Research considerations for more effective groundwater monitoring

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ABSTRACT

Since numerous pathogens occur in feces, water is monitored for fecal contamination using indicator organisms rather than individual pathogens. Although this approach is supported by health effects data in recreational waters, it is questionable when used for drinking water. Most outbreaks in groundwater occur in systems that have not violated the US EPA's maximum contaminant limit (MCL) for total coliforms within 12 months before the outbreak. Additionally, environmentally stable viruses and parasites are often detected in drinking water samples with no detectable indicators. Recent detections of *Escherichia coli* O157:H7 and *Campylobacter jejuni* in groundwaters in the apparent absence of indicators also cast some doubt on the worth of indicators for fecal bacterial pathogens. Individual pathogen monitoring is now technically achievable but currently unreasonable due to the number of possible pathogens and the costs involved. Several alternatives to pathogen monitoring could significantly reduce the frequency at which pathogens occur in waters testing negative for indicators: (i) increasing sample volumes for indicators, (ii) increasing monitoring frequency, (iii) using a suite of indicators, (iv) using a more conservative polymerase chain reaction (PCR) method, (v) sampling when fecal contamination is most likely present or (vi) any combination of these options.

Key words | coliforms, groundwater, indicator monitoring, pathogen monitoring, waterborne outbreaks

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INTRODUCTION

Numerous pathogens of fecal origin can occur in contaminated water; moreover the occurrence of any particular pathogen in contaminated water is random over time and space. There is no way to determine which fecal pathogen or pathogens may be present in water at any given time and it is neither practical nor cost-effective to monitor for all of them. As a result, water quality has been tested by detecting organisms that function as indicators of fecal contamination rather than for specific pathogens (Barrell *et al.* 2000; Payment & Locas 2011). The most commonly used indicators are: total coliforms, fecal (thermotolerant) coliforms, *Escherichia coli* and enterococci (APHA 2005). Other organisms found in feces that have been suggested for use as indicators include *Clostridium perfringens*, *Bacteroides* spp. and coliphages (Savichtcheva & Okabe 2006). The indicators recommended by the US EPA for monitoring recreational water are enterococci for marine waters and either enterococci or *E. coli* for fresh waters. These two particular indicators are recommended for recreational waters because their levels correlated with health effects data in epidemiological studies (Dufour & Ballantine 1986).

Coliforms have traditionally been the indicator of choice for drinking water; although there are there are no health effects data to support this choice. The total coliform rule (TCR) (US EPA 1989) requires testing 100 mL volumes of finished drinking water for total coliforms. If total coliforms are found, the drinking water must be tested for either fecal coliforms or *E. coli*. The sampling plan described in the TCR requires public water systems to collect samples at sites representative of water quality throughout the distribution

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system according to a written sample site plan that is subject to state review and revision. Samples must be collected at regular time intervals throughout the month, except for groundwater systems serving 49,000 persons or fewer, which may collect them on the same day.

The current groundwater rule (GWR) (US EPA 2006) is based entirely on monitoring. The sampling plan for monitoring groundwater is the plan described in the TCR (US EPA 1989). Monthly sampling requirements are based on population served and range from one sample per month for systems serving 25 to 1,000 people to 480 for systems serving more than 3,960,001 people. The adequacy of these existing monitoring practices has often been questioned for groundwaters. This is largely because, historically, almost half of all recognized waterborne outbreaks and illnesses have been caused by consumption of untreated or inadequately treated groundwater (Craun 1979; Craun *et al.* 2002, 2010; Blackburn *et al.* 2004; Yoder *et al.* 2008; McKay 2011). Routine coliform surveillance records for 45 outbreaks during 1991–1998 showed that only 22% of community and only 9% of non-community systems experiencing an outbreak had violated US EPA's maximum contaminant limit (MCL) for total coliforms in the 12 month period before the outbreak (Craun *et al.* 2002).

The effectiveness of current groundwater monitoring practices is questionable for identifying periodic intrusions of fecal contaminants, partly because the amounts of water tested for fecal contamination are minuscule compared to the quantities that pass through even the smallest distribution systems. Periodic monitoring for fecal indicators offers minimal (if any) protection against the presence of fecal pathogens in drinking water, especially in communities that utilize untreated groundwater. Only real-time monitoring of all of the water that passes through a distribution system could fully guarantee drinking water that is free from fecal contamination. Unfortunately, this type of monitoring is not feasible with existing technology and cost constraints. Given detection limits and uncertainty in existing methods, even continuous monitoring would not ensure complete freedom from fecal contamination. The best that we can expect periodic monitoring to accomplish is to allow identification of groundwater systems into which there are recurrent intrusions by contaminated surface water, sewage or leaky septic tanks.

Several possible approaches could be taken, either individually or in combination, to improve water monitoring. These are: (i) test for specific pathogens rather than indicators; (ii) increase the sample volumes; (iii) increase the frequency of monitoring; (iv) utilize targeted sampling, directed toward times when the source waters are most vulnerable to contamination, rather than sampling at set times and frequencies; (v) use a suite of indicators rather than a single indicator for monitoring; and (vi) use quantitative polymerase chain reaction (qPCR), which is a more conservative marker of fecal contamination than cultural methods, to measure indicators.

TARGET ORGANISMS: INDICATORS OR PATHOGENS?

One reason the use of total coliforms as indicators is frequently questioned is that some of the organisms identified as total coliforms are widely distributed in nature and are not necessarily associated with the intestinal tract of warm blooded animals (Dutka 1973). The effectiveness of coliforms as indicators has also been questioned for use in water contaminated by protozoa due to the greater stability of the protozoan cysts and oocysts (Craun *et al.* 1997; Rose 1997). In addition, there is evidence that human viruses survive longer in water than coliforms and have sometimes been observed in groundwater in the absence of bacterial indicators (Cohen & Shuval 1973; Keswick *et al.* 1984; Gerba & Rose 1990; Abbaszadegan *et al.* 2003; Borchardt *et al.* 2003, 2004; Ogorzaly *et al.* 2010). Also, viruses penetrate considerable distances into the soil and into deep wells (Keswick & Gerba 1980) and persist for longer periods in well water than in surface waters incubated at similar temperatures (Yates *et al.* 1985).

Although the efficacy of bacterial indicators has repeatedly been questioned for protection against infection by protozoa (Craun *et al.* 1997; Rose 1997) and enteric viruses (Keswick *et al.* 1984; Yates *et al.* 1985; Abbaszadegan *et al.* 2003; Borchardt *et al.* 2004), there has been a long-standing belief that bacterial indicators provide adequate protection against bacterial enteric pathogens. Recently, however, the adequacy of coliform and *E. coli* monitoring for protection against bacterial pathogens has also been questioned. The

results of a monitoring study of drinking water from private well water supplies in the Netherlands suggested that routine monitoring for total coliforms and *E. coli* using standard membrane filtration methods does not always disclose the presence of pathogenic *E. coli* O157:H7 (Schets *et al.* 2005). This observation could lead to the questionable conclusion that the answer to this dilemma would be to test directly for the pathogen rather than for indicators.

One possible reason for high *E. coli* O157:H7 counts in groundwater in the apparent absence of indicators could be that *E. coli* O157:H7 strains are transported more rapidly through soil into groundwater than most other *E. coli* strains. Recent studies have demonstrated significant variability in cell properties and rates of transport of different strains of *E. coli* in the environment (Yang *et al.* 2004; Bolster *et al.* 2009). Regardless of the reason, *E. coli* O157:H7 should not be present in a 1-L volume of water when indicators are absent.

The results of Schets *et al.* (2005) appear at first glance to support direct pathogen monitoring over indicator monitoring. Although direct pathogen monitoring is now technically possible, it is currently not practical to replace indicator monitoring with pathogen monitoring. This is due to the large number of pathogens known to occur in contaminated drinking water and the cost constraints attached to pathogen monitoring (McKay 2011; Payment & Locas 2011). Some of the methods for pathogens, even bacterial pathogens such as *E. coli* O157:H7, are technically demanding and require analysis of large volumes of water using expensive time-consuming concentration procedures requiring immunomagnetic beads (LeJeune *et al.* 2006). Pathogen monitoring would also require analyses for multiple pathogens, using expensive procedures such as multiplex PCR assays or microarrays. Finally, it is believed that there are still unknown waterborne pathogens (Edberg *et al.* 2000) which obviously cannot be monitored for directly.

Despite these various stability and transport issues, there is some evidence that testing for total coliforms and *E. coli* is effective. Total coliforms have sometimes been epidemiologically associated with waterborne disease outbreaks caused by viruses (Craun *et al.* 1997). Moreover, the results of the two recent studies in Quebec, Canada (Locas *et al.* 2007, 2008) suggested that total coliforms were the best indicator of microbial degradation of water quality and that sampling for total coliforms and *E. coli* remains the best approach to detect contamination of source water by fecal pollution. In both Quebec studies total coliforms were always present at the same time as human enteric viruses. Therefore, indicator monitoring still appears preferable to specific pathogen monitoring.

INCREASING SAMPLE VOLUME

The fact that Schets *et al.* (2005) reported isolating *E. coli* O157:H7 in the absence of bacterial indicators, including total coliforms, is probably because they performed the indicator analyses on the 100 mL water samples but not on the enriched 1 L samples. If they had analyzed the 1 L samples for indicators, total coliforms should have been detected. All *E. coli*, including *E. coli* O157:H7 are coliforms. All coliforms ferment lactose and the analogs of lactose and are detected as total coliforms on media typically used for simultaneous total coliform and *E. coli* analyses. The key metabolic differences between *E. coli* O157:H7 and commensal *E. coli* are the inability of *E. coli* O157:H7 to fully express the β-D-glucuronidase gene and their inability to ferment sorbitol (Wells *et al.* 1983; Ratnam *et al.* 1988). Consequently, the enriched 1 L samples containing *E. coli* O157:H7 would have been positive for total coliforms; although any *E. coli* O157:H7 isolates present would not have appeared to be *E. coli* because they would not have expressed β-D-glucuronidase activity. Schets *et al.* (2005) would likely also have found commensal *E. coli* in their 1 L samples had they looked for them because the pathogenic strains are not likely to outnumber the *E. coli* indicators in the same volume. In domestic sewage the ratio of *E. coli* O157:H7 to fecal coliforms is reported to be 1:1,000 (Blanch *et al.* 2003).

The detection by Schets *et al.* (2005) of *E. coli* O157:H7 in volumes of groundwater as low as 1 L is noteworthy, particularly when fecal indicators were not detected in 100 mL volumes. Estimates of the infectious dose (ID_{50}s) of *E. coli* O157:H7, derived from illness rates observed in foodborne outbreaks, have ranged from 700 colony forming units (CFU) (Tuttle *et al.* 1999) and 31 CFU (Teunis *et al.* 2004) down to as low as 1–10 CFU in an outbreak investigation involving children (Paton & Paton 1998). If we assume the

worst case scenario, an infectious dose of 1 CFU and a Poisson distribution, we calculate approximately a 63% probability of infection in children who consume 1 L of water in a day at an average concentration of 1.0 CFU/L. Other waterborne pathogens, such as *Cryptosporidium* (Dupont *et al.* 1995) and rotaviruses (Ward *et al.* 1986) also have very high probabilities of initiating infections per pathogen. The presence of 1.0 CFU of any pathogen with such a low infectious dose in 1 L would surely present an unacceptable risk.

The results of a recent study (St-Pierre *et al.* 2009) aimed to assess the importance of quantitatively detecting *Campylobacter* spp. in environmental surface water raised some questions concerning the capability of fecal indicator monitoring to identify waters contaminated by *Campylobacter* spp. Overall, 2,471 environmental water samples from rivers and streams in Quebec, Canada were analyzed to determine the prevalence of *Campylobacter* spp., thermotolerant coliforms and *E. coli*. *Campylobacter* spp. were found in 331 of 990 (33%) samples that were negative for thermotolerant coliforms. In addition, five of 53 samples from private wells were positive for *C. jejuni*; however, only two of these samples were positive for thermotolerant coliforms. Again, the sample volumes analyzed for *Campylobacter* were large (up to 2,000 mL in volume) whereas the sample volumes analyzed for the indicators were the standard 100 mL volumes.

Increasing the volume used to test for indicator organisms should significantly decrease the likelihood of finding bacterial pathogens in the absence of indicators. Increasing sample volumes should also reduce the number of sampling events in which viruses or other pathogens are found in the absence of indicators. Mack *et al.* (1972) isolated poliomyelitis virus from 18.9 liter (5 gallon) samples of contaminated well water which contained no coliforms in 100 mL volumes. However, when the five gallon samples were concentrated and analyzed, coliforms, including *E. coli*, were recovered along with the viruses. This observation suggests that the 100 mL volume used to detect indicators was too small.

Further evidence for the value of increasing the sample volumes used for indicators was provided by a comparison of bacterial indicators and sampling programs by Collin *et al.* (1988), who demonstrated that analysis of 300 mL samples instead of 100 mL samples tended to generate better water quality information. They performed assays on 722 water samples using the standard 100 mL volume and duplicate 300 mL assays on the same samples and observed that 84 of the 259 (32%) initially negative samples were positive for coliforms when a 300 mL sample was assayed. Similar results were observed with thermotolerant coliforms; 17% of the negative water samples became positive when a 300 mL volume was analyzed. Hänninen *et al.* (2003) found it necessary to use sample volumes as large as 1,000–2,000 mL to detect fecal indicators in tap water samples after outbreaks of gastroenteritis attributed to *Campylobacter*. The need to use sample volumes that large was probably due to the die-off of the indicators that occurred between the exposures and recognition of a waterborne outbreak and to the fact that contamination was only transient.

INCREASING MONITORING FREQUENCY

Only 22% of public systems that reported outbreaks from 1991 through 1998 had violated US EPA's MCL for total coliforms in the 12-month period before the outbreak. However, coliforms were detected in 73% of these same systems during waterborne outbreak investigations (Craun *et al.* 2002). The fact that coliforms were detected at a greater frequency during outbreak investigations is probably due to the more intensive monitoring that occurs during an outbreak investigation, the infrequent coliform monitoring requirements under the TCR or both. For most of these systems, the TCR had required the collection of only one to three coliform samples each month (Craun *et al.* 2002).

An increase in the frequency of groundwater monitoring is also supported by the results of recent studies undertaken on the virological quality of groundwater in the province of Quebec, Canada (Locas *et al.* 2007, 2008). The results of these studies led the investigators to the conclusion that infrequent analyses for bacterial indicators as well as the use of coliphages as predictors of the presence of human viruses are of limited value whereas frequent monitoring of simple parameters, such as total coliforms and *E. coli*, was the best approach to maximize the probability of detecting water quality changes and the contamination of groundwater.

Grabow (1986) believed that known waterborne outbreaks of viral diseases were always caused by water

which did not conform to conventional bacteriological quality limits, which implies that they could have been prevented if the violations were known early enough. He concluded that quality surveillance should be carried out at the highest possible frequency and the results should be known as soon as possible.

ALTERNATIVE INDICATOR MONITORING METHODS

Use of alternative indicator organisms could also noticeably reduce the frequencies at which pathogens are found in contaminated waters in the assumed absence of fecal indicators. Harwood *et al.* (2005) found that monitoring reclaimed water using a suite of several indicator organisms was more predictive of the presence of enteric viruses and protozoan parasites than monitoring for any single indicator organism. In this study, total coliforms frequently survived the disinfection process; consequently, they tended to be present when pathogens were present. This resulted in a relatively high rate of positive samples in which both total coliforms and pathogens were present. However, samples positive only for total coliforms also resulted in a relatively high rate of samples positive for indicators with no pathogens present. Positive tests for fecal contamination when pathogens are absent (or present below detection limits) are conservative in protecting human health and are unavoidable; however, they are still somewhat undesirable because they represent false alarms. When pathogen-positive and pathogen-negative samples were considered together using the results for all of the indicators, 72% of samples positive for enteric virus, 79% of samples positive for *Giardia*, 75% of samples positive for *Cryptosporidium* oocyst and 71% of samples positive for infectious *Cryptosporidium* were placed in the correct category by discriminate analysis with regard to the presence or absence of the pathogen. In most cases, removal of one variable caused the correct classification rate to decrease by several percentage points. Similarly, Lee *et al.* (2011) found that the positive predictive value of indicators for the presence of norovirus in water was increased by using a combination of chemical, microbial and viral indicators.

Measuring fecal indicators by qPCR rather than by culturing might also reduce the incidences in which infectious viruses are present in the absence of indicators. The results of the most recent EPA epidemiological studies relating swimming-associated gastrointestinal illness to recreational water quality suggest that the qPCR measure may be a truer representation of the risks associated with fecal contamination than cultural methods because it measures all of the enterococci associated with feces, not just the viable cells (Wade *et al.* 2008). The positive qPCR signal persists in the environment longer than the culturable indicators do and is less impacted by processes such as chemical disinfection and possibly solar radiation. Thus, the molecular measurement of *Enterococcus* DNA provides a stable conservative means of quantifying fecal contamination which is not subject to die-off and might also more accurately mirror the dilution and dispersal of feces (Walters *et al.* 2009). The final volumes derived from 100 mL samples and measured by qPCR are miniscule. This could lead to the supposition that qPCR measurements underestimate the numbers or target organisms present. However, indicator measurements by qPCR at beaches actually showed somewhat higher indicator values than those observed in duplicate samples measured by cultural methods (Haugland *et al.* 2005).

REGULAR SAMPLING INTERVALS OR TARGETED SAMPLING?

There have been investigations of waterborne outbreaks in which no coliforms were detected even though large numbers of samples were collected and analyzed. This finding emphasizes the fact that water contamination sufficient to cause an outbreak can be intermittent and short-lived. Thus, the timing of sample collection can be as important as the number of samples collected and selection of appropriate indicators (Craun *et al.* 2002). It is known that groundwater sources are most vulnerable to contamination at specific times, for example, after a snow melt or a large rainstorm (CDC 1999). Targeting sampling times to coincide with those periods during which groundwaters are most vulnerable to contamination would likely lead to the detection of more fecal contamination events than the current monitoring practices of collecting samples at regular but infrequent time intervals throughout the month. This is

most important for karst aquifers, which are particularly vulnerable to contamination (O'Reilly *et al.* 2007; Borchardt *et al.* 2011), and for wells known to be located in an area susceptible to either bovine or human fecal contamination.

A good example of a vulnerable well was demonstrated by the case study of a 16-month-old female child living on an Ontario farm. The child was taken to the hospital suffering from bloody diarrhea caused by *E. coli* O157:H7. She had no known contact with the cattle and did not consume unpasteurized milk. Well water was implicated as the probable source of the pathogen. The *E. coli* O157:H7 isolated from the cattle and the farm water was the same toxin type and phage type as the isolate from the child. Hydrogeological investigation revealed the design and location of the well would allow manure-contaminated surface water to flow into it. Furthermore, the well was shallow, increasing its vulnerability to surface water contamination (Jackson *et al.* 1998). Timely monitoring of this well when it was most vulnerable to contamination could have led to boiling of the water and prevented the child's illness.

The large outbreak of *E. coli* O157:H7 infections that occurred in Alpine, Wyoming in late June of 1998 (Olsen *et al.* 2002) provides another good example of a situation in which targeted sampling at high risk times might have identified vulnerability to fecal contamination and provided an early warning that the source water was compromised. More than 150 cases of acute gastrointestinal illness were identified in this outbreak. These illnesses were significantly associated with drinking municipal water. The un-chlorinated water supply had evidence of fecal organisms as well as the potential for chronic contamination with surface water. Although Alpine was in compliance with the TCR, which requires one negative total coliform result each month for a community of its size, there were several positive readings in April 1998 (1/5 positive), May 1998 (4/7 positive) and June 1998 (2/10 positive). Inspections after the outbreak revealed that the spring supplying Alpine's drinking water was under the influence of surface water. A large pool of water was found in the area over the collection pipes, probably the result of a late snow melt combined with heavy rains and groundwater outfalls. Numerous deer and elk feces were present in the area. Water taken from the storage tank on July 14 contained 108 CFU/100 mL total coliforms. *Enterococcus faecium* was isolated from the same sample, further indicating the presence of fecal contamination; however, *E. coli* O157:H7 was not isolated from that sample. More intensive sampling during the time period in which the system was most vulnerable to contamination could have revealed the fecal contamination and alerted the authorities to either chlorinate or issue a boil water order before the outbreak occurred.

The occurrence of snowmelts and heavy rainfalls in the spring suggests that it would be appropriate to monitor for fecal contamination more frequently during that period. Those communities in which the source waters are susceptible to contamination by cattle have even greater incentive for more intensive monitoring in the spring because longitudinal studies have shown that the monthly prevalence of *E. coli* O157 in cattle is greatest in spring and late summer (Hancock *et al.* 1997; Chapman *et al.* 1997; Mechie *et al.* 1997). Occurrence of *Cryptosporidium* is also seasonal, corresponding with the calving season (Atwill *et al.* 1999).

Targeting sampling toward the most vulnerable times or sites would require different interpretations of the data than are required by the sampling plan incorporated into the current regulations.

DISCUSSION

Although monitoring for fecal contamination can never be completely protective against the occurrence of waterborne disease outbreaks, improvements in the way we monitor could help to identify more groundwater systems that are vulnerable to contamination and potentially reduce the number of outbreaks. The fact that only 22% of public systems reporting outbreaks from 1991 to 1998 had violated the MCL for total coliforms (Craun *et al.* 2002) implies that systems subject to intrusions by fecal contamination are frequently not identified by our current procedures.

There is a need for further research to determine the significance of recoveries of viruses and parasites from water in the absence of indicators. The volumes from which viruses have been recovered are quite variable, ranging from 18.9 L (5 gallons) (Mack *et al.* 1972) to 1,512 L (400 gallons) (Abbaszadegan *et al.* 1999). Recovery of viruses from volumes as small as 18.9 L (5 gallons) is almost certainly

meaningful; however, recovery of viruses from volumes as large as 1,512 L may or may not have public health significance particularly if viruses are detected by PCR, which detects inactivated as well as infective viruses (de Roda Husman *et al.* 2009).

The detection of a bacterial pathogen, such as *E. coli* O157:H7, in a 1 L volume when *E. coli* indicators were absent in 100 mL volumes of the same water would have been considered very unlikely before the discovery that some herds of cattle include 'super-shedders' of *E. coli* O157:H7 (Chase-Topping *et al.* 2007, 2008). The discovery of these super-shedders introduces the possibility that numbers of pathogenic *E. coli* O157:H7 in water contaminated by cattle could occasionally be nearly as high as the numbers of commensal *E. coli*. Most bovine fecal samples positive for *E. coli* O157:H7 contain fewer than 10^2 *E. coli* O157:H7 CFU/g of feces (Chase-Topping *et al.* 2007); these numbers are far fewer than the 10^5 to $>10^7$ CFU/g of commensal *E. coli* characteristically found in bovine feces (Sinton *et al.* 2007). However, feces of super-shedders were observed in various studies to contain high levels of *E. coli* O157:H7 ranging from 10^4–10^5 CFU/g (Zhao *et al.* 1995) to 10^4–10^6 CFU/g (Gansheroff & O'Brien 2000; Omisakin *et al.* 2003) and in one study up to 10^7 CFU/g (Chase-Topping *et al.* 2007). Omisakin *et al.* (2003) reported that feces of 2% of the cattle in the herd they studied contained from 10^5 to 10^6 CFU *E. coli* O157:H7/g feces. If the super-shedders, comprising 2% of the cattle, shed the maximum numbers of the pathogen (10^6–10^7 CFU/g) while the other 98% of the cattle shed the minimum numbers (10^5 CFU/g) of commensal *E. coli*, numbers of *E. coli* O157:H7 in the total fecal content of a herd could be within the same order of magnitude as the commensal *E. coli*. However, this is only feasible if source waters are contaminated by herds containing super shedders which can shed from 10^4 up to 10^7 CFU *E. coli* O157:H7/g feces in the most extreme cases (Zhao *et al.* 1995; Gansheroff & O'Brien 2000; Omisakin *et al.* 2003; Chase-Topping *et al.* 2007).

If we assume the distribution of bovine herds shedding *E. coli* O157:H7 in the US is similar to that observed in Scotland, only 18.9% of farms would even include shedders of *E. coli* O157:H7 and only 2.7% of all farms would be expected to include supershedders (Chase-Topping *et al.* 2007). This expected frequency of occurrence of farms containing supershedders is not sufficient to require all groundwater systems to routinely monitor for *E. coli* O157:H7 in place of indicators. Such routine monitoring for *E. coli* O157:H7 in water (Schets *et al.* 2005) would require cultural enrichment followed by immunomagnetic separation and would be both time-consuming and costly compared to the methods typically used to detect indicators. In place of routine pathogen monitoring it would be more practical to use a more risk-based procedure, including incorporation of a sanitary survey, in developing sampling plans for systems in which the risk of *E. coli* O157:H7 is highest.

The problem of detecting *E. coli* O157:H7 in the absence of indicators could be resolved by using 300–500 mL volumes or possibly the same 1 L volumes to detect the indicators and using one of the newer indicator methods that detect total coliforms and *E. coli* simultaneously, for example Colilert (Edberg *et al.* 1998) or MI agar (Brenner *et al.* 1993). All samples containing *E. coli* O157:H7 would be total coliform-positive by these methods, based on the ability of *E. coli* O157:H7 to ferment lactose (Wells *et al.* 1983; Ratnam *et al.* 1988). It is likely that larger samples containing *E. coli* O157:H7 would also be positive for the commensal *E. coli*, which should outnumber the pathogens even in waters contaminated by bovine herds that include supershedders. *E. coli* O157:H7 have essentially the same stability in the environment as the non-pathogenic strains (Wang & Doyle 1998; Rice & Johnson 2000).

This hypothesis could be verified by performing a field study at one or several sites where groundwater or surface water is subject to contamination by bovine herds that include supershedders. Results of sanitary surveys and hydrogeological assessments should be used to select aquifers that are particularly sensitive to contamination. If such a study were to be performed, it would also offer an opportunity to examine the effectiveness of increasing sample volumes and sampling frequency and scheduling more sampling events during periods when there is more runoff from snow melts or heavy rain and more shedding of *E. coli* O157:H7. The study should be designed to allow a direct comparison between the current sampling plan prescribed in the TCR (US EPA 1989) and modified sampling plans. The two times within one year sampling strategy established in the unregulated contaminant monitoring rule

(UCMR) (US EPA 1999) would not answer the most critical questions regarding the efficacy of indicator organisms to protect against *E. coli* O157:H7. In addition, the sampling strategy developed for UCMR monitoring for *Aeromonas* spp., which consisted of taking a small number of samples from a large number of sites, would not provide the answer to the issue of whether *E. coli* O157:H7 is ever present in the absence of bacterial indicators. Such samples would be consistently negative because few source waters would contain *E. coli* O157:H7. The only practical strategy would be to choose sites known to be vulnerable to contamination by super shedders and sample those sites intensively.

There are similar issues involved in determining the sample size and frequency of sampling necessary to increase the probability that indicators will be detectable whenever viruses or protozoa are present. One difficulty is that we do not really know the proper sample volume to use for these pathogens. Volumes commonly used for viruses and for *Cryptosporidium* are 1,520 L (400 gallons) and 10 L (2.64 gallons), respectively; however, the public health implications of finding one virus or one oocyst in these volumes are unknown. Another complication is the difficulty in determining whether or not viruses, or any other pathogens, are still infective when they are found in groundwater by PCR. Borchardt *et al.* (2003) observed that a sample positive for poliovirus by reverse transcription-PCR (RT-PCR) was negative by cell culture, suggesting that the viruses detected were not infectious at the time of sampling. The fact that we do not know the meaningful sample sizes for viruses, protozoa or pathogenic bacteria is yet another complication.

Although increasing sample volumes and sampling frequency and use of multiple indicators would likely improve our ability to identify groundwater systems that are susceptible to fecal contamination, none of these improvements will allow groundwater systems to rely exclusively on monitoring to prevent waterborne illness. It is important to supplement monitoring with other measures, such as sanitary surveys and hydrogeological analyses. It is also important to recognize that any increases in sample volume or sample frequency or use of multiple indicators will be accompanied by an increase in monitoring costs. A mere doubling of the sample volume to 200 mL or doubling the number of samples could as much as double the cost of monitoring depending on whether membrane filtration or most probable number methods are used. Nevertheless, these increases would still be much less costly than direct pathogen monitoring using the currently available technologies. There is some risk involved in changing to a more conservative indicator monitoring system, i.e. more positive tests for fecal indicators when no pathogens are detected. This could cause more systems to be out of compliance when there is no risk to the public. Additional research is needed to establish a more meaningful relationship between the levels of indicators present and the risk that pathogens will also be present. If investigators in future studies of the occurrence of pathogens relative to indicators in water would test larger volumes for indicators, investigate panels of indicators or use of qPCR for indicator analyses, they should eventually be able to identify a monitoring system that produces better correlations than are found by the traditional tests for coliforms using 100 mL volumes. A cooperative effort involving academic scientists, public utilities and local, state and federal agencies should be undertaken. A federal agency such as the US EPA could serve as the study coordinator and as a repository for the data.

SUMMARY AND CONCLUSIONS

Edberg *et al.* (2000) pointed out that one of the central questions of public health protection is: should one monitor the safety of drinking water for pathogens or indicators? They concluded that the answer in the year 2000 was the same as it had been in 1900: 'Monitoring for indicators better protects human health than monitoring for specific pathogens.' This conclusion is as valid in 2012 as it was in 2000. Monitoring for individual pathogens is too difficult because we do not know which of the many waterborne fecal pathogens will be present at any given time or place and it would be far too expensive to test or all of them. Furthermore, the methods used to measure pathogens are often difficult and costly to perform. Indicator monitoring could be made more effective by increasing sample volumes and/or sampling frequency and sampling at times when the source waters are most vulnerable to incursions by fecal contamination. Use of a suite of indicators or of qPCR instead of culture methods could also potentially make monitoring more effective. It should be kept in mind that monitoring alone is not sufficiently protective. Periodic

sanitary surveys and hydrogeologic assessments are also critical in identifying and eliminating sources of pollution.

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