Assessment of Torque Teno Virus as a Viral Pathogen Indicator in Waters [Project #4288]

ORDER NUMBER: 4288

DATE AVAILABLE: July 2013

PRINCIPAL INVESTIGATORS:
Jeanine D. Plummer and Sharon C. Long

For the past century, public health officials have relied on fecal coliforms to identify and track bacterial contamination. However, several issues arise when using coliforms as indicators for viral pathogen risk. First, there is a lack of association between fecal coliforms and human enteric viruses in the environment. Second, there are differences in the fate of coliforms compared to viral pathogens in surface and groundwater systems. Lastly, the relative resistance of coliforms compared to viral pathogens through engineered treatment processes is not comparable for all viruses and all treatment options.

Despite the potential public health implications, a suitable viral pathogen indicator has yet to be established. Recent research in the drinking water community has focused on modifying coliform monitoring methods or assessing bacteriophages as indicators. There is less current research to develop new, reliable indicator systems for viral pathogen risk. The proposed viral pathogen targets, norovirus and adenovirus, have demonstrated seasonal variations in prevalence. Considering analyst safety, indicator systems remain the best means of assessing pathogen risk. With advances in molecular techniques applied to environmental monitoring, improvements in indicator systems are timely. Therefore, the goal of this research was to examine the value of a potential new tool for assessing viral pathogen risk, specifically Torque teno virus (TTV).

OBJECTIVES

The objective of this research was to determine the value of TTV as an indicator for viral pathogen risk. First, a reliable TTV assay was developed. Then, the occurrence of TTV in fecal sources, wastewaters, and drinking waters was determined. Lastly, these data were compared to those for coliforms, coliphages, and human adenovirus.

BACKGROUND

Despite their wide use and well-developed methods, use of coliform and *E. coli* indicators are not always 100% protective of public health. Outbreaks have occurred in systems for which coliform monitoring indicated the absence of the indicator, and no discernible public health outcomes have resulted from monitoring in violation of the
Total Coliform Rule (TCR) (D’Antonio et al., 1985; Craun et al., 1997; Hrudey and Hrudey, 2004). In a matched triple analysis, Nwachuku et al. (2002) demonstrated that TCR maximum contaminant limit violations did not differ for outbreak and non-outbreak water supplies.

While bacterial indicators are used in regulatory compliance, bacteria and viruses have many differences, and often epidemiological studies fail to show a relationship between viral pathogens and microbial indicators in both environmental systems and treatment processes (Ashbolt et al., 2001). Coliform bacteria are insufficient indicators of viral pathogens because these microorganisms cannot adequately estimate viral pathogen risk in the environment (Skraber et al., 2004). Thermotolerant and total coliforms tend to be poor indicator microorganisms because of their short survival and susceptibility to water treatments (Moe, 2004). Also, pathogenic viruses have been found in waters where the number of coliforms had not exceeded water quality standards (Jiang et al., 2001). Moreover, coliform indicators are not specific to feces and have the ability to grow in natural waters (Ashbolt et al., 2001; Fong and Lipp, 2005; Toranzos et al., 2007).

Viruses that infect coliform bacteria, coliphages, have been investigated as possible viral indicator organisms since as early as the 1980s (Osawa et al., 1981; Furuse, 1987). Recently, coliphages were included as a potential indicator for viral pathogens for use in groundwater systems based on similarities in environmental survival (Donnison and Ross, 1995; Long and Sobsey, 2004; U.S. EPA, 2006). However, they may continue to replicate in surviving bacterial hosts after being shed in feces. Therefore, their quantities and persistence in environmental waters may significantly exceed the quantities of human viruses and incorrectly suggest contamination of water sources (Nasser and Oman, 1999; Pang et al., 2004). Second, survival rates of coliphages vary with temperature and between strains, and thus with season (Chung and Sobsey, 1993; Long and Sobsey, 2004), and enumeration of both male-specific and somatic coliphages may be necessary to fully represent enteroviruses and other human pathogenic viruses (U.S. EPA, 2001a). For example, the somatic phage PRD1 may be most representative of adenovirus and other enveloped virus behavior, while male-specific coliphages such as MS2 may be most representative of other infectious enteroviruses such as hepatitis A virus (Nasser et al., 1993; San Martin et al., 2001). Lastly, it has been demonstrated that while sewage is always positive for coliphages, only a small percentage of individual human or animal feces test positive for coliphages (Long et al., 2002; Long et al., 2005). Therefore, an alternative viral indicator with greater prevalence among individual humans and other zoonotic disease-carrying animals would be a more valuable indicator than coliphages in certain situations.

Based on the shortcomings of coliform bacteria and coliphages as indicators, a viral indicator could be hypothesized to be more appropriate to indicate the health risks of infectious viruses. TTV is a small, unenveloped single stranded DNA (ssDNA) virus first described in 1997. TTV occurs worldwide in humans, occurs in different serotypes between non-human animals and humans, does not appear to be associated with any significant morbidity, and appears to exhibit transport characteristics that are similar to pathogenic enteric viruses (Nishizawa et al., 1997; Abe et al., 1999; Bendinelli et al., 2001). TTV has been associated with various bird species (e.g. chickens, geese, and pigeons), cows, pigs, dogs, cats, and non-human primates as well as humans (Biagini, 2004; Hino and Miyata, 2007). In addition, animal-derived sequences of TTV are not identical to human-derived sequences. It has also been detected through the wastewater stream (Haramoto et al., 2005b). In particular, Haramoto et al. (2005b) found a positive
TTV signal in 97% of wastewater influent samples over a 1-year period. Secondary and final wastewater effluent were positive for TTV 18% and 24% of the time, respectively. The authors note that DNA of chlorine inactivated TTV may have been detected in some final effluent samples. Similar data for drinking water treatment plants are lacking in the literature. Based on current knowledge, TTV fulfills a number of criteria described for ideal viral indicator organisms more fully than coliforms, E. coli, or coliphages (Yates, 2007). In addition, TTV may provide more specificity, geographic stability, and/or temporal stability than direct pathogen monitoring for viruses such as norovirus or adenovirus. Therefore, this research was aimed at assessing the utility of TTV as a potential viral pathogen indicator.

**APPROACH**

The two primary methods in practice today to track viral contamination are cell culture and polymerase chain reaction (PCR). PCR is rapid, sensitive, specific, cost-effective, and simple to perform. Because PCR only indicates the presence/absence of a target sequence, it would yield a positive result for a noninfectious viral particle if the particle’s genetic material was intact (Scott et al., 2002; Griffin et al., 2003; Fong and Lipp, 2005). The presence of viral nucleic acid at a sample site nevertheless indicates that contamination occurred in the recent past and suggests that the site is susceptible to future contamination (Fong et al., 2005). The rapid nature of PCR makes it an ideal tool for periodic monitoring and maintenance of water sources. Therefore, a PCR-based TTV detection assay was developed for use throughout the research project. The assay included the following steps:

1. Initial hollow fiber ultrafiltration (HFUF) concentration (for dilute samples, such as drinking waters);
2. Polyethylene glycol (PEG) precipitation (for HFUF concentrates and concentrated samples, such as wastewaters);
3. Bead-beating extraction (PEG concentrates and solid samples);
4. Kit processing (MoBio PowerSoil DNA Isolation kit) for nucleic acid purification;
5. Amplification of target ssDNA using traditional PCR and touchdown PCR; and
6. Detection of target 63 bp amplicon using gel electrophoresis.

In this project, the occurrence of TTV in animal feces, wastewater, and drinking waters was evaluated by collecting and analyzing samples from four different regions in the United States over a 24 month period. A total of 75 fecal samples (avian, dog, equine, rabbit, and ruminant), 25 wastewater samples (raw, primary, and treated), and 72 drinking water samples (raw, treated, and distribution system) were collected and analyzed. Analyses included turbidity, pH, total and dissolved organic carbon, total coliforms, E. coli, male-specific coliphages, and somatic coliphages using standard methodologies. Samples were diluted or concentrated as appropriate for indicator organism enumerations. Appropriate positive and negative controls were run for all tests. All samples were also analyzed for the presence of TTV. All positive TTV samples and a selected number of negative TTV samples were analyzed for the presence of human adenovirus using a pan-human adenovirus qPCR assay. The data from the field sampling
program was statistically analyzed to determine correlations among and variations between the water quality parameters and indicators.

RESULTS/CONCLUSIONS

The goal of this research was to evaluate a potential new viral indicator, Torque Teno virus, and compare the occurrence of TTV to the occurrence of bacterial indicators, bacteriophages, and human adenovirus. In fecal samples, coliforms and E. coli were found at concentrations ranging from hundreds of most probable number (MPN) per gram in horses to $10^8$ MPN per gram in chickens, as would be expected in healthy animals. Coliphages were detected in approximately half of the fecal samples, which is consistent with prior literature. For the wastewater samples, bacterial indicators (total coliforms and E. coli) were up to $10^8$ MPN per 100 mL in raw wastewater, but decreased by 2 – 3 orders of magnitude through treatment, with levels as low as the hundreds per 100 mL in final wastewater prior to disinfection. For coliphages, raw wastewater had up to $10^5$ plaque forming units (pfu) per 100 mL, but male specific coliphages were below detection limits in 3 of 12 final wastewaters (prior to disinfection). Drinking water samples had significantly lower indicator levels. No groundwater samples (untreated and treated) had detectable levels of coliforms or E. coli. Coliphages were 1.0 pfu/100 mL or less in all groundwater samples. For surface waters, bacterial indicators ranged from 0.10 to 1600 MPN/100 mL in untreated samples and 0.10 to 260 MPN/100 mL in treated samples. It is important to note that treated samples included partially treated samples, thus some were filtered but not yet disinfected. With one exception, all distribution system samples were negative for total coliforms and E. coli, 80% of distribution systems samples were below detection limits for male-specific coliphages, and 80% below detection limits for somatic coliphages. In general, bacterial indicators and coliphages were reduced through treatment of surface waters, all of which underwent filtration and disinfection.

TTV occurrence in fecal samples was rare — three of 75 samples were positive for TTV. This included two domesticated companion dogs and one chicken, and all positive samples were collected in the winter. While these data appear to show agricultural and companion animal carriage rates for TTV on the order of a few percent, the sample size is too small to draw definitive conclusions. For wastewater samples, five of 13 raw/primary samples were positive for TTV and 5 of 12 final effluent (prior to disinfection) samples were positive for TTV. TTV positive and negative samples were distributed among the four sampling regions. Considering season, all raw and treated wastewaters were negative for TTV in summer. In winter, however, 88% of wastewater samples were positive considering all locations. In spring, approximately a third of samples were positive for TTV. While the literature indicates that human serum samples are positive for TTV year round, the results from this research indicate that additional data should be collected to determine if environmental survival may be affected by seasons. In drinking waters, 10 of 72 samples were positive for TTV. The samples that were positive included 1 untreated groundwater and 9 treated/distribution samples (3 groundwaters and 6 surface waters).

The presence of human adenovirus in field samples was determined for a selected number of fecal, wastewater, and drinking water samples. All samples that tested positive for TTV and a selected number of TTV negative samples were included for human adenovirus analysis. Each nucleic acid extract was analyzed in duplicate. For fecal samples, all 75 were tested and were negative for human adenovirus. Both TTV and
adenovirus had a low number of positive results in fecal samples. In wastewaters, all raw sewage samples (10 out of 10) and all primary settled samples (2 out of 2) tested positive for human adenovirus. In addition, 67% of treated wastewaters (8 out of 12) still contained detectable adenovirus. However, the treated wastewaters were all collected prior to disinfection. For the 44 drinking water samples tested for human adenovirus, all were negative.

The quantitative data sets were statistically analyzed using Pearson and Spearman functions to determine correlations. For all data sets, results that were below detection limits were set to one half of the detection limit for analysis. Considering fecal samples, each indicator was correlated to each other indicator; however, the concentrations of somatic coliphages tended to be greater than the concentrations of male-specific coliphages. In the wastewater and drinking water samples, somatic coliphages were correlated to bacterial indicators as well as other water quality parameters. Using analysis of variance, somatic coliphages varied by animal group — demonstrating the potential for this indicator to be used in different seasons and regions. In wastewaters and drinking waters, differences in indicator concentrations were found based on treatment status, as would be expected. Logistic regression was used to evaluate the predictive value of the water quality parameters, or explanatory variables, for predicting the presence of adenovirus. The results indicated that TTV presence at a 95% confidence level (Sig. = 0.05) did not have a significant predictive value for adenovirus (0.30>0.05), and that E. coli had the highest predictive value.

APPLICATIONS/RECOMMENDATIONS

The ultimate goal of the drinking water profession is to provide risk-free drinking water to consumers. A substantial threat is the potential for transmission of waterborne diseases. Recent regulations recognize that viral and protozoan pathogens do not always behave similarly to bacterial indicators. Thus, current monitoring regimes may not be ideal for protecting public health from viral pathogens. This research was designed to determine the utility of TTV as an appropriate viral indicator. However, results demonstrate a small carriage rate among non-human animals, and a high rate of non-detects among drinking water samples. In addition, there were a higher percentage of wastewater samples positive for TTV in the winter than in other seasons. Prior research has indicated TTV does not show seasonal fluctuations and thus additional data are needed to explore this observation. Results also demonstrate that direct pathogen monitoring for human adenovirus may provide useful data, and thus this pathogen could be considered for direct pathogen monitoring based on both study findings and prior literature.