

Microbial Agents

The United States produces some of the cleanest drinking water in the world and yet there are still reports of waterborne disease outbreaks. These outbreaks are produced by microbial agents or pathogens including bacteria, viruses and parasites. These agents can cause acute and chronic illnesses and result in life-threatening conditions for individuals with weakened immune systems. Of the approximately 20 outbreaks reported nationally per year, more than half are related to groundwater consumption (Lee, and others 2002; Yoder and others 2008). Many waterborne outbreaks are not reported or detected.

In Wisconsin, a statewide assessment showed approximately 23 percent of private well water samples tested positive for total coliform bacteria, an indicator species of other biological agents (Warzecha, and others 1995). Approximately 3 percent of private well water samples tested positive for *E. coli*, an indicator of potential water borne disease that originates in the mammalian intestinal tract.

The DNR recommends that private well owners test their water for total coliform bacteria annually or when there is a change in taste, color, or odor of the water. Public drinking water systems that disinfect their water supplies are required to sample, on a quarterly basis, for bacteria from the raw water (before treatment) in each well. These raw water samples are representative of the source from which the wells draw groundwater. The DNR has recently begun tracking total coliform detects in the raw water samples through its Drinking Water System database. Approximately

Manure spreading can contaminate groundwater with bacteria and/or viruses in karst areas and/or where soils are thin. Contamination is more likely when landspreading of manure occurs prior to, or during runoff events. Runoff events occur when precipitation exceeds soil infiltration rates, or snowpack melts during the spring thaw. Runoff risks can be substantially reduced if manure spreading is done according to an approved nutrient management plan which includes a number of restrictions on manure applications to thin soils and locally identified karst features. Currently, however, less than 21 percent of state farmland is covered by a state-approved nutrient management plan. Scores of private wells have had to be replaced due to manure contamination at a cost to the state of over \$500,000

DNR private water staff responds to homeowner complaints regarding private well contamination events, many of which correspond to manure spreading. Until 2007 there were no readily available methods for testing for manure in these wells. Standard methods for testing for bacteria do not show whether the bacteria are derived from human or animal sources. Recently developed laboratory techniques have made it possible to discern whether bacteria are from human, animal or other sources. These microbial source tracking (MST) tools include tests for *Rhodococcus coprophilus* (indicative of grazing animal manure), *Bifidobacteria* (indicative of human waste) and *Bacteriodes* (indicative of recent fecal contamination by either humans and/or grazing animals). Recently, an analysis was developed by Sibley et al. that can successfully detect bovine adenoviruses to indicate bovine fecal contamination of groundwater thus increasing the size of the molecular “toolbox” for better understanding of the origin of fecal contamination. The DNR has been using these tools since 2007 to determine the source of fecal contamination in private wells. Since 2007, in response to private well water quality complaints over 60 groundwater samples have been analyzed. Results indicate that the majority of well water samples were contaminated with grazing animal waste. Less than ten percent of samples collected indicate microbial contamination from human sources. Even more rare were wells contaminated with

both grazing animal and human fecal bacteria. Approximately twenty percent of the well samples had no indication of microbial contamination. DNR's Drinking Water & Groundwater and Runoff Management programs are working with the DATCP nutrient management program to find ways of controlling this significant threat to health.

Some parts of the state are particularly vulnerable to microbial contamination. Microbiological contamination often occurs in areas where the depth to groundwater or depth of soil cover is shallow or in areas of fractured bedrock. In these areas, there is little natural attenuation potential. Door County is one such location where bedrock is fractured and wells are often shallow. Many other parts of Wisconsin contain areas of shallow, fractured bedrock or minor karst features making them very vulnerable to microbial contamination from the land surface.

In a recent survey of 25 private wells in Door County, 18 had detections of total coliform in at least one monthly sample over a 1-year period (Braatz, 2004). Forty percent had detections of a fecal indicator (*E. coli* or enterococci). Significant seasonal trends were also apparent, with higher percentages of wells with fecal indicators in the summer months. There were also waterborne illness outbreaks at two Door County restaurants, one in December 2004 and another in May 2007 (Borchardt, M. A., 2010). The cause of the May 2007 outbreak was a genogroup 1 norovirus, quantified in the restaurant's well water at more than 50 viruses per liter, well above the infectious dose necessary for a widespread outbreak. More than 250 people became ill and 6 people were hospitalized. The nucleic acid sequences of the viruses from the well and stool specimens from ill patrons were identical, providing definitive evidence for the waterborne transmission route. Moreover, a state-of-the-art dye tracer study conducted by the University of Minnesota demonstrated unequivocally a rapid transport route from the restaurant's new septic system to its well. Transport was from both: 1) untreated effluent discovered leaking from a broken pipe fitting near a septic tank; and 2) discharge from the septic drainfield. Groundwater and public health experts believe another outbreak in Door County may be imminent due to the widespread shallow soils and karst bedrock found in the county, which makes it difficult to find an appropriate place for locating septic systems.

There is overwhelming evidence in the state of Wisconsin and nationwide that karst areas have highly vulnerable groundwater requiring special consideration and protection. These findings lead to the conclusion that current requirements for septic systems and associated leach fields are inadequate to protect public health and the environment in areas of Wisconsin where water wells are completed in shallow carbonate aquifers. Wisconsin's requirements for construction of private water wells in these settings, should be revised to require practices that will most likely provide safe, potable groundwater in these settings.

Researchers at the Marshfield Clinic Research Foundation have investigated the association between pathogenic viruses and bacteria in private wells with incidences of infectious diarrhea as indicators of well water contamination (Borchardt, and others 2003b). In general, infectious diarrhea did not correlate with drinking from private wells or drinking from wells that had positive analytical results for total coliform. However, wells which tested positive for enterococci were associated with children having diarrhea of unknown etiology likely caused by noroviruses. A subsequent study of 50 private wells throughout the state indicates that 8percent of private wells may be subject to virus contamination (Borchardt and others 2003a). Wells positive for viruses did not show seasonal trends nor were they associated with commonly used indicators of microbial contamination such as total coliform or fecal enterococci. These studies suggest that increased monitoring and detection methods for viruses are needed to assess the risk of drinking water with potential microbial contamination.

In another study in collaboration with the US Geological Survey, Marshfield researchers found that 50 percent of water samples collected from four La Crosse municipal wells were positive for enteric viruses, including enteroviruses, rotavirus, hepatitis A virus, and norovirus (Borchardt and others 2004). As with the above described private well study, there was no correlation to common indicators of sanitary quality, nor was there a consistent seasonal trend. More surprising, viruses were common even in those wells without any Mississippi River water infiltration (Borchardt and others 2004, Hunt and others 2005), suggesting fecal sources other than those associated with surface waters were contaminating the wells. The most likely source is leaking sanitary sewers. The study did not address whether the viruses are inactivated through disinfection processes, or result in illness in the community.

Leaking sanitary sewers were shown to be a source of infectious viruses to drinking water wells in subsequent work funded by WDNR and the USGS (Hunt and others, in review). Marshfield Clinic and USGS researchers performed a synoptic sampling of over 30 unconfined municipal wells in 14 Wisconsin communities. Groundwater collected was evaluated for surface water contributions and presence of waste-water tracers and human enteric viruses. From this survey 8 wells had surface water contributions, 4 had unambiguous waste-water tracers, and 5 were positive for viruses. These analyses were used to identify 3 well sites used for intensive instrumentation of the shallow groundwater system between the wellhead and suspected sanitary sewer sources. Viruses and waste-water tracers were found in the groundwater at all three instrumented sites. The work showed that concurrent sampling at any one time may not show simultaneous virus and tracer presence due to differences in analytical precision and seasonality of the sources in the waste stream. However, given sufficient sampling over time, a good relation between unambiguous waste-water tracers and virus occurrence was identified such that locations that were characterized by recurring unambiguous tracer occurrence also were found to have enteric viruses present. Moreover, nearby groundwater velocities and presence of infectious viruses at the wellhead demonstrate that high-capacity pumping can induce travel times that are sufficiently short such that viruses are not inactivated during their time in the subsurface. Because sanitary sewers are commonly located near municipal wells and can carry very high numbers of infectious viruses, and very small numbers of infectious viruses in water can constitute a health risk, drinking water wells can be considered vulnerable to fast groundwater flow paths that only contribute a very small amount of virus-laden water to a well. Thus, these results suggest that evaluations of drinking well vulnerability should include low yield-fast transport pathways in addition to traditional high yield-slower transport plume contaminants currently included in wellhead protection. Such evaluations are thought to be important in communities such as the 14 included in the study, as they were chosen because they did not routinely employ chlorination or other disinfection procedures at the time of the study.

Microbial contamination of groundwater is not restricted to aquifers typically regarded as vulnerable or shallow aquifers. In a novel study, researchers at the Marshfield Clinic, Wisconsin Geological and Natural History Survey, and the University of Waterloo, discovered human viruses in the confined aquifer supply Madison's drinking water (Borchardt et al 2007). This finding was completely unexpected because it was believed the 3 to 9 meter shale confining layer protected the aquifer from microbial contamination. Additional research by Marshfield Clinic, WGNHS, and USGS, on the Madison wells has shown virus transport from leaking sanitary sewers to the wells is very rapid, on the order of weeks to months instead of years (Bradbury et al., 2013). The virus transport and contamination levels were particularly high after extreme rainfall events or rapid snowmelt. From a public health perspective, the lesson learned is that all aquifers are potentially vulnerable to microbial contamination. Public water supply systems in cities, towns or villages that supply groundwater are particularly vulnerable to pathogen contamination from leaky sanitary sewer systems. However, as described below, there is no

federal or state requirement for such systems to disinfect their drinking water. Approximately sixty communities in the state do not disinfect the groundwater supplied for drinking water.

Public and private water samples are not regularly analyzed for viruses. Viral testing is expensive and very few labs are capable of conducting the test. The presence of coliform bacteria has historically been used to indicate the water supply is not safe for human consumption. However, virus data complicates this interpretation since the presence of coliform (and other indicators as well) do not always correlate with the presence of enteric viruses. For example, municipal water sampled by Borchardt and others (2004) showed that, even though 50 percent of the samples were positive for viruses, none of the same samples tested positive for coliform or other indicators. Recently, water samples from private residences in Door County found low levels of some viruses but water samples did not contain coliform (Wisconsin DNR). Indicators have a high positive predictive value but a low negative predictive value for pathogen occurrence. In other words, when an indicator is present in drinking water there is a high probability that particular water source will be contaminated with a pathogen at some point in time. However, if an indicator is absent, no inferences can be made about pathogen occurrence. Additional study is needed to determine what virus results mean to human health.

Data from the U.S. EPA shows that the highest percentage of microbial unsafe water is found in small water systems, like transient non-community (TN) systems such as restaurants and convenience stores (Peterson, 2001). There are approximately 9,500 active TN systems in Wisconsin. The mobility of people consuming water at small water systems and general lack of knowledge of illness symptoms hinder waterborne illness outbreak identification.

Nationally, the Center for Disease Control tracks and identifies failures in water systems that lead to illness outbreaks. Because of the increasing evidence for widespread occurrence of microbial contaminants, additional monitoring requirements for vulnerable public water systems are on the horizon.

The U.S. EPA promulgated the Groundwater Rule (GR), on November 8, 2006 which modified Safe Drinking Water Act requirements to increase monitoring for fecal contamination in groundwater and reduce the occurrence of illness from drinking water borne microbial pathogens. Components of the GR include:

1. Sanitary surveys of public systems to identify deficiencies in system construction and operation.
2. Improvement on Safe Drinking Water Act requirements which have focused on sampling for microbial indicators in the distribution system. The GR requires source water monitoring when total coliform is detected in the distribution system.
3. A requirement of corrective action for non-complying features found in the water system and eliminating fecal contamination with treatment or providing an alternative permanent source of water.
4. Monitoring requirements to ensure that treatment equipment is maintained.

Implementation of the deficiency and monitoring requirements of GR began on December 1, 2009. DNR conducts inspections and requires correction of non-complying features. Therefore, the major changes resulting from the GR are additional monitoring of source water and installation of approved treatment devices or a new water source for the wells found to contain fecal contamination. However, the GR does not require testing for all pathogens. Recent research provides adequate evidence to suggest that requiring disinfection of groundwater-sourced public water supply systems would improve protection of public health.

References:

- Borchardt, M. A., P. D. Bertz, S. K. Spencer, and D. A. Battigelli. 2003a. Incidence of enteric viruses in groundwater from household wells in Wisconsin. *Applied and Environmental Microbiology*, 2003, Vol 69, Iss 2, pp 1172-1180.
- Borchardt, M. A., P. H. Chyou, E. O. DeVries, E. A. Belongia. 2003b. Septic system density and infectious diarrhea in a defined population of children. *Environmental Health Perspectives*, 2003, Vol 111, Iss 5, pp 742-748
- Borchardt M.A., Haas N.L., Hunt R.J. 2004. Vulnerability of municipal wells in La Crosse, Wisconsin, to enteric virus contamination from surface water contributions. *Applied and Environmental Microbiology* Vol 70: 5937-5946.
- Borchardt M.A., Bradbury K.R., Gotkowitz M.B., Cherry J.A., and Parker B.L. 2007 Human enteric viruses in groundwater from a confined bedrock aquifer. *Environmental Science and Technology* 41:6606-6612.
- Borchardt, M. A., K.R. Bradbury, E. C. Alexander, R. J. Kolberg, S. C. Alexander, J. R. Archer, L. A. Braatz, B. M. Forest, J. A. Green, and S. K. Spencer, 2010, Norovirus Outbreak Caused by a New Septic System in a Dolomite Aquifer: Ground Water, v. 10.1111/j.1745-6584.2010.00686.x.
- Braatz, L., 2004. A study of fecal indicators and other factors impacting water quality in private wells in Door County, Wisconsin. Master of Science thesis submitted to University of Wisconsin-Green Bay, December 2004. 124 p.
- Bradbury, K.R., Borchardt, M.A., Gotkowitz, M.B., Spencer, S.K., Zhu, J., and Hunt, R.J., 2013, Source and Transport of Human Enteric Viruses in Deep Municipal Water Supply Wells: *Environmental Science & Technology*.
- Hunt, R.J., and M.A. Borchardt. 2003. Susceptibility of La Crosse municipal wells to enteric virus contamination from surface water contributions. Final report submitted to Wisconsin Department of Natural Resources, June 23, 2003.
- Hunt, R.J., T.B. Coplen, N.L. Haas, D.A. Saad, and M.A. Borchardt. 2005. Investigating surface water–well interaction using stable isotope ratios of water, *Journal of Hydrology* vol 302 (1-4):154-172.
- Hunt, R.J., M.A. Borchardt, K.D. Richards, and S.K. Spencer 2010. Assessment of Sewer Source Contamination of Drinking Water Wells Using Tracers and Human Enteric Viruses. *Environ. Sci. Technol.*, 44, 7956–7963
- Lee S.H., Levy D.A., Craun G.F., Beach M.J., Calderon R.L. 2002, Surveillance for waterborne-disease outbreaks--United States, 1999-2000. *MMWR Surveill Summ* 2002;51:1-47.
- Peterson, H. G., 2001. Rural Drinking Water and Waterborne Illness. Safe Drinking Water Foundation, Saskatoon, SK, p. 162-191.

- Sibley, S.D., Goldberg, T.L. and Pederson, J.A. 2011. Detection of Known and Novel Adenoviruses in Cattle Wastes Using Broad-spectrum Primers. Appl and Env. Micro.
- Warzecha, C., R. Gerhardt, and S. Kluender. 1995. Wisconsin private well water quality survey. Wisconsin Department of Health and Social Services, Department of Natural Resources, and State Laboratory of Hygiene. Unpublished report.
- Wisconsin DNR, 2004. Unpublished Door County Files, Sturgeon Bay office.
- Yoder J., V. Roberts, G.F. Craun, V. Hill, L.A. Hicks, N.T. Alexander, V. Radke, R.L. Calderon, M.C. Hlavsa, M.J. Beach, S.L. Roy. 2008. [Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking--United States, 2005-2006](#). Centers for Disease Control and Prevention (CDC). MMWR Surveill Summ. 57:39-62.