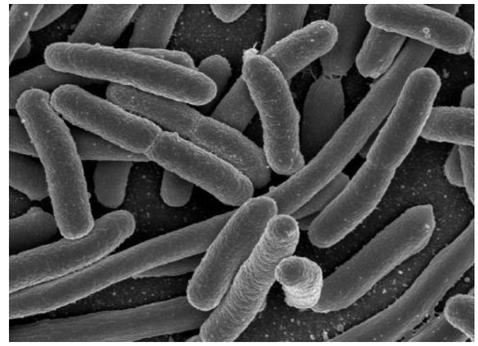
MICROBIAL PATHOGENS

What are they?



E. coli, an indicator of fecal contamination. *Photo: NIAID*

Pathogens are organisms or other agents that can cause disease, including microorganisms such as bacteria, viruses and protozoa that can cause waterborne disease. Groundwater contamination by microbial pathogens can often be traced to human or livestock fecal wastes that seep into the ground from sources such as inappropriately constructed or failing septic systems, leaking sanitary sewers or improperly managed animal manure. Since it is difficult and expensive to test for all pathogenic microorganisms, water samples are usually tested for microbial "indicators" – microbes that are not necessarily harmful themselves, but are a warning sign that other, potentially pathogenic, microorganisms may be present.

Microorganisms are prevalent and abundant in the subsurface and in groundwater (Griebler and Lueders 2009). The United States Geological Survey (USGS Michigan Water Science Center) reports that "Most of the bacterial types found in soils and surface waters have also been found in shallow unconfined and confined aquifers". Virus abundance in an alluvial aquifer in Colorado has been reported as ranging from 80,000 to 1,000,000 cell count per milliliter (Pan et al. 2017). A similar result of on average 71,700 of virus-like particles per milliliter was found for a shallow aquifer in Australia (Roudnew et al., 2013). In that study, in the underlying confined aquifer, with groundwater that entered the subsurface at least 1000 years ago, even higher virus-like particle concentrations were found, averaging from 48,200 virus-like particles per milliliter at 90 meters (295 feet) depth to 883,000 virus-like particles per milliliter at 60 meters (196 feet) depth. While most microorganisms in the subsurface are harmless, pathogenic microbes from human and animal waste sources can be significant sources of groundwater contamination in areas where they can be readily transported to underground drinking water supplies.

There are no groundwater standards for pathogenic microorganisms in Wisconsin, but standards have been established in ch. NR 140 for total coliform bacteria, a microbial pathogen indicator. Both the ch. NR 140 preventive action limit (PAL) and enforcement standard (ES) for total coliform bacteria are zero coliform bacteria present in a tested sample. Public drinking water systems are regularly monitored for total coliform bacteria (WI NR 809.31-809.329) these systems may also be tested for fecal indicators such as *E. coli*, enterococci or coliphages if coliform bacteria are found.

In 2016 the Environmental Protection Agency (EPA) changed its rules related to the use of microbial pathogen indicators in the regulation of public drinking water systems. In 2016 the EPA's Revised Total Coliform Rule (RTCR) for public drinking water systems went into effect. Prior to the RTCR, there was a drinking water maximum contaminant level (MCL) for total coliform bacteria in public drinking water systems. Under the RTCR the total coliform bacteria MCL was removed and replaced with a total coliform treatment technique (TT). If total coliform bacteria are confirmed present in a public drinking water system the total coliform TT requires system assessment and corrective action.

The EPA also established a drinking water MCL for *E. coli* bacteria under the RTCR. Detection of *E. coli* bacteria is considered a more specific indicator of fecal contamination, and the possible presence of harmful pathogens, than just detection of total coliform bacteria. This is because coliform bacteria of both fecal and non-fecal origin exist, and total coliform testing detects any coliform, regardless of its origin. Under the RTCR there is no MCL violation for total coliform bacteria positive samples only, but if *E. coli* indicator bacteria are confirmed present in a public drinking water system there is an MCL violation.

The presence of *E. coli* bacteria in a drinking water supply indicates possible exposure to pathogens, potentially resulting in serious, acute health effects. Detection of *E. coli* bacteria in a public water supply system is considered an immediate public health issue and public notification is required. That notification informs the consumer of the public health threat, and instructs the consumer to either boil water from the public water supply before consuming, or to use bottled water. An assessment of the integrity of the water supply system is also required, with correction of any sanitary defects found.

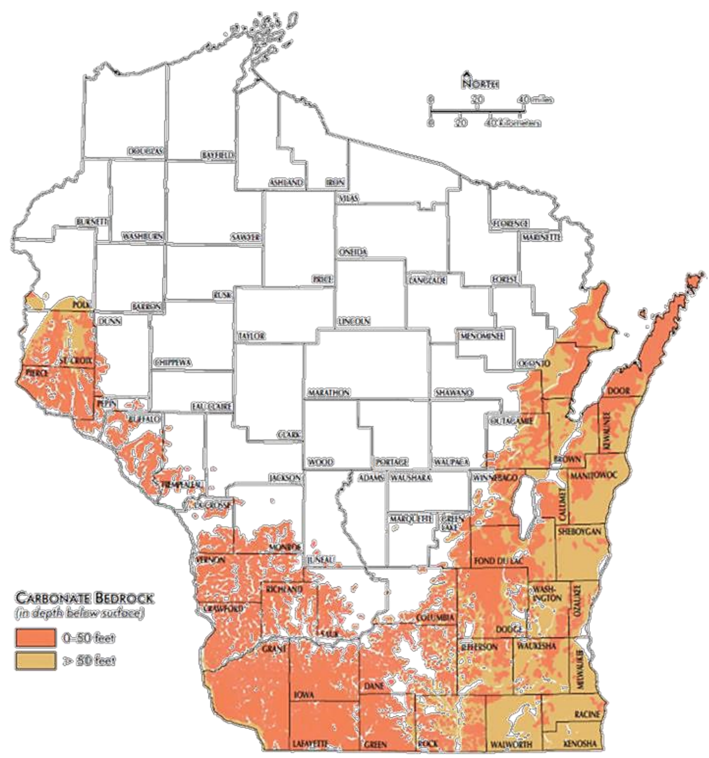
Coliforms are a broad class of bacteria that are naturally present in the environment and are used as an indicator that other potentially harmful microorganisms may be present. Total coliforms, with a few exceptions, are not harmful to humans. Coliforms are abundant in the feces of warm-blooded animals, but can also be found in aquatic environments, soil, and on vegetation*. E.coli* bacteria are a type of coliform bacteria that serve as a fecal indicator in drinking water. Fecal indicators, such as *E. coli*, enterococci and coliphages, are microbes whose presence more specifically indicates that water may be contaminated with human or animal wastes. Pathogenic microorganisms in drinking water can make people very sick and can result in death. Common symptoms include diarrhea, cramps, nausea and headaches. Microbial contamination may pose a special health risk for infants, young children, the elderly and people with severely compromised immune systems.

Microbial pathogen contamination is of particular concern in public water systems, because a large number of people can be exposed to contamination in a short amount of time. In 1993, pathogen contamination at Milwaukee's surface water-sourced drinking water system resulted in 69 deaths and more than 403,000 cases of illness before the epidemic and its source were recognized. In 2007 an outbreak of norovirus, caused by contaminated well water, sickened 229 diners and staff at a Door County restaurant (Borchardt et al. 2011).

Antibiotic resistance, associated with subsurface microorganisms, may also be a significant groundwater contaminant in some situations. Use of antibiotics can result in antibiotic resistance (ineffectiveness of antibiotics in treating infections) spreading into the environment. Groundwater monitoring around swine lagoons in Illinois found that antibiotic resistant genes, associated with lagoon leakage, were present in groundwater (Krapac et al. 2004). In a study of manure at a Wisconsin dairy farm, Walczak et al. (2011) isolated *E. Coli* bacteria that were resistant to four different antibiotics (cephalothin, ampicillin, tetracycline, and erythromycin). The *E. Coli* populations in the manure multiplied on average by about 15 after three days. *E. Coli* may be leached from manure and this might lead to spread of antibiotic resistance to groundwater.

Occurrence in Wisconsin

In Wisconsin, it is well known that groundwater in areas with karst geology – soluble carbonate bedrock, with many large fractures through which water flows rapidly, sometimes with karst surficial features, such as sinkholes, caves and disappearing streams present – is vulnerable to microbial contamination and needs special consideration and protection. In these areas, particularly where there is also thin soil cover and shallow groundwater levels, there is little opportunity for soil to slow and attenuate the transport of microbes. This results in a greater risk that viable pathogens may reach water supply wells. Soluble carbonate bedrock with karst potential can be found in some parts of the state. Door County and parts of Kewaunee County are especially vulnerable since these areas additionally have very thin soils. An estimated 17% of private water supply wells statewide test positive for total coliform bacteria (Knobeloch et al., 2013). Sampling of private water supply wells in Kewaunee County (Kewaunee Co., 2014) has suggested that,



Karst potential in Wisconsin. Areas with carbonate bedrock within 50 feet of the land surface are particularly vulnerable to groundwater contamination. *Figure*: WGNHS in some parts of the county, wells are testing positive for total coliform bacteria at percentages much higher than the statewide average.

A more recent, emerging concern is the potential presence of viruses in drinking water wells, including norovirus, adenovirus and enterovirus. Virus contamination may not necessarily correlate well with total coliform bacteria detection in groundwater (Borchardt et al., 2003b) because viruses have different transport properties than bacteria.

Viruses may be detected in water samples using cell culture methods that measure the cytopathic effect of viruses grown on various cell culture media. Not all types of viruses are culturable, but molecular nucleic acid based methods, such as polymerase chain reaction (PCR), can be used to detect viral genetic material, even from nonculturable viruses. Molecular nucleic acid based methods such as PCR, however, cannot distinguish between genetic material from viable, infectious viruses and genetic material from inactivated, nonviable viruses (Donia et al., 2009).

Research studies, utilizing PCR methods, have detected human enteric virus genomic material in both public and private wells in Wisconsin (Borchardt et al., 2003a, 2004, and 2007). There is limited statewide groundwater virus occurrence data since testing for viral genomic material is expensive, not routinely perform, and levels cannot be reliably inferred from total coliform results. In cities where such studies have been conducted, such as La Crosse and Madison, it has been suggested that transport of viruses from municipal sewer systems to groundwater supplies may be occurring and that this transport might be very rapid (Hunt et al., 2010; Bradbury et al., 2013). These studies suggest that viral contamination of groundwater could potentially occur at other municipal water systems because municipal wells are generally completed in areas with sanitary sewers.

The risk of finding pathogens in groundwater is seasonably variable but typically highest following spring snowmelt or large rainstorms that generate runoff, since these events can create large pulses of water that move quickly through the ground, potentially carrying microbes from septic systems, sewer mains and manure sources (Uejio et al., 2014). Nutrient management plans can help reduce the risk of contamination due to manure spreading, but even with the best manure management practices it is difficult to eliminate occurrences. Over 60 private wells have had to be replaced since 2006 due to manure contamination, at a cost to the state of over $500,000 (Source: DNR Well Compensation Fund records).

There is evidence that disinfection with chlorine or ultraviolet light may reduce the risk of illness from viruses and other microbial sources (Borchardt et al., 2012; Lambertini et al., 2012; Uejio et al., 2014). Continuous disinfection is not dependent on indicator tests to protect human health. Disinfection, however, is not required by law for public water systems that source their drinking water from groundwater. About 60 municipalities in Wisconsin do not disinfect their public water supplies.

GCC Agency Actions

Homeowner complaints about private well *bacterial* contamination events, which often correspond with manure spreading, are an ongoing concern for GCC agencies. Unfortunately, the standard methods for testing for bacteria do not show whether the bacteria are derived from human or animal sources and until 2007 there were no readily available methods for testing for manure.

Funding from the Wisconsin Groundwater Research and Monitoring Program (WGRMP) has supported the development of laboratory techniques that have made it possible to discern whether bacteria are from human, animal or other sources (Pedersen et al., 2008; Long and Stietz, 2009). These microbial source tracking (MST) tools include tests for *Rhodococcus coprophilus* (indicative of grazing animal manure), *Bifidobacteria* (indicative of human waste) and *Bacteroides* (indicative of recent fecal contamination by either humans and/or grazing animals). An analysis can successfully detect bovine adenoviruses to indicate bovine fecal contamination of groundwater (Sibley et al., 2011).

Dr. Sam Sibley, UW-Madison Department of Soil Science, collects a well water sample from a residential home to analyze using new MST tools. Video story at: https://youtu.be/dpE58Rd4i4E. *Photo*: *Carolyn Betz, UW ASC*

The DNR has been using these tools as they become available to determine the source of fecal contamination in private wells. DNR's Drinking Water & Groundwater and Runoff Management programs are working with the DATCP nutrient management program to find ways of controlling this major source of contamination. The DNR, in conjunction with DATCP, are working on revised performance standards and prohibitions related to manure land application in areas of the state with carbonate bedrock and shallow soils.

The DNR developed a rule mandating disinfection of municipal drinking water but this was repealed by the state legislature in 2011. Nationally, the EPA included virus types found in Wisconsin studies on the list of 30 unregulated contaminants that were monitored from 2013 to 2015 in 6,000 public water systems across the United States to gather information to support future drinking water protection. In that sampling, the Unregulated Contaminant Monitoring Rule 3 (UCMR-3) sampling effort, the presence of enterovirus was evaluated using microbial culture methods, and the presence of enterovirus and norovirus genetic material was evaluated using PCR methods. No enteroviruses, or enterovirus or norovirus genetic material, was reported detected in Wisconsin under the UCMR-3 sampling effort.

Future Work

Improving best practices for well construction in the vulnerable karst areas of the state is an ongoing topic of concern. In addition to the potential threat to health posed by manure sources, there are indications that inadequately constructed and maintained septic systems and leach fields could also be sources of microbial groundwater contamination and therefore detrimental to public health and the environment in areas where wells draw from shallow carbonate aquifers. This points to a need to revise the requirements for the construction of private water wells in these areas.

Most of the current data on bacterial contamination in Wisconsin is derived from private well samples. However, public drinking water systems that disinfect their water supplies are also required to sample quarterly for bacteria from the raw water (before treatment) in each well. The DNR began tracking total coliform detects in the raw water sample through its Drinking Water System database, so evaluation of this monitoring data from public wells may enhance understanding of statewide bacterial contamination. This understanding would be further enhanced by an analysis of the equivalence and positive predictive value of the laboratory methods (PCR kits, testing protocols) used to measure concentrations of bacteria and bacterial indicators in groundwater.

Pumping test at one of Madison's municipal wells, part of a WGRMP-funded study to enhance understanding of fractures and virus transport. *Photo*: *Jean Bahr*

There are unanswered questions about viruses in drinking water as well. While previous work has suggested that municipal sanitary sewers may be potential sources of viruses in groundwater, the exact mechanism of entry in cities like Madison is unknown and cannot be explained by normal assumptions about hydrogeology. A study funded by the Wisconsin Groundwater Research and Monitoring Program investigated whether the rapid transport of viruses between the shallow and deep aquifers in Madison can be explained by vertical fractures in the shale layer that separates them. More research is needed on the transport and survival times of various viruses in groundwater aquifers.

Finally, additional public health studies where clinical samples and water samples are collected simultaneously, such as those conducted by GCC researchers in La Crosse, are needed to better describe the relationship between cause of illness and groundwater pathogens.

Further Reading

DNR overview of bacteriological contamination in drinking water

DNR overview of cryptosporidium in drinking water

DHS fact sheet on manure contamination of private wells

WGNHS overview of karst landscapes

WGNHS report on municipal drinking water safety

DNR list of municipal drinking water systems that disinfect

References

Borchardt, M. A., P. D. Bertz, S. K. Spencer, D. A. Battigelli. 2003a. Incidence of enteric viruses in groundwater from household wells in Wisconsin. Applied and Environmental Microbiology, 69(2):1172- 1180. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC143602/

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, 111(5):742-748. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241485/

Borchardt, M.A., N. L. Haas, R. J. Hunt. 2004. Vulnerability of drinking-water wells in La Crosse, Wisconsin, to enteric-virus contamination from surface water contributions. Applied and Environmental Microbiology, 70(10): 5937-5946. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC522136/

Borchardt, M.A., K. R. Bradbury, M. B. Gotkowitz, J. A. Cherry, B. L. Parker. 2007. Human enteric viruses in groundwater from a confined bedrock aquifer. Environmental Science & Technology 41(18):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, S. K. Spencer. 2011. Norovirus outbreak caused by a new septic system in a dolomite aquifer. Ground Water, 49(1):85-97.

Borchardt, M. A., S. K. Spencer, B. A. Kieke, E. Lambertini, F. J. Loge. 2012. Viruses in nondisinfected drinking water from municipal wells and community incidence of acute gastrointestinal illness. Environmental Health Perspectives 120(9):1272:1279. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3440111/

Bradbury, K.R., M. A. Borchardt, M. B. Gotkowitz, S. K. Spencer, J. Zhu, R. J. Hunt. 2013. Source and transport of human enteric viruses in deep municipal water supply wells. Environmental Science & Technology, 47(9):4096-4103.

Donia, D., Bonanni, E., Diaco, L., Divizia, M. 2009. Statistical correlation between enterovirus genome copy numbers and infectious viral particles in wastewater samples. The Society for Applied Microbiology, Letters in Applied Microbiology, 50 (2010): 237-240.

Griebler, C., Lueders, T. 2009. Microbial biodiversity in groundwater ecosystems. Freshwater Biology, 54(4): 649-677. Available at https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2427.2008.02013.x

Hunt, R. J., T. B. Coplen, N. L. Haas, D. A. Saad, M. A. Borchardt. 2005. Investigating surface water–well interaction using stable isotope ratios of water. Journal of Hydrology, 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. Environmental Science and Technology, 44(20):7956–7963.

Kewaunee Co., 2014. Kewaunee County Public Health and Groundwater Protection Ordinance, Ordinance No. 173-9-14. Available at https://www.kewauneeco.org/i/f/files/Ordinances/Chapter%2030.pdf

Knobeloch, L., P. Gorski, M. Christenson, H. Anderson. 2013. Private drinking water quality in rural Wisconsin. Journal of Environmental Health, 75(7):16-20.

Krapac, I. G., Koike, S., Meyer, M. T., et al. 2004. Long-Term Monitoring of the Occurrence of Antibiotic Residues and Antibiotic Resistance Genes in Groundwater near Swine Confinement Facilities. Proceedings of the 4th international conference on pharmaceuticals and endocrine disrupting chemicals in water. Minneapolis, MN. National Groundwater Association. 13-15 Oct. pp. 158-172.

Lambertini, E., M. A. Borchardt, B. A. Kieke, S. K. Spencer, F. J. Loge. 2012. Risk of viral acute gastrointestinal illness from nondisinfected drinking water distribution systems. Environmental Science & Technology 46(17):9299-9307.

Long, S. and J.R. Stietz. 2009. Development and validation of a PCR-based quantification method for Rhodococcus coprophilus. Wisconsin groundwater management practice monitoring project, DNR-206. Available at http://digital.library.wisc.edu/1711.dl/EcoNatRes.LongProject

Pan, D., Nolan, J., Williams, K., Robbins, M., Weber, K. Abundance and Distribution of Microbial Cells and Viruses in an Alluvial Aquifer. 2017. Frontiers in Microbiology. DOI:10.3389/fmicb.2017.01199Corpus ID: 12970321. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5504356/

Pedersen, J. T. McMahon, S. Kluender. 2008. Use of human and bovine adenovirus for fecal source tracking. Wisconsin groundwater management practice monitoring project, DNR-195. Available at http://digital.library.wisc.edu/1711.dl/EcoNatRes.KluenderUse

Roudnew, B., Lavery, T., Seymour, J., Smith, R., Mitchell, J., 2013. Spatially varying complexity of bacterial and virus-like particle communities within an aquifer system. Aquat. Microb. Ecol. 68, 259–266. Available at https://doi.org/10.3354/ame01615

Sibley, S.D., T. L. Goldberg, J. A. Pederson. 2011. Detection of known and novel adenoviruses in cattle wastes using broad-spectrum primers. Applied and Environmental Microbiology, 77(14):5001-5008.

Ueijo, C. K., S. H. Yale, K. Malecki, M. A. Borchardt, H. A. Anderson, J. A. Patz. 2014. Drinking water systems, hydrology, and childhood gastrointestinal illness in central and northern Wisconsin. American Journal of Public Health, 104(4):639-646. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4025711/

USGS, United Sates Geological Survey - Michigan Water Science Center. Bacteria and Their Effects on Ground-Water Quality. Available at https://mi.water.usgs.gov/h2oqual/GWBactHOWeb.html

Walczak, J.J., Xu, S., 2011. Manure as a Source of Antibiotic-Resistant Escherichia coli and Enterococci: a Case Study of a Wisconsin, USA Family Dairy Farm. Water. Air. Soil Pollut. 219, 579–589. https://doi.org/10.1007/s11270-010-0729-x