots and high-use cattle areas	Factors evaluated with respect to their association with the presence of *G. duodenalis* in runoff water: • type of dairy cattle (cow, milking or dry cow calving (calf, 2.1–6.0 months of age; calf, 0.1–2.0 months) • length of vegetated buffer (m) • number of cattle in lot • 24-hour precipitation (mm) • cumulative annual precipitation (mm) • lot area • slope	Increased concentrations and instantaneous loads of *G. duodenalis* associated with: • young calves • absence of vegetative buffer strips • presence areas of high cattle use The following did not have an association with concentrations or instantaneous loads of *G. duodenalis:* • percent slope • area of lot • cattle density • 24-hour precipitation • additional BMPs, such as straw mulching, seeding, removal of manure via scraping, and winter exclusion of cattle *G. duodenalis* concentration in runoff increased monotonically with precipitation up to a threshold precipitation depth, after which concentration decreased slowly with increasing precipitation depth.
Newell et al. (2003)	*Campylobacter* spp.	NA (review article)	Review article evaluating sources of *Campylobacter* in broiler chickens	Prevalence of flock positivity is dependent on flock size and the type of production system. Flock positivity is generally higher (up to 100%) in organic and free-range flocks compared to intensively reared flocks, which presumably reflects the level of environmental exposure of birds as well as the increased age of the birds at slaughter. Most reviewed studies found that water sources were a low-risk factor for positivity and that water contamination usually follows, rather than precedes, colonization of a flock.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Reinstein et al. (2009)	*E. coli* O157:H7	Organically, naturally, and conventionally raised cattle	Prevalence of *E. coli* O157 in organic-, natural-, and conventionally-raised cattle, comparison of antibiotic susceptibility profiles	Prevalences of *E. coli* O157:H7 were 14.8% for organically-raised cattle, 14.2% for naturally-raised cattle, and 11.2% for conventionally raised cattle. Study did not include a statistical comparison of the prevalence data because of a number of differences, particularly in diet, among the three production systems. Organically- and naturally-raised cattle are either required to graze a pasture or fed a forage-based diet. Cattle fed a forage diet have both higher levels and longer durations of fecal shedding of E. coli O157:H7 than cattle fed a grain diet (Van Baale et al. 2004). No major difference in antibiotic susceptibility patterns among the isolates observed.
Sato et al. (2004)	*Campylobacter* spp.	Organic and conventional dairy herds	Prevalence and antimicrobial susceptibilities of *Campylobacter* spp. isolates from bovine feces compared between organic and conventional dairy herds	Prevalence of *Campylobacter* spp. in organic and conventional farms was 26.7 and 29.1%, and the prevalence was not statistically different between the two types of farms *Campylobacter* prevalence was significantly higher in March than in September, higher in calves than in cows, and higher in smaller farms than in large farms. No evidence that restriction of antimicrobial use on dairy farms was associated with prevalence of resistance to ciprofloxacin, gentamicin, erythromycin, and tetracycline.
Trotz-Williams (2008)	*Cryptosporidium parvum*	Dairy farms (Ontario, Canada)	Prevalence of *Cryptosporidium parvum* in feces evaluated with respect to associations with: • number of calves • number of milking cows • perinatal management • management of pre-weaned calves • calf feeding and medications • other factors	30% of calves <1 month old shed *C. parvum* in the study and 77% of farms in the study had at least one shedding calf. Predictors associated with increased in-farm prevalence of shedding were the use of calf scour prophylaxis in cows and calves and feeding of milk replacer to young calves; predictors <u>not</u> associated with *C. parvum* shedding were number of calves, number of milking cows, and others.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Trotz-Williams et al. (2007)	*Cryptosporidium parvum*	Dairy farms (Ontario, Canada)	Prevalence of *Cryptosporidium parvum* in feces evaluated with respect to associations with: • calf age • calf birth season • calf birth in multi-cow calving pen • calf feeding regimen • calf and dam housing • other factors	Calves born in summer months were more likely to shed *C. parvum* than those born in summer months. Age of calves at sampling and the time calves remained with their dams after birth were both associated with prevalence of fecal shedding of *C. parvum.*
USDA (1997)	*E. coli* O157	Beef cattle feedlots	Factors evaluated with respect to their association with *E. coli* O157 in feces from: • Length of time cattle are on feed • Feeding barley Refer to USDA (1995) for sampling design details.	Pens for cattle that had been on feed <20 days were 3.4× more likely to have a positive *E. coli* O157 sample. Possible reasons include stress from transportation to the feedlot or animal mixing with the feedlot populations. Pens receiving some portion of barley were 2.75× more likely to have a positive *E. coli* O157 sample than pens receiving no barley. Possible explanation is the way barley is digested in cattle. Feeding barley is regional practice. States in this study feeding barley with positive feedlots were AZ, CA, ID, TX, and WA. Pens with cattle weighing >700 lbs upon entry to the feedlot were less likely to have a positive *E. coli* O157 sample. Possible reasons include larger cattle may handle transportation stress and a new environment better than smaller/younger cattle. Pens with at least 85% heifers (females) were less likely to have positive *E. coli* O157 samples; authors do not have an explanation. Other factors not found to be associated with positive samples: • ionophore use • feeding antibiotics, coccidiostats, probiotics, urea, and other food additives • animal density within pens • previous health status of cattle

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Van Overbeke et al. (2006)	*Campylobacter* spp. and *Salmonella enterica*	Conventional and organic broiler farms	Conventional vs. organic farms in Belgium	No significant differences could be found in prevalence of *Salmonella* between organic and conventional broilers; in contrast, *Campylobacter* infections were significantly higher in organic flocks (however, organic flocks were slaughtered at 12 weeks compared to 6 weeks for conventional flocks and age is a known important factor). In organic broilers, the following *Salmonella* serotypes were found: *Virchow*, *Hadar*, and *Livingstone*; serotypes *Mbandaka* and *Virchow* found on the conventional farms *C. coli* and *C. jejuni* found in both production systems. No statistically significant differences found between organic and conventional meat; these results are similar to other previous related studies.
Warnick et al. (2003)	*Salmonella* spp.	Dairy farms in the Midwest and northeast U.S.	Factors evaluated with respect to their impact on shedding prevalence, including: • cattle group (lactating, cows to be culled, cows to calf within 2 weeks, calves) • antibiotic treatment within two weeks • sick within previous week	Within-herd *Salmonella* prevalence and serotype prevalence varied widely over short time periods. In general, cows nearing calving exhibited greater *Salmonella* shedding than other groups, though high variability was observed in shedding from all groups.
Wesley et al. (2000)	*Campylobacter* spp.	Dairy farms throughout the U.S.	Factors evaluated with respect to herd prevalence and individual sample prevalence of *Campylobacter* in feces, including: • region • farm size • chlorination of drinking water	Region not associated with herd- or individual animal prevalence of *Campylobacter.* Farm size not associated with herd-level prevalence of *Campylobacter* shedding, but was with increased shedding prevalence on the animal level. Other factors associated with increased shedding included manure management practices. Drinking water chlorination not associated with a decrease in shedding prevalence.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Wilhem et al. (2009)	*Campylobacter*, *E. coli*, *Salmonella* spp., *Staphyloccus aureus*	Organic and conventional dairy farms	Review article with stated objective is to identify, evaluate, and summarize all primary research investigating the prevalence of zoonotic bacteria in organic dairy production or comparing organic and conventional dairy production	Bacterial outcomes reported in 17 studies. *Campylobacter*, *E. coli*, and *Salmonella* spp. were reported in 2, 7, and 4 studies, respectively. Contradictory findings reported for differences in bacterial outcomes between dairy production types (organic vs. conventional); these findings may result from geographic differences in organic regulations, baseline prevalences, laboratory methods used, or methods of analysis. Specifically, no significant difference in *Campylobacter* prevalence in fecal samples was found between organic and conventional farms. No significant differences in herd-level prevalence of (STEC) in the U.S. Greater individual prevalence on organic dairy farms. No differences in STEC prevalence was found between organic and conventional farms in Switzerland or the Netherlands. No significant difference was observed in the prevalence of *Salmonella* spp. in fecal samples, either at the farm or individual levels.

Study	Organism(s)	Farm Type(s)	Factor(s) and Notes	Major Findings
Young et al (2009)	*Campylobacter*, *E. coli*, *Salmonella* spp., *Staphyloccus aureus*	Organic and conventional farms. Chicken, swine and beef farms.	Review article summarizing published prevalences of zoonotic and potentially zoonotic bacteria in organic and conventional chicken, swine and beef production using systematic review and meta-analysis methodology	In 37 studies, specific bacterial and AMR outcomes were compared between organic and conventional chicken, swine or beef production. The prevalence of *Campylobacter* was higher in organic broiler chickens at slaughter (in 3/5 studies, in the others no difference was noted) *Campylobacter* isolates from conventional retail chicken were more likely to be ciprofloxacin-resistant. Bacteria isolated from conventional animal production exhibited a higher prevalence of resistance to antimicrobials; however, the recovery of some resistant strains was also identified in organic animal production. Limited or inconsistent research was identified in studies examining the prevalence of zoonotic and potentially zoonotic bacteria in other food-animal species. In four studies, researchers investigated *Salmonella* spp. in broiler chickens on farms or at slaughter in the U.S., Belgium, and Italy, and found very few or no positive samples in both organic and conventional populations. Conflicting results were reported in six studies that examined the prevalence of *Salmonella* spp. in swine on farms and at slaughter in the U.S., Denmark, and Germany. Studies conducted in the U.S. showed higher *Salmonella* prevalence in organic farms; international studies showed contrary results.