Detection and Monitoring of Microbiological Contaminants

Protecting groundwater from microbial contamination is a top public health priority. The United States and Canada experience significant levels of gastrointestinal disease from drinking water, more than 70 percent of which is associated with contaminated well water. The GCC has solicited research projects that attempt to improve understanding of microbiological aspects of groundwater contamination.

Bacteria

Several projects focused on developing new techniques for detecting, quantifying, and monitoring microorganisms in groundwater and soils. Researchers at the UW-Madison Soil Science Department developed a rapid molecular method using the polymerase chain reaction (PCR) to assay soils for the presence of specific sewage-borne pathogens (Hickey 1998). PCR-based methods eliminate the need to culture organisms for detection, and remedy shortcomings of traditional techniques by allowing rapid, sensitive, and specific identification of the pathogens of concern rather than indicator organisms. The PCR protocol Hickey developed was designed to detect DNA originating from E. coli, which is one of the major species of bacteria associated with human waste. This method is capable of distinguishing E. coli DNA from that of its closest relative, Shigella and detecting the DNA equivalent to about 20 cells.

Because they have the capacity to co-metabolize a wide variety of organic chemicals, including halogenated compounds, methanotrophic bacteria have significant potential for bioremediation. The UW- Milwaukee Department of Biological Sciences developed methods for quantification of methanotrophs in groundwater (Collins 1998, 2000). These methods, that include competitive PCR and direct PCR, provide approaches to monitoring bioremediation and natural attenuation. In addition, this work provided the basis of another study that applied direct PCR to the detection of pathogens in groundwater (Collins 2002).

A study by the Wisconsin State Laboratory of Hygiene (WSLH) investigated storage and handling requirements for water samples submitted for coliform and E. coli analysis (Degnan and others, 2003). As of 2015, the US EPA has no guidelines for sample holding times and shipping temperatures for drinking water samples submitted for E. coli testing. The study provided evidence to expand the allowable storage time of water samples submitted for E. coli analysis beyond the current eight hour limit as well as supporting a single preservation protocol for both surface waters and drinking water samples. A change to a maximum holding time of chilled samples for up to 30 hours could easily be supported by the data presented in this study. The data also called into question the current practice of allowing up to 48 hours for submitting drinking water samples with no attempt to cool them. A reduction in the time period to 30 hours, or a requirement to ship the samples at less than 10 degrees C, could be supported by the data.

Another WSLH study developed a culture method for detecting Helicobacter pylori from a heterogeneous microbial population in water, and then use this method to establish a data base for its occurrence in Wisconsin groundwater (Degnan and others 2003). Prior to this study, there were no reliable methods for detecting viable H. pylori in environmental samples (water, manure, vegetables, etc.). H. pylori is recognized by the World Health Organization to be the primary cause of peptic ulcers, chronic gastritis and stomach cancer. About 50% of the U.S. population is thought to be symptomatic or asymptomatic carriers, even though the source of human infection is not well understood. The efforts of this study resulted in the development of a high quality plating media for selecting viable H. pylori from mixed microbial populations. Samples from over 400 private wells were H.

pylori-absent, including wells used by infected residents. These results suggest that the route of H. pylori to humans in Wisconsin probably does not involve private well water.

WSLH researchers in the Water Microbiology Unit completed testing of a hollow fiber ultrafiltration method for concentrating low levels of microorganisms from large volumes (up to 100 L) of drinking water. Acceptable levels of organism recoveries were demonstrated for bacteria (E. coli and enterococci), viruses (MS2 coliphage) and parasites (Cryptosporidium and Giardia). Quantitative recoveries were recorded for concentrations as low as 0.3 organisms per 100 ml. Establishing testing with lower detection limits for pathogens and indicators adds an additional margin of safety in the protection of public health from waterborne diseases.

A study conducted at the WSLH (Long, 2009), and funded by the DNR, developed a Real-Time PCR assay for the molecular detection of Rhodococcus coprophilus. Detection of Rhodococcus coprophilus is an indicator of fecal pollution from grazing animals. This data is useful as part of the WSLH's "toolbox" of microbial source tracking methods to determine the source of fecal contamination of groundwater.

Other assays performed as part of the microbial source tracking (MST) toolbox are; genotyping of male- specific coliphages, detection of sorbitol-fermenting Bifidobacteria and detection of Bacteroides using different primer and probe sets to distinguish between human and animal sources of fecal pollution. As of 2015 there have been 49 groundwater samples collected for analysis. One sample was from a drain tile and the others were from 40 different private wells (with 8 wells sampled twice). Results indicate 28 of the 49 samples were positive for contamination from grazing animals, 3 samples tested positive for bacteria associated with human waste, 10 samples tested positive for recent but inconclusive fecal contamination, and 9 samples tested clean. The use of these analyses has proven valuable to DNR in granting Well Compensation awards for replacement wells for wells contaminated with livestock waste (manure).

A powerful microbial source tracking tool was developed by Sibley et.al that enables scientists to detect bovine adenovirus. This assay determines environmental fecal contamination from those animals. These viruses were detected from both stool and urine (Pedersen, 2008).

A UW Water Resources Institute project examined the strengths and weaknesses of 10 enzyme-based tests approved by the U.S. EPA for detecting total coliform and E. coli in drinking water (Olstadt and others, 2007). The results suggest these tests differ significantly in their ability to detect/enumerate total coliforms and E. coli and to suppress false positive results from Aeromonas ssp., a non-coliform organism. The most significant of these findings was the inability of some test method/sample matrix combinations to even detect E. coli in high concentrations.

The release of antibiotics into our water resources is driving efforts to characterize the occurrence, fate, and transport of resistant bacteria in the environment. In a WRI-sponsored project, onsite-wastewater treatment systems were evaluated as a potential source of genes that encode antibiotic resistance in bacteria (McMahon, 2006). The concentrations of resistance genes in the septic tanks were several orders of magnitude higher than those observed in treated municipal wastewater effluent. The investigators hypothesize that past agricultural activity may have contributed to the presence of resistance genes in subsurface bacteria, but long term sampling with higher spatial resolution is required to adequately confirm the hypothesis.

Methods for the detection of the presence of toxigenic E. coli in a water sample require

several days to complete (USEPA, 2010). In the case of an emergency outbreak, the source of infection remaining unproven for this amount of time may result in continued exposure, increased incidence of infection and possibly deaths to vulnerable populations. Research is underway at the WSLH for development and optimization of a quantitative polymerase chain reaction method to detect generic E. coli and toxigenic E. coli for recreational water as well as drinking water. This method will be challenged by experimentation with effects of PCR inhibitors commonly found in drinking and surface water, effects of competing organisms and performance on a wide range of groundwater samples with a variety of water chemistries. This development will increase the already broad array of testing offered by the WSLH and assist with expediency in a potential outbreak situation involving toxigenic E. coli.

The WSLH had taken part in a USEPA/CSC study involving the validation of a culture method for Vibrio cholera as a tool in an emergency response situation. The method consists of a mock hurricane "occurrence" resulting in multiple deaths and illnesses. The Water Microbiology unit was tasked with analyzing water for Vibrio cholerae from targeted sites from hurricane affected areas. The lab was able to use the V. cholerae culture method to detect V. cholerae from the spiked samples and effectively report our data to the EPA/CSC.

The WSLH is taking part in a validation study overseen by USEPA/CSC evaluating a method to characterize "Human Fecal Pollution in Water by TaqMan Quantitative Polymerase Chain Reaction (qPCR)." The validation entails evaluation of multiple primer/probe sets for human-associated Bacteroides in a multiplex reaction incorporating an internal amplification control. The purpose of this study is to standardize a method for laboratories to detect the presence of human-associated Bacteroides in an effort to source track human fecal contamination of groundwater and surface/recreational water.

Viruses

The Marshfield Clinic Research Foundation has investigated the association of pathogenic viruses and bacteria in private wells with incidences of infectious diarrhea and indicators of well water contamination (Borchardt 1998, 2000). In general, infectious diarrhea was not associated with drinking from private wells, nor was it associated with drinking from wells positive for total coliform. However, wells positive for enterococci were associated with children having diarrhea of unknown etiology, which was likely caused by Norwalk-like viruses. Final results indicate that the incidence of virus contamination in private wells may affect 4-12% of private wells. Of concern to drinking water regulators is the seasonal variability of the virus occurrences and lack of correspondence between viral presence and common microbial indicators.

In another study with the US Geological Survey, Marshfield researchers found that 50% of water samples collected from four La Crosse municipal wells were positive for enteric viruses, including enteroviruses, rotavirus, hepatitis A virus, and Norwalk-like virus (Hunt, 2003; Borchardt, 2004). As with the private well study, there was no correspondence to common indicators of sanitary quality. More surprising, there was no relationship between presence of surface water in the well water samples as determined by isotope analysis and virus occurrence. Recent work between Marshfield Clinic and USGS targeted the source and transport of viruses to drinking water wells. This work was funded by the DNR and USGS, and involved field investigation using physical measurements, wastewater tracers, and virus analyses. Water sampling screening in 14 Wisconsin communities again documented virus occurrence in wells without surface water sources, and a second sanitary sewer source was supported by wastewater tracer presence. Using more

intensive characterization at one municipal well in three Wisconsin communities, the relation between high wastewater tracer and virus occurrence was documented, and also demonstrated sufficiently short travel times such that viruses would be expected to remain infectious even in a 400 foot deep municipal well. Given the wide extent and age of infrastructure, these findings suggest that viruses may be more common than previously expected in Wisconsin drinking water. Work by Marshfield Clinic has begun to evaluate whether the viruses are inactivated through disinfection processes, or result in illness in the community. This type of research into the link between virus occurrence and human health will provide the overall context to this extensive Wisconsin research topic.

Viruses have also been found in deep bedrock wells that were thought to be protected by low permeability confining units. Studies funded by AWWARF and DNR examined virus occurrence in three deep (>400 feet) confined bedrock wells serving Madison. The surprising result was that infectious viruses were repeatedly present in two of three wells sampled. Examination of potential virus sources and pathways was inconclusive, but sampling results suggest that the deep groundwater is more vulnerable to virus contamination than previously thought (Borchardt, 2007). A follow-up study (Bradbury and others, 2010) funded through the Wisconsin Joint Solicitation found viruses in each of seven deep wells sampled over a period of two years, with many samples positive for infectivity. Correlation between viral serotypes found in sewage, lakes, and groundwater suggests very rapid transport, on the order of weeks, from the source(s) to wells. If such rapid transport exists, then deeply-cased municipal wells may be much more vulnerable to shallow contamination than previously assumed. One outcome of the initial study was the use of increased disinfection by the Madison Water Utility in order to assure public health.

A combined microbial and chemical target toolbox is being tested, validated and applied at WSLH to conduct microbial source tracking. The toolbox uses microbial and chemical tracers that are specific or unique to waste sources to determine sources of contamination and allows for a weight-of-evidence approach for identifying sources of contamination. Methodology discriminates between human sewage-related sources and animal fecal contamination and can identify grazing animal contamination. This suite of tests has been applied to contamination events in Dodge and Door Counties, among others. In one instance, an improperly installed septic system was the culprit. In another instance, farm field manure runoff during heavy rains was identified. By identifying the source of microbial contamination, remediation or correctional actions can be targeted and the spending limited funds on "false sources" can be avoided. Research to improve on the methods in this toolbox is being funded by the DNR and UWS.

After several years of development and validation, researchers at the Marshfield Clinic Research Foundation now possess the capacity for high-throughput testing of waterborne viruses. Virus tests include six common human enteric virus groups and six common bovine viruses. The number of tests that used to take three months to complete can now be accomplished in an afternoon. Recently, these researchers completed a study involving more than 20,000 virus analyses of the groundwater supplying drinking water in 14 Wisconsin communities. This level of laboratory capacity relies on three major advances:

1. Inexpensive and effective concentration of waterborne viruses using glass wool filtration, a method developed and fully validated at Marshfield Clinic (Lambertini, 2008);
2. Virus detection by real- time quantitative polymerase chain reaction (qPCR) using recently developed high-throughput platforms and highly specific fluorescent probes; and
3. Development at Marshfield Clinic of a unique Laboratory Information Management System (LIMS) for quality assurance, quality control, and data management of analyses for waterborne pathogens. Contingent on several more advances, the researchers believe

it will be possible to screen a water sample for all common waterborne pathogens using an approach that is inexpensive, efficient, and reliable.

The sole use of bacterial fecal markers is not adequately protective of human health or indicative of the presence of other microorganisms, including viruses. Therefore, the fecal source tracking toolbox available to WSLH has been expanded to with the conception and optimization of novel species-specific PCR assays for distinguishing human from bovine adenoviruses in groundwater samples (Pedersen, 2008, 2010 and 2011). These viruses are widespread in human and bovine populations, and have already proven useful for indicating the presence and source of wastes in groundwater. Because the environmental fate and transport behaviors and prevalence of enteric viruses can differ, we are evaluating additional species-specific virus targets, polyomaviruses and Torque Teno Viruses. The additional of these viral targets will provide the WSLH with unique source tracking capacity and with a robust set of makers for describing the presence of fecal contamination. The interrogation of samples for multiple viral and bacterial targets is especially important for situations where contamination is suspected in private wells.

Protozoans

The WSLH Flow Cytometry unit completed the final phase of a multi-year project to develop and round-robin validate methods for genotyping Cryptosporidium from microscope slides. This method will aid water utilities, states, and EPA regions by providing information regarding the human health risk when water tests positive for the presence of Cryptosporidium. The method currently mandated by the U.S. EPA (method 1622/1623) does not speciate human infectious from non-human infectious species. This "add-on" method can be used to provide supplemental information which will aid water treatment and source water protection decision-making processes.

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