

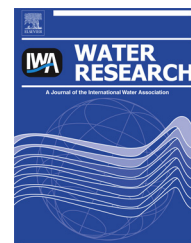


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Bacteria, viruses, and parasites in an intermittent stream protected from and exposed to pasturing cattle: Prevalence, densities, and quantitative microbial risk assessment

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ABSTRACT

Over 3500 individual water samples, for 131 sampling times, targeting waterborne pathogens/fecal indicator bacteria were collected during a 7-year period from 4 sites along an intermittent stream running through a small livestock pasture system with and without cattle access-to-stream restriction measures. The study assessed the impact of cattle pasturing/riparian zone protection on: pathogen (bacterial, viral, parasite) occurrence, concentrations of fecal indicators, and quantitative microbial risk assessments (QMRA) of the risk of *Cryptosporidium*, *Giardia* and *Escherichia coli* O157:H7 infection in humans. Methodologies were developed to compute QMRA mean risks on the basis of water samples exhibiting potentially human infectious *Cryptosporidium* and *E. coli* based on genotyping *Cryptosporidium*, and *E. coli* O157:H7 presence/absence information paired with enumerated *E. coli*. All *Giardia* spp. were considered infectious. No significant pasturing treatment effects were observed among pathogens, with the exception of *Campylobacter* spp. and *E. coli* O157:H7. *Campylobacter* spp. prevalence significantly decreased downstream through pasture treatments and *E. coli* O157:H7 was observed in a few instances in the middle of the unrestricted pasture. Densities of total coliform, fecal coliform, and *E. coli* reduced significantly downstream in the restricted pasture system, but not in the unrestricted system. Seasonal and flow conditions were associated with greater indicator bacteria densities, especially in the summer. Norovirus GII was detected at rates of 7–22% of samples for all monitoring sites, and rotavirus in 0–7% of samples for all monitoring sites; pasture treatment trends were not evident, however. Seasonal and stream flow variables (and their

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interactions) were relatively more important than pasture treatments for initially stratifying pathogen occurrence and higher fecal indicator bacteria densities. Significant positive associations among fecal indicator bacteria and *Campylobacter* spp. detection were observed. For QMRA, adjusting for the proportion of *Cryptosporidium* spp. detected that are infectious for humans reduces downstream risk estimates by roughly one order of magnitude. Using QMRA in this manner provides a more refined estimate of beneficial management practice effects on pathogen exposure risks to humans.

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1. Introduction

Restricting cattle access to water courses has been shown to reduce nutrient, sediment, and bacteria loads, and improve stream and riparian zone integrity (Line, 2003; Mayer et al., 2007). Yet, how these practices influence the prevalence of zoonotic pathogens and viruses that can result from implementation of stream/riparian zone protection practices is not well documented. Schwarte et al. (2011) reported bovine enterovirus in ~8–17% of the runoff simulations from bare unrestricted stream access pasture sites under rainfall simulation, while bovine coronavirus and rotavirus group A, and *Escherichia coli* O157:H7 were rarely detected. Billington et al. (2011) suggest that uncontrolled stock access to waterways results in elevated public health risks from pathogens based on hazard identification, exposure assessment, dose-response analysis, and risk characterisation of stock accessing waterways upstream of drinking water off-takes. Aarons and Gourley (2012) suggest improved riparian zone protection approaches and strategies are needed, and Miller et al. (2010) indicate that not all water quality endpoints are improved with riparian zone fencing.

A complicating factor in the assessment of the water quality benefits imposed by riparian zone protection (a beneficial management practice (BMP)), is that pasturing systems, regardless of whether they are fenced or not, are variably susceptible to livestock, wildlife, and human fecal inputs (Wilkes et al., 2013). Varied fecal inputs have the potential to confound the true nature of BMP impact on, in particular, loading of microorganisms in water and the prevalence and nature of zoonotic pathogens of differing virulence potential to humans.

It is becoming increasingly important to gauge the effectiveness of an agricultural BMP in the context of a breadth of potential services the BMP can provide. Most often, agricultural BMPs that are protective of water quality are evaluated for environmental and production benefits, but their potential human health risk implications are not. Schmidt et al. (2013a) recently demonstrated that quantitative microbial risk assessment (QMRA) could be used to quantify human potential health risks from *Campylobacter* spp. associated with tile drainage management BMPs imposed *en masse* at a watershed scale. However, even QMRA approaches must consider true relative and absolute risks in the context of differences in pathogen species, genotype, serovars, strains or lineage occurrence, because not all of these pathogen subtypes necessarily have the same virulence potential to humans (see Afchain et al.'s (2008) accounting of differing strains of *Bacillus cereus* in QMRA of manufactured food, for example). Parsing

out risks on the basis of human pathogenicity better represents true exposure risks in the framework of BMP assessment.

This study evaluated whether restricting cattle access to streams can reduce the prevalence of zoonotic pathogens, parasites, and viruses within a stream discharge and seasonal context. It also assessed the utility of fecal indicator organisms to predict pathogen prevalence. The underpinning study hypothesis is that cattle associated pathogens will decrease or remain constant within the restricted cattle access pasture (RCA), and then increase within a downstream unrestricted cattle access pasture (URCA). A Bayesian QMRA approach was employed to calculate human health risks from a hypothetical recreational exposure to these waters for selected pathogens.

2. Methods and materials

2.1. Study site

Details of the study site and RCA and URCA are given in Sunohara et al. (2012) (Fig. 1). The stream is intermittent (Strahler order = 2) with low and no-flows typically occurring in summer. The stream drains a watershed of roughly 482 ha above the experimental site (above the RCA and URCA area) (Schmidt et al., 2013a). The study framework is an upstream downstream design (USDA, 1996). The RCA area is ~2 ha in size with a fence buffering the stream from pasturing adult cattle (3–5 m buffer). Stream flow length in the RCA is ~356 m with a flow gradient of ~0.002 m m⁻¹. The RCA is situated upstream of the ~2 ha URCA where adult cattle have unrestricted access to the stream. The URCA possesses a ~348 m stream flow length and gradient of ~0.004 m m⁻¹. Holstein cattle densities were

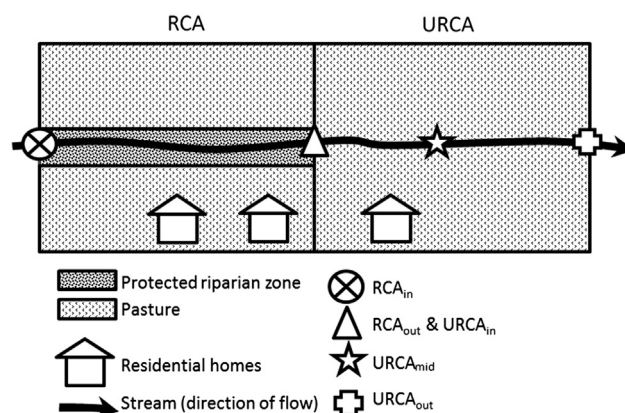


Fig. 1 – Diagrammatical layout of study site.

maintained at ~ 2.5 animals ha^{-1} in both systems; a density that is not uncommon for pasturing operations in the study region. Cattle were generally allowed to graze from May–June to November–December, beginning in 2005. Adjacent to the RCA and URCA experimental pastures are several residential homes on the southern bank of the stream; small septic system leakages have been observed on occasion. Other prospective fecal inputs along the experimental pastures include house pets, and observed wildlife including small birds, muskrat, vole, turtle, duck, Canada goose, and wild turkey. Fecal inputs upstream of the RCA_{in} could be impacted potentially by a small hobby farm located immediately upstream of RCA_{in} . The farm consists of penned animals of very low and variable seasonal intensity (10–20 goats, 1 donkey, and 2 horses). Yet, it is unclear if there was significant impact of the farm on stream water quality given that many of these livestock were penned in areas where lot drainage to the stream would have been minimal. Liquid swine manures were also applied on fields west of the study area that could have contaminated the roadside ditch that drained near the RCA_{in} sample site in spring and/or fall, but these applications were not frequent.

2.2. Water sampling, microbiological methods and flow monitoring

Water samples were collected (2004–2010) on a bi-weekly basis beginning April to May until late November (Wilkes et al., 2011). Samples were collected at RCA_{in} (serving as a baseline of water quality input into the restricted cattle access experimental region), $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$ (representing the water quality output from the RCA experimental region, and also serving as a baseline of water quality input into the unrestricted cattle access region), at a midpoint of the URCA region (URCA_{mid}), and finally at the output of the URCA region (URCA_{out}) (Fig. 1). Water quality data collected at these sample sites were divided into two groups:

Group 1) *Temporally Concurrent Data*: data collected concurrently (in synchrony, on the same day) from 2005 to 2010 at RCA_{in} , $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$; and URCA_{out} for bacteria, virus, and coliphage, plus data collected from 2005 to 2010 concurrently at RCA_{in} , URCA_{out} for parasites (due to limited concurrent samples for parasites at output of RCA and input to URCA ($\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$) this data was excluded in the temporally concurrent grouping); and Group 2) *All Available Data*: all data collected at each monitoring site from 2004 to 2010 including URCA_{mid} irrespective of temporal concurrence of sampling (i.e., one site may have more data support than another depending on the microorganism).

Water sampling, hydrological monitoring, and microbiological analysis (pathogenic bacteria, fecal indicator bacteria, coliphage, *Giardia* and *Cryptosporidium* (oo)cyst densities) approaches have been provided in other companion papers (Wilkes et al., 2009; Sunohara et al., 2012), with the exception of *Aeromonas*. *Aeromonas* (monitoring began in 2009) was processed and enumerated following USEPA Method 1605 (USEPA, 2001) and Havelaar et al. (1987). *Cryptosporidium* were enumerated, sequenced, and genotyped using phylogenetic analysis (Ruecker et al., 2012).

For group A rotavirus, hepatitis E virus, and norovirus GII, 500 mL of water were processed in accordance with the

OPFLP-04 standard method for the recovery and concentration of viruses present in artificially and naturally contaminated water following Health Canada's compendium of analytical methods (Brassard et al., 2005, 2007; see Supp. Table 1 for detection limits for viruses and other microbiological targets). Negative controls (experimental assay with a non-inoculated water sample as a negative control) were included and processed following the same method applied for virus detection. Viral nucleic acids were extracted using the QIAamp RNA Viral Mini Kit (Qiagen, Mississauga, ON) according to the manufacturer's recommendations. Real-time TaqMan RT-PCR and PCR assays for the detection of norovirus GII and hepatitis E virus, rotavirus, and feline calicivirus (as sample process control) were performed in 25 μL with the 1-step Brilliant II QRT-PCR core reagent kit for RNA viruses (Agilent Technologies Canada, Mississauga, ON) and the amplification conditions were carried out according to previously described procedures (Kageyama et al., 2003; Zeng et al., 2008; Ward et al., 2009).

Stream discharge at the RCA_{in} was monitored using a 4150 Area-Velocity Flow Logger (Teledyne Isco, Inc.) and a low-profile area-velocity sensor; an area-velocity calculation was applied to determine stream discharge as described by Sunohara et al. (2012). Due to there being no fixed structure at the $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$ and the other downstream sites, we linearly scaled discharge at these sites by applying a proportional amount of discharge based on its contributing area relative to that of RCA_{in} (as the distance between the RCA_{out} and the downstream sites was relatively small); results were verified independently by discharge measurements made by Flow Probe (Global Water Instrumentation, Gold River, CA). Discharge was then grouped based on specific flow conditions measured at the RCA_{in} using percentile classes. Discharge greater than $0.018 \text{ m}^3 \text{ s}^{-1}$ was considered "high flow" (defined as the 75th percentile for discharge measurements), discharge between 0.002 and $0.018 \text{ m}^3 \text{ s}^{-1}$ (defined as discharge between the 25th and 75th percentiles) was considered "low flow," and discharge $< 0.002 \text{ m}^3 \text{ s}^{-1}$ (defined as discharge less than the 25th percentile) was considered "no flow."

2.3. Statistical analysis

Statistical differences in the presence/absence (P/A) of pathogens in water on the basis of sample site and stream flow, and sample site and season, were assessed using Fisher's exact tests. Interactions among site-season-flow characteristics in terms of pathogen P/A were examined in classification mode using CART (Classification and Regression Tree Analysis) (V. 6.6, Salford Systems, CA) (Wilkes et al., 2013). Optimal cross-validated models with a maximum of 3 tree-levels are presented. Site, season, and flow interactions for densities of fecal indicator organism, parasite, and F-coliphage were examined in CART using the least absolute deviation regression tree approach (Wilkes et al., 2011). Differences in the distributions of F-coliphage and fecal indicator bacteria densities within pathogen P/A groupings were assessed by Mann-Whitney U (MWU) tests. Also, F-coliphage and indicator bacteria density thresholds by which pathogen P/A data could be split into similar groups with one binary split were examined using CART in a manner consistent with Wilkes et al. (2009).

2.4. Quantitative microbial risk assessment

It was assumed that all pathogen data from each site are representative of the site-specific variability in pathogen concentrations, and it was assumed that the concentration of total *Cryptosporidium* oocysts, *Giardia* cysts, and culturable *E. coli* at a monitoring site varies temporally according to a gamma distribution. Bayes' theorem and Markov Chain Monte Carlo were used to generate a set of parameter values that is representative of a posterior distribution describing uncertainty in the estimated parameters. For *Cryptosporidium* and *Giardia*, the distribution was fit to the raw count and volume data using a probabilistic model that accounts for random measurement errors (Schmidt and Emelko, 2011). Variability in the analytical recovery of the enumeration method was evaluated using matrix spike recovery data (see Supplement ary Content, QMRA method).

An assumption in this model is that only a fraction of the (oo)cysts or *E. coli* colonies that were enumerated are capable of initiating infection in a human host (Table 1A and B). In the case of *Cryptosporidium*, site-specific information about the fraction of sequenced oocysts that were either *Cryptosporidium parvum* or *Cryptosporidium hominis* (Wilkes et al., 2013) was used to correct for infectivity (disregarding the viability of the oocysts). Uncertainty in this fraction at each site was described using a simple posterior distribution from a

hierarchical model (see Supp. Cont.). Infectivity information was not available for *Giardia*, so all enumerated cysts were assumed infectious. *E. coli* O157:H7 P/A data were used to adjust total *E. coli* that were presumed to be infectious, and uncertainty in this fraction was evaluated using Bayes' theorem and Markov Chain Monte Carlo with a uniform prior (see Supp. Cont.).

Novel dose-response modelling approaches were used herein. For *Cryptosporidium*, a hierarchical Bayesian model based upon the U.S. EPA's Economic Analysis for the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA, 2005) that included beta-distributed strain-to-strain variation in the exponential dose-response model parameters of six human feeding studies, was used (see Supp. Cont.). The exponential dose-response model for *Giardia* (based upon the data of Rendtorff (1954)) and the actual beta-Poisson, or hypergeometric, dose-response model for *E. coli* (based upon the data of DuPont et al. (1971)) were used with Bayesian analysis of parameter uncertainty (Schmidt et al., 2013b).

Because no systematic human exposures (e.g., swimming, drinking water) are known to exist at the study sites, a single hypothetical consumption value of 45 mL was used (representing accidental ingestion during a swim) (Dufour et al., 2006). A second-order risk characterization procedure comparable to that of Schmidt et al. (2013a) was used to separate variability from uncertainty in, 1) the parameters of the

Table 1 – Pathogenic sequences of *Cryptosporidium* observed at sample sites for All Available Data (Table A) and the quantity and type of occurrence data used in QMRA for each pathogen (Table B).

Table A			
Monitoring site	Total number of <i>Cryptosporidium</i> sequences	Number of pathogenic sequences	Notes
RCA _{in}	76	0	
RCA _{out} /URCA _{in}	9	1	1 <i>C. hominis</i>
URCA _{out}	89	4	2 <i>C. parvum</i> ; 2 <i>C. hominis</i>
*	13	2	2 <i>C. parvum</i>
*	7	0	
*	9	0	
*	25	0	
*	63	4	4 <i>C. hominis</i>
*	68	0	
*	31	0	
*	19	0	
*	88	6	4 <i>C. parvum</i> ; 2 <i>C. hominis</i>
*	48	0	
*	8	0	
*	44	0	
*	51	1	1 <i>C. parvum</i>
*	9	0	
Table B			
Monitoring site	<i>Cryptosporidium</i> / <i>Giardia</i> enumeration data ^a	<i>E. coli</i> plating data ^b	<i>E. coli</i> O157:H7 presence/absence data ^c
RCA _{in}	40	109	79 (0)
RCA _{out} /URCA _{in}	8	87	66 (0)
URCA _{mid}	—	66	50 (3)
URCA _{out}	51	92	75 (0)

*Other sites in South Nation River Basin not included in this study (see Marti et al., 2013, for reference to other sites).
a (Oo)cyst counts and corresponding enumerated volumes.
b Reported concentration estimates.
c Total number of data (and number of positive results).

gamma distribution describing temporal concentration variability, 2) the fraction of enumerated microorganisms that were infectious, and 3) the parameter(s) of the dose-response model. In this approach, mean risk for a particular set of parameter values was evaluated using numerical integration, and 30,000 such sets of parameters (collectively representing uncertainty in the estimated parameters) were used to evaluate the uncertainty in mean risk. The resulting risk information was summarized using 95% credible intervals for mean risk (illustrating the anticipated probability of infection for the hypothetical exposure and effect of all sources of uncertainty). The posterior probability that the mean risk at one site was greater than that at another was used as the Bayesian analog to significance in a two-tailed hypothesis test. This does not provide a measure of statistical significance (*p*-value): however, strong support for a substantial difference exists when computed posterior probabilities exceed 0.975, which is the analog of a two-tailed *p*-value of 0.05 (Schmidt et al., 2013a).

All Bayesian Markov Chain Monte Carlo analyses were performed with the open-source software OpenBUGS (V.3.2.1, rev 781). Three chains with relatively variable initial states were used to assess convergence (often including use of the built-in Gelman-Rubin statistic feature) and history plots were used to evaluate mixing. A burn-in of 5000 iterations was used

to ensure that the first recorded iteration was minimally influenced by the initial state of the chain, and 30,000 iterations (usually every fifth of 150,000 iterations) were recorded in most cases as a representative random sample from the posterior distribution of interest.

3. Results

3.1. Prevalence

Cryptosporidium oocysts (with sample site prevalence ranging between 46 and 63%) and *Campylobacter* spp. (29–55%) were the most frequently observed pathogens at the study site for Temporally Concurrent Data (Fig. 2, ‘all data’) and when All Available Data were considered (Fig. 3, ‘all data’). *Giardia* cysts (19–27%) and norovirus GII (7–22%) were observed relatively less frequently on a sample site prevalence basis, and *Salmonella* spp. (3–8%), rotavirus (0–7%), hepatitis E virus (0–4%), and *E. coli* O157:H7 (0–6%) were rarely observed in water samples (Figs. 2 and 3, ‘all data’). F-RNA (26–42% samples) and F-DNA (28–41%) coliphage detections were observed from moderately to occasionally. The range of site medians for fecal indicator bacteria and *Aeromonas* spp. densities (CFU

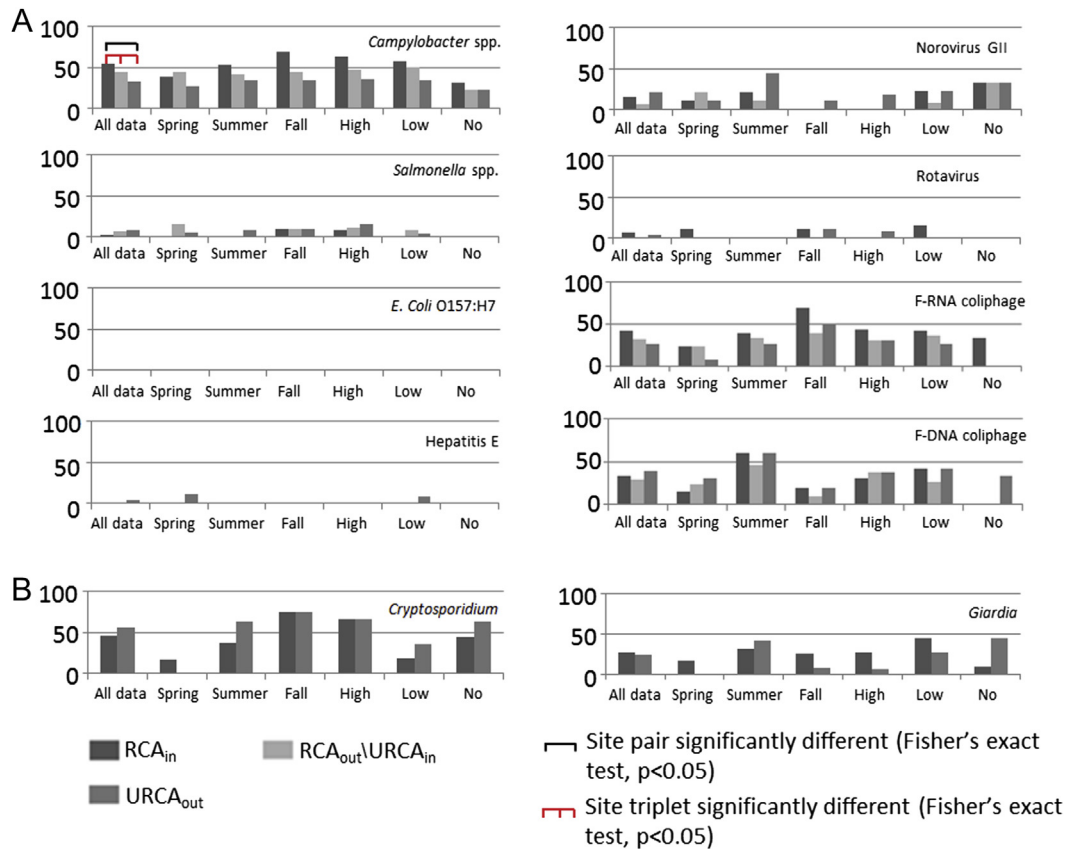


Fig. 2 – The percentage (%) of samples under specific site, season, and flow condition positive for detection of microorganisms. Temporally Concurrent Data were used for: (A) the RCA_{in}, RCA_{out}/URCA_{in}, and URCA_{out} for bacteria and viruses, and (B) *Cryptosporidium* and *Giardia* which were collected in a temporally concurrent manner at sites RCA_{in} and URCA_{out} only. See Fig. 3 for parasite occurrence at RCA_{out}/URCA_{in}. High flow $\geq 0.018 \text{ m}^3 \text{ s}^{-1}$; low flow $\geq 0.002 \text{ m}^3 \text{ s}^{-1}$ and $< 0.018 \text{ m}^3 \text{ s}^{-1}$, and no flow $< 0.002 \text{ m}^3 \text{ s}^{-1}$. Black bar represents significant Fisher's exact test *p*-value (< 0.05) from site pair 2 × 2 comparison. Red bar represents significant Fisher's exact test *p*-value (< 0.05) from site triplet 2 × 3 comparison.

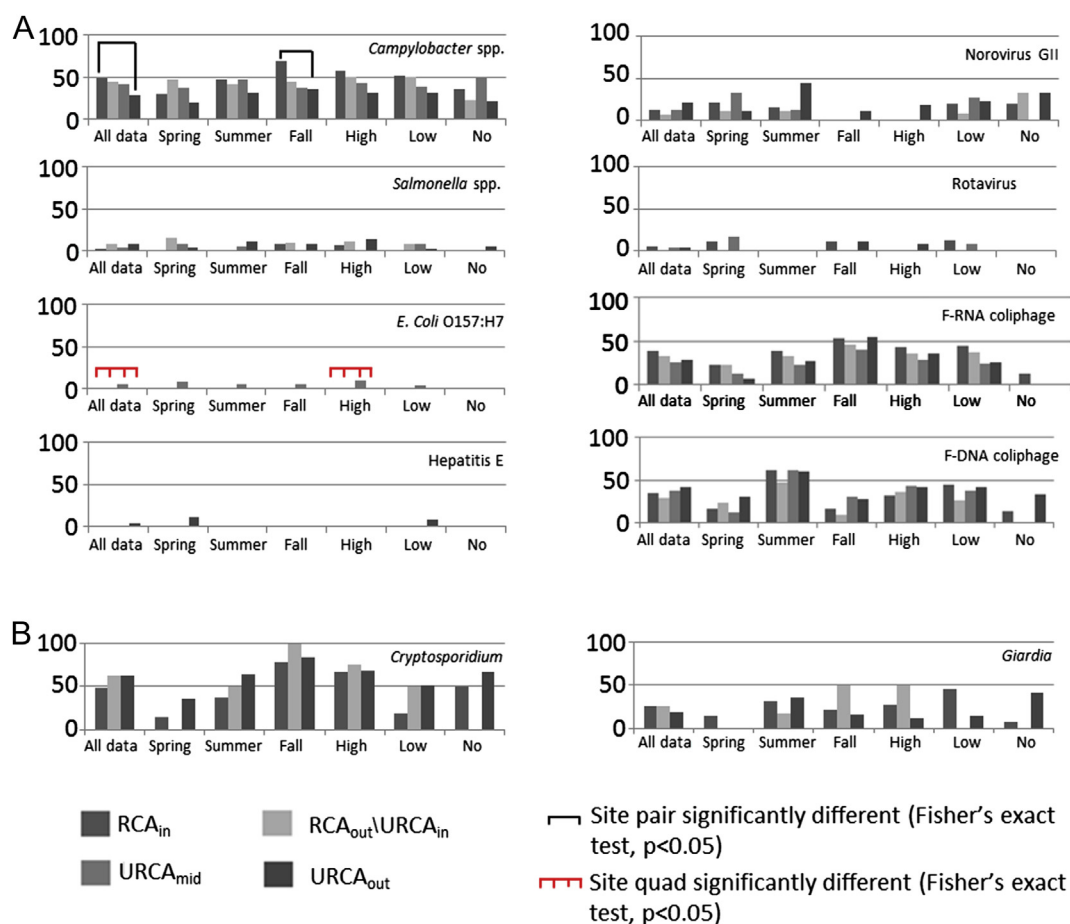


Fig. 3 – The percentage (%) of samples under specific site, season, and flow condition positive for detection of microorganisms. Data represented here were based on All Available Data for (A) bacteria and viruses and (B) parasites. High flow $\geq 0.018 \text{ m}^3 \text{ s}^{-1}$; low flow $\geq 0.002 \text{ m}^3 \text{ s}^{-1}$ and $< 0.018 \text{ m}^3 \text{ s}^{-1}$, and no flow $< 0.002 \text{ m}^3 \text{ s}^{-1}$. Black bar represents significant Fisher’s exact test p -value (< 0.05) from site pair 2×2 comparison. Red bar represents significant Fisher’s exact test p -value (< 0.05) from site quad 2×4 comparison.

100 mL^{-1}) were: total coliform (375–710), fecal coliform (290–460), *E. coli* (106–146), Enterococci (134–240), *Aeromonas* spp. (7550–9550) and *Clostridium perfringens* (4–7) (Figs. 4 and 5, ‘all data’).

3.2. Temporally concurrent data

Unlike the All Available Data datasets, the Temporally Concurrent Data allowed for the evaluation of the stream/riparian zone protection treatment (site) effects on water quality targets. The only significant differences (p -value, Fisher’s exact test) in pathogen prevalence for these data occurred for *Campylobacter* spp. (‘all data’ between RCA_{in} and URCA_{out} sites) (Fig. 2). Here, *Campylobacter* spp. detections reduced by 11% between the RCA_{in} and $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$, and another 11% between $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$ and URCA_{out} .

CART classification tree analysis indicated, overall, no site, season, and flow interactions occurred within tree growing constraints, except for *Cryptosporidium* (see Supp. Tbl. 2). For *Campylobacter* spp., CART classified P/A groups by, no flow (presence:absence ratio = 0.35) vs. high AND low flow (0.94). For *Salmonella* spp., classification was also based on flow: no flow

AND low flow (0.03) vs. high flow (0.13). For *Cryptosporidium* oocyst detects, there was an interaction identified. These were: summer AND fall AND high flow (3.33), and summer AND fall AND no flow AND low flow (0.90), and spring (0.09). For *Giardia* cyst detection, season was the most important classifier: spring AND fall (0.16) vs. summer (0.58). Too few detects for *E. coli* O157:H7 precluded CART analysis for this pathogen.

Significant differences among site flow groupings of fecal indicator bacteria were observed, specifically for low flow ($p < 0.05$) (Fig. 4). For low stream flow, fecal coliform densities were significantly higher at the RCA_{in} relative to $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$, and *E. coli* densities were significantly higher at RCA_{in} , relative to $\text{RCA}_{\text{out}}/\text{URCA}_{\text{in}}$ and URCA_{out} (Fig. 4). For CART regression tree models (Supp. Tbl. 3), for specific microorganisms (total coliforms, fecal coliforms, *E. coli*, *Enterococcus*, *C. perfringens*, *Aeromonas*, and *Cryptosporidium*), the data initially subdivided higher and lower density data on the basis of season (except for *C. perfringens* data which initially split into higher and lower densities by flow and no-flow conditions). Overall, with the exception of *C. perfringens* and *Cryptosporidium* (these microorganisms having higher relative densities in fall and to a lesser extent spring), summer, under various

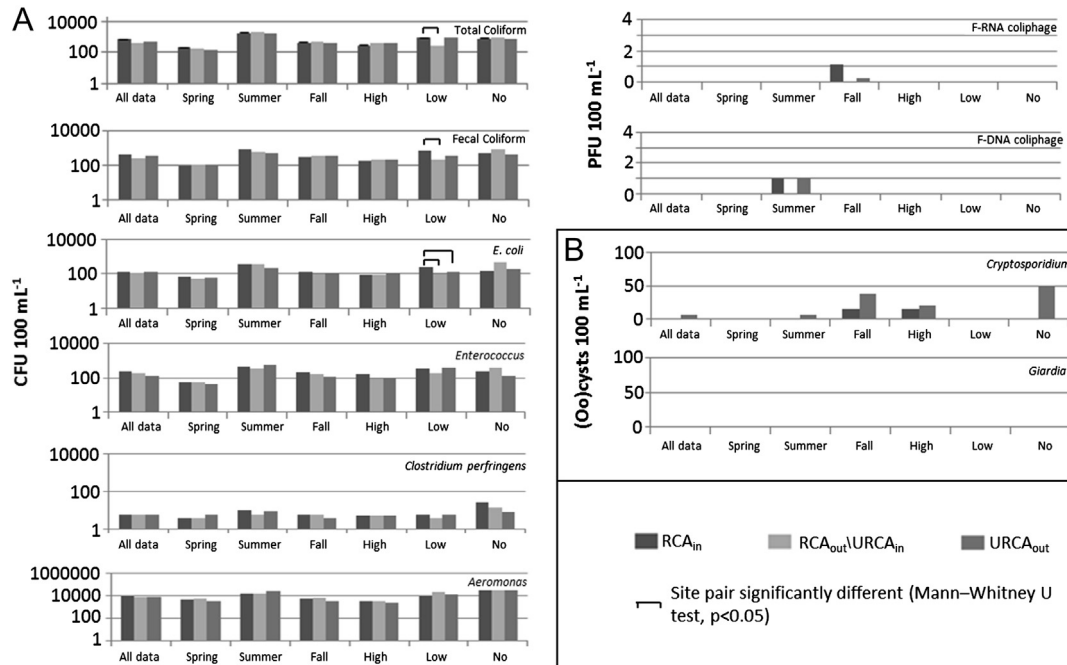


Fig. 4 – Median densities of fecal indicator bacteria, *Aeromonas*, parasites and F-coliphage for samples collected under specific site, season, and flow condition. Temporally Concurrent Data were used for: (A) the RCA_{in}, RCA_{out}/URCA_{in}, and URCA_{out} for bacteria and coliphage, and (B) parasite data which were collected in a temporally concurrent manner at sites RCA_{in} and URCA_{out} only. See Fig. 5 for parasite median density at RCA_{out}/URCA_{in}. High flow $\geq 0.018 \text{ m}^3 \text{ s}^{-1}$; low flow $\geq 0.002 \text{ m}^3 \text{ s}^{-1}$ and $< 0.018 \text{ m}^3 \text{ s}^{-1}$, and no flow $< 0.002 \text{ m}^3 \text{ s}^{-1}$.

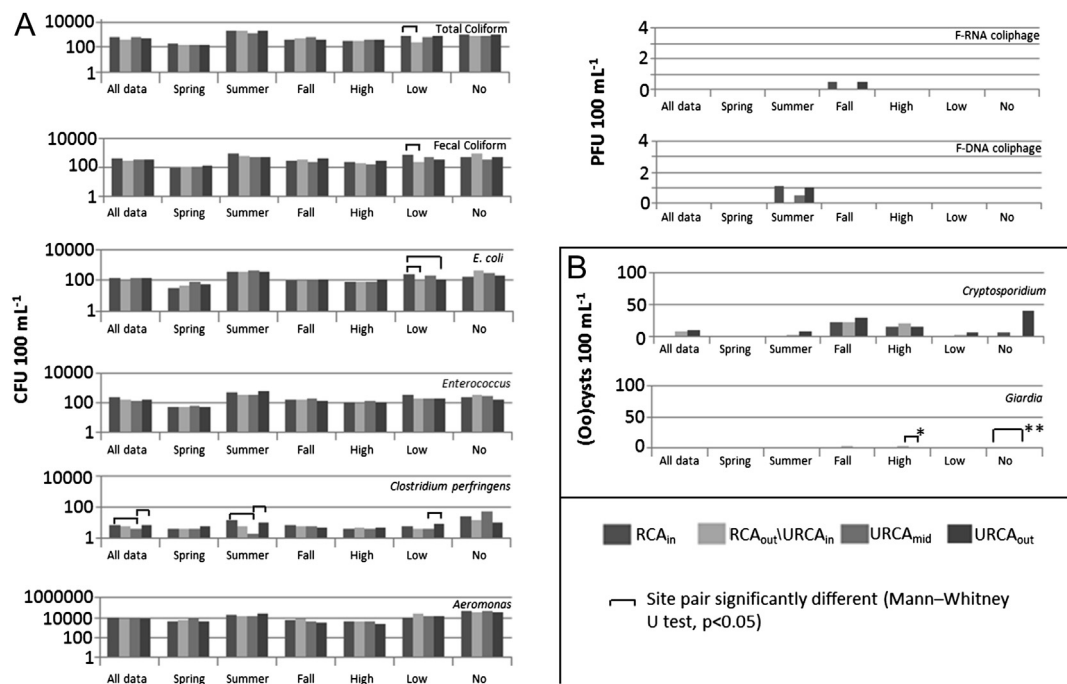


Fig. 5 – Median densities of fecal indicator bacteria, *Aeromonas*, parasites and F-coliphage for samples collected under specific site, season, and flow conditions for All Available Data. (A) bacteria and F-coliphage, (B) parasites. * RCA_{out}/URCA_{in} vs. URCA_{out}; ** RCA_{in} vs. URCA_{out}. High flow $0.018 \text{ m}^3 \text{ s}^{-1}$; low flow $0.002 \text{ m}^3 \text{ s}^{-1}$ and $< 0.018 \text{ m}^3 \text{ s}^{-1}$, and no flow $< 0.002 \text{ m}^3 \text{ s}^{-1}$.

flows, was a season that was associated most strongly with higher median densities of the microorganisms in the CART regression tree analysis (Supp. Tbl. 3).

There were some noteworthy data trends, albeit not statistically significant, in the pathogen prevalence data when RCA_{in} , $RCA_{out}/URCA_{in}$, and $URCA_{out}$ were concurrently monitored. *Salmonella* spp. tended to increase in prevalence from the RCA_{in} to the $URCA_{out}$, most markedly at higher flow (Fig. 2). Norovirus GII was, overall, most prevalent in summer and at the $URCA_{out}$. Rotavirus was most prevalent at the RCA_{in} relative to other sites. Hepatitis E virus occurrence was detected at the $URCA_{out}$ exclusively with spring and low flow being key conditions associated with the occurrence of this virus. F-RNA coliphage occurred most frequently in fall at RCA_{in} . Prevalence of F-DNA coliphage was at or over ~47% in summer, representing the greatest overall prevalence rate, with RCA_{in} and $URCA_{out}$ having the highest rate during this season. Fall and high flow were conditions where the occurrence of *Cryptosporidium* was highest, and moreover, prevalence was generally higher at the $URCA_{out}$ relative to the RCA_{in} (Fig. 2b). For *Giardia*, trends were opposite of that observed for *Cryptosporidium*, with higher *Giardia* occurrence in summer and low flow. *E. coli* O157:H7 was not found during conditions associated with concurrent site sampling despite significant numbers of samples collected.

3.3. All available data

Campylobacter spp. and *E. coli* O157:H7 detections were significantly different among sites ($p < 0.05$) (Fig. 3), with significant site differences for *Campylobacter* spp. for 'all data' and 'fall data' between RCA_{in} and $URCA_{out}$ (2×2 , Fisher's exact test), and for *E. coli* O157:H7 for 'all data' and 'high flow' (2×4 contingency table, Fisher's exact test). *E. coli* O157:H7 was observed more often at $URCA_{mid}$ ($n = 3$, out of 50 samples), and was not detected at any other site (Fig. 3). Also, rotavirus and norovirus GII were detected at $URCA_{mid}$ in 4 and 13% of samples, respectively, with equivalent or greater detection rates at $URCA_{out}$ (4 and 22% for rotavirus and norovirus GII, respectively); detection rates for rotavirus and norovirus GII were 6 and 13%, respectively, at RCA_{in} (Fig. 3). Hepatitis E virus was detected at rates of 4% at $URCA_{out}$ for 'all data', and was detected in spring (11%) and no flow (8%) there, while no detections were observed upstream.

Trends in indicator bacteria median values (Fig. 5) showed at low flow, there were significant differences in total coliform, fecal coliform, and *E. coli* densities between RCA_{in} and $RCA_{out}/URCA_{in}$ (with lower median densities at $RCA_{out}/URCA_{in}$ versus RCA_{in}), and similar but non-significant reduction trends between RCA_{in} and $RCA_{out}/URCA_{in}$ for 'all data' (Fig. 5). This reduction in densities downstream is further illustrated with significantly lower *C. perfringens* densities at $URCA_{mid}$.

F-RNA and F-DNA coliphage densities showed no significant trends. However, F-RNA median densities were greater in the fall only (Fig. 5) at RCA_{in} (0.5 PFU 100 mL⁻¹), decreasing within $RCA_{out}/URCA_{in}$ and $URCA_{mid}$ (0 PFU 100 mL⁻¹), and increasing to 0.5 PFU 100 mL⁻¹ at $URCA_{out}$. In summer, median densities of F-DNA coliphage were greatest at RCA_{in} (1.1 PFU 100 mL⁻¹), reducing to 0 PFU 100 mL⁻¹ at $RCA_{out}/URCA_{in}$, and

increasing to 0.5 and 1.0 PFU 100 mL⁻¹ at $URCA_{mid}$ and $URCA_{out}$, respectively.

3.4. Fecal indicator thresholds related to pathogen presence/absence

Relationships between fecal indicator organisms and pathogens can be variable (Yates, 2007; Wilkes et al., 2009); however, they are useful for helping determine conditions and pollution drivers that may impose higher risk for human infection by pathogens, as well as help explain the efficacy of BMPs in reducing surface water impairment by fecal pollution. For this analysis, all available data were used. Distributions of indicator bacteria densities were significantly different in the presence vs. the absence (P.vs.A.) of *Campylobacter* spp. (MWU tests; $p < 0.05$), with higher medians in *Campylobacter* spp. presence (Table 2). Distributions of fecal coliforms were significantly different in the P.vs.A. of *Cryptosporidium* (medians of 485 CFU 100 mL⁻¹ in presence, vs. 320 CFU 100 mL⁻¹ in absence of *Cryptosporidium*), and distributions of *Enterococcus* were significantly different in the P.vs.A. of *Giardia* (medians of 320 CFU 100 mL⁻¹ in presence, versus 144 CFU 100 mL⁻¹ in absence of *Giardia*). Distributions of *Aeromonas* spp. were significantly different in *Salmonella* spp. P.vs.A.; counter intuitively, however, where medians were greater in the absence of *Salmonella* spp.

CART was able to help quantify threshold fecal indicator and *Aeromonas* spp. densities that delineated groups of greater detection percentages of targeted pathogens (Supp. Tbl. 4). For approximately 75% (18/24) of the fecal indicator bacteria vs. pathogen classification results that cross-validated, there was greater percentage of pathogen occurrence associated with the > indicator density threshold classification group. For the fecal indicator bacteria vs. bacterial pathogens this value was 75%, for the fecal indicator bacteria vs. parasite results this value was 90%, if only for a few. Coliphage vs. pathogens classification results indicated coliphage was not as specific for discriminating pathogen occurrence as were the fecal indicator bacteria.

3.5. Quantitative microbial risk assessment

Ninety-five percent credible intervals for mean risk (Fig. 6) ranged from 10^{-4.5} to 10⁻² for total *Cryptosporidium* spp., 10⁻¹² to 10⁻³ for pathogenic *Cryptosporidium* spp., 10⁻⁵ to 10^{-2.5} for total *Giardia*, and 10⁻¹⁰ to 10^{-3.5} for *E. coli* O157:H7. Considering only the fraction of the total *Cryptosporidium* oocysts that are deemed to be pathogenic resulted in mean risks being reduced by approximately an order of magnitude or more.

Since this risk analysis is comparative rather than predictive, mean risks are compared in a relative way to compute posterior probabilities that the risk at one site is greater than that at another. For total *Cryptosporidium*, $URCA_{out}$ had greater mean risk than RCA_{in} or $RCA_{out}/URCA_{in}$ with pair-wise posterior probabilities of 0.9993 and 0.9974, respectively. The $RCA_{out}/URCA_{in}$, however, had lower mean risk than the RCA_{in} with a posterior probability of 0.8531. These results indicated a slight decrease in mean risk from total *Cryptosporidium* across the RCA followed by an increase in mean risk across the URCA.

Table 2 – Associations between the densities of fecal indicator microorganisms, including *Aeromonas*, and the occurrence of pathogens in surface water (All Available Data).

Pathogen	Indicator microorganism (*unit)	Pathogen presence grouping			Pathogen absence grouping			Mann-Whitney U P value
		Total number of microorganism samples in presence of pathogen	Median microorganism	Mean rank sum	Total number of microorganism samples in absence of pathogen	Median microorganism	Mean rank sum	
<i>Campylobacter</i> spp.	F-RNA coliphage*	82	0	77.6	63	0	67.0	0.069
<i>Campylobacter</i> spp.	F-DNA coliphage*	82	0	76.6	64	0	69.5	0.245
<i>Campylobacter</i> spp.	Total Coliforms**	109	750	144.5	155	400	124.0	0.032‡
<i>Campylobacter</i> spp.	Fecal Coliforms**	103	480	140.3	152	340	119.6	0.028‡
<i>Campylobacter</i> spp.	<i>E. coli</i> **	109	164	144.0	155	121	124.4	0.040‡
<i>Campylobacter</i> spp.	<i>Enterococcus</i> **	109	230	145.6	155	162	123.3	0.020‡
<i>Campylobacter</i> spp.	<i>C. perfringens</i> **	107	10	144.8	154	4	121.4	0.013‡
<i>Campylobacter</i> spp.	<i>Aeromonas</i> **	62	11900	57.4	44	7300	48.0	0.125
<i>Cryptosporidium</i>	F-RNA coliphage*	24	1	16.9	9	50	17.2	0.953
<i>Cryptosporidium</i>	F-DNA coliphage*	24	0	16.1	9	0	19.4	0.392
<i>Cryptosporidium</i>	Total Coliforms**	56	485	53.2	45	500	48.2	0.393
<i>Cryptosporidium</i>	Fecal Coliforms**	56	485	56.9	45	320	43.6	0.023‡
<i>Cryptosporidium</i>	<i>E. coli</i> **	56	145	53.4	45	122	48.0	0.353
<i>Cryptosporidium</i>	<i>Enterococcus</i> **	56	164	51.5	45	196	50.4	0.852
<i>Cryptosporidium</i>	<i>C. perfringens</i> **	54	5	53.8	45	3	45.4	0.147
<i>Cryptosporidium</i>	<i>Aeromonas</i> **	5	6500	4.4	2	4505	3.0	0.571
<i>Giardia</i>	F-RNA coliphage*	6	26	15.5	27	0.5	17.3	0.699
<i>Giardia</i>	F-DNA coliphage*	6	0	20.5	27	0	16.2	0.348
<i>Giardia</i>	Total Coliforms**	20	675	59.6	81	470	48.9	0.145
<i>Giardia</i>	Fecal Coliforms**	20	680	59.2	81	370	49.0	0.165
<i>Giardia</i>	<i>E. coli</i> **	20	186	56.3	81	122	49.7	0.371
<i>Giardia</i>	<i>Enterococcus</i> **	20	320	62.6	81	144	48.1	0.047‡
<i>Giardia</i>	<i>C. perfringens</i> **	19	3	49.2	80	4	50.2	0.891
Norovirus GII	F-RNA coliphage*	15	0	49.0	88	0	52.5	0.674
Norovirus GII	F-DNA coliphage*	15	0	47.6	89	0	53.3	0.503
Norovirus GII	Total Coliforms**	15	290	47.1	88	515	52.8	0.496
Norovirus GII	Fecal Coliforms**	13	230	44.7	81	370	48.0	0.689
Norovirus GII	<i>E. coli</i> **	15	130	54.4	88	129	51.6	0.735
Norovirus GII	<i>Enterococcus</i> **	15	190	49.2	88	200	52.5	0.694
Norovirus GII	<i>C. perfringens</i> **	15	16	46.4	88	14	52.9	0.439
Norovirus GII	<i>Aeromonas</i> **	15	11200	52.7	88	9500	51.9	0.930
Rotavirus	F-RNA coliphage*	4	0	41.5	99	0	52.4	0.493
Rotavirus	F-DNA coliphage*	4	0	36.5	100	0	53.1	0.296
Rotavirus	Total Coliforms**	4	495	49.3	99	500	52.1	0.863
Rotavirus	Fecal Coliforms**	4	505	55.0	90	323	47.2	0.594
Rotavirus	<i>E. coli</i> **	4	230	66.5	99	130	51.4	0.340
Rotavirus	<i>Enterococcus</i> **	4	284	50.5	99	200	52.1	0.928
Rotavirus	<i>C. perfringens</i> **	4	44	78.8	99	12	50.9	0.068
Rotavirus	<i>Aeromonas</i> **	4	7550	49.5	99	9500	52.1	0.876
<i>Salmonella</i> spp.	F-RNA coliphage*	9	0	49.5	136	0	74.6	0.083

Salmonella spp.	F-DNA coliphage*	9	0	77.6	137	0	73.2	0.773
Salmonella spp.	Total Coliforms**	15	1160	144.4	250	520	132.3	0.558
Salmonella spp.	Fecal Coliforms**	13	330	118.0	243	408	129.1	0.602
Salmonella spp.	E. coli**	15	130	147.7	250	133	132.1	0.448
Salmonella spp.	Enterococcus**	15	110	122.1	250	200	133.7	0.576
Salmonella spp.	C. perfringens**	15	10	153.8	247	6	130.1	0.244
Salmonella spp.	Aeromonas**	8	475	27.0	98	950	55.7	0.009#

*PFU per 100 mL⁻¹, **CFU 100 mL⁻¹, # Significant $p < 0.05$.

When only pathogenic *Cryptosporidium* are considered, the mean risk at the URCA_{out} site is still greater than that of the RCA_{in} and RCA_{out}/URCA_{in} sites (with posterior probabilities of 0.9990 and 0.9750 respectively). The mean risk at the RCA_{out}/URCA_{in} site is also greater than that of the RCA_{in} site with posterior probability 0.8425. These results indicate a slight increase in mean risk from pathogenic *Cryptosporidium* across the RCA (attributed to a single detection of human source classed *C. hominis* at RCA_{out}/URCA_{in}) and a further increase across the URCA (attributed to a detection of both *C. hominis* and *C. parvum*).

For total *Giardia*, the mean risk at URCA_{out} is greater than that of the RCA_{in} and RCA_{out}/URCA_{in} (with posterior probabilities of 0.9541 and 0.8658 respectively). The overall average mean risk at the RCA_{out}/URCA_{in} site is greater than that of the RCA_{in} site (Supp. Fig. 1), but the corresponding posterior probability is only 0.3960. This peculiar result is attributable to the relatively small number of available data and corresponding increase in uncertainty. Collectively, these results indicate little change in mean risk from total *Giardia* across the RCA, followed by an increase in mean risk across the URCA.

In the case of *E. coli* O157:H7 (for which risk results are also available for URCA_{mid}), URCA_{mid} was found to have greater mean risk than the RCA_{in}, RCA_{out}/URCA_{in}, and URCA_{out} with posterior probabilities of 0.9952, 0.9648, and 0.9189, respectively. This is attributable to the detection of *E. coli* O157:H7 on three occasions at this site, while it was undetected at all other sites. The mean risk at RCA_{out}/URCA_{in} was greater than that of RCA_{in} with a posterior probability of 0.6735 and lower than that of URCA_{out} with a posterior probability of 0.6180; thus there is a slight increasing *E. coli* risk trend across both the RCA and URCA. An interesting finding in this risk assessment (unlike *Cryptosporidium* and *Giardia*) is that close to two orders of magnitude of uncertainty in the computed mean risk values are attributable to uncertainty in the parameters of the dose–response model (results not shown here). This is attributable to the relatively small number of subjects and range of mean doses in the *E. coli* dose–response experiment by DuPont et al. (1971).

4. Discussion

It is recognized that water quality surveillance (WHO, 2004; Thomas et al., 2013) can contribute to a better understanding of fecal pollution sources and drivers, and therefore appropriate mitigation measures. Overall, there were not many strong statistically significant trends in pathogen prevalence or densities associated with restricting cattle access to the water course (protecting riparian zone). However, indicator bacteria densities (total coliform, fecal coliform, *E. coli*) were higher at the RCA_{in} site compared to RCA_{out}/URCA_{in} site ($p < 0.05$, MWU), illustrating an apparent riparian zone treatment effect. Restricting cattle access did not reduce, statistically speaking, *Campylobacter* spp. occurrence relative to upstream (site input) prevalence, and surprisingly, *Campylobacter* spp. occurrence was even lower at the URCA_{out} relative to RCA_{in}. Schmidt et al. (2013a) found that *Campylobacter* spp. densities were relatively high in these watersheds at RCA_{in}, and upstream of the RCA_{in}, where the only potential for

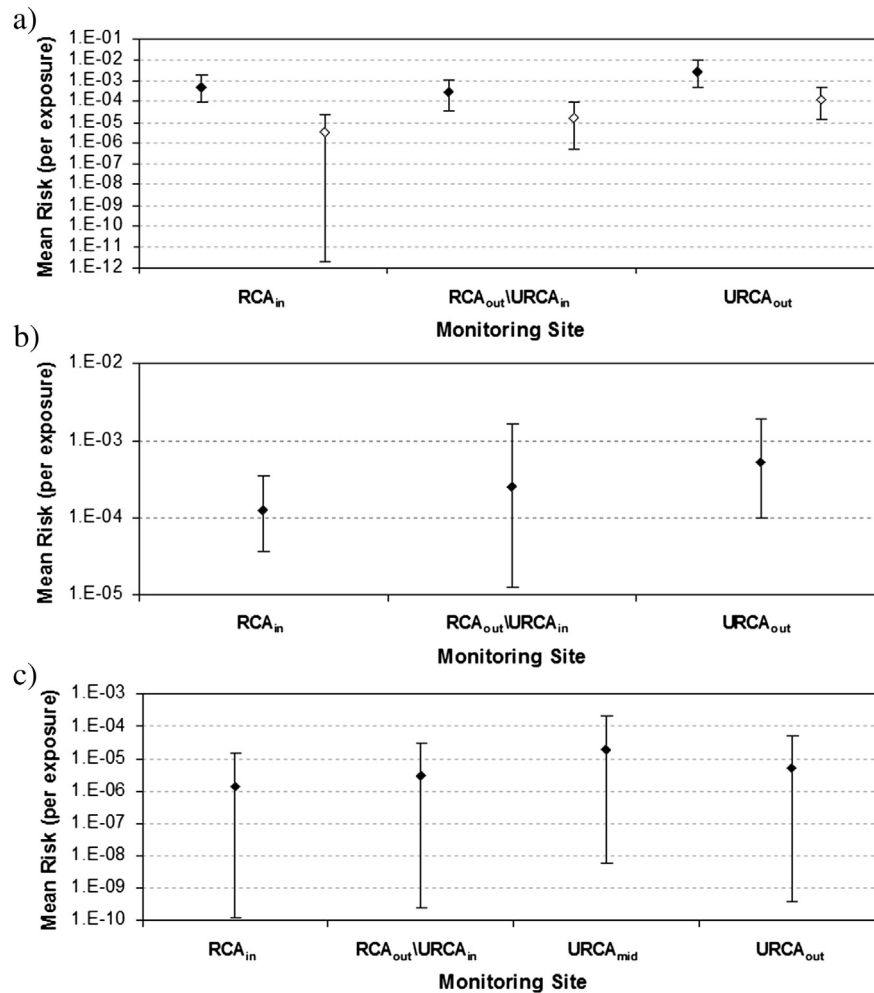


Fig. 6 – Plots of the uncertainty in the mean risk at each site for a) total *Cryptosporidium* (black points) and pathogenic *Cryptosporidium* (white points), b) *Giardia*, and c) *E. coli* O157:H7. The points show the posterior mean (of the mean risk) and the error bars show the 95% equal-tailed credible intervals.

agricultural inputs of *Campylobacter* spp. would have been related to land applications of manure and subsequent stream inputs via, primarily, subsurface tile drainage. The overall decreasing trend in *Campylobacter* spp. detection downstream from RCA_{in} to URCA_{out} was most pronounced during fall at higher flows; a time when manure is applied to fields and manure contaminants can be transported off site via tile drainage possibly resulting in overall higher prevalence at RCA_{in}. Sunohara et al. (2012) described the RCA as functioning as an in-line wetland, where loads of many fecal pollution indicators and nutrients were found to decrease significantly from RCA_{in} to RCA_{out}/URCA_{in} and increase again from RCA_{out}/URCA_{in} to URCA_{out}. If the source of *Campylobacter* spp. was primarily from livestock, the RCA effect may have been formative in terms of reducing the prevalence of this pathogen in water. The light pasturing in the URCA and lack of cattle related manure land applications on fields adjacent to the RCA and URCA may have helped promote the steady downstream decreases in prevalence observed. Moreover, in fall, the pasturing cattle are often trucked back to barns for the winter, which would further reduce potential livestock-based fecal inputs in both pasture treatments. However, *Campylobacter*

spp. has been shown to be associated with the occurrence of avian-sourced *Cryptosporidium* in the area (Wilkes et al., 2013), which may partially explain site prevalence differences.

The only other noteworthy trend amongst the sites for a pathogen was for *E. coli* O157:H7, resulting solely from 3 positive detections out of 50 at URCA_{mid}. Infected livestock likely introduced the *E. coli* serotype (Renter et al., 2003) into the watercourse via fecal contamination in close proximity to the sampling site, given that cattle have direct access to the water course at this site and that there was no detection of this pathogen at URCA_{out} just downstream. When predicting downstream public health risks from exposure to *E. coli* O157:H7, it was demonstrated that cattle access, most likely, has a direct influence on increasing risk, and although not significant, it is important to differentiate *E. coli* detected in a surface water system (many of which are not human pathogens) from those that are capable of initiating human infections (like *E. coli* O157:H7). Unlike *Cryptosporidium* and *Giardia*, the breadth of the credible intervals in predicted mean risk shrank by roughly two orders of magnitude when relative mean risks were used instead of mean risks. This suggests that roughly two orders of magnitude of uncertainty are

attributable to uncertainty in the parameters of the dose–response model. This illustrates the importance of using relative mean risks to understand a system, given that dose–response data will continue to remain the limiting factor in our ability to more precisely quantify risk.

No significant differences in densities of F-RNA and F-DNA coliphage distribution were observed among the sample sites either, which could be due to, among other things, differential survival rates of subtypes of F- coliphage. Cole et al. (2003) observed seasonal differences in the proportions of F-DNA and F-RNA (higher relative occurrence of F-DNA in summer, complimenting the occurrence results observed in this study), attributing it to survival characteristics, host excretion patterns, or landuse change, and Brion et al. (2002) has observed differential survival rates of subtypes of F-RNA in water. For this study, greatest median densities of F-RNA coliphage occurred in fall while greatest median densities of F-DNA coliphage occurred in spring. Nevertheless, the issue of variable environmental fitness of a microorganism, or strain, can make it additionally difficult to observe the effects of landscape factors on densities (Anderson et al., 2005; van Elsas et al., 2011).

Overall data mining analysis via CART revealed that (Supp. Tbl. 2–3) cattle exclusion measures did not appear to be as important for delineating the occurrence of pathogens, or for parsing of ‘higher’ and ‘lower’ densities of fecal indicator organisms, relative to the more discriminating season and stream flow criteria. This is consistent with findings of Wilkes et al. (2009, 2013). Seasonal attributes could be important in the timing and intensity of fecal inputs from various sources, the seasonal growth of filtering vegetation in the riparian zone, and the accumulation of fecal material over time in and along the stream system. Further, season is interrelated with the timing of critical hydrological events for moving contaminants in water, and season is connected to temperature and moisture factors that affect the survivability of pathogens in soil/water environments.

In Ruecker et al. (2012) and Wilkes et al. (2013), wildlife and livestock were dominant genotypes/species of *Cryptosporidium* observed in surface waters in the study area. Yet many of the wildlife species/genotypes observed were not a known human health risk. *Cryptosporidium* QMRA results underscore the importance of incorporating the proportion of human pathogenic oocysts (including *C. hominis* (human host source)) in the final risk estimates, resulting in a reduction in mean risk by at least one order of magnitude. These results, in the context of the detection of norovirus GII and rotavirus (which can be of human origin) at these sites, suggest there could be an influence of human waste in this system (e.g., septic leakage). Regarding *Giardia*, the lack of additional subtyping data to differentiate between human pathogenic *Giardia* among all *Giardia* detected, limits how we interpret the influence of the URCA on the *Giardia* levels detected at all three sample sites. Based solely on *Giardia* cyst concentrations, the QMRA analyses illustrate an increasing risk from RCA_{in} to URCA_{out}, although not statistically significant. These QMRA results underscore the importance of understanding the many potential sources of fecal inputs in an open system such as a watershed when interpreting the impact of a land use management practice on public health endpoints.

Norovirus was the most commonly detected virus in surface water in this experiment. Recently Thomas et al. (2013) estimated that Norovirus was the number one ranked foodborne illness in Canada. While extensions in this context cannot be made directly to waterborne exposure, a high norovirus prevalence was observed in this study in relation to other viral targets (greater than ~40% of samples for URCA_{out} in summer). Perhaps small order streams helped reduce dilution effects. Norovirus genogroup II is one of the currently 5 identified genogroups (GI–GV) of norovirus, for which GII genotypes are the most commonly known for infecting humans (Lindesmith et al., 2008), but some are also found in swine. Norovirus GII and group A rotavirus (found in human, swine, and bovine) tended to be higher at RCA_{in}, decrease in prevalence at RCA_{out}/URCA_{in}, and increase again at the URCA_{out}. These prevalence trends did underscore the significant and documented RCA effect found in the fecal indicator bacteria (Fig. 4) and nutrient and fecal indicator loads given in Sunohara et al. (2012). Liquid swine manure has been applied on fields that can, under high rainfall situations, drain into roadside ditches that could impact the RCA_{in}, as a result, swine inputs cannot be refuted. The contributions from septic systems from homes located along the stream (Sunohara et al., 2012), as well as pets and a few hobby farm animals associated with them, is not clearly explicit from the data reported in this study (outside of the *C. hominis* observations regarding human source). But these sources could have contributed, along with inhibited dilution effects in this intermittent system, to the higher relative rates of occurrence of this virus in the water course.

A component of this study included a global examination of pathogen associations with indicator bacteria and F- coliphage densities in surface water for the RCA and URCA sites. Assessment of BMP impact on surface water quality within a public health context would currently require monitoring of a suite of pathogens and their densities for different treatments at great monetary and logistical expense. Ideally, fecal indicator organisms could serve as pathogen proxies or surrogates in this capacity (Wilkes et al., 2009). For the pathogenic viruses, there were no strong relationships with F-RNA or F-DNA coliphage density or occurrence which would appear to reduce potential for coliphage data in this small surface water system to serve as proxies for viral pathogens. There were, however, multiple indicator microorganisms that could serve as modest pathogen proxies, especially for *Campylobacter* spp. and to a lesser extent *Cryptosporidium* spp., thereby complimenting the results observed in Wilkes et al. (2009). For assessing the effects of pasturing BMPs such as those here, the fact that fecal indicator bacteria demonstrated a ‘low flow’ pasture treatment effect indicates they could potentially serve as adequate, and considerably less expensive organisms to gauge effects of riparian zone protection on net fecal pollution (and potentially serve as a proxy for the occurrence of *Campylobacter* spp.).

5. Conclusions

Some of the key conclusions that can be drawn from this study include:

- *E. coli* O157:H7 was infrequently detected, but apparently, light pasturing operations with livestock having the capacity to interact with the water course can increase the likelihood of this pathogen occurring in water, and thereby increase relative mean risks of exposure to this pathogen.
- For the unrestricted and restricted pasture systems, relative exposure risks were ranked in decreasing order as: total *Cryptosporidium*, total *Giardia* spp., human pathogenic *Cryptosporidium* and *E. coli* O157:H7. Adjusting for the proportion of *Cryptosporidium* spp. detected that are infectious for humans reduces downstream risk estimates by roughly one order of magnitude. The QMRA indicated that relative risk increased across the RCA and URCA region for *E. coli* O157:H7; human-pathogenic *Cryptosporidium* relative risk slightly increased across the RCA due to the occurrence of *C. hominis* (associated with human and likely of septic origin from homes next to the BMP) at $RCA_{out}/URCA_{in}$ and further increased in the URCA (due to increased occurrence of *C. hominis* and *C. parvum* (normally associated with cattle) in tandem at $URCA_{out}$), and little change in relative risk was observed across the RCA for *Giardia*, but *Giardia* relative risk subsequently increased in the URCA. From a relative risk perspective, the fencing BMP did provide improved risk protection for *Giardia* whereas risks for *E. coli* and human-pathogenic *Cryptosporidium* (likely partly due to septic inputs from homes) increased downstream to URCA; the URCA region overall provides an increased risk from background, whereas the RCA region (and the BMP) results varied depending on the targeted pathogen. Using QMRA in this manner provides a more refined estimate of BMP effects on human health.
- The cattle exclusion measures did not have an equal effect on all microorganisms. *Campylobacter* spp. had high relative prevalence in this agricultural watershed but detections decreased significantly through the restricted pasture and unrestricted pasture exhibiting a pasturing/riparian protection treatment effect (Sunohara et al., 2012). In contrast, *Cryptosporidium* had high relative prevalence for all sites (both restricted and unrestricted, no reduction) plus subtyping attributes helped to more accurately enumerate human infectivity risks and host specificity (see Ruecker et al., 2012).
- Density distribution differences of total coliform, fecal coliform, *E. coli*, *Enterococcus*, and *C. perfringens* concentrations were associated with *Campylobacter* spp. detection; greater median densities of indicator organisms were observed in *Campylobacter* spp. presence. These indicator bacteria show promise as a conservative proxy for this pathogen in agriculturally dominated surface waters.
- Overall, data mining found seasonal and stream flow variables and interactions to be important for delineating pathogen occurrence and higher indicator densities relative to site pasturing treatments. Consistent with other studies, empirical models that may attempt to predict prevalence of pathogen or fecal indicators should at least assess seasonal and flow variable interactions.
- Indicator microorganisms exhibited no statistically significant distribution differences amongst the pasture sites except for total coliform, fecal coliform, and *E. coli* differences between RCA_{in} and $RCA_{out}/URCA_{in}$ at low flow; where relevant

densities for this flow regime reduced from RCA_{in} to $RCA_{out}/URCA_{in}$ demonstrating a riparian zone treatment effect. Seasonal and flow conditions were associated more strongly with greater indicator bacteria densities, especially in the summer. Norovirus GII was detected at rates of 7–22%, and rotavirus 0–7% for monitoring sites, and no statistically significant associations were found with indicator bacteria or F-coliphage densities. It is speculated that at least house septic issues are potential sources of viruses (as supported by occurrence of *C. hominis*), but other sources (swine) may have contributed as well.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2013.07.041>.

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