Bacterial and viral indicators of fecal contamination in drinking water

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Coliform indicators are used as an index of waterborne disease risk. Coliforms respond differently to environmental stressors and engineered treatment processes than viral pathogens and thus are imperfect indicators. Bacteriophages, which are similar in structure and morphology to human enteric viruses, may be more highly correlated with the presence of viral pathogens. In this research, coliforms, Escherichia coli, male-specific coliphages, and somatic coliphages were quantified in feces and in wastewater and drinking water samples collected in multiple regions and seasons. Physical and chemical water quality were also measured. Bacterial indicators varied with animal type in feces and varied with treatment stage in wastewater and drinking water. Samples analyzed for coliphages had a high number of nondetects (49% in fecal samples and up to 79% in drinking water samples), but coliphages can be analyzed in addition to bacteria to provide complementary information on fecal contamination in water and public health risk. In some cases, turbidity and organic carbon may provide a rapid indication of pollution that could trigger targeted microbiological monitoring.

Keywords: bacterial indicators, male-specific coliphages, somatic coliphages, water quality correlations

For more than a century, coliform bacteria, fecal coliform bacteria, and Escherichia coli have been used as indicators of the microbiological safety of water supplies (Griffin et al, 2008). This approach is based on the assumption that there is a quantifiable relationship between the concentration of coliform indicators and the potential health risks from pathogens. Today, in developed countries such as the United States and the United Kingdom, the practice of using coliforms and other indicator organisms is still widely accepted (Yates, 2007). However, the fate of coliforms is different from that of viral pathogens in natural water systems and engineered treatment processes (Fong & Lipp, 2005; Griffin et al, 1999).

In natural water systems, enteric viruses can tolerate changing environmental conditions better than coliforms (Espinosa et al, 2009). Coliform bacteria are more susceptible than enteric viruses to extremes in pH, salinity, and temperature (Fong & Lipp, 2005). Griffin et al (1999) found that coliforms were not adequate predictors of fecal contamination and public health risks from protozoa and enteric viruses in the Florida Keys. Inconsistencies between coliforms and pathogens have been found in both wastewater (Harwood et al, 2003) and drinking water systems (LeChevallier et al, 1996). Coliforms have been found in water distribution systems with no apparent negative public health outcomes (Geldreich & Rice, 1987), and, conversely, disease outbreaks have occurred in water systems that were not in violation of coliform regulations. In water systems with disease outbreaks, coliforms were detected in only half of the systems (Craun et al, 1997). Further, a 2004 report by the Centers for Disease Control and Prevention stated that only 10 of 17 waterborne disease outbreaks of infectious etiologies were associated with positive total or fecal coliform counts (Blackburn et al, 2004). In Wisconsin, Borchardt et al (2003) found that bacterial indicators were not statistically associated with the occurrence of viruses in groundwater wells.

Coliphages, which have been investigated as possible viral indicator organisms since the 1980s, may be more appropriate for monitoring the fate of viruses in water (Furuse, 1987). Certain strains of coliphages are small, icosahedral, and nonenveloped viruses, making them structurally similar to many human enteric viruses. They also exhibit similarities to enteric viruses with regard to environmental transport and survival; however, coliphage survival characteristics vary by season and by coliphage group. Male-specific coliphages, also known as F-specific or F+ coliphages, infect E. coli through the F pilus on the host. Male-specific coliphages appear to be present in feces and sewage and also seem to be present at low concentrations in uncontaminated environmental settings (Cole et al, 2003). Somatic coliphages, which infect E. coli through the host cell wall, are the most abun-
dant group of bacteriophages and are likely to be more persistent in water than male-specific ribonucleic acid (RNA) coliphages (Lee & Sobsey, 2011).

Relationships among coliphages, coliforms, and pathogens vary under different conditions. Borrego et al (1990) found that coliphages were good indicators of fecal pollution in both river and marine waters. Ogórzalý et al (2009) studied a river in an urbanized watershed with recognized anthropogenic influences and found that bacterial indicators were correlated with somatic coliphages. Likewise, Jiang et al (2007) investigated the occurrence and distribution of fecal indicator bacteria, male-specific coliphages, and polymerase chain reaction (PCR)–detectable human adenoviruses and enteroviruses at 15 locations around the Newport Bay, Calif., watershed. Among 206 samples, fecal indicator bacteria and coliphages had similar seasonal and freshwater-to-saltwater distribution patterns, suggesting they share similar environmental sources. In addition, coliphages were correlated with PCR-detectable human viral genomes. Borrego et al (1987) evaluated the relationship between E. coli and its parasitic phages in the vicinity of sewage outfalls, river water contaminated by domestic and industrial sewage discharges, and estuarine waters. Coliphages were a good indicator of the presence of the pathogenic microorganisms studied, and coliphages may be better indicators of fecal pollution than classical bacterial indicator systems.

However, bacteriophages may continue to replicate in surviving bacterial hosts after being shed in feces, and male-specific coliphages are present in human feces infrequently, are relatively scarce, and have die-off rates that vary with water temperature (Lee & Sobsey, 2011). Payment and Locas (2011) observed non- enteric indicators (total coliforms and aerobic endospores) more frequently than E. coli, male-specific coliphages, or somatic coliphages in virus-positive groundwater samples. In a study of groundwater in Massachusetts, the presence of coliforms and coliphages was uncorrelated (Long & Dewar, 2008). Carducci et al (1999) found no relationship between coliphages and viral contamination in sewage treatment plants. Also, somatic coliphages have been found to have higher concentrations than male-specific phages in wastewater and raw water sources (Stewart-Pullaro et al, 2006). Payment and Locas (2011) found that coliphages were not good predictors of the presence or absence of viruses because coliphages occurred in low numbers and less frequently than bacterial indicators. In a study of four French rivers, Hot et al (2003) found no statistical correlation between somatic coliphages and enteroviruses, human adenoviruses, or Norwalk I and II viruses. Because of these shortcomings, male-specific coliphages have most often been used as source-tracking indicators rather than generic indicators of fecal contamination (Cole et al, 2003).

The purpose of this research was to directly compare coliforms and coliphages as indicator organisms in wastewater and drinking water systems. Although previous studies have evaluated these indicators, this study used identical methodologies to measure these indicators and a number of water quality parameters in feces and in wastewater and drinking water samples collected from four US regions during a 24-month period. Finally, comprehensive statistical analyses were applied to the dataset in order to identify water quality and indicator relationships. The strength of these relationships could be used in the design of more protective monitoring strategies than monitoring for coliforms alone.

**EXPERIMENTAL PROTOCOLS**

**Sampling overview.** Samples were collected from the Northeast, South, Midwest, and West regions of the United States to allow spatial variability to be assessed. Wastewater, drinking water, and fecal samples were collected by volunteer samplers in Massachusetts, North Carolina, Florida, Wisconsin, Colorado, Nevada, and Washington. Fecal samples were collected at farms or private residences; wastewater and drinking water samples were obtained from municipal treatment facilities. To allow assessment of temporal variability, samples were collected during multiple seasons. Fecal and wastewater sampling was conducted from June 2010 to April 2011 (12 events). Drinking water sampling was conducted from May 2011 to March 2012 (16 events).

Fresh fecal samples were collected from five animal groups: chicken, dog, equine (horse and donkey), rabbit, and ruminant (cow, sheep, goat, and llama). Samplers monitored the animals and collected feces in sterile containers immediately after the animals defecated. Wastewater samples, collected as grab samples at municipal treatment facilities, included raw sewage and final effluent before disinfection. Drinking water samples, collected as grab samples from municipal treatment facilities, represented both groundwater and surface water systems. For indicator enumerations in drinking water samples, 20-L samples were collected and concentrated using hollow-fiber ultrafiltration (HFUF) at remote laboratory sites proximate to the sampling locations to reduce detection limits. HFUF has been shown to quantitatively concentrate a variety of microorganisms from source water and drinking water matrices (Olstad et al, 2005; Hill et al, 2005). This system, based on a 30,000-Da-molecular-weight cutoff, has been demonstrated to be effective for MS2 coliphages, noroviruses, and adenoviruses (Sibley, 2008; Hill et al, 2007). Details can be found in Plummer and Long (2013). The procedure concentrated each 20-L sample to approximately 400 mL (up to 50 x concentration factor). All samples were then placed in coolers with icepacks and transported by overnight shipment to the laboratory for processing.

**Analytical methods.** Indicator organism enumerations were performed on all samples. Samples were diluted, undiluted, or concentrated as needed. Feces were resuspended in buffered water according to method 9050c.1a (Standard Methods, 2005). Total coliforms and E. coli were enumerated in accordance with method 9223, the enzyme substrate test (Standard Methods, 2005), using defined substrate technology\(^1\) in the multiple-well format.\(^2\) Results from duplicate tests were averaged, and the values were adjusted based on dilution or concentration of the sample to determine of the most probable number per 100 mL in the original sample. One positive control (a proprietary E. coli sample\(^3\)) and one negative control (buffered water) were analyzed for total coliforms and E. coli for each sampling event.
Male-specific and somatic coliphages were enumerated using method 1602, the single agar layer method (USEPA, 2001). Fecal samples were resuspended in phosphate-buffered saline (PBS), 1 g in 9 mL of PBS, and allowed to disperse for 4 h at 4°C before processing. Fecal and wastewater sample volumes of 1 mL were plated on triplicate 100-mm Petri dishes with host and tryptic soy agar (TSA). Log-phase \textit{E. coli} F amp \textsuperscript{4} and TSA with magnesium chloride (MgCl\textsubscript{2}) and streptomycin–ampicillin were used to enumerate male-specific coliphages; log-phase \textit{E. coli} CN-13\textsuperscript{5} and TSA with MgCl\textsubscript{2} and nalidixic acid were used to enumerate somatic coliphages. Solidified plates were incubated at 36°C for 18–24 h, and the number of plaques on each plate was counted. Results from the triplicate plates were averaged. Drinking water samples were plated on 150-mm Petri dishes, on which a 100-mL sample (as collected or concentrated by HFUF) was mixed with double strength tryptic soy broth + agar, media additives, and \textit{E. coli} host and distributed among five plates. After 16–24 h, the plaque-forming units were counted. The total plaque count for each sample was the sum of counts from the five plates. In addition to the samples, the following controls were prepared: agar-negative controls, host-positive controls, stock coliphage–positive controls, and matrix spikes. Full details can be found in Plummer and Long (2013).

The wastewater and drinking water samples were analyzed for physical and chemical parameters, including turbidity, pH, and total and dissolved organic carbon (TOC and DOC). Turbidity and pH were measured in duplicate in accordance with methods 2130 and 4500-H\textsuperscript{4}, respectively (\textit{Standard Methods}, 2005). TOC and DOC concentrations were measured in accordance with method 5310B (\textit{Standard Methods}, 2005). All instruments were calibrated and quality-checked according to standard quality assurance–quality control procedures.

**Statistical analysis.** Statistical analyses were conducted with predictive analytics software.\textsuperscript{6} First, data from the field-sampling program were statistically analyzed to determine correlations and variations among the water quality parameters and indicators. The Pearson Correlation, which is conducted on true values, assumes the data are normally distributed and measures the strength of the linear relationship between two variables. This correlation was used to compare study results with prior literature; however, the normality of the datasets was tested using the Shapiro-Wilk Test, and all datasets were not normally distributed, except for data on drinking water pH. The Spearman Rank Correlation is a nonparametric test that is computed on ranks and depicts monotonic relationships. The individual water quality parameters, bacterial indicator values, and coliphage values were compared in these analyses. Critical \(r\)-values were determined for the appropriate degrees of freedom at the 95% confidence level (\(\alpha = 0.05\)) for each dataset comparison, and calculated \(r\)-values larger than the critical \(r\)-values were considered significant. Although \(r\)-values may be interpreted to indicate the strength of the relationship between two variables, these values cannot be used to determine whether the relationship is causal or associative.

Two-way analysis of variance (ANOVA) was conducted to determine variances by animal type (fecal dataset) and by season and region (fecal, wastewater, and drinking water datasets). Seasons were defined by the solar calendar in which spring begins at the end of March, summer begins at the end of June, fall begins at the end of September, and winter begins at the end of December. For this analysis, \(p\)-values < 0.05 were considered statistically significant.

**RESULTS**

**Fecal results.** A total of 75 samples from five animal groups were collected and tested for traditional bacterial indicators and coliphages (Table 1). Coliphages were detected in approximately half of the fecal samples, male-specific coliphages were below detection limits in 41 of the 75 samples, and somatic coliphages were below detection limits in 32 of the 75 samples. In samples with detectable concentrations of indicator organisms, coliforms and \textit{E. coli} ranged from tens of MPN/g in horses to 10\textsuperscript{8} MPN/g in chickens. Coliphages also had wide-ranging values, from a low of 5 pfu/g in dogs, horses, and cows to much higher values in chickens (> 2.5 \times 10\textsuperscript{7} pfu/g). In addition to microbiological measurements, the pH of fecal resuspensions was determined and ranged from 5.18 (chickens) to 8.94 (rabbits). The greatest pH range in fecal samples from one animal type was in chicken feces (5.18–8.57), followed by ruminant feces (5.23–8.34). Equine feces had the smallest pH range (6.41–7.71). No seasonal or regional trends were apparent.

**Wastewater results.** Physical and chemical water quality parameters for the wastewater samples are shown in Figure 1.
Turbidity in raw wastewater was typically tens to hundreds of nephelometric turbidity units, with concentrations as high as 900 ntu. Concentrations were reduced through treatment, with final wastewater effluent turbidities typically on the order of single-digit nephelometric turbidity units. No major differences were observed in pH, which ranged from 6.15 to 7.62. TOC and DOC concentrations were also reduced through treatment. Raw wastewater TOC concentrations ranged from 42.3 to 194 mg/L, whereas TOC concentrations in final wastewater effluent averaged 18.3 mg/L. Raw wastewater DOC concentrations ranged from 29.4 to 97.3 mg/L, whereas DOC concentrations in final wastewater effluent ranged from 6.04 to 15.6 mg/L. Bacterial indicator counts (Table 2) were up to $10^8$ MPN/100 mL in raw wastewater but decreased by two to three orders of magnitude through treatment, with values as low as hundreds of MPN/100 mL in final wastewater effluent before disinfection. Coliphage numbers in raw wastewater were as high as $10^5$ pfu/100 mL but as low as below detection limits in final wastewater effluent before disinfection.

**Drinking water results.** A total of 72 samples from both groundwater and surface water sources were collected and tested. Measurements of physical and chemical parameters are shown in Figure 1. Turbidity in untreated drinking water averaged 3.02 ntu, with concentrations as high as 9.69 ntu. These concentrations were reduced through treatment, with treated drinking water turbidity averaging 0.58 ntu. No major differences in pH were observed through treatment; it ranged from 6.03 to 8.89. TOC and DOC concentrations were reduced through treatment. TOC concentrations in untreated drinking water ranged from 0.96 to 15.94 mg/L, whereas TOC concentrations in treated water ranged from 0.26 to 10.77 mg/L. DOC ranges were similar.

Microbial measurements in the drinking water samples are shown in Table 3, categorized by source water type and treatment status. Bacterial indicators and coliphages were below detection limits.
limits in numerous samples of both treated and untreated water. Samples of untreated surface water had total coliform counts of up to 1,610 MPN/100 mL, and treatment reduced these counts by one or more orders of magnitude. The greatest E. coli count in untreated drinking water was 35.2 MPN/100 mL, and treatment decreased this value by two orders of magnitude. Both male-specific and somatic coliphages were below detection limits in samples in every category (surface water, groundwater, raw water, and treated water). The maximum concentration of male-specific coliphages was 882 pfu/100 mL in a surface water sample; however, most detectable quantities of coliphages were in the tenths to ones of plaque-forming units per 100 mL. All groundwater samples were negative for coliforms and E. coli, and coliphage concentrations were < 1 pfu/100 mL.

**Correlation analysis.** All correlation analyses were evaluated at the 95% confidence level. Eliminating data or using zeros for results below detection limits introduces significant bias into statistical analyses (Fu & Wang, 2011; Helsel, 1990). Consistent with methods applied to groundwater samples (in which concentrations below detection limits can be frequent), results that were below detection limits were set to one half of the detection limit to reduce, although not eliminate, bias (Alley, 1990; McBean & Rovers, 1984). Critical r-values were determined from statistical tables on the basis of the number of matched samples in each dataset. Results for the fecal dataset are shown in Table 4. Although the Pearson Correlation requires data to be normally distributed, results are shown for comparison because the Pearson Correlation was used in many prior studies. Linear correlations were demonstrated with coliforms and E. coli, coliforms and male-specific coliphages, and E. coli and male-specific coliphages. In contrast, somatic coliphages were not linearly correlated with any other indicator organisms. The nonparametric Spearman Correlation, which does not depend on type of data distribution, yielded different results. This test showed significant correlations between somatic coliphages and all other indicators (coliforms, E. coli, and male-specific coliphages). However, the Spearman test indicated that male-specific coliphages were not correlated with any other microbial measure. Because results were different for the two correlation tests, the linear Pearson test is not appropriate for use with this dataset.

Tables 5 and 6 show the results of correlation analyses for wastewater and drinking water samples, respectively. When all wastewater samples were analyzed, 11 linear correlations were found with the Pearson Correlation (which requires normally distributed data) and 21 were found with the Spearman Correlation (which is nonparametric). Analytical results from the Pearson Correlation showed that all indicators except somatic coliphages exhibited some statistical correlation with another parameter; however, there were no correlations between coliforms and the coliphages. Analytical results from the Spearman Correlation showed that all parameters were correlated with all other parameters with the one exception of pH, which was not correlated with any other measure. As with the fecal dataset, somatic coliphages

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Concentrations of indicator organisms in wastewater samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater Type</td>
<td>Coliforms MPN/100mL</td>
</tr>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Raw</td>
<td>11</td>
</tr>
<tr>
<td>Primary settled effluent</td>
<td>2</td>
</tr>
<tr>
<td>Final effluent before disinfection</td>
<td>12</td>
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</tbody>
</table>

BDL—below detection limits, n—number of samples

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Concentrations of indicator organisms in drinking water samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Source Water and Treatment Status</td>
<td>Coliforms MPN/100mL</td>
</tr>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>SW untreated</td>
<td>15</td>
</tr>
<tr>
<td>SW treated</td>
<td>42</td>
</tr>
<tr>
<td>GW untreated</td>
<td>4</td>
</tr>
<tr>
<td>GW treated</td>
<td>11</td>
</tr>
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</table>
TABLE 4  Results of correlation analyses for fecal samples

<table>
<thead>
<tr>
<th>Correlation Test</th>
<th>Parameter</th>
<th>pH</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Male-specific Coliphages</th>
<th>Somatic Coliphages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>pH</td>
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<td>1</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Coliforms</td>
<td>0.061</td>
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<td>1</td>
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</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>0.054</td>
<td>0.974</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Male-specific coliphages</td>
<td>0.014</td>
<td>0.584</td>
<td>0.584</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Somatic coliphages</td>
<td>0.096</td>
<td>0.094</td>
<td>0.071</td>
<td>-0.013</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>pH</td>
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<td>1</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Coliforms</td>
<td>0.151</td>
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<tr>
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<td>E. coli</td>
<td>0.081</td>
<td>0.936</td>
<td>1</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>Male-specific coliphages</td>
<td>0.205</td>
<td>0.138</td>
<td>0.077</td>
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<tr>
<td></td>
<td>Somatic coliphages</td>
<td>0.323</td>
<td>0.557</td>
<td>0.447</td>
<td>0.473</td>
<td>1</td>
</tr>
</tbody>
</table>

E. coli—Escherichia coli

Number of samples = 75; two-tailed testing; 95% confidence level; critical r-value for Pearson Correlation = 0.228; critical r-value for Spearman Correlation = 0.227

Statistically significant correlations are shown in bold.

In wastewater samples, there were no differences in any water quality measures or indicator organisms by season or by region. This may be expected because domestic wastewater is relatively consistent throughout the year and in different areas of the United States. Although organic carbon, total coliforms, E. coli, and male-specific coliphages varied by treatment stage, somatic coliphages did not. Reductions in these parameters through treatment would account for the variability. In drinking water samples, type of treatment resulted in differences in all parameters except pH and male-specific coliphages. Because the treatment process is designed to reduce the concentration of organics, indicator organisms, and pathogens, statistically significant differences in these parameters at different stages of treatment are consistent with expectations. Seasonal differences in organic carbon concentrations were observed, as were regional differences in pH, organic carbon, and total coliforms. These differences may reflect seasonal changes in foliage and differences in watershed characteristics for individual water systems.

**DISCUSSION**

Frequency of detection is an important consideration when the usefulness of a potential indicator organism is evaluated. Coliforms and E. coli were detected in the majority of fecal samples. This was expected because healthy ruminants harbor E. coli and thus coliforms in their gastrointestinal tracts. However, bacterial indicators were below detection limits in some fecal samples. These results are likely attributable to a collection or sampling error. One fecal sample from one cow (collected in Wisconsin in the spring) contained coliforms and E. coli below detection limits. The two additional cow fecal samples collected on this same date had counts on the order of $10^4$ and $10^7$ MPN/g, suggesting a collection or processing error for the cow fecal sample with a zero count. Similarly, one chicken fecal sample (collected in Colorado in the spring) contained coliforms and E. coli below detection limits. The other chicken fecal sample collected on this day had coliform and E. coli counts of 100 MPN/g. In contrast, all other samples of chicken feces had coliform and E. coli values in the range of $5.75 \times 10^3$ to $6.05 \times 10^8$ MPN/g. These results suggest that this one chicken fecal sample may have been compromised during collection or processing.

Coliphage results ranging from below detection limits to $>10^7$ pfu/g in chicken feces are consistent with the results of previous studies. Leclerc et al (2000) analyzed fecal samples from dogs, sheep, goats, ducks, geese, chickens, cows, hogs, and horses and found male-specific coliphage counts ranging from $8.6 \times 10^2$ pfu/g (dog) to $1.9 \times 10^7$ pfu/g (horses). Long et al (2005) found somatic coliphage counts as high as $3.6 \times 10^6$ pfu/g in cow feces and $1.9 \times 10^7$ pfu/g in horse feces. These investigators detected male-specific and somatic coliphages in 45 and 57% of fecal samples, respectively. Long et al (2005) measured coliphages in 36 fecal samples from grazing and agricultural animals in different geographic locations during different seasons to assess male-specific coliphages as delineators of microbial pollution sources in surface water. Male-specific coliphages in all fecal samples from grazing animals were below the detection limit of 3.0 pfu/g. Somatic coliphages in some fecal samples from cows, horses, sheep, and pigs were below detection limits.

demonstrated correlations with bacterial indicators only when examined with the nonparametric Spearman test.

In the drinking water samples (Table 6), all indicators exhibited some statistical correlations. Male-specific coliphages were correlated with coliforms, E. coli, and somatic coliphages (Spearman Correlation); and somatic coliphages were correlated with coliforms, E. coli, and male-specific coliphages (the former two by both correlations and the latter by the Spearman Correlation). With regard to general water quality, TOC and DOC were correlated with each other, and organic carbon was correlated with coliforms and E. coli but not with coliphages. As noted with regard to the fecal samples, the two correlation tests gave different results and thus the Spearman Correlation—which did not depend on the data being normally distributed—was the appropriate test in this instance.

**Analysis of variance.**ANOVA was used to determine differences in indicator organism concentrations by season, region, animal type (feces), source (groundwater versus surface water), and treatment type (wastewater and drinking water). Results are shown in Table 7. In fecal samples, coliforms, E. coli, and somatic coliphages differed statistically by animal group. Although regional differences in bacterial indicators were found in feces, this may have been related to which animals were sampled in each region rather than regional differences within specific animal groups. Somatic coliphages did not vary by region and thus may be used as a means to differentiate animal fecal sources. No seasonal differences were observed. Fecal samples were collected immediately after the animal defecated and thus represent conditions in the gastrointestinal tract, which may not be affected by season.
limits. Calci et al (1998) found a high percentage of horse, cow, and sheep feces to have male-specific coliphage concentrations below 10 pfu/g. However, most of the 11 animal types in their study shed relatively low numbers of male-specific coliphages, despite the fact that all of these animals harbor male-specific coliphages. Calci et al (1998) reported that more than 53% of chickens in their study shed < 10 pfu/g of male-specific coliphages. Jones and Johns (2009) concluded that certain properties of coliphages prevent their full recovery from fecal samples. These investigators observed male-specific coliphage nondetects ranging from 30 to 96% in fecal samples from pigs, cattle, and poultry. Jones and Johns (2009) think the 1 g of fecal material used in the coliphage enumeration process is insufficient. Male-specific coliphages were detected in only 9 of 25 fecal samples when the sample size was 1 g; however, when the sample size was increased to 10 g, male-specific coliphages were detected in 16 of 25 samples. The frequency of coliphage nondetects is a concern with regard to use of this organism as an indicator.

Male-specific and somatic coliphages attach themselves to and infect coliform bacteria; therefore, positive correlations between male-specific coliphages and bacterial indicators are expected (Cole et al, 2003). These investigators found that in fecal samples with detectable coliphages, somatic coliphages tended to have higher concentrations than male-specific coliphages. In most cases when both types of coliphages were detected, the concentration of somatic coliphages was one to three orders of magnitude greater than that of male-specific coliphages. Cole et al (2003) found that concentrations of male-specific coliphages differed in different animal types: samples from cattle and swine contained coliphages more frequently than samples from waterfowl and companion animals. However, swine and waterfowl were not tested in the current study. Variations in carriage rates (positivity rates and fecal concentrations) are factors that likely contributed to inconsistent correlations between indicator organisms and pathogens reported for environmental water supplies (Wu et al, 2011).

Results for wastewater samples analyzed in the current study were consistent with those of previous studies. Raw wastewater turbidity can range from < 1 ntu to thousands of nephelometric turbidity units. The typical pH range for untreated domestic wastewater is 6.5–8.5; analytical results for the majority of raw and final wastewater effluent samples in the current study fell within this range. Outside of this range, aquatic organisms can become physiologically stressed. Typical concentrations of organic carbon in untreated wastewater range from 50 to 300 mg/L. Indicator values in wastewater samples in this study were on the order of $10^4$–$10^6$ MPN/100 mL, whereas coliphage values were on the order of $10^3$–$10^5$ pfu/100 mL (Table 2). Claydong et al (2001) found that total coliform numbers in raw domestic wastewater ranged from $4.3 \times 10^6$ to $1.1 \times 10^8$ MPN/100 mL.

### Table 5: Results of correlation analyses for wastewater samples

<table>
<thead>
<tr>
<th>Correlation Test</th>
<th>Parameter</th>
<th>Turbidity</th>
<th>pH</th>
<th>TOC</th>
<th>DOC</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Male-specific Coliphages</th>
<th>Somatic Coliphages</th>
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<td>Pearson</td>
<td>Turbidity</td>
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<td>Male-specific coliphages</td>
<td>0.702</td>
<td>-0.204</td>
<td>0.656</td>
<td>0.642</td>
<td>0.669</td>
<td>0.712</td>
<td>0.589</td>
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<td>Somatic coliphages</td>
<td>0.702</td>
<td>-0.204</td>
<td>0.656</td>
<td>0.642</td>
<td>0.669</td>
<td>0.712</td>
<td>0.589</td>
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</table>

DOC—dissolved organic carbon, E. coli—Escherichia coli, TOC—total organic carbon

Number of samples = 25; two-tailed testing, 95% confidence level; critical r-value for Pearson Correlation = 0.396; critical r-value for Spearman Correlation = 0.398

Statistically significant correlations are shown in bold.
and decreased by 95% or more through treatment. In the current study, coliphage numbers in raw wastewater were as high as 10^5 pfu/100 mL but as low as below detection limits in final wastewater effluent before disinfection. Other researchers have found similar results, with coliphage concentrations in raw wastewater ranging from 10^4 to 10^6 pfu/100 mL (Calci et al, 1998). Because coliphages can tolerate wastewater treatment, they are suitable indicators of fecal contamination (Espinosa et al, 2009).

In wastewater samples, the Spearman test showed that organic carbon concentrations were correlated with bacterial indicators and coliphages, a finding that is consistent with trends in previous studies of surface water influenced by septic systems (Plummer & Long, 2009). It can be hypothesized that more concentrated wastewater would contain higher amounts of both organic carbon and microorganisms. Previous work by Claydong et al (2001) found a significant correlation between male-specific coliphages and total coliforms, a result that was also observed in the current study when correlations were assessed with the Spearman test. Consistent with the correlation between E. coli and male-specific coliphages reported here, a study of wastewater-affected catchment samples found a strong correlation (r = 0.842) between E. coli and male-specific RNA coliphages (Sundram et al, 2002). The lack of correlation between somatic coliphages and other indicators shown with the Pearson Correlation has been observed in previous studies. Imamovic et al (2010) found negative or very low correlation coefficients when comparing bacterial indicators (E. coli strains or coliforms) with somatic coliphages (r = –0.10 to 0.41). It is important to consider whether correlations cited in the literature depict linear or monotonic relationships, because these relationships yielded very different results in the current work.

With regard to season, Long et al (2005) found no seasonal trend in male-specific coliphage densities in wastewater. Season may not have played a role in wastewater concentrations of indicator organisms because sewage is typically transported to the treatment facility in subsurface, closed piping systems where atmospheric temperatures have little effect. Regional effects on E. coli have been shown in other studies. Parveen et al (2006) found that region played a moderately significant role in the resistance of E. coli to certain antibiotics. However, no regional differences were observed in the data in the current study. Thus, it appears that coliphage concentrations in domestic sewage may be consistent year-round.

Drinking water concentrations of bacterial indicators in the current study were consistent with average coliform and E. coli concentrations found in previous studies (LeChevallier et al, 1996). The maximum concentration of male-specific coliphages

### TABLE 6

Results of correlation analyses for drinking water samples

<table>
<thead>
<tr>
<th>Correlation Test</th>
<th>Parameter</th>
<th>Turbidity</th>
<th>pH</th>
<th>TOC</th>
<th>DOC</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Male-specific Coliphages</th>
<th>Somatic Coliphages</th>
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<td>DOC</td>
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<td>0.040</td>
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<td>0.558</td>
<td>0.530</td>
<td>0.633</td>
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</table>

DOC—dissolved organic carbon, E. coli—Escherichia coli, TOC—total organic carbon

Number of samples = 54–72, depending on parameter; two-tailed testing, 95% confidence level; critical r-value for Pearson Correlation = 0.232–0.269; critical r-value for Spearman Correlation = 0.232–0.268

Statistically significant correlations are shown in bold.
in an untreated drinking water sample was 1.83 pfu/100 mL, whereas the maximum value in a treated water sample was 882 pfu/100 mL. This likely can be explained because the treated water sample with the high value was collected from the Northeast in the spring, whereas the untreated water sample with the high value was collected from the West in the winter; thus these results do not indicate an increase in male-specific coliphages in a particular sample through treatment. Calci et al (1998) suggested that wastewater treatment plants are the principal contributors of male-specific coliphages to drinking water sources. The highest quantity of somatic coliphages in untreated drinking water was 5.80 pfu/100 mL, whereas the highest quantity in treated water was 1.10 pfu/100 mL. Average somatic coliphage concentrations were reduced by one order of magnitude through treatment—from $10^{-1}$ to approximately $10^{-2}$ pfu/100 mL. Stewart-Pullaro et al (2006) found similar coliphage concentrations in drinking water sources. In comparisons of water quality and microbial indicators, the correlation between organic carbon and coliforms has been observed before. Boualam et al (2002) observed a positive relationship between DOC and the culturability of coliform bacteria in treated drinking water from surface water sources. Garzio-Hadzick et al (2010) found that an increase in organic carbon in surface water led to slower inactivation of *E. coli*. Lucena and Jofre (2010) found somatic coliphages to be correlated with fecal coliforms, a result that is consistent with the correlation of somatic coliphages and *E. coli* shown by the Spearman test in the current study. Lucena and Jofre (2010) suggested that enumerating one bacterial indicator and somatic coliphages would be more informative about the presence of pathogens in freshwater supplies than enumerating two bacterial indicators. The advantage of using multiple indicators was supported by subsequent work and recent review and re-evaluation of indicator organisms (Payment & Locas, 2011; Yates, 2007).

SUMMARY

This study evaluated physical and chemical water quality parameters, bacterial indicators, and viral indicators in feces and in wastewater and drinking water samples. Coliforms and *E. coli* were detected in the majority of fecal samples, all wastewater samples, and some drinking water samples, depending on treatment status. Neither bacterial indicator varied by season, nor did they vary by region in drinking water. However, both bacterial indicators varied by animal type and region in fecal samples. Coliforms and *E. coli* were detected in treated wastewater; however, samples were collected before final disinfection. Correlations between bacterial indicators and coliphages depended on whether a linear or nonparametric test was used. Thus, statistical relationships need to be considered for validity because datasets with a significant number of nondetects are
unlikely to be normally distributed. Male-specific and somatic coliphages showed no seasonal or regional variations in the samples studied. The ability of coliphages to survive various environmental and treatment conditions in different regions and seasons without variance suggests they may be good indicators of fecal contamination. However, the frequency of nondetects was high; coliphages were not detected in approximately 49% of fecal samples and in up to 79% of drinking water samples. Drinking water samples were concentrated up to 25 times in this study. Concentrating samples to a higher degree could increase the detection of coliphages.

Epidemiological data demonstrate that coliforms and *E. coli* are not always perfect indicators of waterborne disease risk. Although the value added by coliphage testing has been supported scientifically, such testing requires additional laboratory capacity and funding. This study re-evaluated both bacterial and viral indicators in sources of fecal contamination (feces and domestic wastewater) and in drinking water supplies, and it included multiple statistical measurements of geographic and seasonal variability to demonstrate contemporary relevance. Results showed that traditional bacterial indicators and coliphages each have many qualities of ideal indicators. Male-specific and somatic coliphages can be analyzed in addition to bacterial indicators to provide complementary data on fecal contamination in drinking water, potentially by providing additional information about the fecal source or by indicating a fecal contamination event that may not be captured by bacterial results. In addition, turbidity and organic carbon, which showed multiple correlations with microbial indicators, may be useful in providing a rapid measure of degraded water quality that can trigger more intensive microbiological monitoring. Last, statistical analysis must be tailored to particular datasets to ensure that associations between parameters are valid.

**ACKNOWLEDGMENT**

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**ABOUT THE AUTHORS**

Jeanine D. Plummer (to whom correspondence should be addressed) is an associate professor of civil and environmental engineering and director of environmental engineering at Worcester Polytechnic Institute (WPI), 100 Institute Rd., Worcester, MA 01609 USA; jplummer@wpi.edu. She has 20 years of experience in the drinking water profession, 14 of those at WPI, where she teaches classes on environmental engineering, laboratory methods, drinking water quality and treatment, and water quality modeling. Her research focuses on drinking water quality, source water protection, fecal pollution, microbial source tracking, indicator organisms, and alternative disinfection strategies. She holds MS and PhD degrees from the University of Massachusetts, Amherst, and a BS degree from Cornell University, Ithaca, N.Y. Sharan C. Long is a professor and director of environmental microbiology at the University of Wisconsin, Madison. Abigail J. Charest is a graduate research assistant at WPI, and Daniel O. Roop is a staff engineer at Tighe & Bond in Worcester, Mass.

**FOOTNOTES**

1. Colilert®, IDEXX Laboratories Inc., Westbrook, Maine
2. Quant-Tray®, IDEXX Laboratories Inc., Westbrook, Maine
3. ATCC #11775, American Type Culture Collection (ATCC), Manassas, Va.
4. ATCC #700891, ATCC, Manassas, Va.
5. ATCC #700609, ATCC, Manassas, Va.
6. Statistical Package for the Social Sciences, IBM, Armonk, N.Y.

**REFERENCES**


