Supplemental Content

# Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to pasturing cattle: prevalence, densities, and quantitative microbial risk assessment

by

Wilkes, G., Brassard, J., Edge, T.A., Gannon, V., Jokinen, C.C., Jones, T.H., Neumann, N., Pintar, K.D.M., Ruecker, N., Schmidt, P.J., Sunohara, M., Topp, E., and Lapen, D.R.

Manuscript: Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to pasturing cattle: prevalence, densities, and quantitative microbial risk assessment

QMRA Method: Probabilistic modelling and OpenBUGS code

This section provides the OpenBUGS code used in the evaluation of mean risk for *Cryptosporidium* spp., *Giardia*, and *E. coli* O157:H7, as well as some additional information about the utilized models. Details on the following modules are provided:

A) Non-constant analytical recovery analysis for *Cryptosporidium*/*Giardia* matrix spike data
B) Temporal concentration variability analysis for *Cryptosporidium*/*Giardia* enumeration data
C) Hierarchical infectivity analysis for *Cryptosporidium*
D) Temporal concentration variability and infectivity analysis for *E. coli* and *E. coli* O157:H7
E) Strain-to-strain variability analysis for the exponential *Cryptosporidium* dose-response model

Bayesian procedures used to evaluate parameter uncertainty for the exponential dose-response model (*Giardia*) and the actual beta-Poisson dose-response model (pathogenic *E. coli*) are provided in Schmidt *et al.* (2013).

The numerical integration approach used to evaluate mean risk ($P^*$) at each site uses 1) parameters of a gamma distribution ($\rho$,$\lambda$) describing temporal concentration variability for the target microorganism, 2) a constant fraction of target microorganisms that are infectious ($I$), 3) a constant volume of surface water that is consumed during a single swimming exposure ($V$), and 4) the parameter(s) of the exponential ($r$) or actual beta-Poisson dose-response model ($\alpha$,$\beta$). Schmidt *et al.* (Accepted) provided details on a similar numerical integration and how uncertainty in the specified parameter values was incorporated into a second-order risk assessment. This work differs only in the addition of a constant infectivity value (except in the case of *Giardia*) and use of an exponential dose-response model for *Cryptosporidium* spp. and *Giardia*. The numerical integration equations with exponential and actual beta-Poisson dose-response models, respectively, are as follows:

$$P^*(\rho,\lambda,I,V,r)=\sum_{d=1}^{\infty}\left[\frac{\Gamma(d+\rho)}{d!\Gamma(\rho)}\left(\frac{\lambda PV}{\lambda PV+1}\right)^d\left(\frac{1}{\lambda PV+1}\right)^{\rho}\right]\left[1-(1-r)^d\right]$$

$$P^*(\rho,\lambda,I,V,\alpha,\beta)=\sum_{d=1}^{\infty}\left[\frac{\Gamma(d+\rho)}{d!\Gamma(\rho)}\left(\frac{\lambda IV}{\lambda IV+1}\right)^d\left(\frac{1}{\lambda IV+1}\right)^{\rho}\right]\left[1-\frac{\Gamma(\alpha+\beta)\Gamma(d+\beta)}{\Gamma(\beta)\Gamma(d+\alpha+\beta)}\right]$$

**A) Non-constant Analytical Recovery Analysis for *Cryptosporidium*/*Giardia* Matrix Spike Data**

loga: logarithm (base 10) of shape parameter of beta dist. describing non-constant analytical recovery
- STOCHASTIC (unknown)

logb: logarithm (base 10) of shape parameter of beta dist. describing non-constant analytical recovery
- STOCHASTIC (unknown)

a: shape parameter of beta distribution describing non-constant analytical recovery
- DETERMINISTIC (parameter of interest)

b: shape parameter of beta distribution describing non-constant analytical recovery
- DETERMINISTIC (parameter of interest)

logC[i]: logarithm (base 10) of (oo)cyst conc. during $i^{th}$ sampling event - STOCHASTIC (unknown)

C[i]: (oo)cyst concentration during $i^{th}$ sampling event in (oo)cysts/L- DETERMINISTIC

Pf[i]: analytical recovery of $i^{th}$ field sample - STOCHASTIC (nuisance parameter)

VEf[i]: enumerated volume of $i^{th}$ field sample in litres - CONSTANT

EXf[i]: expected number of observed (oo)cysts in enumerated volume of $i^{th}$ field sample
- DETERMINISTIC

Xf[i]: number of observed (oo)cysts in enumerated volume of $i^{th}$ field sample
- STOCHASTIC (measured)

Pm[i]: analytical recovery of $i^{th}$ matrix spike sample - STOCHASTIC (nuisance parameter)

VTm[i]: total volume of $i^{th}$ matrix spike sample in litres - CONSTANT

ENative[i]: expected number of native (oo)cysts contained in total volume of $i^{th}$ matrix spike sample
- DETERMINISTIC

Native[i]: number of native (oo)cysts contained in total volume of $i^{th}$ matrix spike sample
- STOCHASTIC (nuisance parameter)

Seed[i]: number of seeded (oo)cysts added to total volume of $i^{th}$ matrix spike sample - CONSTANT

N[i]: number of total (oo)cysts contained in total volume of $i^{th}$ matrix spike sample - DETERMINISTIC

Q[i]: enumerated fraction of $i^{th}$ matrix spike sample - CONSTANT

PEm[i]: effective analytical recovery of $i^{th}$ matrix spike sample - DETERMINISTIC

Xm[i]: number of observed (oo)cysts in enumerated volume of $i^{th}$ matrix spike sample
- STOCHASTIC (measured)

```
model {
    # Prior g(a,b) = 1/ab with 0.000001 < a < 1000000, 0.000001 < b < 1000000
    loga ~ dunif(-6, 6)
    logb ~ dunif (-6, 6)
    # Transform loga and logb to a and b
    a <- pow(10, loga)
    b <- pow(10, logb)
    for (i in 1:m) {
        # Matrix (oo)cyst concentrations
        logC[i] ~ dunif(-6, 6) # Prior g(C[i]) = 1/C[i] with 0.000001 < C[i] < 1000000
        C[i] <- pow(10, logC[i])
        # Model for field sample count
        Pf[i] ~ dbeta(a, b)
        EXf[i] <- C[i] * VEf[i] * Pf[i]
        Xf[i] ~ dpois(EXf[i])
        # Model for matrix spike sample count
        Pm[i] ~ dbeta(a, b)
        ENative[i] <- C[i] * VTm[i]
        Native[i] ~ dpois(ENative[i])
        N[i] <- Native[i] + Seed[i]
        PEm[i] <- Pm[i] * Q[i]
        Xm[i] ~ dbin(PEm[i], N[i])
    }
}
```

In the analysis of *Cryptosporidium* and *Giardia* matrix spike recovery, it was assumed that there was a single beta distribution describing sample-to-sample variation in analytical recovery for each type of protozoan among all surface water monitoring sites. Matrix spike recovery data from 13 sites throughout the South Nation River basin were used in this analysis. The modelling approach is modified from Teunis and Havelaar (1999) and Schmidt (2010). It accounts for random measurement errors in the enumeration of both the field and matrix spike samples, which are not assumed to have equal analytical recovery. A uniform prior on the base 10 logarithm of concentration was used for the concentration of indigenous (oo)cysts in each matrix spike analysis. The numbers of seeded (oo)cysts were assumed to be precisely known.

**B) Temporal Concentration Variability Analysis for *Cryptosporidium*/*Giardia* Enumeration Data**

logmu: logarithm (base 10) of mean concentration - STOCHASTIC (unknown)
logsigma: logarithm (base 10) of standard deviation of concentration - STOCHASTIC (unknown)
mu: mean concentration - DETERMINISTIC
sigma: standard deviation of concentration - DETERMINISTIC
rho: shape parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC (parameter of interest)
lambda: scale parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC (parameter of interest)
tau: reciprocal of scale parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC
a: shape parameter of the beta distribution describing non-constant analytical recovery – CONSTANT
b: shape parameter of the beta distribution describing non-constant analytical recovery – CONSTANT
C[i]: concentration during $i^{th}$ sampling event in (oo)cysts/L - STOCHASTIC (nuisance parameter)
P[i]: analytical recovery of enumeration method for $i^{th}$ sample - STOCHASTIC (nuisance parameter)
V[i]: enumerated volume of $i^{th}$ sample in litres - CONSTANT
theta[i]: expected number of (oo)cysts in $i^{th}$ sample - DETERMINISTIC
X[i]: number of (oo)cysts observed in $i^{th}$ sample - STOCHASTIC (measured)

```
model {
    # Standard normal prior on logarithm (base 10) of mean and std. dev. of conc. in (oo)cysts/L
    logmu ~ dnorm(0, 1)
    logsigma ~ dnorm(0, 1)
    # Transform logmu and logsigma to rho and lambda
    mu <- pow(10, logmu)
    sigma <- pow(10, logsigma)
    rho <- mu * mu / (sigma * sigma)
    lambda <- sigma * sigma / mu
    tau <- 1 / lambda
    #   Probabilistic model
    for (i in 1:m) {
        C[i] ~ dgamma(rho, tau)
        P[i] ~ dbeta(a, b)
        theta[i] <- C[i] * V[i] * P[i]
        X[i] ~ dpois(theta[i])
    }
}
```

This procedure is based upon Schmidt and Emelko (2011). The principal difference is that independent standard normal priors are assumed for the mean and standard deviation of concentration in units of (oo)cysts $L^{-1}$.

The mean and standard deviation of analytical recovery were approximately 40% and 10% respectively for both *Cryptosporidium* and *Giardia*. The base-10 logarithms of the mean and standard deviation were assigned independent normal priors, as per Schmidt *et al.* (2013). The priors for *Cryptosporidium* and *Giardia* suggest that the mean and standard deviation of the (oo)cyst concentration were most likely near 1 (oo)cyst $L^{-1}$ and were unlikely to exceed 1000 (oo)cysts $L^{-1}$ or to fall below 0.001 (oo)cysts $L^{-1}$. Cumulative *Cryptosporidium* oocyst counts per cumulative enumerated volume at monitoring sites throughout the South Nation River watershed (with adjustment for 40% mean analytical recovery) ranged from 0.068-0.885 oocysts $L^{-1}$, so this prior is somewhat conservatively high. It was assumed that the standard deviations and means would have similar ranges of values and also that *Giardia* should have the same priors as were used for *Cryptosporidium*.

**C) Hierarchical Infectivity Analysis for *Cryptosporidium***

loga: logarithm (base 10) of shape parameter of beta dist. describing infectivity variation among sites
- STOCHASTIC (unknown)

logb: logarithm (base 10) of shape parameter of beta dist. describing infectivity variation among sites
- STOCHASTIC (unknown)

a: shape parameter of beta distribution describing infectivity variation among sites - DETERMINISTIC

b: shape parameter of beta distribution describing infectivity variation among sites - DETERMINISTIC

I[i]: Fraction of oocysts that are pathogenic at ith surface water monitoring site
- STOCHASTIC (parameter of interest - selected sites)

X[i]: number of sequences from ith surface water monitoring site classified as pathogenic
- STOCHASTIC (measured)

N[i]: number of sequences from ith surface water monitoring site – CONSTANT

```
model {
    #   Prior g(a,b) = 1/ab with 0.001 < a < 1000, 0.001 < b < 1000
    loga ~ dunif(-3,3)
    logb ~ dunif(-3,3)
    # Transform loga and logb to a and b
    a <- pow(10,loga)
    b <- pow(10,logb)
    #   Probabilistic model for fraction of sequences that are pathogenic
    for (i in 1:m) {
        I[i] ~ dbeta(a,b)
        X[i] ~ dbin(I[i],N[i])
    }
}
```

In the analysis of *Cryptosporidium* infectivity, it was assumed that each monitoring site has a constant fraction of total oocysts that are pathogenic and that this fraction varies from site to site according to a beta distribution. Data from 17 sites throughout the South Nation River basin (of which only four are addressed in this study) were used in the analysis.

**D) Temporal Concentration Variability and Infectivity Analysis for *E. coli* and *E. coli* O157:H7**

logmu: logarithm (base 10) of mean concentration - STOCHASTIC (unknown)
logsigma: logarithm (base 10) of standard deviation of concentration - STOCHASTIC (unknown)
mu: mean concentration - DETERMINISTIC
sigma: standard deviation of concentration - DETERMINISTIC
rho: shape parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC (parameter of interest)
lambda: scale parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC (parameter of interest)
tau: reciprocal of scale parameter of gamma distribution describing temporal concentration variability
- DETERMINISTIC
I: constant fraction of E. coli that are O157:H7
C[i]: concentration during $i^{th}$ sampling event in CFU/100mL - STOCHASTIC (measured)
theta[i]: expected number of CFU in $i^{th}$ sample - DETERMINISTIC
X[i]: O157:H7 presence/absence - STOCHASTIC (measured)

```
model {
    # Normal prior on logarithm (base 10) of mean and std. dev. of concentration in CFU/100mL
    logmu ~ dnorm(3, 1)
    logsigma ~ dnorm(3, 1)
    # Transform logmu and logsigma to rho and lambda
    mu <- pow(10, logmu)
    sigma <- pow(10, logsigma)
    rho <- mu * mu / (sigma * sigma)
    lambda <- sigma * sigma / mu
    tau <- 1 / lambda
    # Uniform prior on I
    I ~ dunif(0, 1)
    # Probabilistic model
    for (i in 1:m) {
        C[i] ~ dgamma(rho, tau)
        theta[i] <- 1 - exp(- C[i] * 5 * I)
        X[i] ~ dbern(theta[i])
    }
}
```

The distributions for *E. coli* were fit directly to colony forming unit (CFU) concentration estimates because raw data (i.e., counts, volumes, dilutions) were unavailable. A gamma distribution was fit to the concentration estimates without consideration of random measurement error in the plate count enumerations. For *E. coli*, it was assumed that the mean and standard deviation of the concentration were most likely near 1000 CFU 100 $mL^{-1}$ and were unlikely to exceed $10^6$ CFU 100 $mL^{-1}$ or to fall below 1 CFU 100 $mL^{-1}$. These priors are considered to be relatively uninformative due to the broad range of values that are considered to be highly plausible (6 orders of magnitude). In the case of *E. coli*, mean concentration estimates in the range of 73-1119 CFU 100 $mL^{-1}$ were obtained from the sites considered in Schmidt *et al.* ( 2013), and the prior on the mean is centred around 1000 CFU 100 $mL^{-1}$.

Each monitoring site was assumed to have a constant fraction of total *E. coli* that were *E. coli* O157:H7. A uniform prior on the interval (0,1) was used. A truncated uniform prior on the interval (0,0.5) was considered, but would not have changed the outcome given that values exceeding 0.5 were not supported by the available data. In this model, the probability of a positive *E. coli* O157:H7 presence-absence result is linked to the corresponding total *E. coli* concentration estimate.

**E) Strain-to-Strain Variability Analysis for the Exponential *Cryptosporidium* Dose-response Model**

loga: logarithm of shape parameter of beta distribution describing variation among strains
- STOCHASTIC (unknown)
logb: logarithm of shape parameter of beta distribution describing variation among strains
- STOCHASTIC (unknown)
a: shape parameter of beta distribution describing variation among strains - DETERMINISTIC
b: shape parameter of beta distribution describing variation among strains - DETERMINISTIC
R[i]: probability of infection by a single strain i oocyst - STOCHASTIC (nuisance parameter)
I[j]: strain index variable for jth consumer - CONSTANT
D[j]: mean dose consumed by jth consumer - CONSTANT
P[j]: probability of infection for jth consumer, who consumes mean dose D[j] of strain I[j] oocysts
- DETERMINISTIC
X[j]: infection indicator variable for jth consumer - STOCHASTIC (measured)
mu: mean of beta dist. describing variation among strains - DETERMINISTIC (parameter of interest)

```
model {
    # Prior g(a,b)=1/ab with each parameter constrained to [0.000001,1000000]
    loga ~ dunif(-6, 6)
    logb ~ dunif(-6, 6)
    a <- pow(10, loga)
    b <- pow(10, logb)
    mu <- a / (a + b)
    for (i in 1:m) {
        R[i] ~ dbeta(a, b)
    }
    for (j in 1:n) {
        P[j] <- 1 - exp(-D[j] * R[I[j]])
        X[j] ~ dbern(P[j])
    }
}
```

This procedure is modified from a modelling approach considered in U.S. EPA (2005). It assumes that each of 6 *Cryptosporidium* dose-response studies using different strains/species can be modelled by an exponential dose-response model, and that the parameters of these models are random values from a beta-distribution describing overall variation in dose-response among pathogenic *Cryptosporidium* strains/species. The mean of this distribution is then used as the parameter of an exponential dose-response model because variation among individual microorganisms (*i.e.* different strains/species of oocysts in a water body to which a consumer might be exposed) is averaged out in an exponential dose-response model (Schmidt *et al.*, 2013).

Schmidt, P.J., 2010. Addressing the Uncertainty Due to Random Measurement Errors in Quantitative Analysis of Microorganism and Discrete Particle Data. PhD Thesis, University of Waterloo, Waterloo, Ontario, Canada, 2010.

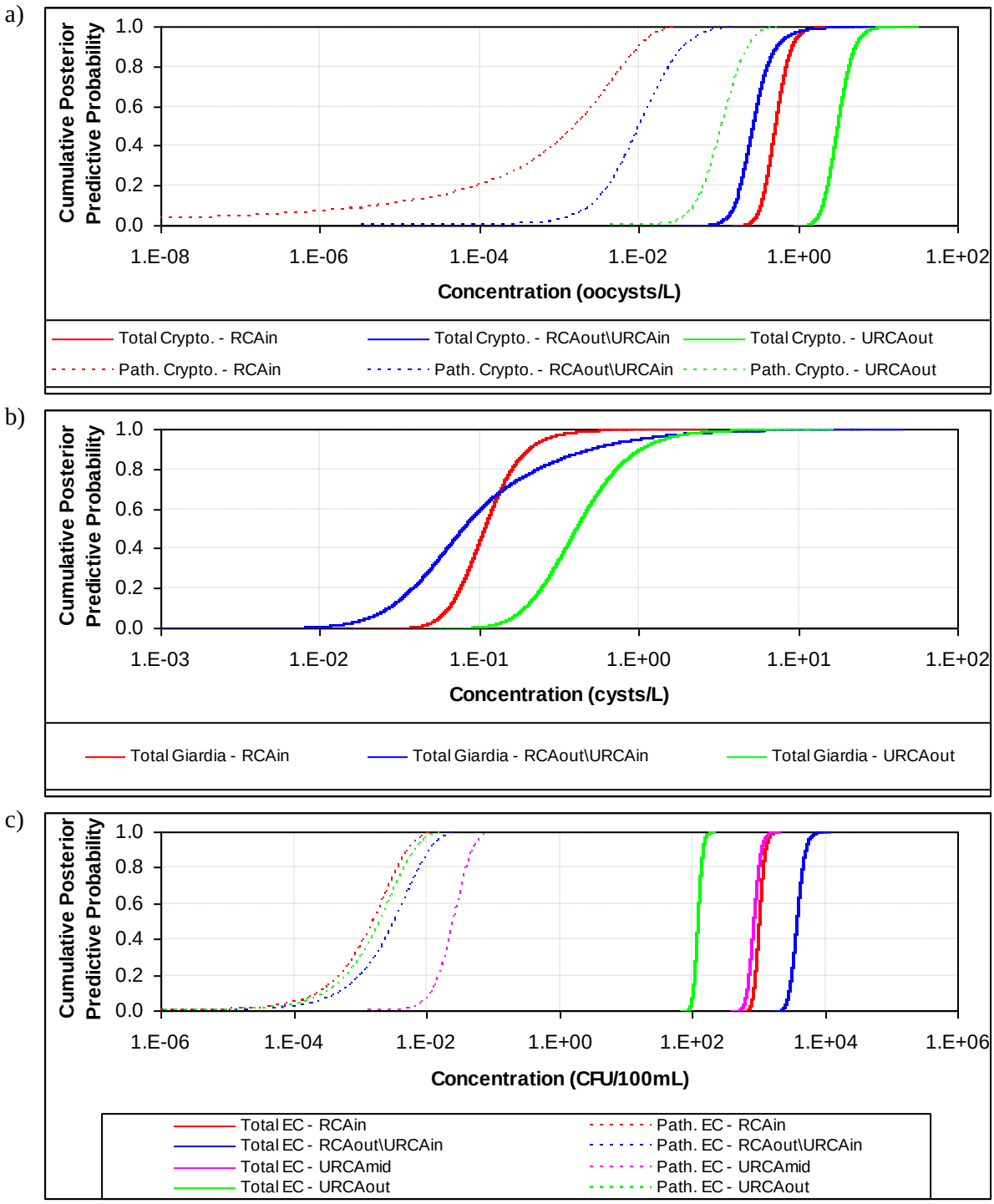
Schmidt, P.J., Emelko, M.B., 2011. QMRA and decision-making: Are we handling measurement errors associated with pathogen concentration data correctly? *Wat. Res.* 45, 427-438.

Schmidt, P.J., Pintar, K.D.M, Fazil, A.M., 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* http://dx.doi.org/10.1111/risa.12006.

Schmidt, P.J., Pintar, K.D.M., Fazil, A.M., Flemming, C.A., Lanthier, M., Laprade, N., Sunohara, M., Simhon, A., Thomas, J.L., Topp, E., Wilkes, G., Lapen, D.R., Accepted. Using *Campylobacter* spp. and *Escherichia coli* data and Bayesian microbial risk assessment to examine public health risks in agricultural watersheds under tile drainage management. *Wat. Res.* 47, 3255-3272.

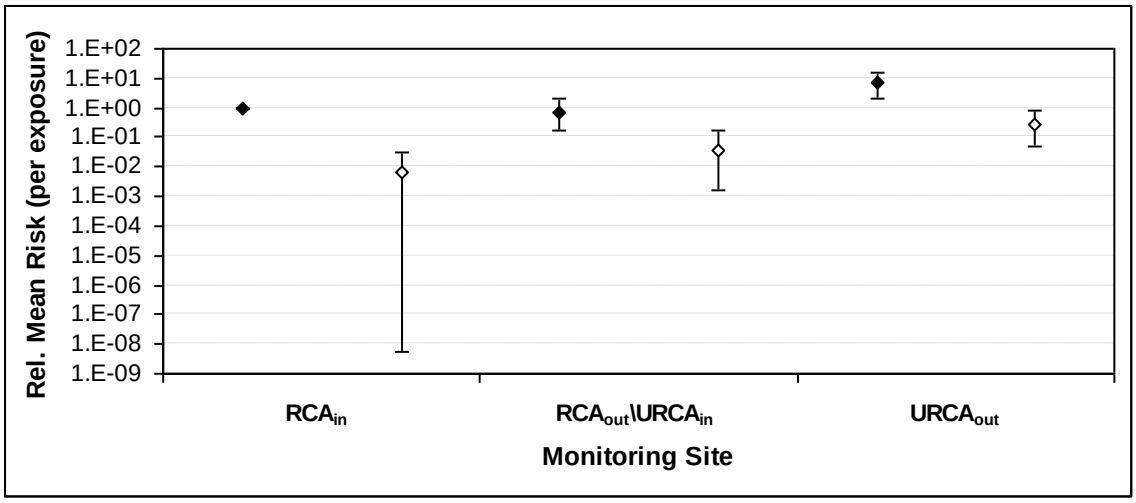
Teunis, P.F.M., Havelaar, A.H., 1999. *Cryptosporidium* in drinking water: Evaluation of the ILSI/RSI quantitative risk assessment framework. RIVM Report no. 284 550 006, RIVM, Bilthoven, The Netherlands.

United States Environmental Protection Agency (U.S. EPA), 2005. Economic Analysis for the Final Long Term 2 Enhanced Surface Water Treatment Rule; EPA 815-R-06-001, U.S. Environmental Protection Agency, Office of Water, Washington, DC.

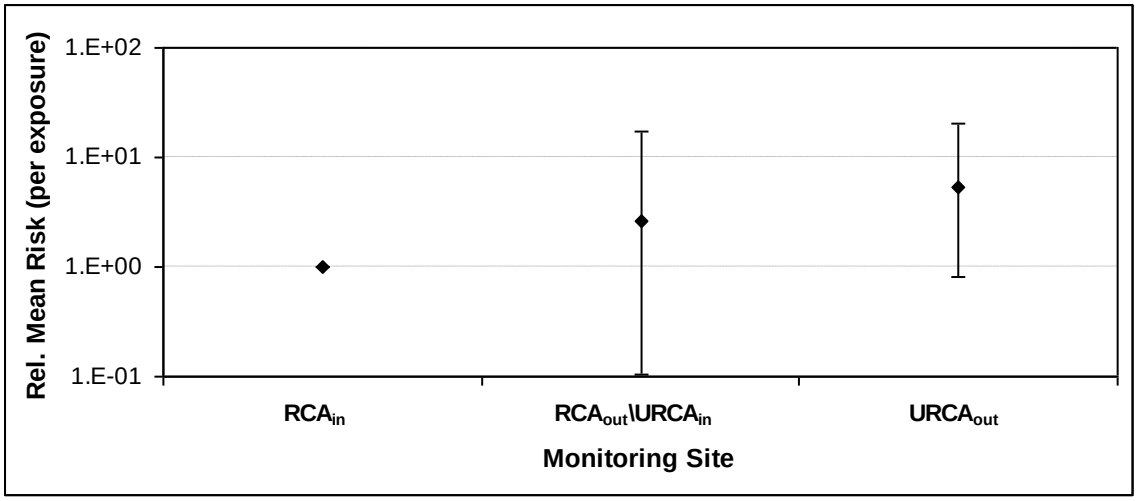



Supp. Cont. Fig. 1. These plots illustrate uncertainty in the mean concentration at each site for a) total and pathogenic *Cryptosporidium* oocysts, b) total *Giardia* cysts, and c) total *E. coli* and *E. coli* O157:H7. Uncertainty in each mean concentration was evaluated using 30,000 sets of plausible parameter values generated by Markov Chain Monte Carlo.

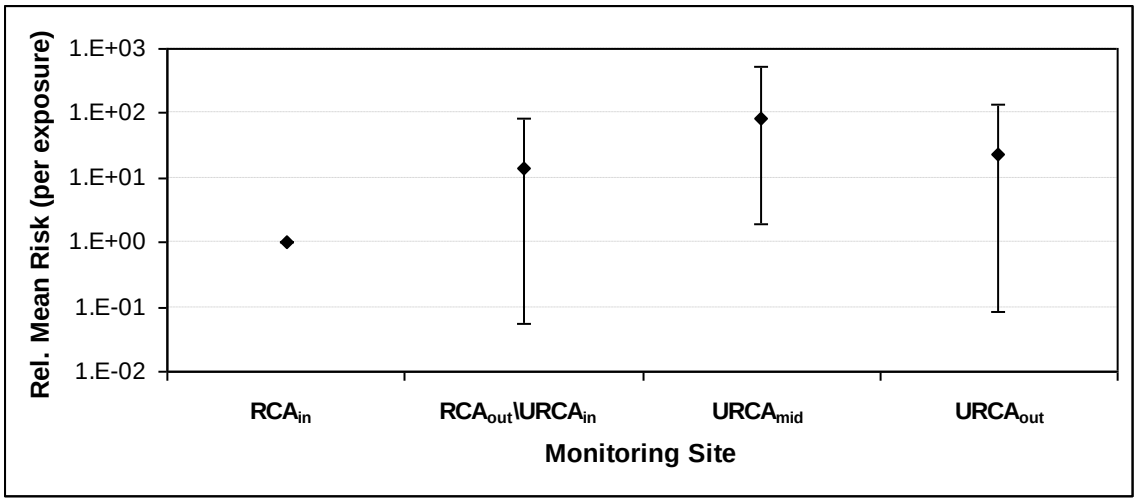
a)




b)




c)




Supp. Cont. Fig. 2. These plots illustrate the uncertainty in the relative mean risk (relative to the $RCA_{in}$ site) at each site for a) *Cryptosporidium*, b) *Giardia*, and c) *E. coli* O157:H7. The points show the posterior mean (of the relative mean risk) and the error bars show the 95% equal-tailed credible intervals. Results for both total *Cryptosporidium* (black). Relative risks, relative to the mean risk for each pathogen at RCAin, were calculated using the same dose-response model parameters for each site. Uncertainty in relative mean risk was evaluated using 30,000 iterations, in which the same dose-response parameters were used for each site within a single iteration, but the values of these parameters varied among iterations. This incorporates dose-response parameter uncertainty in a way that acknowledges that dose-response does not differ among sites.

Manuscript: Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to pasturing cattle: prevalence, densities, and quantitative microbial risk assessment

Supp. Cont. Table 1. Detection limits of various pathogens, parasites, viruses and indicator bacteria targeted in this experiment.

| | Target | Limit of quantification [Laboratory] |
|---|---|---|
| Fecal indicators | *E. coli* | 1 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| | *Enterococcus* spp. | 1 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| | *C. perfringens* | <2 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| | Fecal Coliforms | <2 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| | Total Coliforms | <2 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| | *Aeromonas* | 10 CFU 100 $mL^{-1}$ [AAFC LONDON, ON] |
| Pathogens | *Cryptosporidium* spp. | 1 oocyst 0.63 to 22.75 $L^{-1}$ [CALGARY, AB] |
| | *Giardia* spp. | 1 cyst 0.63 to 22.75 $L^{-1}$ [CALGARY, AB] |
| | *Campylobacter* spp. | 1-10 CFU 500 $mL^{-1}$ [LETHBRIDGE, AB] |
| | *Salmonella spp.* | 1-10 CFU 500 $mL^{-1}$ [LETHBRIDGE, AB] |
| | *E. coli* O157:H7 | 1-10 CFU 500 $mL^{-1}$ [LETHBRIDGE, AB] |
| Viruses | Hepatitis E | 48 viral genomic copies 500 $mL^{-1}$ [AAFC ST. HYACINTHE, QC] |
| | Norovirus GII | 48 viral genomic copies 500 $mL^{-1}$ [AAFC ST. HYACINTHE, QC] |
| | Rotavirus | 48 viral genomic copies 500 $mL^{-1}$ [AAFC ST. HYACINTHE, QC] |
| Coliphage | F-RNA | 0.5 PFU 100 $mL^{-1}$ [AAFC LACOMBE, AB] |
| | F-DNA | 0.5 PFU 100 $mL^{-1}$ [AAFC LACOMBE, AB] |

Supp. Cont. Table 2. Presence and absence of pathogens associated with final CART classification models, using as independent variables sample site (pasture treatment), season, and flow regime for *Temporally Concurrent Data*. High flow ≥ 0.018 $m^3 s^{-1}$; low flow ≥ 0.002 $m^3 s^{-1}$ and < 0.018 $m^3 s^{-1}$; and no flow < 0.002 $m^3 s^{-1}$.

| Microorganism | Criteria | N | Presence | Absence | Detections as percent of total samples | Presence: absence ratio |
|---|---|---|---|---|---|---|
| *Campylobacter* spp. | All data | 192 | 84 | 108 | 44% | 0.78 |
| *Campylobacter* spp. | No flow | 39 | 10 | 29 | 26% | 0.35 |
| *Campylobacter* spp. | (High flow) AND (Low flow) | 153 | 74 | 79 | 48% | 0.94 |
| *Salmonella* spp. | All data | 195 | 12 | 183 | 6% | 0.07 |
| *Salmonella* spp. | (Low flow) AND (No flow) | 117 | 3 | 114 | 3% | 0.03 |
| *Salmonella* spp. | High flow | 78 | 9 | 69 | 12% | 0.13 |
| *Cryptosporidium* oocysts | All data | 74 | 38 | 36 | 51% | 1.06 |
| *Cryptosporidium* oocysts | (Summer, Fall) AND (High flow) | 26 | 20 | 6 | 77% | 3.33 |
| *Cryptosporidium* oocysts | (Summer, Fall) AND (No flow, low flow) | 36 | 17 | 19 | 47% | 0.90 |
| Cryptosporidium oocysts | Spring | 12 | 1 | 11 | 8% | 0.09 |
| *Giardia* cysts | All data | 74 | 19 | 55 | 26% | 0.35 |
| *Giardia* cysts | (Spring) AND (Fall) | 36 | 5 | 31 | 14% | 0.16 |
| *Giardia* cysts | Summer | 38 | 14 | 24 | 37% | 0.58 |

Supp. Cont. Table 3. Median and mean absolute deviation (MAD) of fecal indicator bacteria (CFU 100 $mL^{-1}$) and parasites ((oo)cysts 100 $L^{-1}$) associated with site, season and flow regime, as determined using least absolute deviation regression tree analysis using CART. Analysis uses *Temporally Concurrent Data*. High flow ≥ 0.018 $m^3 s^{-1}$; low flow ≥ 0.002 $m^3 s^{-1}$ and < 0.018 $m^3 s^{-1}$; and no flow < 0.002 $m^3 s^{-1}$. N, number of samples in CART grouping.

| **Microorganism** | Criteria | **Median ± MAD, N** |
|---|---|---|
| Total Coliform | All data | 500 ± 19127, 255 |
| Total Coliform | (Summer) AND (No flow) | 1800 ± 132402, 30 |
| Total Coliform | (Summer) AND (Low flow) | 1000 ± 6429, 48 |
| Total Coliform | (Summer) AND (High flow) | 3700 ± 9057, 12 |
| Total Coliform | Fall | 430 ± 1033, 72 |
| Total Coliform | Spring | 150 ± 3944, 93 |
| Fecal Coliform | All data | 370 ± 3910, 246 |
| Fecal Coliform | (Summer) AND (No flow) | 1200 ± 16467, 30 |
| Fecal Coliform | (Summer) AND (High flow, Low flow) | 650 ± 493, 57 |
| Fecal Coliform | Fall | 360 ± 976, 69 |
| Fecal Coliform | Spring | 108 ± 3954, 90 |
| *E. coli* | All data | 128 ± 1621, 258 |
| *E. coli* | Summer | 320 ± 2996, 90 |
| *E. coli* | (Spring) AND (Fall) | 88 ± 860, 168 |
| *Enterococcus* | All data | 184 ± 1424, 258 |
| *Enterococcus* | (Summer) AND (No flow) | 370 ± 8245, 30 |
| *Enterococcus* | (Summer) AND (High flow, Low flow) | 530 ± 780, 60 |
| *Enterococcus* | Fall | 174 ± 284, 72 |
| *Enterococcus* | Spring | 50 ± 429, 96 |
| *C. perfringens* | All data | 6 ± 121, 252 |
| *C. perfringens* | (No flow) AND (Summer) | 11 ± 23, 30 |
| *C. perfringens* | (No flow) AND (Spring, Fall) | 30 ± 25, 18 |
| *C. perfringens* | (High flow) AND (Low flow) | 6 ± 143, 204 |
| *Aeromonas* | All data | 8700 ± 15146, 84 |
| *Aeromonas* | Summer | 22000 ± 18663, 27 |
| *Aeromonas* | (High Flow) AND (Spring) | 440 ± 639, 12 |
| *Aeromonas* | (High Flow) AND (Fall) | 4600 ± 3653, 18 |
| *Aeromonas* | (Spring, Fall) AND (Low flow, No flow) | 6600 ± 18911, 27 |
| *Cryptosporidium* oocysts | All data | 6 ± 134, 74 |
| *Cryptosporidium* oocysts | Fall | 27 ± 39, 24 |
| *Cryptosporidium* oocysts | (Spring) AND (Summer) | 0 ± 176, 50 |
| *Giardia* cysts | No useful split was found. No tree created | |
| F-RNA coliphage | No useful split was found. No tree created | |
| F-DNA coliphage | No useful split was found. No tree created | |

Supp. Cont. Table 4. Associations between the densities of fecal indicator microorganisms and the presence and absence of pathogens. CART was used in classification mode defining indicator densities that best split target pathogen observations into homogeneous presence and absence groups (for *All Available Data*) (see Wilkes et al., 2009).

| | | | | For fecal indicator microorganism densities ≤ CART split value | | | For fecal indicator microorganism densities > CART split value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Pathogen | Fecal indicator microorganism | Optimal fecal indicator split value | CART cross validated? Yes(Y)/ No(N) | Presence of pathogen | Absence of pathogen | Positive samples (%) | Presence of pathogen | Absence of pathogen | Positive samples (%) | Is the percentage positive in this grouping greater than ≤ grouping? |
| *Campylobacter* spp. | F-RNA coliphage * | 3.35 | Y | 91 | 151 | 38 | 20 | 7 | 74 | Yes, by 36% |
| *Campylobacter* spp. | F-DNA coliphage * | 0.65 | Y | 81 | 142 | 36 | 30 | 16 | 65 | Yes, by 29% |
| *Campylobacter* spp. | Total Coliforms ** | 245 | Y | 24 | 64 | 27 | 87 | 94 | 48 | Yes, by 21% |
| *Campylobacter* spp. | Fecal Coliforms ** | 17 | Y | 2 | 19 | 10 | 109 | 139 | 44 | Yes, by 34% |
| *Campylobacter* spp. | *E. coli* ** | 57 | Y | 17 | 52 | 25 | 94 | 106 | 47 | Yes, by 22% |
| *Campylobacter* spp. | *Enterococcus* ** | 45 | Y | 8 | 36 | 18 | 103 | 122 | 46 | Yes, by 28% |
| *Campylobacter* spp. | *C. perfringens* ** | 7.5 | Y | 49 | 97 | 34 | 62 | 61 | 50 | Yes, by 16% |
| *Campylobacter* spp. | *Aeromonas* ** | 49500[i] | Y | 102 | 158 | 39 | 9 | 0 | 100 | Yes, by 61% |
| *Cryptosporidium* | F-RNA coliphage * | 300 | Y | 58 | 46 | 56 | 2 | 0 | 100 | Yes, by 44% |
| *Cryptosporidium* | F-DNA coliphage * | 27.5[i] | Y | 55 | 44 | 56 | 5 | 5 | 50 | No, lower by 6% |
| *Cryptosporidium* | Total Coliforms ** | 35 | Y | 0 | 7 | 0 | 60 | 39 | 61 | Yes, by 61% |
| *Cryptosporidium* | Fecal Coliforms ** | 43 | Y | 0 | 10 | 0 | 60 | 36 | 63 | Yes, by 63% |
| *Cryptosporidium* | *E. coli* ** | 3 | Y | 1 | 6 | 14 | 59 | 40 | 60 | Yes, by 46% |
| *Cryptosporidium* | *Enterococcus* ** | 15.5 | Y | 4 | 8 | 33 | 56 | 38 | 60 | Yes, by 27% |
| *Cryptosporidium* | *C. perfringens* ** | 0.5 | Y | 8 | 14 | 36 | 52 | 32 | 62 | Yes, by 26% |
| *Cryptosporidium* | *Aeromonas* ** | - | N | - | - | - | - | - | - | |
| *Giardia* | F-RNA coliphage * | 100 | Y | 23 | 80 | 22 | 0 | 3 | 0 | No, lower by 22% |
| *Giardia* | F-DNA coliphage * | - | N | - | - | - | - | - | - | |
| *Giardia* | Total Coliforms ** | 3800[i] | Y | 16 | 76 | 17 | 7 | 7 | 50 | Yes, by 33% |
| *Giardia* | Fecal Coliforms ** | 285 | Y | 4 | 35 | 10 | 19 | 48 | 28 | Yes, by 18% |
| *Giardia* | *E. coli* ** | 919 | Y | 19 | 79 | 19 | 4 | 4 | 50 | Yes, by 31% |
| *Giardia* | *Enterococcus* ** | 213 | Y | 6 | 50 | 11 | 17 | 33 | 34 | Yes, by 23% |
| *Giardia* | *C. perfringens* ** | 83 | Y | 23 | 80 | 22 | 0 | 3 | 0 | No, lower by 22% |
| Norovirus GII | F-RNA coliphage * | - | N | - | - | - | - | - | - | |
| Norovirus GII | F-DNA coliphage * | 1450 | Y | 14 | 93 | 13 | 1 | 0 | 100 | Yes, by 87% |
| Norovirus GII | Total Coliforms ** | - | N | - | - | - | - | - | - | |
| Norovirus GII | Fecal Coliforms ** | - | N | - | - | - | - | - | - | |
| Norovirus GII | *E. coli* ** | 1075 | Y | 15 | 82 | 15 | 0 | 11 | 0 | No, lower by 15% |
| Norovirus GII | *Enterococcus* ** | - | N | - | - | - | - | - | - | |
| Norovirus GII | *C. perfringens* ** | 49[i] | Y | 15 | 74 | 17 | 0 | 19 | 0 | No, lower by 17% |
| Norovirus GII | *Aeromonas* ** | - | N | - | - | - | - | - | - | |
| Rotavirus | F-RNA coliphage * | 1.75 | Y | 4 | 78 | 5 | 0 | 26 | 0 | No, lower by 5% |
| Rotavirus | F-DNA coliphage * | 0.25 | Y | 4 | 72 | 5 | 0 | 32 | 0 | No, lower by 5% |
| Rotavirus | Total Coliforms ** | - | N | - | - | - | - | - | - | |
| Rotavirus | Fecal Coliforms ** | - | N | - | - | - | - | - | - | |
| Rotavirus | *E. coli* ** | - | N | - | - | - | - | - | - | |
| Rotavirus | *Enterococcus* ** | - | N | - | - | - | - | - | - | |
| Rotavirus | *C. perfringens* ** | 19[i] | Y | 0 | 61 | 0 | 4 | 43 | 9 | Yes, by 9% |
| Rotavirus | *Aeromonas* ** | 6000 | Y | 0 | 38 | 0 | 4 | 66 | 6 | Yes, by 6% |
| *Salmonella* spp. | F-RNA coliphage * | 0.25 | Y | 15 | 208 | 7 | 0 | 47 | 0 | No, lower by 7% |
| *Salmonella* spp. | F-DNA coliphage * | - | N | - | - | - | - | - | - | |
| *Salmonella* spp. | Total Coliforms ** | 65 | Y | 0 | 35 | 0 | 15 | 220 | 6 | Yes, by 6% |
| *Salmonella* spp. | Fecal Coliforms ** | 1545 | Y | 15 | 214 | 7 | 0 | 41 | 0 | No, lower by 7% |
| *Salmonella* spp. | *E. coli* ** | - | N | - | - | - | - | - | - | |
| *Salmonella* spp. | *Enterococcus* ** | 115 | Y | 9 | 85 | 10 | 6 | 170 | 3 | No, lower by 7% |
| *Salmonella* spp. | *C. perfringens* ** | - | N | - | - | - | - | - | - | |
| *Salmonella* spp. | *Aeromonas* ** | 950[i] | Y | 5 | 10 | 33 | 10 | 245 | 4 | No, lower by 29% |

i, CART selected primary split criteria amongst all possible indicator microorganism (F-RNA coliphage, F-DNA coliphage, total coliform, fecal coliform, *E. coli*, Enterococcus, *C. perfringens*) and *Aeromonas* criteria that cross validated.

*PFU 100 $mL^{-1}$.

**CFU 100$mL^{-1}$.

Supp. Cont. Table 5. Number of observations for *Temporally Concurrent Data* for different season and flow regimes.

| Microorganism | Site | All data | Spring | Summer | Fall | High flow | Low flow | No flow |
|---|---|---|---|---|---|---|---|---|
| | | N | N | N | N | N | N | N |
| *Campylobacter* spp. | $RCA_{in}$ | 64 | 18 | 26 | 20 | 25 | 26 | 13 |
| *Campylobacter* spp. | $RCA_{out}/URCA_{in}$ | 64 | 18 | 26 | 20 | 25 | 26 | 13 |
| *Campylobacter* spp. | $URCA_{out}$ | 64 | 18 | 26 | 20 | 25 | 26 | 13 |
| *Salmonella* spp. | $RCA_{in}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *Salmonella* spp. | $RCA_{out}/URCA_{in}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *Salmonella* spp. | $URCA_{out}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *E. coli* O157:H7 | $RCA_{in}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *E. coli* O157:H7 | $RCA_{out}/URCA_{in}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *E. coli* O157:H7 | $URCA_{out}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *Cryptosporidium* oocysts | $RCA_{in}$ | 37 | 6 | 19 | 12 | 15 | 11 | 11 |
| *Cryptosporidium* oocysts | $URCA_{out}$ | 37 | 6 | 19 | 12 | 15 | 11 | 11 |
| *Giardia* cysts | $RCA_{in}$ | 37 | 6 | 19 | 12 | 15 | 11 | 11 |
| *Giardia* cysts | $URCA_{out}$ | 37 | 6 | 19 | 12 | 15 | 11 | 11 |
| Norovirus GII | $RCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Norovirus GII | $RCA_{out}/URCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Norovirus GII | $URCA_{out}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Rotavirus | $RCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Rotavirus | $RCA_{out}/URCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Rotavirus | $URCA_{out}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| F-RNA coliphage | $RCA_{in}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| F-RNA coliphage | $RCA_{out}/URCA_{in}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| F-RNA coliphage | $URCA_{out}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| F-DNA coliphage | $RCA_{in}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| F-DNA coliphage | $RCA_{out}/URCA_{in}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| F-DNA coliphage | $URCA_{out}$ | 38 | 13 | 15 | 10 | 16 | 19 | 3 |
| Total Coliform | $RCA_{in}$ | 85 | 31 | 30 | 24 | 37 | 32 | 16 |
| Total Coliform | $RCA_{out}/URCA_{in}$ | 85 | 31 | 30 | 24 | 37 | 32 | 16 |
| Total Coliform | $URCA_{out}$ | 85 | 31 | 30 | 24 | 37 | 32 | 16 |
| Fecal Coliform | $RCA_{in}$ | 82 | 30 | 29 | 23 | 37 | 29 | 16 |
| Fecal Coliform | $RCA_{out}/URCA_{in}$ | 82 | 30 | 29 | 23 | 37 | 29 | 16 |
| Fecal Coliform | $URCA_{out}$ | 82 | 30 | 29 | 23 | 37 | 29 | 16 |
| *E. coli* | $RCA_{in}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *E. coli* | $RCA_{out}/URCA_{in}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *E. coli* | $URCA_{out}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *Enterococcus* | $RCA_{in}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *Enterococcus* | $RCA_{out}/URCA_{in}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *Enterococcus* | $URCA_{out}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| *Clostridium perfringens* | $RCA_{in}$ | 84 | 31 | 30 | 23 | 36 | 32 | 16 |
| *Clostridium perfringens* | $RCA_{out}/URCA_{in}$ | 84 | 31 | 30 | 23 | 36 | 32 | 16 |
| *Clostridium perfringens* | $URCA_{out}$ | 84 | 31 | 30 | 23 | 36 | 32 | 16 |
| *Aeromonas* | $RCA_{in}$ | 28 | 11 | 9 | 8 | 12 | 13 | 3 |
| *Aeromonas* | $RCA_{out}/URCA_{in}$ | 28 | 11 | 9 | 8 | 12 | 13 | 3 |
| *Aeromonas* | $URCA_{out}$ | 28 | 11 | 9 | 8 | 12 | 13 | 3 |

Supp. Cont. Table 6. Number of observations for *All Available Data* for different season and flow regimes.

| Microorganism | Site | All data | Spring | Summer | Fall | High flow | Low flow | No flow |
|---|---|---|---|---|---|---|---|---|
| | | N | N | N | N | N | N | N |
| *Campylobacter* spp. | $RCA_{in}$ | 79 | 23 | 33 | 23 | 28 | 29 | 22 |
| *Campylobacter* spp. | $RCA_{out}/URCA_{in}$ | 65 | 19 | 26 | 20 | 26 | 26 | 13 |
| *Campylobacter* spp. | $URCA_{mid}$ | 50 | 13 | 21 | 16 | 21 | 23 | 6 |
| *Campylobacter* spp. | $URCA_{out}$ | 75 | 25 | 28 | 22 | 28 | 29 | 18 |
| *Salmonella* spp. | $RCA_{in}$ | 79 | 23 | 33 | 23 | 28 | 29 | 22 |
| *Salmonella* spp. | $RCA_{out}/URCA_{in}$ | 66 | 20 | 26 | 20 | 27 | 26 | 13 |
| *Salmonella* spp. | $URCA_{mid}$ | 50 | 13 | 21 | 16 | 21 | 23 | 6 |
| *Salmonella* spp. | $URCA_{out}$ | 75 | 25 | 28 | 22 | 28 | 29 | 18 |
| *E. coli* O157:H7 | $RCA_{in}$ | 79 | 23 | 33 | 23 | 28 | 29 | 22 |
| *E. coli* O157:H7 | $RCA_{out}/URCA_{in}$ | 66 | 20 | 26 | 20 | 27 | 26 | 13 |
| *E. coli* O157:H7 | $URCA_{mid}$ | 50 | 13 | 21 | 16 | 21 | 23 | 6 |
| *E. coli* O157:H7 | $URCA_{out}$ | 75 | 25 | 28 | 22 | 28 | 29 | 18 |
| *Cryptosporidium* oocysts | $RCA_{in}$ | 40 | 7 | 19 | 14 | 15 | 11 | 14 |
| *Cryptosporidium* oocysts | $RCA_{out}/URCA_{in}$ | 8 | 0 | 6 | 2 | 4 | 4 | 0 |
| *Cryptosporidium* oocysts | $URCA_{mid}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cryptosporidium* oocysts | $URCA_{out}$ | 58 | 17 | 22 | 19 | 25 | 21 | 12 |
| *Giardia* cysts | $RCA_{in}$ | 40 | 7 | 19 | 14 | 15 | 11 | 14 |
| *Giardia* cysts | $RCA_{out}/URCA_{in}$ | 8 | 0 | 6 | 2 | 4 | 4 | 0 |
| *Giardia* cysts | $URCA_{mid}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Giardia* cysts | $URCA_{out}$ | 58 | 17 | 22 | 19 | 25 | 21 | 12 |
| Hepatitis E | $RCA_{in}$ | 31 | 9 | 13 | 9 | 11 | 15 | 5 |
| Hepatitis E | $RCA_{out}/URCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Hepatitis E | $URCA_{mid}$ | 23 | 6 | 8 | 9 | 9 | 11 | 3 |
| Hepatitis E | $URCA_{out}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Norovirus GII | $RCA_{in}$ | 31 | 9 | 13 | 9 | 11 | 15 | 5 |
| Norovirus GII | $RCA_{out}/URCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Norovirus GII | $URCA_{mid}$ | 23 | 6 | 8 | 9 | 9 | 11 | 3 |
| Norovirus GII | $URCA_{out}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Rotavirus | $RCA_{in}$ | 31 | 9 | 13 | 9 | 11 | 15 | 5 |
| Rotavirus | $RCA_{out}/URCA_{in}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| Rotavirus | $URCA_{mid}$ | 23 | 6 | 8 | 9 | 9 | 11 | 3 |
| Rotavirus | $URCA_{out}$ | 27 | 9 | 9 | 9 | 11 | 13 | 3 |
| F-RNA coliphage | $RCA_{in}$ | 44 | 13 | 18 | 13 | 16 | 20 | 8 |
| F-RNA coliphage | $RCA_{out}/URCA_{in}$ | 39 | 13 | 15 | 11 | 17 | 19 | 3 |
| F-RNA coliphage | $URCA_{mid}$ | 31 | 8 | 13 | 10 | 14 | 16 | 1 |
| F-RNA coliphage | $URCA_{out}$ | 39 | 13 | 15 | 11 | 17 | 19 | 3 |
| F-DNA coliphage | $RCA_{in}$ | 44 | 13 | 18 | 13 | 16 | 20 | 8 |
| F-DNA coliphage | $RCA_{out}/URCA_{in}$ | 39 | 13 | 15 | 11 | 17 | 19 | 3 |
| F-DNA coliphage | $URCA_{mid}$ | 32 | 9 | 13 | 10 | 14 | 16 | 2 |
| F-DNA coliphage | $URCA_{out}$ | 39 | 13 | 15 | 11 | 17 | 19 | 3 |
| Total Coliforms | $RCA_{in}$ | 108 | 36 | 42 | 29 | 41 | 35 | 32 |
| Total Coliforms | $RCA_{out}/URCA_{in}$ | 86 | 32 | 30 | 24 | 38 | 32 | 16 |
| Total Coliforms | $URCA_{mid}$ | 65 | 24 | 23 | 18 | 28 | 29 | 8 |
| Total Coliforms | $URCA_{out}$ | 104 | 32 | 36 | 36 | 43 | 40 | 21 |
| Fecal Coliforms | $RCA_{in}$ | 105 | 35 | 40 | 29 | 41 | 32 | 32 |

| Microorganism | Site | All data | Spring | Summer | Fall | High flow | Low flow | No flow |
|---|---|---|---|---|---|---|---|---|
| Fecal Coliforms | $RCA_{out}/URCA_{in}$ | 83 | 31 | 29 | 23 | 38 | 29 | 16 |
| Fecal Coliforms | $URCA_{mid}$ | 62 | 23 | 23 | 16 | 28 | 27 | 7 |
| Fecal Coliforms | $URCA_{out}$ | 103 | 32 | 36 | 35 | 43 | 39 | 21 |
| *E. coli* | $RCA_{in}$ | 109 | 37 | 42 | 29 | 42 | 35 | 32 |
| *E. coli* | $RCA_{out}/URCA_{in}$ | 87 | 33 | 30 | 24 | 39 | 32 | 16 |
| *E. coli* | $URCA_{mid}$ | 66 | 25 | 23 | 18 | 29 | 29 | 8 |
| *E. coli* | $URCA_{out}$ | 105 | 33 | 36 | 36 | 44 | 40 | 21 |
| *Enterococcus* | $RCA_{in}$ | 109 | 37 | 42 | 29 | 42 | 35 | 32 |
| *Enterococcus* | $RCA_{out}/URCA_{in}$ | 87 | 33 | 30 | 24 | 39 | 32 | 16 |
| *Enterococcus* | $URCA_{mid}$ | 66 | 25 | 23 | 18 | 29 | 29 | 8 |
| *Enterococcus* | $URCA_{out}$ | 105 | 33 | 36 | 36 | 44 | 40 | 21 |
| *Clostridium perfringens* | $RCA_{in}$ | 107 | 36 | 42 | 28 | 40 | 35 | 32 |
| *Clostridium perfringens* | $RCA_{out}/URCA_{in}$ | 85 | 32 | 30 | 23 | 37 | 32 | 16 |
| *Clostridium perfringens* | $URCA_{mid}$ | 64 | 24 | 23 | 17 | 27 | 29 | 8 |
| *Clostridium perfringens* | $URCA_{out}$ | 104 | 32 | 36 | 36 | 43 | 40 | 21 |
| *Aeromonas* | $RCA_{in}$ | 32 | 11 | 13 | 8 | 12 | 15 | 5 |
| *Aeromonas* | $RCA_{out}/URCA_{in}$ | 28 | 11 | 9 | 8 | 12 | 13 | 3 |
| *Aeromonas* | $URCA_{mid}$ | 22 | 7 | 7 | 8 | 9 | 11 | 2 |
| *Aeromonas* | $URCA_{out}$ | 28 | 11 | 9 | 8 | 12 | 13 | 3 |