DES MOINES WATER TRAILS: HEALTH RISK FROM WATERBORNE PATHOGENS DURING RECREATIONAL WATER USE

August 14, 2023

Tucker Burch	U.S. Department of Agriculture, Agricultural Research Service, Laboratory for Infectious Disease and the Environment, Marshfield, WI
Joel Stokdyk	U.S. Geological Survey, Upper Midwest Water Science Center, Laboratory for Infectious Disease and the Environment, Marshfield, WI
Aaron Firnstahl	U.S. Geological Survey, Upper Midwest Water Science Center, Laboratory for Infectious Disease and the Environment, Marshfield, WI
Sarah Opelt	U.S. Department of Agriculture, Agricultural Research Service, Laboratory for Infectious Disease and the Environment, Marshfield, WI
Rachel Cook	U.S. Department of Agriculture, Agricultural Research Service, Laboratory for Infectious Disease and the Environment, Marshfield, WI
Mark Borchardt	U.S. Department of Agriculture, Agricultural Research Service, Laboratory for Infectious Disease and the Environment, Marshfield, WI

Background

Recreational sites are being developed on waterways in the Des Moines metro area as part of the Iowa Water Trails (Iowa Confluence Water Trails 2023). Waterways can be contaminated by enteric pathogens that cause acute gastrointestinal illness (AGI) when ingested during recreation (Garcia-Aljaro et al. 2018). Waterborne enteric pathogens originate in wastewater, livestock manure, and wildlife feces and can enter waterways through various pathways, including runoff, failing sanitary infrastructure, and combined sewer overflows (Garcia-Aljaro et al. 2018; Alegbeleye & Sant'Ana 2020; Whitman et al. 2011). The agricultural and urban land use surrounding the recreation sites represent potential sources of waterborne pathogens, so water quality assessments are needed to determine the suitability of these waterways for recreation.

Recreational water quality is commonly assessed using the bacteria *Escherichia coli*, which is an indicator of fecal contamination that generally does not cause illness (USEPA 2012; Saleem et al. 2022; Ashbolt et al. 2001). However, fecal indicator bacteria like *E. coli* may not be suitable for characterizing health risk in all situations because they have weak correlation with the presence of disease-causing pathogens and they originate in various fecal sources that vary in the degree of health risk posed to recreators (Soller et al. 2010; Korajkic et al. 2018). The disparity between *E. coli* and illness may be greater in areas where multiple pollution sources occur, like where human wastewater, livestock manure, and wildlife feces are common (Soller et al. 2010). In such situations, risk can be evaluated by measuring the pathogens and fecal sources that are present at individual recreational sites.

Sources of fecal pollution in water can be assessed using microbial source tracking (MST) (Harwood et al. 2014). MST uses the presence of host-specific fecal microorganisms to associate contamination with the host. For example, some microbes are specific to the gastrointestinal tract of humans and do not grow in animals, so they are only shed in human fecal material (Shanks et al. 2010). When these human-specific fecal microbes are found in water samples, it indicates that human fecal contamination is present. MST markers have been established for various hosts, including humans, livestock, and birds.

The risk of waterborne illness is commonly assessed using quantitative microbial risk assessment (QMRA; Haas et al. 2014). QMRA is a predictive approach that uses pathogen levels in water to determine risk, so it is useful for estimating risk prior to documented cases of illness, like when planning the development of recreational sites. It can also be used to evaluate factors that affect the level of risk (e.g., Burch et al. 2021; Corsi et al. 2016).

For six recreational sites in the Des Moines metro area, Iowa, the health risk posed to recreators during activities like swimming, kayaking, and fishing was characterized. Water samples collected over two years were tested for pathogens, and health risk was estimated using QMRA. Risk was categorized by fecal source (human and animal) and compared to standard *E. coli* measurements.

Objectives

The report includes four study objectives:

1. Collect water samples from six stream sites over two recreational water seasons. Test samples for waterborne pathogen genes and MST markers that distinguish between human, bovine, porcine, and avian fecal sources.
2. Estimate the average risk of AGI per exposure across the recreational season (May – October) from exposure to pathogens during various water recreation activities at each site.

3. Categorize health risk by fecal source using microbial tests that distinguish between human, bovine, porcine, and avian fecal sources.
4. Evaluate the performance of *E. coli* for identifying elevated risk of illness.

Objective 1. Occurrence of pathogens and identification of fecal sources

Approach: Sites, sample collection, and sample analysis

Six stream sites in the Des Moines, Iowa, metro area that are existing or planned recreation areas were selected for inclusion in the study (Fig. 1). Sites were located in Polk County, where agriculture is the primary land use, with 51% of land area in farms (NASS 2019). Sites included Beaver Creek, the Des Moines River, Fourmile Creek North, Fourmile Creek South, the Raccoon River, and Walnut Creek. Recreation activities at these sites may include swimming, wading, fishing, rafting, and kayaking. Semi-monthly samples were collected over two water recreation seasons (May – October 2020 and 2021); 23 to 26 samples were collected per site (147 total). A Teledyne ISCO automated sampler collected one bottle of water every 12 minutes over approximately 4 hours (mean: 3.85 hrs, range: 0.2 – 4.0 hrs), and then 100 mL from each bottle (n = 20) was composited. Grab samples (n = 20) were collected when automated sampling was not possible.

Samples were tested for pathogen genes using quantitative polymerase chain reaction (qPCR), a genetic testing technique. Samples positive for *Cryptosporidium* spp. were further tested by microscopy and genetic sequencing. Fecal sources were identified by microbial source tracking (MST), in which genetic tests identify microbes that are found in the feces of certain animals. Samples were analyzed using nine qPCR tests for genes of microbes that are specific to human, cattle/ruminant, pig, or bird feces. Negative controls were analyzed to ensure that laboratory contamination was absent, and positive controls verified method performance. Laboratory procedures are described in Appendix A.

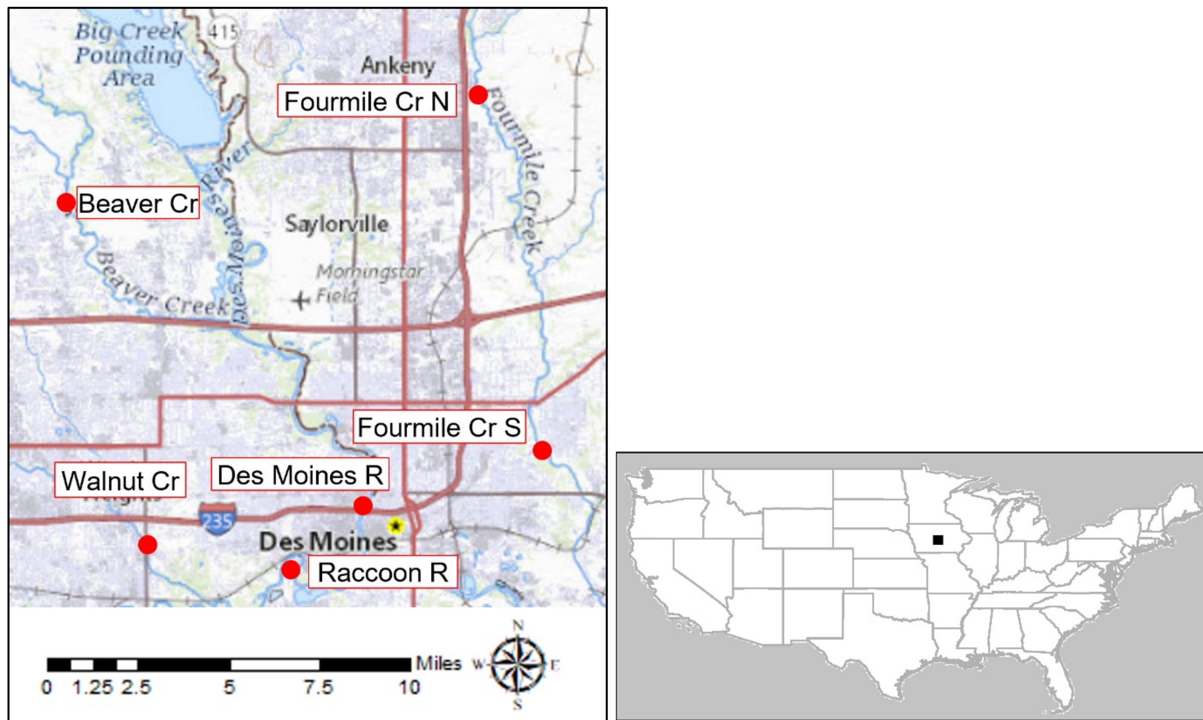

Figure 1. Locations of study sites in the Des Moines metro area; inset shows study location in Polk County, Iowa, USA (USGS 2022). Station numbers for sites are 05482000, 05484900, 05484800, 05481950, 05485605, and 05485640 (USGS 2023).

Key findings

- Pathogen genes were detected at all 6 sites and in 97 of 147 samples (66%) (Table 1.1).
- Many pathogen types were detected, including viruses, bacteria, and protozoa. Some can only infect humans, while others, known as zoonotic pathogens, can be shared by humans and animals.
- *Cryptosporidium* spp. was the most common pathogen detected (61 of 147 samples), and it was detected at all sites (Table 1.1). *Cryptosporidium* oocysts were observed in 54 of 61 qPCR-positive samples using immunofluorescent microscopy.
- Since not all *Cryptosporidium* spp. cause illness in humans, follow-up analyses were conducted using qPCR and genetic sequencing to identify the *Cryptosporidium* species detected. Follow-up analyses were successful for 23 of 61 samples, and 3 species were detected: *C. bovis*, *C. meleagridis*, and *C. parvum* (Table 1.1).
- Human (56% samples), bovine/ruminant (45%), avian (41%), and porcine (10%) fecal sources were detected, including 70 samples (48%) that were positive for multiple fecal sources.

Context and interpretation

Recreational water quality is commonly assessed using the indicator *E. coli*, which detects fecal contamination but does not identify the type or quantity of pathogens that may be present. Studies that rely only on indicators must assume the type and quantity of pathogens when estimating risk (e.g., Boehm & Soller 2020). In contrast, pathogen measurements provide site-specific data to improve the representativeness of risk estimates. For example, *Cryptosporidium* species known to infect humans were detected and therefore contributed to health risk, while other waterborne pathogens like *Giardia duodenalis* and hepatitis E virus were not included in risk estimates because they were not detected at these sites.

At least one pathogen gene was detected in 66% of all samples, and the occurrence of individual pathogens was intermittent. For example, *Salmonella* spp. was detected in 5 of 147 samples (3%; Table 1.1). The sporadic presence of pathogens observed at these sites is consistent with other studies (Corsi et al. 2014; Lenaker et al. 2018) and reflects variation in factors like weather and land use (e.g., rainfall, stream flow, manure application). Potential exposure to pathogens during water recreation can therefore change over time as the quantity and type of pathogens change.

Genetic markers for many pathogen types were detected. The pathogens tested originate in fecal sources, like wastewater or manure, so their presence depends on the proximity of fecal sources and whether the fecal sources contain pathogens. The pathogens that only infect humans originated in human feces, but many of the pathogens can be found in fecal material from humans, livestock, or wildlife (Jorgensen et al. 2015). Inclusion of MST with the pathogen measurements allowed an assessment of the fecal sources that contributed to pathogen presence.

Human, bovine, avian, and porcine fecal sources were identified at these sites based on MST analysis of water samples, and it was common for a single sample to be positive for multiple MST markers. Along with the variety of pathogens detected, the MST results indicate that multiple contamination sources affect water quality at these sites. Leaking sanitary sewers, wastewater discharges, septic systems, livestock manure, and wildlife feces are possible sources of the pathogens and MST markers detected. The relative occurrence of specific fecal sources differed by site, but no single fecal source dominated overall (Table 1.1).

The study design and methods inform interpretation of results for Objective 1. The goal of this objective was to characterize pathogen presence and fecal sources across the swimming season. The

number of samples per site is sufficient to achieve that goal, but temporal trends at shorter timescales (e.g., weeks or months) cannot be reliably assessed. Likewise, the pathogens and fecal sources identified during the study may change in response to factors that influence water quality, like weather and land use.

Use of qPCR facilitated the analysis of many pathogens and allowed analysis of fecal source-specific microbes (Table 1.1). Quantitative PCR is a genetic technique and does not distinguish between living and dead microorganisms. However, this characteristic is not relevant for its use with MST markers (results indicate the fecal source regardless of microbes being alive or dead), and it is accounted for when estimating health risk using published dose harmonization factors (see Appendix B). In addition, microscopy confirmed the presence of oocysts for 89% of samples positive for *Cryptosporidium* spp., which was the most frequently detected qPCR target. Samples positive for *Cryptosporidium* spp. by qPCR that were negative by microscopy may reflect the greater analytical sensitivity of qPCR, detection of non-viable genetic material, or other methodological differences.

Table 1.1. Occurrence of pathogens and microbial source tracking markers by site for samples collected May – October of 2020 and 2021.

		Number of positive samples [a]						
Microbe type	Microbe	Beaver Cr (*n* = 25)	Des Moines R (*n* = 25)	Fourmile Cr N. (*n* = 23)	Fourmile Cr S. (*n* = 24)	Raccoon R (*n* = 26)	Walnut Cr (*n* = 24)	All sites (*n* = 147)
Avian fecal microbe	Avian associated *Bacteroidales*	11	7	7	12	10	14	61
Bovine/ruminant fecal microbe	*Bacteroidales*-like Cow M2	2	0	0	2	0	0	4
	Bacteroidales-like Cow M3	0	0	1	1	0	1	3
	Ruminant *Bacteroides*	18	3	13	9	11	12	66
	(Any bovine/ruminant microbe)	18	3	13	9	11	12	66
Porcine fecal microbe	Pig-Bac-1	7	2	1	1	1	0	12
	Pig-Bac 2	0	2	1	3	0	0	6
	(Any porcine microbe)	7	3	1	3	1	0	15
Human fecal microbe	*Bacteroidales*-like HumM2	14	7	5	2	1	3	32
	Human *Bacteroides*	21	14	10	14	7	12	78
	Pepper mild mottle virus [b]	17	4	4	3	1	4	33
	(Any human microbe) [c]	22	14	11	14	8	13	82
Pathogen	Norovirus genogroup 1 [d]	1	0	0	0	0	0	1
	Norovirus genogroup 2 [d]	4	0	0	0	0	2	6
	Human adenovirus groups A-F [d]	4	0	0	0	0	0	4
	Enteropathogenic *E. coli* [d]	1	0	1	0	0	1	3
	Shiga toxin 1-producing bacteria [d]	1	0	0	0	0	1	2
	Shiga toxin 2-producing bacteria [d]	2	0	1	0	1	2	6
	Campylobacter jejuni [d]	0	0	2	4	2	5	13
	Salmonella [d]	1	0	2	1	1	0	5
	Cryptosporidium spp. [d]	10	14	5	9	9	14	61
	Cryptosporidium bovis [d,e]	4	4	1	3	3	7	22
	Cryptosporidium meleagridis [d,e]	0	0	0	0	1	0	1
	Cryptosporidium parvum [d,e]	0	0	1	0	1	0	2
	Human polyomavirus	0	0	0	0	0	1	1
	Rotavirus A	7	2	9	9	6	9	42
	Staphylococcus aureus	0	3	0	0	0	2	5
	(Any pathogen)	15	17	13	18	17	17	97

Note: While tests identified microbe-specific genes, microbe names are listed for clarity of presentation. The number of positive samples for any bovine microbe, any porcine microbe, any human microbe, and any pathogen does not equal the sum of individual microbes because some samples were positive for multiple microbes.

[a] 10 microbes were not detected: *Cryptosporidium hominis*, *C. baileyi*, *C. galli*, *C. suis*, *C. ubiquitum*, *Giardia duodenalis*, hepatitis E virus, enterovirus, rotavirus C, and *Shigella*/enteroinvasive *E. coli*.

[b] The plant pathogen "pepper mild mottle virus" is common in human wastewater and often used as a marker of human fecal contamination, but it may originate in other sources and was therefore not included in the count of samples positive for any human microbe.

[c] In addition to *Bacteroidales*-like HumM2 and human *Bacteroides*, counts of any human microbe include the human-specific pathogens norovirus genogroup 1, human adenovirus groups A-F, and human polyomavirus.

[d] Designates pathogen included in QMRA.

[e] *Cryptosporidium bovis, meleagridis,* and *parvum* were identified via follow-up analyses after initial qPCR tests detected *Cryptosporidium* spp. *C. bovis* was identified by a species-specific qPCR analysis, while *C. meleagridis* and *C. parvum* were identified by Sanger sequencing. See Appendix A for additional details.

Objective 2. Risk estimates for AGI during water recreation

Approach: Quantitative microbial risk assessment (QMRA)

QMRA was used to estimate the risk of AGI for accidental consumption of water during recreation. The QMRA estimated risk for each of the six study sites. The primary QMRA inputs were 1) pathogen concentrations measured in water, 2) dose-response models that predict the probability of illness for individual pathogens, and 3) water ingestion volumes for each recreational activity. Pathogen concentrations come from the current study as described under Objective 1. Dose-response parameters and water ingestion volumes come from previous studies published in the scientific literature (Appendix B). QMRA followed the standard steps of hazard identification, exposure assessment, dose-response assessment, and risk characterization (Haas et al. 2014), and the QMRA was organized with respect to three main factors: site, activity, and pathogen.

The QMRA considered four recreational water activities to provide information on a range of possible uses for each site:

- <u>Swimming – all ages</u>: Swimming for a population composed of all age groups. This provides a representative risk estimate for a population of average demographic characteristics (e.g., typical age structure and approximately equal numbers of males and females). This is the most common type of risk estimate found in the scientific literature, so it provides a useful reference point to previous studies.
- <u>Swimming – children:</u> Swimming for children ages 6-12 years old. This provides a conservatively high risk estimate because children 6-12 years old swallow the most water while swimming (compared to other age groups), and differences in risk across activities are determined by the amount of water swallowed.
- <u>Limited contact recreation without head immersion</u>: Limited contact activities represent many of the non-swimming activities planned for development at these sites, like kayaking and fishing. These activities do not involve as much direct contact with the water as swimming, but they can still result in very small amounts of water ingestion (e.g., hand-to-mouth contact after touching water).
- <u>Limited contact recreation with head immersion:</u> Same as above, but this activity type assumes a person's head was immersed in the water (e.g., tipping over in a kayak).

The QMRA was completed for the pathogens designated in Table 1.1. Based on the dose-response models used, norovirus genogroups 1 and 2 were combined for QMRA, and the shiga toxin-producing bacteria were combined with enteropathogenic *E. coli* (EPEC). Thus, QMRA included adenovirus, norovirus, *Campylobacter jejuni*, EPEC, *Salmonella* spp., *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium bovis*, and ungenotyped *Cryptosporidium* spp. Pathogens that were not detected at a given site lacked the requisite concentration data to perform QMRA and were excluded from risk estimates for that site. Exclusion in this case implicitly assumes that risk from undetectable pathogens is minimal compared to risk from detectable pathogens.

Risk was estimated for each combination of site, activity, and pathogen. Pathogen-specific risk estimates were also combined to estimate total risk across all pathogens. These total risk estimates represent the overall risk expected for each activity at each site. Also, because they represent risk due to all detected enteric pathogens, total risk estimates can be compared to the EPA's benchmark for acceptable risk during water recreation (USEPA 2012). This benchmark varies by state. For Iowa, it is 36 illnesses per 1,000 water recreation events (e.g., 36 illnesses per 1,000 swimmers in a day; USEPA 2012). Results represent average risk across the water recreation season (defined as May – October). Average

risk is composed of individual simulated recreation events that varied in terms of water ingestion and pathogen concentration. Technical details of the QMRA are described in Appendix B.

Key findings

- Risk varied by site and activity (Figure 2.1), which reflects differences in pathogen levels by site and differences in the volume of water ingested by activity.
- For swimming, average risk over the water recreation season was below the benchmark (36 illnesses per 1,000 exposure events) for four of the sites; average risk for limited contact activities is below the benchmark for five of the sites (Figure 2.1).
- Risk also varied depending on water consumption and pathogen levels for individual simulated recreation events, and risk for the majority of individual recreation events was below USEPA's benchmark of 36 AGI cases per 1,000 events (Table 2.1).
- Many pathogens contributed to risk, though some pathogens were never detected at particular sites (Table 2.2).

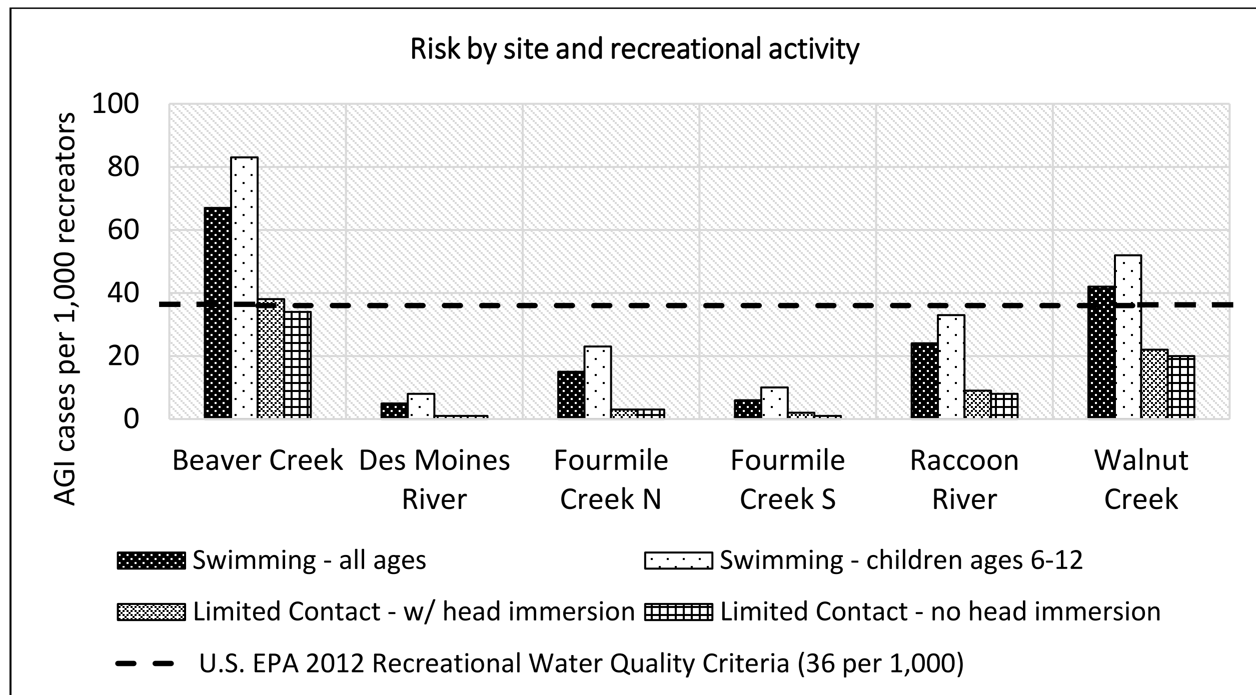

Figure 2.1. Average risk for the recreation season by study site and recreational water activity. AGI, acute gastrointestinal illness

Table 2.1. Average risk for the recreation season for swimming – all ages and percentage of individual recreational events above and below USEPA's risk benchmark.

	Risk per swimming event, all ages		
Site	< 36 AGI cases per 1,000	> 36 AGI cases per 1,000	Average Risk (AGI cases per 1,000)
Beaver Creek	77%	23%	67
Des Moines River	97%	3%	5
Fourmile Creek N	90%	10%	15
Fourmile Creek S	95%	5%	6
Raccoon River	88%	12%	24
Walnut Creek	84%	16%	42

AGI, acute gastrointestinal illness; benchmark of 36 AGI cases per 1,000 events is from USEPA 2012.

Table 2.2. Percent of total risk by pathogen for swimming – all ages.

Pathogen	Beaver Creek	Des Moines River	Fourmile Creek N	Fourmile Creek S	Raccoon River	Walnut Creek
Adenovirus	9%	-	-	-	-	-
Norovirus (GI + GII)	70%	-	-	-	-	66%
Campylobacter jejuni	- [a]	-	6%	85%	19%	10%
EPEC	12%	-	26%	-	11%	20%
Salmonella	7%	-	53%	8%	3%	-
Cryptosporidium bovis	2%	100%	1%	7%	9%	4%
Cryptosporidium meleagridis	-	-	-	-	0.02%	-
Cryptosporidium parvum	-	-	13%	-	59%	-
Unknown *Cryptosporidium* spp.	-	-	-	-	-	-
Total	100%	100%	100%	100%	100%	100%

[a] Dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated and does not contribute to total risk. Detectable risk for each pathogen is reported in Table B.4 of Appendix B.

Context and interpretation

The risk of AGI associated with recreational water activities is often interpreted with respect to the USEPA benchmark of 36 illnesses per 1,000 water recreation events (USEPA 2012). In general, risk was higher at Beaver Creek and Walnut Creek compared to the other four sites, which reflects differences in pathogen contamination. Average risk for swimming (all ages and children ages 6-12) exceeded the benchmark at Beaver Creek and Walnut Creek, and limited contact with head immersion exceeded the benchmark at Beaver Creek (Fig. 2.1). By activity, risk was higher for swimming than limited contact activities. Swimming exposures involve larger volumes of water ingestion than limited contact activities and are therefore expected to produce higher risk estimates.

The risk estimates presented in Figure 2.1 are averages across an entire recreational season for each activity. The averages are useful for characterizing overall risk, but it is important to recognize that risk varies day-to-day and from one person to another. Risk is not always equal to the average for a given site, and average risk includes exposure events with both minimal and elevated risk. In general, risk can vary by the pathogen(s) present on a given day and by the amount of water that different people ingest during recreation. The site averages are based on detected pathogens from samples

collected over two recreation seasons, and changes to contamination at these sites may result in changes in risk.

The six sites were compared in terms of risk because the recreational water quality criteria are based on risk, which is the probability of AGI given exposure during water recreation. However, the total burden of illness (i.e., how many recreators get sick) is based not only on risk, but also on the number of recreators. Therefore, if a disproportionate number of recreators use a site with relatively low risk, then the total burden of illness may be greater than for a site with relatively high risk but few recreators. The number of expected recreators per site was not defined, so the burden of illness was not estimated; if site use is known or estimated, then the burden of illness can be estimated using the risk data presented here.

The QMRA estimated risk of AGI by accounting for pathogen concentrations, water ingestion, and each pathogen's dose-response characteristics. Uncertainty in each of these inputs was incorporated into risk estimates using Monte Carlo simulations (see Appendix B), and sensitivity analyses allowed examination of uncertainty (see Appendix C). Uncertainty in risk estimates stems from uncertainty in the QMRA inputs and was consistent with levels of uncertainty in other QMRAs for recreational water (e.g., Gholipour et al. 2023; Boehm & Soller 2020; Schoen et al. 2020). The dose-response model for unknown *Cryptosporidium* spp. was an important source of uncertainty at all sites (Tables C.11-C.16) because *Cryptosporidium* spp. was detected at all sites; the *Cryptosporidium* species could not be identified in all samples (Table 1.1); and infectivity varies broadly by species (see Appendix B). Other important factors during sensitivity analysis were concentrations of one or more pathogens detected at each site (Tables C.11-C.16). The specific pathogens of importance varied by site because different pathogens were detected at different sites. Uncertainty in pathogen concentrations is a function of the number of samples collected at each site, and this study is based on a total of 147 samples collected across six sites.

A particular strength of the approach taken here is the use of site-specific pathogen data for estimating risk. Sites were repeatedly sampled for pathogens over two recreation seasons, and samples were frequently negative for individual pathogens (Table 1.1). For example, *Salmonella* and *Campylobacter jejuni* were present in 5 samples (3%) and 13 samples (9%), respectively, and were therefore not detected in more than 90% of samples. Because pathogens that are absent from water do not pose a risk to recreators, risk estimates included pathogen-negative exposures to reflect their sporadic presence at the study sites. This approach represents variability in water quality and is consistent with intermittent pathogen presence reported for other studies (Corsi et al. 2014; Kishida et al. 2012; Korajkic et al. 2018; Lenaker et al. 2018). Samples may also be negative when pathogens are present in water at low concentrations that produce a false negative test result. The methods used for pathogen detection are sufficiently sensitive to detect average risk that is below the USEPA benchmark (Table B.4), making them suitable for the current study's goals. The low pathogen doses that can produce a false negative test result may increase total risk for the study sites, but such doses are lower than detected doses and therefore pose less risk to recreators. The pathogen doses that can be reliably detected by the analysis methods therefore present the greatest risk to recreators.

The goal of Objective 2 of was to characterize the risk of illness across the recreation season, and risk estimates were based on pathogens detected in samples collected over two recreation seasons, capturing variability in water quality conditions. Results describe average risk for each activity and the expected frequency of elevated-risk events at each site. Results therefore inform broader level questions, like which sites or activities exceed the USEPA's risk benchmark. The study was not designed

to define the specific risk for any single recreation event or to assess temporal variability within the recreational season (e.g., differences in risk by week or month). Also, the study could not address the risk of infection by rotavirus A, which was detected 56 times, because the laboratory analyses do not distinguish wild-type rotavirus from vaccine, which can be shed in stool or manure (Higashimoto et al. 2018; Matthijnssens et al. 2010). Finally, note that differences in swimming risk between children and all ages reflect differences in water ingestion volume, but they do not reflect differences in the infectivity of pathogens for children versus adults because dose-response studies are usually conducted on adults (Haas et al. 2014).

Objective 3. Examining risk by fecal source

Approach: Microbial source tracking in QMRA

The pathogen exposures in QMRA were categorized by fecal source using the microbial source tracking markers reported in Table 1.1 (e.g., avian associated *Bacteroidales*, *Bacteroidales*-like Cow M2, etc.). For example, if *Campylobacter jejuni* was detected with human *Bacteroides* in the same sample and no other fecal source markers were present, then the resulting risk would be categorized as "human only." The fecal source was categorized as "multiple" when multiple fecal sources were detected in a sample. Risk was characterized for each fecal source as the average over the water recreation season and as the percentage of individual exposures that exceeded USEPA's benchmark of 36 illnesses per 1,000 recreation events.

Key findings

- All fecal sources were associated with some water recreation exposures, and no single fecal source dominated risk (Tables 3.1 – 3.6).
- Associations between fecal source and risk varied by site. Across sites, risk was generally greatest when multiple fecal sources were present. The majority of exposures for which risk exceeded 36 AGI cases per 1,000 were associated with the presence of multiple fecal sources (Tables 3.1 – 3.6).
- Although human wastewater frequently contaminated waterways, average risk was less than 36 AGI cases per 1,000 exposures when human wastewater was detected in the absence of other fecal sources (Tables 3.1 – 3.6).

Context and interpretation

Human and animal fecal contamination were detected at all sites, and risk was greatest when multiple fecal sources were present. For example, at the Beaver Creek site multiple fecal sources were present for all exposures with risk greater than the USEPA benchmark, and average risk was 87 AGI cases per 1,000 exposure events (Table 3.1). In contrast, average risk at Beaver Creek was < 1 AGI case per 1,000 exposure events when only one individual fecal source was present.

While human wastewater was detected more frequently than livestock manure and bird feces (Table 1.1), it was rarely detected alone: 76% of samples positive for human wastewater were also positive for animal feces. The frequent co-occurrence of human, livestock, and bird fecal contamination precludes identification of a single dominant fecal source that contributed to risk. Instead, risk was predominantly associated with the presence of multiple fecal sources at these sites, which may indicate that efforts to reduce risk that address multiple fecal sources may be most effective. Likewise, factors that influence pathogen transport regardless of source, like rainfall and stream flow, may be important to further characterize times of elevated contamination and risk (e.g., Corsi et al. 2014; 2016).

Detection of MST markers indicates the presence of fecal contamination and the possible presence of feces-borne pathogens, but MST markers may be present when pathogens are absent and vice versa (e.g., Lenaker et al. 2018; Korajkic et al. 2018). For example, human MST markers are consistently detected in wastewater while the presence of specific pathogens can be intermittent (e.g., Kitajima et al. 2014; Lenaker et al. 2018). As a result, a particular fecal source may be present at a site, but there is no risk associated with that fecal source category if pathogens are absent from water. For example, "avian only" (avian fecal material present in the absence of other fecal sources) contributed to 21% of exposures at Walnut Creek, but there was no detectable risk associated with these exposures during the study period (Table 3.6).

Table 3.1. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Beaver Creek.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	0%	Not applicable [b]	0%
Cow only	4%	- [c]	0%
Pig only	0%	Not applicable [b]	0%
Human only	16%	0.0064	0%
Multiple	76%	90	100%
None detected	4%	-	0%
	Total: 100%	Average: 67	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Table 3.2. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Des Moines River.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	8%	- [c]	0%
Cow only	0%	Not applicable [b]	0%
Pig only	4%	-	0%
Human only	24%	8.0	100%
Multiple	32%	-	0%
None detected	32%	0.065	0%
	Total: 100%	Average: 5	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Table 3.3. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Fourmile Creek North.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	0%	Not applicable [b]	0%
Cow only	9%	-[c]	0%
Pig only	0%	Not applicable [b]	0%
Human only	0%	Not applicable [b]	0%
Multiple	52%	13	56%
None detected	39%	10	44%
	Total: 100%	Average: 15	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Table 3.4. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Fourmile Creek South.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	8%	-[c]	0%
Cow only	8%	9	17%
Pig only	0%	Not applicable [b]	0%
Human only	17%	3	3%
Multiple	46%	4	80%
None detected	21%	0.047	0%
	Total: 100%	Average: 6	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Table 3.5. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Raccoon River.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	15%	- [c]	0%
Cow only	12%	12	14%
Pig only	0%	Not applicable [b]	0%
Human only	12%	14	23%
Multiple	31%	32	63%
None detected	31%	-	0%
	Total: 100%	Average: 24	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Table 3.6. Distribution of swimming exposures, risk, and risk exceedances by fecal source at Walnut Creek.

Fecal source	Percentage of site's swimming exposures	Average risk per swimming exposure (AGI cases/1,000 exposures)	Percentage of site's risk exceedances [a]
Bird only	21%	- [c]	0%
Cow only	8%	44	20%
Pig only	0%	Not applicable [b]	0%
Human only	13%	0.32	0%
Multiple	50%	48	80%
None detected	8%	0.40	0%
	Total: 100%	Average: 42	Total: 100%

Note: Results are for swimming – all ages. Results for other recreational activity types were similar. AGI, acute gastrointestinal illness.

[a] An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 cases per 1,000 exposures.

[b] Since this fecal source was never present on its own, risk per swimming event cannot be estimated for it independently of other sources.

[c] Dashes indicate that no pathogens were detected in association with the given fecal source at this site.

Objective 4. Evaluating performance of *E. coli* for identifying health risk

Approach: E. coli *measurements in QMRA*

Companion samples were collected along with those analyzed for pathogens and MST markers and were analyzed for *E. coli* by Des Moines Water Works using the standard enzyme substrate test (9223B-QT; Rice et al. 2012). QMRA results were categorized with respect to *E. coli* exceedances, where an *E. coli* exceedance is defined as a concentration greater than 235 most probable number (MPN) per 100 mL. Assuming 1 MPN equals 1 colony-forming unit (CFU), this value corresponds to Iowa's beach action value (BAV) for recreational water use (235 CFU per 100 mL).

Comparison of QMRA risk estimates to the BAV allows us to assess how well the BAV identifies high-risk conditions at these sites, where high-risk conditions are defined as those greater than the USEPA benchmark of 36 AGI cases per 1,000 exposure events. More specifically, data were analyzed to determine whether an exceedance of the BAV (*E. coli* concentration > 235 MPN per 100 mL) corresponded to an exceedance of USEPA's risk benchmark (risk > 36 AGI cases per 1,000 exposures). Two questions were addressed regarding *E. coli* exceedances:

1) What percentage of exposures above the risk benchmark had a matching *E. coli* exceedance? This question is addressed by the true positive rate.
2) What percentage of exposures *below* the risk benchmark had an *E. coli* exceedance? This question is addressed by the false positive rate.

Assessments were conducted for each of the four activities considered in the QMRA. Results for swimming – all ages are reported in Table 4.1, and results for other activities are reported in Appendix C (Table C.17).

Key findings

- Performance of the *E. coli* BAV for detecting risk exceedances varied across sites (Table 4.1)
- True positive rates were generally high (66 – 100%), indicating that the *E. coli* BAV reliably identified conditions with elevated risk at most sites (Table 4.1).
- However, false positive rates were also relatively high (40 – 70%), indicating that *E. coli* routinely exceeded the BAV during low-risk conditions (Table 4.1).

Table 4.1. Diagnostic performance of *E. coli* as an indicator for risk exceedances. Performance is shown for swimming – all ages; results for other activities were similar (see Table C.17 in Appendix C).

Site	Samples	*E. coli* exceedances (> 235 MPN/100 mL)	Swimming – all ages	
			True positive rate [a]	False positive rate [b]
Beaver Creek	25	19	100%	70%
Des Moines River	25	11	100%	42%
Fourmile Creek N	23	17	100%	72%
Fourmile Creek S	24	17	93%	70%
Raccoon River	26	12	98%	40%
Walnut Creek	24	14	66%	58%

Note: An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 AGI cases per 1,000 exposures.

[a] True positive rate is the percentage of exposures where risk results matched the BAV (risk > 36 AGI cases per 1,000 and *E. coli* > 235 MPN per 100 mL).

[b] False positive rate is the percentage of exposures where risk results did not match the BAV (risk < 36 AGI cases per 1,000 and *E. coli* > 235 MPN per 100 mL).

Context and interpretation

The true positive rate varied by site but was generally high. At most sites, the BAV identified nearly all exposures that exceeded the USEPA benchmark. The true positive rate for Walnut Creek was lowest and indicated that 66% of exposures above USEPA's benchmark were identified by an *E. coli* exceedance; 34% of risk exceedances were therefore not identified by the BAV. Various site-level factors may produce risk exceedances that are not identified by the BAV. For example, the relative quantities of *E. coli* and pathogens differ by fecal source (e.g., Soller et al. 2010), and fecal contamination at these study sites may differ from sites used to establish the BAV (USEPA 2012). Alternatively, risk exceedances may result from the presence of pathogens that persist longer in water than *E. coli* or from the detection of non-viable pathogens. Regardless, the relatively low true positive rate indicates that *E. coli* is less effective as a public health tool at Walnut Creek compared to other sites in the study.

The false positive rate also varied by site. False positives occur when *E. coli* exceeds the BAV but risk does not exceed the USEPA benchmark. Indicators like *E. coli* can be present in the absence of disease-causing pathogens and can originate in fecal sources that vary in the degree of health risk posed to recreators (Soller et al. 2010; Korajkic et al. 2018). Overall, results show that *E. coli* typically identified high-risk conditions at these sites but tended to overestimate the frequency of risk exceedances.

The risk benchmark of 36 illnesses per 1,000 recreational events was originally developed for swimming at sites primarily influenced by human wastewater (USEPA 2012). Compliance with this benchmark is usually assessed by measuring *E. coli* and comparing to concentration thresholds that correspond to USEPA's risk benchmark. However, the sites studied here were not influenced solely by human fecal material (see Objective 3), so alternative BAVs for these sites may be applicable (USEPA 2012). QMRA is a possible tool for developing alternative BAVs (USEPA 2012), which may be designed to decrease the false positive rate and thereby improve *E. coli*'s overall performance as an indicator of risk to recreators at these sites. As noted for risk estimates under Objective 2, *E. coli*'s estimated diagnostic performance in this study is based on pathogens detected by qPCR in samples collected over two

recreation seasons. Changes to contamination at these sites may change the estimated diagnostic performance of *E. coli*.

Summary and Conclusion

Recreational water activities like swimming, wading, and kayaking present a risk of illness due to accidental ingestion of water containing pathogens. This study characterized the risk of AGI for recreation at six sites on the Iowa Water Trails in the Des Moines, Iowa, metro area. Risk varied by site and activity. Estimated average swimming risk exceeded USEPA's benchmark for two of six sites, whereas average risk associated with limited contact activities was relatively low for all sites. The estimated risk values describe average risk across the recreation season and include individual exposure events both above and below USEPA's benchmark.

Many pathogen types were detected using qPCR, including viruses, bacteria, and protozoa. The pathogens can originate in various fecal sources, and microbial source tracking identified human, bovine, porcine, and avian fecal contamination of waterways. No single fecal source dominated risk estimates, and risk was generally highest when multiple fecal sources were present.

Iowa's beach action value (BAV), which is based on the general fecal indicator *E. coli*, reliably identified recreation exposures that exceeded the USEPA benchmark at most sites. However, it also produced a high number of false positive results (i.e., high *E. coli* concentrations during low-risk conditions). It may be possible to optimize *E. coli*'s diagnostic performance at these sites based on pre-defined standards for acceptable sensitivity and specificity.

Acknowledgements

Claire Hruby (Iowa Department of Natural Resources) and Amanda Brown (Polk County Conservation) contributed to study design, collected samples, and provided *E. coli* data. Susan Spencer (U.S. Department of Agriculture-Agriculture Research Service, retired) assisted with study design and sample analyses. Chris Jones (University of Iowa) provided access to automated samplers used during field sampling. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Data are available at U.S. Geological Survey National Water Information System (USGS 2023).

References

Alegbeleye OO, Sant'Ana AS. 2020. Manure-borne pathogens as an important source of water contamination: An update on the dynamics of pathogen survival/transport as well as practical risk mitigation strategies. International Journal of Hygiene and Environmental Health 227: 113524.

Ashbolt NJ, Grabow WO, Snozzi M. 2001. Indicators of microbial water quality, p. 289-316 in Fewtrell L and Bartram J, Water quality: guidelines, standards, and health. IWA Publishing, London.

Boehm AB, Soller JA. 2020. Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination. Microbial Risk Analysis. 16:100139.

Burch TR, Stokdyk JP, Spencer SK, Kieke Jr BA, Firnstahl AD, Muldoon MA, Borchardt MA. 2021. Quantitative microbial risk assessment for contaminated private wells in the fractured dolomite aquifer of Kewaunee County, Wisconsin. Environmental Health Perspectives. 129(6):067003.

Burch TR, Stokdyk JP, Rice N, Anderson AC, Walsh JF, Spencer SK, Firnstahl AD, Borchardt MA. 2022. Statewide quantitative microbial risk assessment for waterborne viruses, bacteria, and protozoa in public water supply wells in Minnesota. Environmental Science & Technology. 56(10):6315-24.

Corsi SR, Borchardt MA, Spencer SK, Hughes PE, Baldwin AK. 2014. Human and bovine viruses in the Milwaukee River watershed: hydrologically relevant representation and relations with environmental variables. Science of the Total Environment. 490:849-860.

Corsi SR, Borchardt MA, Carvin RB, Burch TR, Spencer SK, Lutz MA, McDermott CM, Busse KM, Kleinheinz GT, Feng X, Zhu J. 2016. Human and bovine viruses and bacteria at three Great Lakes beaches: Environmental variable associations and health risk. Environmental Science & Technology. 50:987-995.

Garcia-Aljaro C, Blanch AR, Campos C, Jofre J, Lucena F. 2018. Pathogens, faecal indicators and human-specific microbial source-tracking markers in sewage. Journal of Applied Microbiology 126: 701-717. https://doi.org/doi:10.1111/jam.14112.

Gholipour S, Nikaeen M, Rabbani D, Mohammadi F, Manesh RM, Besharatipour N, Bina B. 2023. Occurrence of enteric and non-enteric microorganisms in coastal waters impacted by anthropogenic activities: A multi-route QMRA for swimmers. Marine Pollution Bulletin. 188:114716.

Haas CN, Rose JB, Gerba CP. 2014. *Quantitative Microbial Risk Assessment*. John Wiley & Sons, Hoboken, New Jersey.

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes. FEMS Microbiology Reviews. 38:1–40.

Higashimoto Y, Ihira M, Miyazaki Y, Kuboshiki A, Yoshinaga S, Hiramatsu H, Suzuki R, Miyata M, Miura H, Komoto S, Yukitake J. 2018. Monitoring shedding of five genotypes of RotaTeq vaccine viruses by genotype-specific real-time reverse transcription-PCR assays. Journal of Clinical Microbiology. 56:e00035-18.

Iowa Confluence Water Trails. 2023. <https://www.iconwatertrails.com/> Accessed 14 Aug 2023.

Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW. 2015. *Manual of Clinical Microbiology*, 11th Edition. ASM Press, Washington, D.C.

Kishida N, Morita H, Haramoto E, Asami M, Akiba M. 2012. One-year weekly survey of noroviruses and enteric adenoviruses in the Tone River water in Tokyo metropolitan area, Japan. Water Research. 46:2905-2910.

Kitajima M, Iker BC, Pepper IL, Gerba CP. 2014. Relative abundance and treatment reduction of viruses during wastewater treatment processes—identification of potential viral indicators. Science of the Total Environment. 488:290-296.

Korajkic A, McMinn BR, Harwood VJ. 2018. Relationships between Microbial Indicators and Pathogens in Recreational Water Settings. International Journal of Environmental Research and Public Health 15(2842).

Lenaker PL, Corsi SR, McLellan SL, Borchardt MA, Olds HT, Dila DK, Spencer SK, Baldwin AK. 2018. Human-associated indicator bacteria and human-specific viruses in surface water: a spatial assessment with implications on fate and transport. Environmental Science & Technology. 52:12162-12171.

Matthijnssens J, Joelsson DB, Warakomski DJ, Zhou T, Mathis PK, van Maanen MH, Ranheim TS, Ciarlet M. 2010. Molecular and biological characterization of the 5 human-bovine rotavirus (WC3)-based reassortant strains of the pentavalent rotavirus vaccine, RotaTeq®. Virology. 403:111-127.

National Agriculture Statistics Service (NASS). 2019. 2017 Census of agriculture. AC-17-A-51. U.S. Department of Agriculture. Washington, D.C.

Rice EW, Baird RB, Eaton AD, Clesceri LS, Eds. 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed.; American Public Health Association, Washington, DC.

Saleem F, Edge TA, Schellhorn HE. 2022. Validation of qPCR method for enterococci quantification at Toronto beaches: Application for rapid recreational water monitoring. Journal of Great Lakes Research 48(3): 707-716.

Schoen ME, Boehm AB, Soller J, Shanks OC. 2020. Contamination scenario matters when using viral and bacterial human-associated genetic markers as indicators of a health risk in untreated sewage-impacted recreational waters. Environmental Science & Technology. 54:13101-13109.

Shanks OC, White K, Kelty CA, Sivaganesan M, Blannon J, Meckes M, Varma M, Haugland RA. 2010. Performance of PCR-based assays targeting *Bacteroidales* genetic markers of human fecal pollution in sewage and fecal samples. Environmental Science & Technology. 44:6281-6288.

Soller JA, Schoen ME, Bartrand T, Ravenscroft JE, Ashbolt NJ. 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research. 44(16):4674-91.

U.S. Environmental Protection Agency (USEPA). 2012. Recreational water quality criteria. EPA 820-F-12-058; Office of Water: Washington, D.C.

U.S. Geological Survey (USGS). 2022. Topo Base Map, The National Map.

U.S. Geological Survey (USGS). 2023. USGS water data for the Nation: U.S. Geological Survey National Water Information System database, accessed at https://doi.org/10.5066/F7P55KJN. [Site number = 05482000, 05484900, 05484800, 05481950, 05485605, 05485640; Date range = 2020-06-09 to 2021-10-27].

Whitman RL, Nevers MB, Przybyla-Kelly K, Byappanahalli MN. 2011. Physical and biological factors influencing environmental sources of fecal indicator bacteria in surface water. In Sadowsky MJ and Whitman RL (Ed), The Fecal Bacteria. 2010. ASM Press, Washington, D.C.

APPENDICES

Appendix A: Sample collection and laboratory analyses

Semi-monthly samples were collected over two water recreation seasons (May - October 2020 and 2021); 23 to 26 samples were collected per site (147 total). Samples were either grab samples (*n* = 20) or collected via an automated ISCO sampler (*n* = 127). Sampler was housed in a metal cabinet and samples were not exposed to direct sunlight; samples were removed immediately. Average sample volume was 465 mL (range: 150 – 1,000 mL). Samples were processed within 48 hours of collection by adding 10.1 mL/L of 2.5 M $MgCl_2$ prior to filtration through a 0.45-micron mixed cellulose ester filter (Millipore) (Haramoto et al. 2012, Katayama et al. 2002). Filters were eluted using 5 mL of solution containing tetrasodium pyrophosphate decahydrate (Sigma-Aldrich), EDTA trisodium salt trihydrate (Sigma-Aldrich), and Tween 80 (Fisher Scientific). Beef extract (Gibco Bacto) was added, and all final concentrated sample volumes (FCSV) were stored at -80 °C until DNA extraction.

A QIAcube and QIAamp DNA blood mini kit with buffer AVL (Qiagen) were used to extract nucleic acids from 280 µL of FCSV and elute into 140 µL AE Buffer (Qiagen). Three total extractions were performed for each sample to supply sufficient material for testing.

Viral RNA was reverse transcribed by combining 21.5 µL of extracted sample, 21.5 µL water, and 1.75 µL random hexamers (ProMega) and denaturing at 5 min at 95 °C. Immediately afterwards, 80.25 µL of reverse transcription master mix was added and incubated at 42 °C for 60 min followed by 5 min at 95 °C. The composition of reverse transcription master mix was as follows: 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 0.6 nM dithiothreitol, 70 µM of each deoxynucleoside triphosphate (ProMega), 0.72 U RNAsin (ProMega), and 50 U SuperScript III reverse transcriptase (Invitrogen Life Technologies).

Thirty qPCR assays were completed for pathogens and microbial source tracking markers (see Appendix A reference list for qPCR assays citations). Each qPCR reaction contained 6 µL of extracted sample and 14 µL of LightCycler 480 Probes Mastermix (Integrated DNA Technology). Analyses were performed on a Roche LightCycler 480 II instrument (Roche Diagnostics Corporation) at 95 °C for 5 min followed by 45 cycles of 10 s at 95 °C and 1 min at 60 °C. Duplicate analyses were performed for all targets in all samples. After accounting for all sample processing steps (sub-sampling, concentrations, and dilutions), the mean effective sample volume analyzed by qPCR for DNA targets was 1.8 mL (range: 0.065 – 4.4 mL). For RNA targets, it was 0.29 mL (range: 0.023 - 0.75 mL).

Samples were tested for PCR and reverse transcription (RT)-PCR inhibition as described in Gibson et al. (2012) using salmon testes DNA (Sigma-Aldrich) and hepatitis G Ultramer RNA Oligo (Integrated DNA Technologies), respectively. Samples with cycle of quantification values at least 2 or 6 cycles greater than the control were diluted 1:5 or 1:10 with AE buffer, respectively, prior to reverse transcription or qPCR analysis. Fifty-seven samples exhibited inhibition.

Standard curves were created from MiniGene and Ultramer oligos (Integrated DNA Technologies) in AE buffer containing 0.02% (w/v %) bovine serum albumin. The Roche LightCycler 480 software calculated the cycle of quantification values using the second derivative maximum method and regressed them against the decimal logarithm of target concentration. Standard curve efficiencies ranged from 1.86 to 2.23, mean square errors from 0.0032 to 0.13, and highest Cq measured from 36.98 to 40. Standards were quantified by the vendor (Integrated DNA Technologies), and standard curves covered a dynamic range of 5 orders of magnitude. Standard curves were fit using the built-in algorithm from the Roche LightCycler software.

Negative controls were included with each qPCR plate (PCR-grade water), DNA extraction (AE buffer), and periodically during the sample concentration and field collection processes (sterile water). Data were rejected for any instances where corresponding negative controls exhibited fluorescence over baseline values. Four samples each for pepper mottle mild virus and ruminant *Bacteroides* were rejected due to the associated negative filter control exhibiting fluorescence above baseline. As this is a step that cannot be repeated, the data were removed. All nucleic acid extraction, reverse transcription, and qPCR negative controls passed. All negative controls were run in duplicate at the qPCR step.

Positive qPCR controls (Ultramers or Minigenes, IDT) were included in each run and fell within a 0.5 cycle range of the expected value for all but three ruminant *Bacteroides* detections, which were only reported qualitatively. Positive reverse transcription and DNA extraction controls (bovine herpes virus and bovine respiratory syncytial virus vaccines) were included and evaluated qualitatively for each batch. All positive controls met the criteria for data acceptance.

Additional analyses were performed to aid in species identification and confirm positive results for the pan-*Cryptosporidium* target detections. Direct immunofluorescence assay (IFA) was performed on 100 µL FCSV using the MeriFluor *Cryptosporidium*/*Giardia* Detection Kit (Meridian Biosciences, Inc.) as described in Stokdyk et al. (2019). Sanger sequencing was performed to aid in species and subtype identification using the 18s rRNA gene and GP60 genes, respectively, also as described in Stokdyk et al. (2019) with the addition of an enzymatic purification for secondary PCR products (ExoSAP-IT, ThermoFisher Scientific). Six additional qPCR assays were performed as described previously for all pan-*Cryptosporidium* detections: *C. baileyi*, *C. bovis*, *C. galli*, *C. meleagridis*, *C. suis*, and *C. ubiquitum* (Li et al. 2015, Mary et al. 2013, Nakamura et al. 2014, Stroup et al. 2006).

References for Appendix A

Baque RH, Gilliam AO, Robles LD, Jakubowski W, Slifko TR. 2011. A real-time RT-PCR method to detect viable *Giardia lamblia* cysts in environmental waters. Water Research 45:3175-3184.

Best E, Powell EJ, Swift C, Grant KA, Frost JA. 2003. Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. FEMS Microbiology Letters 229:237-241.

Boxus M, Letellier C, Kerkhofs P. 2005. Real time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. Journal of Virological Methods. 125:125-130.

Chang KO, Nielsen PR, Ward LA, Saif LJ. 1999. Dual infection of gnotobiotic calves with bovine strains of group A and porcine-like group C rotaviruses influences pathogenesis of the group C rotavirus. 1999. Journal of Virology. 73(11): 9284-93.

De Leon R, Shieh C, Baric R S, Sobsey MD. 1989. Detection of enterovirus and hepatitis A virus in environmental samples by gene probes and polymerase chain reaction, p. 833-853. Proceedings of the Advances of water analysis and treatment proceedings Water Quality Technology Conference, American Water Works Association, Denver, CO.

Deer DM, Lampel KA. 2010. Development of a Multiplex Real-Time PCR Assay with Internal Amplification Control for the Detection of *Shigella* Species and Enteroinvasive *Escherichia coli*. Journal of Food Protection. 73(9):1618-1625

Derzelle S, Grine A, Madic J, de Garam CP, Vingadassalon N, Dilasser F, Jamet E, Auvray F. 2011. A quantitative PCR assay for the detection and quantification of Shiga toxin-producing *Escherichia coli* (STEC) in minced beef and dairy products. International Journal of Food Microbiology. 151:44-51.

Garson JA, Ferns RB, Grant PR, Ijaz S, Nastouli E, Szypulska R, Tedder RS. 2012. Minor groove binder modification of widely used TaqMan probe for hepatitis E virus reduces risk of false negative real-time PCR results. Journal of Virological Methods. 186:157-160.

Gibson, KE, Schwab, KJ, Spencer, SK, Borchardt, MA. 2012. Measuring and mitigating inhibition during quantitative real time PCR analysis of viral nucleic acid extracts from large-volume environmental water samples. Water Research. 46 (13):4281– 4291.

Green HC, Dick LK, Gilpin B, Samadpour M, Field KG. 2012. Genetic markers for rapid PCR-based identification of gull, Canada goose, duck, and chicken fecal contamination in water. Applied and Environmental Microbiology. 78(2):503-510

Green HC, Haugland RA, Varma M, Millen HT, Borchardt MA, Field KG, Walters WA, Knight R, Sivaganesan M, Kelty CA, Shanks OC. 2014. Improved HF183 quantitative real-time PCR assay for characterization of human fecal pollution in ambient surface water samples. Applied and Environmental Microbiology. 80:3086-3094.

Haramoto E, Kitajima M, Kishida N, Konno Y, Katayama H, Asami M, Akiba M. 2013. Occurrence of pepper mild mottle virus in drinking water sources in Japan. Applied and Environmental Microbiology. 79:7413-7418.

Haramoto E, Katayama H, Asami M, Akiba M. 2012. Development of a novel method for simultaneous concentration of viruses and protozoa from a single water sample. Journal of Virological Methods. 182(1-2):62-9.

Haugland, R.A., S.C. Siefring, L.J. Wymer, K.P. Brenner, and A. P. Dufour. 2005. Comparison of *Enterococcus* Density Measurements by Quantitative Polymerase Chain Reaction and Membrane Filter Analysis at Two Freshwater Recreational Beaches. Water Research. 39:559--568.

Hoorfar J**,** Ahrens P, Rådström P. 2000. Automated 5' nuclease PCR assay for identification of *Salmonella enterica*. Journal of Clinical Microbiology. 38:3429-3435.

Ibekwe AM, Watt PM, Shouse PJ, Grieve CM. 2004. Fate of *Escherichia coli* O157:H7 in irrigation water on soils and plants as validated by culture method and real-time PCR. Canadian Journal of Microbiology. 50:1007-1014.

Jothikumar N, Cromeans TL, Hill VR, Lu X, Sobsey MD, Erdman DD. 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Applied and Environmental Microbiology. 71:3131-3136.

Jothikumar N, Lowther JA, Henshilwood K, Lees DN, Hill VR, Vinjé J. 2005. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. Applied and Environmental Microbiology. 71:1870-1875.

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, Takeda N, Katayama K. 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. Journal of Clinical Microbiology. 41:1548-1557.

Katayama H, Shimasaki A, Ohgaki S. Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. 2002. Applied and Environmental Microbiology. 68:1033-1039.

Li N, Neumann NF, Ruecker N, Alderisio KA, Sturbaum GD, Villegas EN, Chalmers R, Monis P, Feng Y, Xiao L. 2015. Development and Evaluation of Three Real-Time PCR Assays for Genotyping and Source Tracking *Cryptosporidium* spp. in Water. Applied and Environmental Microbiology. 81:5845-5854.

Malorny B, Paccassoni E, Fach P, Bunge C, Martin A, Helmuth R. 2004. Diagnostic real-time PCR for detection of *Salmonella* in food. Applied and Environmental Microbiology. 70:7046-7052.

Mary C, Chapey E, Dutoit E, Guyot K, Hasseine L, Jeddi F, Menotti J, Paraud C, Pomares C, Rabodonirina M, Rieux A, Derouin F. 2013. Multicentric evaluation of a new real-time PCR assay for quantification of *Cryptosporidium* spp. and identification of *Cryptosporidium parvum* and *Cryptosporidium hominis*. Journal of Clinical Microbiology. 51:2556-2563.

McQuaig SM, Scott TM, Lukasik JO, Paul JH, Harwood VJ. 2009. Quantification of human polyomaviruses JC Virus and BK Virus by TaqMan quantitative PCR and comparison to other water quality indicators in water and fecal samples. Applied and Environmental Microbiology. 75:3379-3388.

Meleg E, Bányai K, Martella V, Jiang B, Kocsis B, Kisfali P, Melegh B, Szũcs G. 2008. Detection and quantification of group C rotaviruses in communal sewage. Applied and Environmental Microbiology. 74:3394-3399.

Mieszkin S, Furet JP, Corthier G, Gourmelon M. 2009. Estimation of pig fecal contamination in a river catchment by real-time PCR using two Pig-specific *Bacteroidales* 16S rRNA Genetic Markers. Applied and Environmental Microbiology. 75:3045-3054.

Mieszkin S, Yala JF, Joubrel R, Gourmelon M. 2010. Phylogenetic analysis of *Bacteroidales* 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. Journal of Applied Microbiology. 108:974-984.

Monpoeho S, Dehée A, Mignotte B, Schwartzbrod L, Marechal V, Nicolas JC, Billaudel S, Férré V. 2000. Quantification of enterovirus RNA in sludge samples using single tube real-time RT-PCR. Biotechniques. 29:88-93.

Nakamura AA, Homem CG, da Silva AMJ, Meireles MV. 2014. Diagnosis of gastric cryptosporidiosis in birds using a duplex real-time PCR assay. Veterinary Parasitology. 205:7-13.

Rutledge RG, Stewart D. 2010. Assessing the performance capabilities of LRE-based assays for absolute quantitative real-time PCR. PLoS One. 5:e9731.

Schlueter V, Schmolke S, Stark K, Hess G, Ofenloch-Haehnle B, Engel AM. 1996. Reverse transcription PCR detection of hepatitis G virus. Journal of Clinical Microbiology. 34:2660-2664.

Schwab KJ, De Leon R, Sobsey MD. 1995. Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, hepatitis A virus, and Norwalk virus by reverse transcription-PCR. Applied and Environmental Microbiology. 61:531-537.

Shanks OC, Atikovic E, Blackwood AD, Lu J, Noble RT, Domingo JS, Seifring S, Sivaganesan M, Haugland RA. 2008. Quantitative PCR for detection and enumeration of genetic markers of bovine fecal pollution. Applied and Environmental Microbiology. 74:745-752.

Shanks OC, Kelty CA, Sivaganesan M, Varma M, Haugland RA. 2009. Quantitative PCR for genetic markers of human fecal pollution. Applied and Environmental Microbiology. 75:5507–5513.

Stokdyk JP, Spencer SK, Walsh JF, de Lambert JR, Firnstahl AD, Anderson AC, Rezania LW, and Borchardt MA. 2019. *Cryptosporidium* incidence and surface water influence of groundwater supplying public water systems in Minnesota, USA. Environmental Science & Technology. 53:3391-3398.

Stroup SE, Roy S, Mchele J, Maro V, Ntabaguzi S, Siddique A, Kang G, Guerrant RL, Kirkpatrick BD, Fayer R, Herbein J, Ward H, Haque R, Houpt ER. 2006. Real-time PCR detection and speciation of *Cryptosporidium* infection using Scorpion probes. Journal of Medical Microbiology. 55:1217-1222.

Wang HY, Kim S, Kim J, Park SD, Uh Y, Lee H. 2014. Multiplex real-time PCR assay for rapid detection of methicillin-resistant staphylococci directly from positive blood cultures. Journal of Clinical Microbiology. 52:1911-20.

Wang J, O'Keefe J, Orr D, Loth L, Banks M, Wakeley P, West D, Card R, Ibata G, Van Maanen K, Thoren P, Isaksson M, Kerkhofs P. 2007. Validation of a real-time PCR assay for the detection of bovine herpesvirus 1 in bovine semen. Journal of Virological Methods. 144:103–108.

Zeng SQ, Halkosalo A, Salminen M, Szakal ED, Puustinen L, Vesikari T. 2008. One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. Journal of Virological Methods. 153:238-340.

Zhang T, Breitbart M, Lee WH, Run JQ, Wei CL, Soh SW, Hibberd ML, Liu ET, Rohwer F, Ruan Y. 2006. RNA viral community in human feces: Prevalence of plant pathogenic viruses. PLoS Biology. 4:e3.

Zheng W, Porter E, Noll L, Stoy C, Lu N, Wang Y, Liu X, Purvis T, Peddireddi L, Lubbers B, Hanzlicek G, Henningson J, Liu Z, Bai J. 2019. A multiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens. Journal of Microbiological Methods. 160:87-92.

Appendix B: Quantitative microbial risk assessment (QMRA)

QMRA was used to estimate the average risk of acute gastrointestinal illness (AGI) across the water recreation season (May-October) for accidental consumption of surface water during each of four different types of recreational activities. These activities included swimming for a general population composed of all age groups, swimming for children ages 6-12 years old, limited contact recreation with head immersion, and limited contact recreation without head immersion. The primary QMRA inputs were pathogen concentrations measured in water samples at each site, dose-response parameters that predict the probability of illness for individual pathogens, and water ingestion volumes for each the four activities. QMRA followed the standard framework of hazard identification, exposure assessment, dose-response assessment, and risk characterization (Haas et al. 2014). Each step is described below.

Hazard identification was based on pathogens detected in water samples from each site. The QMRA included nine pathogens that were detected in study samples: adenovirus, norovirus, *Campylobacter jejuni*, enteropathogenic *E. coli*, *Salmonella* spp., *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium bovis*, and ungenotyped *Cryptosporidium* spp. Three pathogens were analyzed for but never detected: enterovirus, *Cryptosporidium hominis*, and *Giardia*. These three were excluded from further consideration in the QMRA. By only considering those pathogens detected in study samples, hazard identification represents pathogens relevant to the specific study sites in question. Additional pathogens excluded from QMRA were rotavirus A, human polyomavirus, and *Staphylococcus aureus*. Rotavirus A was excluded because its qPCR assays cannot distinguish wild-type rotavirus from vaccine shed in stool. The latter two pathogens were measured as indicative of water contamination in general, but they were excluded from QMRA because they do not cause AGI.

Exposure assessment was based on measured pathogen concentrations in water samples from each site. These concentrations were combined with water ingestion data for each of the four activities to estimate pathogen dose (D). Dose is calculated as the product of concentration (C) and volume (V), i.e., $D = C \times V$. Average ingestion volumes for the four activities were assumed to be 35 mL, 55 mL, 5 mL, and 4 mL per recreational event for swimming-all ages, swimming-children, limited contact-head immersion, and limited contact-no immersion, respectively (Dorevitch et al. 2011; DeFlorio-Barker et al. 2018). Ingestion volumes for swimming activities are the latest available for freshwater environments and represent equal mixtures of male and female swimmers (DeFlorio-Barker et al. 2018). Ingestion volumes for limited contact activities represent the only empirical data available based on published studies (Dorevitch et al. 2011).

During exposure assessment, pathogen concentrations obtained by qPCR were related to infectious units through dose harmonization (Burch et al. 2021; 2022). Dose harmonization converts qPCR-measured units of gene copies to the infectious units used in published dose-response studies for each pathogen. Dose harmonization factors (denoted as H) were based on previous studies and are summarized in Table B.1. Because these factors are generally extracted from *in situ* environmental measurements, they implicitly account for viability as well as the unit conversion from genomic copies to infectious units. Whenever possible, we used factors extrapolated from measurements in other surface water samples; dose harmonization factors specific to the current study's sites are unavailable, so values extrapolated from other surface water environments represent the best available data. In some cases, dose harmonization factors had to be extrapolated from non-surface water environments because no surface water values were available (Table B.1).

Table B.1. Summary of dose harmonization factors.

Pathogen	Dose harmonization factor, *H*		Reference
	Units [a]	Point estimate [b]	
Adenovirus	gene copies/$TCID_{50}$	700	Kundu et al. 2013
Norovirus	gene copies/gene copy	1	Not Applicable
Campylobacter jejuni	gene copies/CFU	7.4	Corsi et al. 2016
EPEC	gene copies/CFU	5.6 [c]	Corsi et al. 2016
Salmonella	gene copies/CFU	5.6	Corsi et al. 2016
Cryptosporidium (all species)	gene copies/oocyst	14	Stokdyk et al. 2019

[a] Abbreviations: $TCID_{50}$ is 50% tissue culture infectious dose; CFU is colony forming units; EPEC, enteropathogenic *E. coli*.

[b] Risk estimates were generated using Monte Carlo simulations. See Table B.3 for a detailed description of distributions used in simulating each pathogen's value of *H*.

[c] Dose harmonization factors have not been published for EPEC; the value indicated is extrapolated from *Salmonella* based on physiological similarity (both organisms are gram negative).

Dose-response models calculate the probability of infection (or illness) from the pathogen dose estimated during exposure assessment. For models that predict probability of infection, morbidity ratios are used to convert to probability of illness. Dose-response model parameters and morbidity ratios vary by pathogen. Models used in this study are summarized in Table B.2 using each model's characteristic "single-hit" probability of illness (i.e., the probability that ingestion of 1 infectious unit causes an illness), and model parameters are presented in detail in Table B.3. Dose-response assessment used established models from previous studies when available; this included models for adenovirus, norovirus, *Campylobacter jejuni*, EPEC, *Salmonella*, and *Cryptosporidium parvum*.

Models for *Cryptosporidium bovis* and *Cryptosporidium meleagridis* have not been applied in prior QMRAs and were developed for the current study based on published dose-response data. The approach used differed between the two based on available data. For *Cryptosporidium meleagridis*, published dose-response data are available (Chappell et al. 2011). These data were analyzed using maximum likelihood estimation to obtain an estimate of the applicable dose-response parameter. On the other hand, while *Cryptosporidium bovis* is known to infect humans (Ryan et al. 2021), published dose-response data are unavailable for it. As a result, we estimated dose-response parameters for *Cryptosporidium bovis* based on the range of other *Cryptosporidium* species known to infect humans at relatively low rates, using the infectivity of *Cryptosporidium muris* as a lower bound and *Cryptosporidium cuniculus* as an upper bound (Puleston et al. 2014; Chappell et al. 2015; Ryan et al. 2021).

Because we could not successfully identify the species of all *Cryptosporidium* that we detected, we also developed a new model for unknown *Cryptosporidium* spp. This model is based on consideration of two factors: 1) the proportion of *Cryptosporidium* spp. known to infect humans (21 of 44 species = 48%) (Zahedi & Ryan 2020; Ryan et al. 2021) and 2) for infectious species with published dose-response data, the broad variability in infectivity across *Cryptosporidium* species. More specifically, it was assumed that the 23 species of *Cryptosporidium* not known to infect humans had dose-response parameters equal to 0. In contrast, dose-response parameters for infective species were assumed to be greater than 0. The range of values used in the latter case was defined based on the least and most infective *Cryptosporidium* spp. known at present. These are *Cryptosporidium muris* and *Cryptosporidium hominis*, respectively (Chappell et al. 2006; 2015).

Table B.2. Summary of dose-response models.

Pathogen	Probability of illness for exposure to 1 infectious unit [a]		Reference(s)
	Infectious unit [b]	**Point estimate [c]**	
Adenovirus	$TCID_{50}$	21%	Haas et al. 1993 Crabtree et al. 1997
Norovirus	gene copy	0.029% - 42% [d]	Lindesmith et al. 2003 Teunis et al. 2008b Messner et al. 2014 Schmidt 2015
Campylobacter jejuni	CFU	0.27%	Medema et al. 1996 Schmidt et al. 2013
EPEC	CFU	0.57%	Teunis et al. 2008a
Salmonella	CFU	0.27%	WHO 2002
Cryptosporidium parvum	oocyst	2%	USEPA 2005 DuPont et al. 1995
Cryptosporidium bovis	oocyst	0.01%	This study Puleston et al. 2014 Chappell et al. 2015
Cryptosporidium meleagridis	oocyst	0.0016%	This study Chappell et al. 2011
Unknown *Cryptosporidium* spp.	oocyst	0% - 9% [d]	This study Chappell et al. 2006 Chappell et al. 2015 Zahedi and Ryan 2020 Ryan et al. 2021

[a] Also referred to as "single-hit" probabilities of illness (Haas et al. 2014), which can be derived from standard formulas for each model using the parameter values listed in Table B.3.

[b] Refers to the units used in available dose-response models. Abbreviations: $TCID_{50}$ is 50% tissue culture infectious dose; CFU is colony forming units; EPEC, enteropathogenic *E. coli*.

[c] Risk estimates were generated using Monte Carlo simulations. See Table B.3 for a detailed description of distributions used in simulating each pathogen's dose-response model.

[d] Dose-response for norovirus and unknown *Cryptosporidium* species are highly uncertain, so ranges are provided instead of point estimates.

Risk was characterized as the probability of AGI per recreational event for each combination of pathogen (n = 9), site (n = 6), and activity (n = 4). Total risk across all pathogens was also calculated for each combination of site and activity, yielding a total of 240 individual risk estimates. These estimates are summarized under Objective 2 of the main text above and reported comprehensively in Appendix C. Risk was also characterized with respect to fecal source based on the co-occurrence of pathogen detections with source-specific MST markers. Finally, risk was characterized with respect to concentrations of the fecal indicator organism *E. coli* using sample-level *E. coli* data from companion samples collected simultaneously with pathogen and MST samples. These companion samples were analyzed for *E. coli* using the standard enzyme substrate test (9223B-QT; Rice et al. 2012) by Des Moines Water Works.

Risk estimates were generated using 2-dimensional Monte Carlo simulations in R version 4.1.1 with the package “mc2d” (Pouillot and Delignette-Muller 2010; R Core Team 2021). This approach incorporates the effects of variability and uncertainty in QMRA inputs on risk estimates. Inputs with variable components included pathogen, MST marker, and *E. coli* concentrations as well as water ingestion volumes. Inputs with uncertain components included pathogen, MST marker, and *E. coli* concentrations, most dose harmonization factors, water ingestion volumes, and dose-response parameters. Variability and uncertainty in pathogen, MST, and *E. coli* concentrations were estimated using a bootstrapping procedure described previously (Burch et al. 2021; 2022). Variability and uncertainty in all other QMRA inputs were defined based on distributions reported in the published literature. Table B.3 summarizes QMRA inputs with respect to their representation in 2-dimensional Monte Carlo simulations.

The level of risk that can be detected with a stated level of certainty is reported in Table B.4. Values represent the risk of illness for exposure to pathogens that are present in water at theoretical 95%, 90%, and 50% limits of detection (Stokdyk et al. 2016). By definition, the limit of detection is a probabilistic parameter, so multiple levels of certainty are presented. At a given level of certainty, the probability of detecting a pathogen decreases as the concentration decreases, which means that lower pathogen concentrations are detected less often. Samples less than a given limit of detection can be detected, but there is a lower probability of doing so (e.g., < 95% probability). At the 95% probability level for all tested pathogens, the analysis methods are capable of detecting risk that is less than the US EPA benchmark of 36 AGI cases per 1,000 recreation events. For example, there is a 95% probability of detecting an adenovirus risk equal to 0.73 AGI cases per 1,000 recreation events, and lower levels of risk can be detected with lower levels of certainty. In comparison, the average risk of illness from adenovirus that was actually detected at Beaver Creek was 4 AGI cases per 1,000 swimming events (the average includes the many exposures with dose equal to zero), which is 5 times greater than the risk associated with the 95% limit of detection.

Table B.3. Inputs for 2-dimensional Monte Carlo (2DMC) simulations.

Input	Value(s) [a]	2DMC Type	Reference(s)
Pathogen concentrations, C_{ijk} • i indexes site • j indexes pathogen within i • k indexes observation within ij • K total observations for each combination of site and pathogen	k ~ Empirical (via bootstrap sampling with replacement)	Uncertain	This report
	$\{C_{ijk} \mid C_{ij1}, C_{ij2}, \ldots, C_{ijK}\}$ ~ Empirical (gc/L) (via qPCR analyses in dead-end ultrafiltration samples)	Variable	
Adenovirus dose harmonization, H_{adeno}	H_{adeno} = 700 gc/TCID$_{50}$	Constant	Kundu et al. 2013
Campylobacter jejuni dose harmonization, H_{campy}	H_{campy} ~ Normal(7.4, 1.2) gc/CFU	Uncertain	Corsi et al. 2016
EPEC dose harmonization, H_{EPEC}	H_{EPEC} ~ Normal(5.6, 0.67) gc/CFU	Uncertain	Corsi et al. 2016
Salmonella dose harmonization, H_{sal}	H_{sal} ~ Normal(5.6, 0.67) gc/CFU	Uncertain	Corsi et al. 2016
Cryptosporidium dose harmonization (all species), H_{crypto}	H_{crypto} ~ Normal(14, 0.4) gc/oocyst	Uncertain	Stokdyk et al. 2019
Water consumption per person per recreational event – swimming, all ages, $V_{swim\text{-}all}$ • V_{low} = low estimate • V_{mid} = mid-range estimate • V_{high} = high estimate • $\omega_{swim\text{-}all}$ = mixing weights for low, mid, high	V_{low} ~ Trunc-Ln-normal(2.92, 1.43, -Inf, 4.09) mL	Variable	Dufour et al. 2006 Soller et al. 2017
	V_{mid} ~ Ln-normal(2.59, 1.43) mL	Variable	DeFlorio-Barker et al. 2018
	V_{high} ~ Log$_{10}$-normal(1.20, 0.68) mL	Variable	Boehm and Soller 2020
	$\{\omega_{swim\text{-}all} \mid \omega_{low}, \omega_{mid}, \omega_{high}\} = \{1/3, 1/3, 1/3\}$	Uncertain	Assumed
	$V_{swim\text{-}all}$ ~ Mixture($\{V_{low}, V_{mid}, V_{high}\}$, $\omega_{swim\text{-}all}$) mL	Variable and Uncertain	Derived from inputs
Water consumption per person per recreational event – swimming, children 6-12 years old, $V_{swim\text{-}kids}$	$V_{swim\text{-}kids}$ ~ Ln-normal(3.41, 1.10) mL (uncertainty defined by re-sampling distribution in uncertainty dimension)	Variable and Uncertain	DeFlorio-Barker et al. 2018

Water consumption per person per recreational event – limited contact without head immersion, V_{LC} • $\mu\text{-}Ln_{LC}$ = mean of Ln-normal distribution • $\sigma\text{-}Ln_{LC}$ = std. dev. of Ln-normal distribution • ρ_{LC} = correlation between $\mu\text{-}Ln_{LC}$ and $\sigma\text{-}Ln_{LC}$	$\mu\text{-}Ln_{LC}$ ~ Uniform(0.69, 0.83)	Uncertain	Dorevitch et al. 2011
	$\sigma\text{-}Ln_{LC}$ ~ Uniform(1.03, 1.08)	Uncertain	
	ρ_{LC} = -0.99	Constant	
	V_{LC} ~ Ln-normal($\mu\text{-}Ln_{LC}$, $\sigma\text{-}Ln_{LC}$) mL	Variable and Uncertain	
Water consumption per person per recreational event – limited contact with head immersion, $V_{LC\text{-}Imm}$ • $\mu\text{-}Ln_{LC\text{-}Imm}$ = mean of Ln-normal distribution • $\sigma\text{-}Ln_{LC\text{-}Imm}$ = std. dev. of Ln-normal distribution • $\rho_{LC\text{-}Imm}$ = correlation between $\mu\text{-}Ln_{LC\text{-}Imm}$ and $\sigma\text{-}Ln_{LC\text{-}Imm}$	$\mu\text{-}Ln_{LC\text{-}Imm}$ ~ Uniform(0.69, 1.28)	Uncertain	Dorevitch et al. 2011
	$\sigma\text{-}Ln_{LC\text{-}Imm}$ ~ Uniform(1.01, 1.06)	Uncertain	
	$\rho_{LC\text{-}Imm}$ = -0.93	Constant	
	$V_{LC\text{-}Imm}$ ~ Ln-normal($\mu\text{-}Ln_{LC\text{-}Imm}$, $\sigma\text{-}Ln_{LC\text{-}Imm}$) mL	Variable and Uncertain	
Adenovirus illness dose-response (exponential with morbidity) • r_{adeno} = exponential dose-response parameter for adenovirus infection • m_{adeno} = adenovirus morbidity ratio (probability of illness given infection)	r_{adeno} = 0.42	Constant	Crabtree et al. 1997
	m_{adeno} ~ Uniform(0, 1)	Uncertain	Haas et al. 1993
Norovirus illness dose-response (exponential with immunity and morbidity). Mixture of two models. • $r_{noro,1}$ = exponential dose-response parameter for norovirus infection, model 1 • $\varphi_{imm,1}$ = immunity parameter for norovirus infection among seropositive hosts, model 1 • $r_{noro,2}$ = exponential dose-response parameter for norovirus infection, model 2	$r_{noro,1}$ ~ Beta(2.91, 2734) (uncertainty defined by re-sampling distribution in uncertainty dimension)	Variable and Uncertain	Schmidt 2015
	$\varphi_{imm,1}$ = 0.7246	Constant	
	$r_{noro,2}$ = 1	Constant	Messner et al. 2014

• $\varphi_{imm,2}$ = immunity parameter for norovirus infection among seropositive hosts, model 2 • ω_{noro} = mixing weights for norovirus model 1 and model 2 • r_{noro} = exponential dose-response parameter for norovirus infection • φ_{noro} = immunity parameter for norovirus infection among seropositive hosts • φ_{sero+} = proportion seropositive in population, applied to both models • m_{noro} = norovirus morbidity ratio, applied to both models	$\varphi_{imm,2} = 0.722$	Constant	Messner et al. 2014
	$\{\omega_{noro} \mid \omega_{model\ 1}, \omega_{model\ 2}\} = \{0.50, 0.50\}$	Constant	Assumed
	$r_{noro} \sim \text{Mixture}(\{r_{noro,1}, r_{noro,2}\}, \omega_{noro})$	Variable and Uncertain	Derived from inputs
	$\varphi_{noro} \sim \text{Mixture}(\{\varphi_{noro,1}, \varphi_{noro,2}\}, \omega_{noro})$	Uncertain	Derived from inputs
	$\varphi_{sero+} = 0.8$	Constant	Lindesmith et al. 2003
	$m_{noro} \sim \text{Beta}(24, 16)$	Uncertain	Teunis et al. 2008a
Campylobacter jejuni illness dose-response (exact beta-Poisson with morbidity) • α_{campy} = hypergeometric dose-response parameter for *Campylobacter jejuni* infection • β_{campy} = hypergeometric dose-response parameter for *Campylobacter jejuni* infection • m_{campy} = morbidity ratio for *Campylobacter jejuni*	$\alpha_{campy} \sim$ Empirical	Uncertain	Schmidt et al. 2013
	$\beta_{campy} \sim$ Empirical	Uncertain	
	$m_{campy} = 0.22$	Constant	Medema et al. 1996
EPEC illness dose-response (exact beta-Poisson) • α_{EPEC} = hypergeometric dose-response parameter for EPEC illness • β_{EPEC} = hypergeometric dose-response parameter for EPEC illness	$\alpha_{EPEC} \sim$ Empirical	Uncertain	Teunis et al. 2008b
	$\beta_{EPEC} \sim$ Empirical	Uncertain	
Salmonella illness dose-response (approximate beta-Poisson)[c]	$\ln(\alpha_{sal}) \sim \text{Triangular}(-2.57, -2.02, -1.48)$	Uncertain	WHO 2002

<table>
<tr><td>α_{sal} = approximate beta-Poisson dose-response parameter for Salmonella illness
β_{sal} = approximate beta-Poisson dose-response parameter for Salmonella illness</td><td>$\ln(\beta_{sal})$ ~ Triangular(3.65, 3.94, 4.06)</td><td>Uncertain</td><td>WHO 2002</td></tr>
<tr><td>Cryptosporidium bovis illness dose-response (exponential)
r_{bovis} = exponential dose-response parameter for Cryptosporidium bovis illness</td><td>$\log_{10}(r_{bovis})$ ~ Uniform(-5.39, -2.57)</td><td>Uncertain</td><td>This study
Puleston et al. 2014
Chappell et al. 2015</td></tr>
<tr><td>Cryptosporidium meleagridis illness dose-response (exponential)
$r_{meleagridis}$ = exponential dose-response parameter for Cryptosporidium meleagridis illness</td><td>$r_{meleagridis} = 1.6\times10^{-5}$</td><td>Constant</td><td>This study
Chappell et al. 2011</td></tr>
<tr><td rowspan="8">Cryptosporidium parvum illness dose-response (exponential with morbidity). Exponential model constructed from mixture of 4 models in USEPA (2005): Models 1, 1b, 2, and 2b. Those models are each constructed from mixtures of uniform distributions based on respective percentiles of r reported in USEPA (2005).
$r_{model\ 1,5}$ = exponential dose-response parameter, Model 1, 5th percentile
$r_{model\ 1,25}$ = exponential dose-response parameter, Model 1, 25th percentile
$r_{model\ 1,50}$ = exponential dose-response parameter, Model 1, 50th percentile
$r_{model\ 1,75}$ = exponential dose-response parameter, Model 1, 75th percentile
$r_{model\ 1,95}$ = exponential dose-response parameter, Model 1, 95th percentile</td><td>$\text{logit}(r_{model\ 1,5})$ ~ Uniform(-10, -5.4)</td><td>Uncertain</td><td rowspan="8">USEPA 2005</td></tr>
<tr><td>$\text{logit}(r_{model\ 1,25})$ ~ Uniform(-5.4, -4.1)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1,50})$ ~ Uniform(-4.1, -3.2)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1,75})$ ~ Uniform(-3.2, -2.2)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1,95})$ ~ Uniform(-2.2, -1.1)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1,100})$ ~ Uniform(-1.1, 5.0)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1b,5})$ ~ Uniform(-10, -4.8)</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{model\ 1b,25})$ ~ Uniform(-4.8, -3.9)</td><td>Uncertain</td></tr>
</table>

<table>
<tr><td rowspan="14">• $r_{\text{model 1,100}}$ = exponential dose-response parameter, Model 1, 100th percentile
• $r_{\text{model 1b,5}}$ = exponential dose-response parameter, Model 1b, 5th percentile
• $r_{\text{model 1b,25}}$ = exponential dose-response parameter, Model 1b, 25th percentile
• $r_{\text{model 1b,50}}$ = exponential dose-response parameter, Model 1b, 50th percentile
• $r_{\text{model 1b,75}}$ = exponential dose-response parameter, Model 1b, 75th percentile
• $r_{\text{model 1b,95}}$ = exponential dose-response parameter, Model 1b, 95th percentile
• $r_{\text{model 1b,100}}$ = exponential dose-response parameter, Model 1b, 100th percentile
• $r_{\text{model 2,5}}$ = exponential dose-response parameter, Model 2, 5th percentile
• $r_{\text{model 2,25}}$ = exponential dose-response parameter, Model 2, 25th percentile
• $r_{\text{model 2,50}}$ = exponential dose-response parameter, Model 2, 50th percentile
• $r_{\text{model 2,75}}$ = exponential dose-response parameter, Model 2, 75th percentile
• $r_{\text{model 2,95}}$ = exponential dose-response parameter, Model 2, 95th percentile
• $r_{\text{model 2,100}}$ = exponential dose-response parameter, Model 2, 100th percentile
• $r_{\text{model 2b,5}}$ = exponential dose-response parameter, Model 2b, 5th percentile
• $r_{\text{model 2b,25}}$ = exponential dose-response parameter, Model 2b, 25th percentile
• $r_{\text{model 2b,50}}$ = exponential dose-response parameter, Model 2b, 50th percentile</td>
<td>$\text{logit}(r_{\text{model 1b,50}}) \sim \text{Uniform}(-3.9, -3.0)$</td><td>Uncertain</td><td rowspan="14">USEPA 2005</td></tr>
<tr><td>$\text{logit}(r_{\text{model 1b,75}}) \sim \text{Uniform}(-3.0, -2.0)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 1b,95}}) \sim \text{Uniform}(-2.0, -0.67)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 1b,100}}) \sim \text{Uniform}(-0.67, 5.0)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,5}}) \sim \text{Uniform}(-10, -4.6)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,25}}) \sim \text{Uniform}(-4.6, -3.5)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,50}}) \sim \text{Uniform}(-3.5, -2.7)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,75}}) \sim \text{Uniform}(-2.7, -2.0)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,95}}) \sim \text{Uniform}(-2.0, -0.94)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2,100}}) \sim \text{Uniform}(-0.94, 5.0)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2b,5}}) \sim \text{Uniform}(-10, -4.6)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2b,25}}) \sim \text{Uniform}(-4.6, -3.5)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2b,50}}) \sim \text{Uniform}(-3.5, -2.7)$</td><td>Uncertain</td></tr>
<tr><td>$\text{logit}(r_{\text{model 2b,75}}) \sim \text{Uniform}(-2.7, -1.9)$</td><td>Uncertain</td></tr>
</table>

• $r_{\text{model 2b,75}}$ = exponential dose-response parameter, Model 2b, 75th percentile • $r_{\text{model 2b,95}}$ = exponential dose-response parameter, Model 2b, 95th percentile • $r_{\text{model 2b,100}}$ = exponential dose-response parameter, Model 2b, 100th percentile • $\omega_{\text{percentile}}$ = mixing weights for percentiles in each model • $r_{\text{model 1}}$ = exponential dose-response parameter, Model 1 • $r_{\text{model 1b}}$ = exponential dose-response parameter, Model 1b • $r_{\text{model 2}}$ = exponential dose-response parameter, Model 2 • $r_{\text{model 2b}}$ = exponential dose-response parameter, Model 2b • ω_{model} = mixing weights for Models 1, 1b, 2, and 2b • r_{parvum} = exponential dose-response parameter for *Cryptosporidium parvum* infection • m_{crypto} = *Cryptosporidium* morbidity ratio	logit($r_{\text{model 2b,95}}$) ~ Uniform(-1.9, -0.70)	Uncertain	USEPA 2005
	logit($r_{\text{model 2b,100}}$) ~ Uniform(-0.70, 5.0)	Uncertain	
	$\{\omega_{\text{percentile}} \mid \omega_{5}, \omega_{25}, \omega_{50}, \omega_{75}, \omega_{95}, \omega_{100}\}$ = {0.05, 0.2, 0.25, 0.25, 0.2, 0.05}	Constant	
	$r_{\text{model 1}}$ ~ Mixture($\{r_{\text{model 1,5}}, r_{\text{model 1,25}}, r_{\text{model 1,50}}, r_{\text{model 1,75}}, r_{\text{model 1,95}}, r_{\text{model 1,100}}\}, \omega_{\text{percentile}}$)	Uncertain	Derived from inputs
	$r_{\text{model 1b}}$ ~ Mixture($\{r_{\text{model 1b,5}}, r_{\text{model 1b,25}}, r_{\text{model 1b,50}}, r_{\text{model 1b,75}}, r_{\text{model 1b,95}}, r_{\text{model 1b,100}}\}, \omega_{\text{percentile}}$)	Uncertain	Derived from inputs
	$r_{\text{model 2}}$ ~ Mixture($\{r_{\text{model 2,5}}, r_{\text{model 2,25}}, r_{\text{model 2,50}}, r_{\text{model 2,75}}, r_{\text{model 2,95}}, r_{\text{model 2,100}}\}, \omega_{\text{percentile}}$)	Uncertain	Derived from inputs
	$r_{\text{model 2b}}$ ~ Mixture($\{r_{\text{model 2b,5}}, r_{\text{model 2b,25}}, r_{\text{model 2b,50}}, r_{\text{model 2b,75}}, r_{\text{model 2b,95}}, r_{\text{model 2b,100}}\}, \omega_{\text{percentile}}$)	Uncertain	Derived from inputs
	$\{\omega_{\text{model}} \mid \omega_{\text{model1}}, \omega_{\text{model1b}}, \omega_{\text{model2}}, \omega_{\text{model2b}}\}$ = {0.25, 0.25, 0.25, 0.25}	Constant	Assumed
	r_{parvum} ~ Mixture($\{r_{\text{model 1}}, r_{\text{model 1b}}, r_{\text{model 2}}, r_{\text{model 2b}},\}, \omega_{\text{model}}$)	Uncertain	Derived from inputs
	m_{crypto} ~ Beta(7, 11)	Uncertain	DuPont et al. 1995
Illness dose-response for unknown *Cryptosporidium* spp. (exponential). Mixture of two models. • $r_{\text{unknown 1}}$ = exponential dose-response parameter for unknown *Cryptosporidium* spp. (assumed non-infectious) • $r_{\text{unknown 2}}$ = exponential dose-response parameter for unknown *Cryptosporidium* spp. (assumed infectious)	$r_{\text{unknown 1}} = 0$	Constant	Assumed
	$\log_{10}(r_{\text{unknown 2}})$ ~ Uniform(-5.39, -0.85)	Uncertain	This study Chappell et al. 2006 Chappell et al. 2015
	$\{\omega_{\text{unknown}} \mid \omega_{\text{unknown 1}}, \omega_{\text{unknown 2}}\}$ = {0.52. 0.48}	Constant	This study Zahedi and Ryan 2020 Ryan et al. 2021

• ω_{unknown} = mixing weights for non-infectious and infectious *Cryptosporidium* spp. • r_{unknown} = exponential dose-response parameter for unknown *Cryptosporidium* spp. (infectious and non-infectious combined)	r_{unknown} ~ Mixture({$r_{\text{unknown 1}}$, $r_{\text{unknown 2}}$}, ω_{unknown})	Uncertain	Derived from inputs

[a] Statistical distributions were parameterized as Beta(shape1, shape2), Normal(mean, SD), Ln-normal(meanln, SDln), Log_{10}-normal(meanlog_{10}, SDlog_{10}), Uniform(min, max), Mixture(elements, mixing weights), Triangular(min, mode, max), and Trunc-Ln-normal(meanln, SDln, min, max). The logit function is defined as $\ln[x / (1 - x)]$.

[c] Approximate beta-Poisson and exact beta-Poisson produce equivalent results for *Salmonella* dose-response parameters (Burch et al. 2022).

Table B.4. Average detectable swimming risk per site by pathogen and probability level. Values in table are AGI cases per 1,000 water recreation events, calculated as point estimates from the given theoretical limits of detection (LOD) per total effective sample volume analyzed (Stokdyk et al. 2016).

Pathogen	95% detectable risk (LOD = 3.0 genomic copies)	90% detectable risk (LOD = 2.3 genomic copies)	50% detectable risk (LOD = 0.7 genomic copies)
Adenovirus	0.73	0.56	0.17
Norovirus [a]	5.5 – 350	4.0 – 350	1.3 – 340
Campylobacter jejuni	0.88	0.67	0.21
EPEC	3.8	2.9	0.89
Salmonella	1.1	0.88	0.27
Cryptosporidium parvum	3.5	2.7	0.82
Cryptosporidium bovis	0.019	0.015	0.0044
Cryptosporidium meleagridis	0.0031	0.0024	0.00073
Unknown *Cryptosporidium* spp. [a]	0 – 24	0 – 18	0 – 5.6

[a] Detectable risks for norovirus and unknown *Cryptosporidium* spp. are notably uncertain and therefore presented as ranges. Uncertainty for norovirus stems from a lack of definitive information in the scientific literature on its dose-response behavior (Van Abel et al. 2017). Uncertainty for unknown *Cryptosporidium* spp. results from incomplete species information in the current study (Objective 1) and broad variability in the dose-response behavior of those *Cryptosporidium* species known to infect humans (Zahedi & Ryan 2020; Ryan et al. 2021).

References for Appendix B

Boehm AB, Soller JA. 2020. Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination. Microbial Risk Analysis. 16:100139.

Burch TR, Stokdyk JP, Spencer SK, Kieke Jr BA, Firnstahl AD, Muldoon MA, Borchardt MA. 2021. Quantitative microbial risk assessment for contaminated private wells in the fractured dolomite aquifer of Kewaunee County, Wisconsin. Environmental Health Perspectives. 129:067003.

Burch TR, Stokdyk JP, Rice N, Anderson AC, Walsh JF, Spencer SK, Firnstahl AD, Borchardt MA. 2022. Statewide quantitative microbial risk assessment for waterborne viruses, bacteria, and protozoa in public water supply wells in Minnesota. Environmental Science & Technology. 56:6315-6324.

Chappell CL, Okhuysen PC, Langer-Curry R, Widmer G, Akiyoshi DE, Tanriverdi S, Tzipori S. 2006. *Cryptosporidium hominis*: experimental challenge of healthy adults. The American Journal of Tropical Medicine and Hygiene. 75:851-857.

Chappell CL, Okhuysen PC, Langer-Curry RC, Akiyoshi DE, Widmer G, Tzipori S. 2011. *Cryptosporidium meleagridis*: Infectivity in healthy adult volunteers. The American Journal of Tropical Medicine and Hygiene. 85:238-242.

Chappell CL, Okhuysen PC, Langer-Curry RC, Lupo PJ, Widmer G, Tzipori S. 2015. *Cryptosporidium muris*: Infectivity and illness in healthy adult volunteers. The American Journal of Tropical Medicine and Hygiene. 92:50-55.

Corsi SR, Borchardt MA, Carvin RB, Burch TR, Spencer SK, Lutz MA, McDermott CM, Busse KM, Kleinheinz GT, Feng X, Zhu J. 2016. Human and bovine viruses and bacteria at three Great Lakes beaches: Environmental variable associations and health risk. Environmental Science & Technology. 50:987-995.

Crabtree KD, Gerba CP, Rose JB, Haas CN. 1997. Waterborne adenovirus: A risk assessment. Water Science and Technology. 35:1-6.

DeFlorio-Barker S, Arnold BF, Sams EA, Dufour AP, Colford JM, Weisberg SB, Schiff KC, Wade TJ. 2018. Child environmental exposures to water and sand at the beach: Findings from studies of over 68,000 subjects at 12 beaches. Journal of Exposure Science & Environmental Epidemiology. 28:93-100.

Dorevitch S, Panthi S, Huang Y, Li H, Michalek AM, Pratap P, Wroblewski M, Liu L, Scheff PA, Li A. 2011. Water ingestion during water recreation. Water Research. 45:2020-2028.

Dufour AP, Evans O, Behymer TD, Cantu R. 2006. Water ingestion during swimming activities in a pool: A pilot study. Journal of Water and Health. 4:425-430.

DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. New England Journal of Medicine. 332:855-859.

Haas CN, Rose JB, Gerba C, Regli S. 1993. Risk assessment of virus in drinking water. Risk Analysis. 13:545-552.

Haas CN, Rose JB, Gerba CP. 2014. *Quantitative Microbial Risk Assessment*. John Wiley & Sons. Hoboken, New Jersey.

Kundu A, McBride G, Wuertz S. 2013. Adenovirus-associated health risks for recreational activities in a multi-use coastal watershed based on site-specific quantitative microbial risk assessment. Water Research. 47:6309-6325.

Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang XI, Lindblad L, Stewart P, LePendu J, Baric R. 2003. Human susceptibility and resistance to Norwalk virus infection. Nature Medicine. 9:548-553.

Medema GJ, Teunis PF, Havelaar AH, Haas CN. 1996. Assessment of the dose-response relationship of *Campylobacter jejuni*. International Journal of Food Microbiology. 30:101-111.

Messner MJ, Berger P, Nappier SP. 2014. Fractional Poisson—A simple dose-response model for human norovirus. Risk Analysis. 34:1820-1829.

Pouillot R, Delignette-Muller ML. 2010. Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages. International Journal of Food Microbiology. 142:330-340.

Puleston RL, Mallaghan CM, Modha DE, Hunter PR, Nguyen-Van-Tam JS, Regan CM, Nichols GL, Chalmers RM. 2014. The first recorded outbreak of cryptosporidiosis due to *Cryptosporidium cuniculus* (formerly rabbit genotype), following a water quality incident. Journal of Water and Health. 12:41-50.

R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org.

Rice EW, Baird RB, Eaton AD, Clesceri LS, Eds. 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed.; American Public Health Association: Washington, DC.

Ryan U, Zahedi A, Feng Y, Xiao L. 2021. An update on zoonotic *Cryptosporidium* species and genotypes in humans. Animals. 11:3307.

Schmidt PJ, Pintar KD, Fazil AM, Topp E. 2013. Harnessing the theoretical foundations of the exponential and beta-Poisson dose-response models to quantify parameter uncertainty using Markov chain Monte Carlo. Risk Analysis. 33:1677-1693.

Schmidt PJ. 2015. Norovirus dose–response: Are currently available data informative enough to determine how susceptible humans are to infection from a single virus? Risk Analysis. 35:1364-1383.

Soller JA, Schoen M, Steele JA, Griffith JF, Schiff KC. 2017. Incidence of gastrointestinal illness following wet weather recreational exposures: Harmonization of quantitative microbial risk assessment with an epidemiologic investigation of surfers. Water Research. 121:280-289.

Stokdyk JP, Firnstahl AD, Spencer SK, Burch TR, Borchardt MA. 2016. Determining the 95% limit of detection for waterborne pathogen analyses from primary concentration to qPCR. Water Research. 96:105-113.

Stokdyk JP, Spencer SK, Walsh JF, de Lambert JR, Firnstahl AD, Anderson AC, Rezania LI, Borchardt MA. 2019. *Cryptosporidium* incidence and surface water influence of groundwater supplying public water systems in Minnesota, USA. Environmental Science & Technology. 53:3391-3398.

Teunis PF, Ogden ID, Strachan NJ. 2008a. Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. Epidemiology & Infection. 136:761-770.

Teunis PF, Moe CL, Liu P, E. Miller S, Lindesmith L, Baric RS, Le Pendu J, Calderon RL. 2008b. Norwalk virus: How infectious is it? Journal of Medical Virology. 80:1468-1476.

U.S. Environmental Protection Agency (USEPA). 2005. Appendices to the Economic Analysis for the Final Long Term 2 Enhanced Surface Water Treatment Rule, Volume II (H - U). EPA 815-R-06-001. Office of Water, Washington, DC.

Van Abel N, Schoen ME, Kissel JC, Meschke JS. 2017. Comparison of risk predicted by multiple norovirus dose–response models and implications for quantitative microbial risk assessment. Risk Analysis. 37:245-264.

World Health Organization. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. http://www.fao.org/3/a-y4392e.pdf [accessed September 11, 2019].

Zahedi A, Ryan U. 2020. *Cryptosporidium* – An update with an emphasis on foodborne and waterborne transmission. Research in Veterinary Science. 132:500-512.

Appendix C: Supplementary results tables

Table C.1. Total risk across all pathogens by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming - all ages		Swimming - children		Limited contact w/ head immersion		Limited contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	67	16 – 215	83	22 – 263	38	4 – 140	34	3 – 132
Des Moines River	5	0.0030 – 142	8	0.0056 – 183	0.80	0.00046 – 75	0.67	0.00034 – 70
Fourmile Creek N	15	2 – 95	23	4 – 123	3	0.41 – 37	3	0.38 – 28
Fourmile Creek S	6	0.66 – 131	10	1 – 165	2	0.12 – 64	1	0.091 – 59
Raccoon River	24	2 – 128	33	3 – 179	9	0.43 – 55	8	0.38 – 49
Walnut Creek	42	4 – 198	52	7 – 225	22	0.88 – 143	20	0.73 – 137

Table C.2. Adenovirus risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	4	0.056 – 24	7	0.10 – 34	0.77	0.010 – 4	0.64	0.0076 – 3
Des Moines River	-[a]	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek S	-	- – -	-	- – -	-	- – -	-	- – -
Raccoon River	-	- – -	-	- – -	-	- – -	-	- – -
Walnut Creek	-	- – -	-	- – -	-	- – -	-	- – -

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.3. Norovirus risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	34	4 – 105	39	6 – 105	23	0.94 – 105	22	0.76 – 105
Des Moines River	-[a]	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek S	-	- – -	-	- – -	-	- – -	-	- – -
Raccoon River	-	- – -	-	- – -	-	- – -	-	- – -
Walnut Creek	13	0 – 68	15	0 – 68	4	0 – 68	3	0 – 68

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.4. *Campylobacter jejuni* risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	-[a]	- – -	-	- – -	-	- – -	-	- – -
Des Moines River	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	0.48	0 – 4	0.81	0 – 6	0.092	0 – 1	0.078	0 – 1
Fourmile Creek S	3	0.14 – 11	4	0.26 – 14	0.72	0.023 – 5	0.60	0.022 – 3
Raccoon River	2	0 – 9	3	0 – 11	0.65	0 – 5	0.56	0 – 4
Walnut Creek	2	0.12 – 10	3	0.22 – 13	0.44	0.019 – 4	0.36	0.015 – 3

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.5. Enteropathogenic *E. coli* (EPEC) risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	6	0.12 – 100	9	0.17 – 127	1	0.035 – 41	1	0.029 – 35
Des Moines River	-[a]	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	2	0 – 46	3	0 – 66	0.40	0 – 14	0.34	0 – 12
Fourmile Creek S	-	- – -	-	- – -	-	- – -	-	- – -
Raccoon River	1	0 – 54	2	0 – 68	0.43	0 – 26	0.39	0 – 22
Walnut Creek	4	0 – 72	6	0 – 92	0.95	0 – 30	0.80	0 – 26

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.6. *Salmonella* risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	3	0 – 13	5	0 – 18	0.95	0 – 4	0.81	0 – 3
Des Moines River	-[a]	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	4	0 – 15	6	0 – 22	0.96	0 – 4	0.81	0 – 3
Fourmile Creek S	0.27	0 – 1	0.48	0 – 2	0.045	0 – 0.20	0.037	0 – 0.15
Raccoon River	0.33	0 – 2	0.59	0 – 2	0.057	0 – 0.25	0.046	0 – 0.21
Walnut Creek	-	- – -	-	- – -	-	- – -	-	- – -

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.7. *Cryptosporidium bovis* risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	0.88	0.0006 – 30	1	0.0012 – 44	0.13	0.00010 – 7	0.10	0.000092 – 5
Des Moines River	1	0.0012 – 46	2	0.0024 – 61	0.16	0.00019 – 15	0.13	0.00016 – 13
Fourmile Creek N	0.079	0 – 9	0.14	0 – 15	0.011	0 – 1	0.0096	0 – 1
Fourmile Creek S	0.26	0 – 16	0.41	0 – 25	0.035	0 – 3	0.028	0 – 3
Raccoon River	0.92	0 – 30	1	0 – 43	0.12	0 – 7	0.10	0 – 6
Walnut Creek	0.69	0.019 – 19	1	0.034 – 27	0.092	0.0025 – 3	0.072	0.0023 – 2

Table C.8. *Cryptosporidium meleagridis* risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	-[a]	- – -	-	- – -	-	- – -	-	- – -
Des Moines River	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek S	-	- – -	-	- – -	-	- – -	-	- – -
Raccoon River	0.0022	0 – 0.020	0.0040	0 – 0.025	0.00033	0 – 0.0021	0.00028	0 – 0.0016
Walnut Creek	-	- – -	-	- – -	-	- – -	-	- – -

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.9. *Cryptosporidium parvum* risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	-[a]	- – -	-	- – -	-	- – -	-	- – -
Des Moines River	-	- – -	-	- – -	-	- – -	-	- – -
Fourmile Creek N	1	0 – 17	2	0 – 24	0.20	0 – 5	0.16	0 – 4
Fourmile Creek S	-	- – -	-	- – -	-	- – -	-	- – -
Raccoon River	6	0 – 37	8	0 – 43	2	0 – 25	2	0 – 23
Walnut Creek	-	- – -	-	- – -	-	- – -	-	- – -

[a] Short dashes indicate that the given pathogen was not detected at a site; risk for that pathogen was therefore not estimated.

Table C.10. Unknown *Cryptosporidium* spp. risk by site and activity. Values in table are acute gastrointestinal illness cases per 1,000 water recreation events. CI, confidence interval.

Site	Swimming – all ages		Swimming – children		Limited Contact w/ head immersion		Limited Contact w/out head immersion	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
Beaver Creek	0	0 – 140	0	0 – 178	0	0 – 77	0	0 – 68
Des Moines River	0	0 – 130	0	0 – 172	0	0 – 72	0	0 – 67
Fourmile Creek N	0	0 – 67	0	0 – 89	0	0 – 24	0	0 – 21
Fourmile Creek S	0	0 – 126	0	0 – 160	0	0 – 63	0	0 – 57
Raccoon River	0	0 – 91	0	0 – 126	0	0 – 31	0	0 – 26
Walnut Creek	0	0 – 174	0	0 – 197	0	0 – 123	0	0 – 119

Table C.11. Sensitivity analysis on QMRA inputs for Beaver Creek. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Adenovirus – concentration	0.44	0.43	0.44	0.43
Norovirus – concentration	0.43	0.42	0.43	0.42
Unknown *Cryptosporidium* spp. – dose-response	0.41	0.44	0.27	0.26
Norovirus – dose-response	0.34	0.23	0.61	0.64
Cryptosporidium bovis – concentration	0.34	0.36	0.28	0.27
EPEC – dose-response	0.31	0.35	0.19	0.17
EPEC – concentration	0.21	0.25	0.10	0.09
Unknown *Cryptosporidium* spp. – concentration	0.18	0.18	0.15	0.15
Water ingestion volume	0.14	0.03	0.04	0.03
Cryptosporidium bovis – dose-response	0.09	0.11	0.02	0.02
EPEC – dose harmonization	-0.05	N.S.	-0.06	-0.06
Cryptosporidium spp. – dose harmonization	N.S.	N.S.	N.S.	N.S.
Salmonella – dose harmonization	N.S.	N.S.	N.S.	N.S.
Adenovirus – dose-response	N.S.	N.S.	N.S.	N.S.
Salmonella – dose response	N.S.	N.S.	N.S.	N.S.
Salmonella – concentration	N.S.	N.S.	N.S.	N.S.

EPEC, enteropathogenic *E. coli*

Table C.12. Sensitivity analysis on QMRA inputs for Des Moines River. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Unknown *Cryptosporidium* spp. – dose-response	0.66	0.67	0.65	0.65
Cryptosporidium bovis – concentration	0.42	0.42	0.41	0.41
Cryptosporidium bovis – dose-response	0.36	0.36	0.37	0.37
Water ingestion volume	0.11	0.03	0.02	0.00
Unknown *Cryptosporidium* spp. – concentration	0.06	0.05	0.07	0.07
Cryptosporidium spp. – dose harmonization	-0.05	N.S.	-0.05	-0.05

Table C.13. Sensitivity analysis on QMRA inputs for Fourmile Creek North. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Unknown *Cryptosporidium* spp. - dose-response	0.38	0.40	0.35	0.36
EPEC - dose-response	0.34	0.35	0.36	0.36
EPEC - concentration	0.34	0.34	0.32	0.33
Cryptosporidium parvum - concentration	0.32	0.33	0.31	0.32
Salmonella - concentration	0.31	0.31	0.35	0.35
Water ingestion volume	0.20	0.06	0.16	0.06
Cryptosporidium parvum - dose-response	0.15	0.15	0.17	0.17
Cryptosporidium bovis - dose-response	0.09	0.11	0.06	0.06
Campylobacter jejuni - dose-response	0.09	0.10	0.11	0.11
Salmonella - dose-response	0.06	0.07	0.07	0.07
Cryptosporidium bovis - concentration	0.06	0.07	N.S.	N.S.
Unknown *Cryptosporidium* spp. - concentration	N.S.	N.S.	N.S.	N.S.
Salmonella - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - dose harmonization	N.S.	N.S.	N.S.	N.S.
EPEC - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - concentration	N.S.	N.S.	N.S.	N.S.
Cryptosporidium spp. - dose harmonization	N.S.	N.S.	N.S.	N.S.

EPEC, enteropathogenic *E. coli*

Table C.14. Sensitivity analysis on QMRA inputs for Fourmile Creek South. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Unknown *Cryptosporidium* spp. - dose-response	0.64	0.65	0.59	0.59
Campylobacter jejuni - dose-response	0.35	0.32	0.46	0.48
Cryptosporidium bovis - dose-response	0.26	0.28	0.21	0.20
Campylobacter jejuni - concentration	0.23	0.23	0.25	0.24
Cryptosporidium bovis - concentration	0.19	0.21	0.14	0.14
Water ingestion volume	0.14	0.04	0.10	0.03
Unknown *Cryptosporidium* spp. - concentration	0.08	0.08	0.08	0.08
Salmonella - dose-response	-0.05	-0.05	-0.06	-0.06
Salmonella - dose harmonization	-0.05	-0.05	N.S.	-0.04
Salmonella - concentration	N.S.	N.S.	N.S.	N.S.
Cryptosporidium spp. - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - dose harmonization	N.S.	N.S.	N.S.	N.S.

Table C.15. Sensitivity analysis on QMRA inputs for Raccoon River. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Unknown *Cryptosporidium* spp. - dose-response	0.38	0.41	0.27	0.26
Cryptosporidium parvum - concentration	0.37	0.34	0.48	0.48
EPEC - dose-response	0.26	0.25	0.27	0.27
EPEC - concentration	0.26	0.27	0.22	0.21
Cryptosporidium bovis - dose-response	0.26	0.28	0.17	0.17
Cryptosporidium bovis - concentration	0.22	0.25	0.18	0.17
Water ingestion volume	0.16	0.02	0.09	0.04
Cryptosporidium parvum - dose-response	0.15	0.10	0.27	0.29
Campylobacter jejuni - dose-response	0.11	0.09	0.13	0.13
Salmonella - dose harmonization	-0.04	N.S.	N.S.	N.S.
Salmonella - concentration	N.S.	N.S.	N.S.	N.S.
Cryptosporidium spp. - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - concentration	N.S.	N.S.	N.S.	N.S.
EPEC - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - dose harmonization	N.S.	N.S.	N.S.	N.S.
Salmonella - dose-response	N.S.	N.S.	N.S.	N.S.
Unknown *Cryptosporidium* spp. - concentration	N.S.	N.S.	N.S.	N.S.
Cryptosporidium meleagridis - concentration	N.S.	N.S.	N.S.	N.S.

EPEC, enteropathogenic *E. coli*

Table C.16. Sensitivity analysis on QMRA inputs for Walnut Creek. Values in table are Spearman's rank correlation coefficients between the given input and risk, sorted by absolute value for swimming – all ages. N.S. indicates "not significant" (p-value ≥ 0.05).

QMRA Input	Activity			
	Swimming – all ages	Swimming – children	Limited Contact w/ head immersion	Limited Contact w/out head immersion
Unknown *Cryptosporidium* spp. - dose-response	0.57	0.59	0.49	0.47
Norovirus - concentration	0.37	0.37	0.36	0.36
Norovirus - dose-response	0.29	0.21	0.47	0.49
EPEC - concentration	0.27	0.30	0.18	0.18
EPEC - dose-response	0.26	0.29	0.19	0.18
Unknown *Cryptosporidium* spp. - concentration	0.25	0.26	0.21	0.20
Water ingestion volume	0.14	0.05	0.03	0.03
Cryptosporidium bovis - dose-response	0.12	0.16	0.05	0.05
Campylobacter jejuni - dose-response	0.10	0.11	0.09	0.09
EPEC - dose harmonization	-0.09	-0.08	-0.09	-0.09
Campylobacter jejuni - concentration	0.05	0.06	0.03	0.03
Cryptosporidium bovis - concentration	N.S.	N.S.	-0.05	-0.06
Cryptosporidium spp. - dose harmonization	N.S.	N.S.	N.S.	N.S.
Campylobacter jejuni - dose harmonization	N.S.	N.S.	N.S.	N.S.

EPEC, enteropathogenic *E. coli*

Table C.17. Diagnostic performance of *E. coli* as an indicator for risk exceedances for activities other than swimming – all ages (see Table 4.1).

Site	Samples	*E. coli* exceedances (> 235 MPN/100 mL)	Swimming - children		Limited contact w/ head immersion		Limited contact w/out head immersion	
			True positive rate	False positive rate	True positive rate	False positive rate	True positive rate	False positive rate
Beaver Creek	25	19	100%	69%	100%	72%	100%	73%
Des Moines River	25	11	100%	42%	100%	44%	100%	44%
Fourmile Creek N	23	17	100%	70%	100%	73%	100%	74%
Fourmile Creek S	24	17	89%	70%	100%	71%	100%	71%
Raccoon River	26	12	95%	39%	100%	43%	100%	43%
Walnut Creek	24	14	64%	58%	76%	58%	79%	58%

* An exceedance is defined as a swimming event where risk was estimated to be greater than a probability of 36 acute gastrointestinal illness cases per 1,000 exposures.