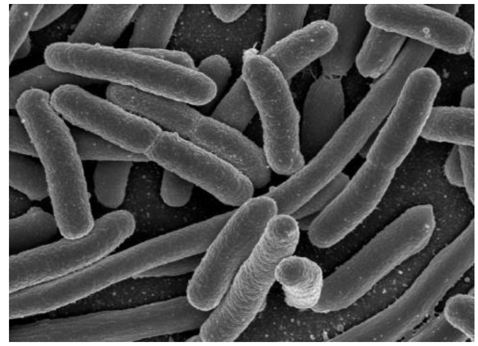
MICROBIAL PATHOGENS

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 pathogen "indicators", such as total coliform bacteria, fecal coliform bacteria, *E. coli* bacteria, enterococci bacteria or coliphage viruses. These indicator microbes 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). While most microorganisms in the subsurface are harmless, pathogenic microbes from human and animal fecal waste sources can contaminate groundwater in areas where they can be readily transported through the subsurface to underground drinking water supplies.

There are no specific groundwater quality standards for pathogenic microorganisms in Wisconsin, but standards have been established in ch. NR 140 for total coliform bacteria, an indicator of possible microbial pathogen contamination. Both the ch. NR 140 preventive action limit (PAL) and enforcement standard (ES) for total coliform bacteria are 0 coliform bacteria present in a tested sample. Public drinking water systems are regularly monitored for total coliform bacteria (WI NR 809.31-809.329), and these systems are tested for *E. coli*, and possibly other fecal indicators such as enterococci or coliphages, if coliform bacteria are found to be present.

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. Under the RTCR the existing total coliform bacteria drinking water maximum contaminant level (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.

Total coliform bacteria include bacteria that naturally occur in the environment, and total coliform are, with a few exceptions, not harmful to humans. Under the RTCR, detection of total coliform bacteria is used as an indicator of possible microbial pathways into a public drinking water system. *E. coli* bacteria are a sub-group of coliform bacteria considered to be a more specific indicator of fecal contamination and the potential for pathogens to be present in drinking water. Under the RTCR, detection of *E. coli* bacteria in a public water supply system is an MCL violation. Public notification is required for a public drinking water system *E. coli* MCL violation. This notification instructs the public to either boil water from the public system before consuming, or to use bottled water.

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 in 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 at large animal feeding operations for growth promotion can result in antibiotic resistance (ineffectiveness of antibiotics in treating infections) spreading into the environment (Gilchrist et al. 2007). Groundwater monitoring around swine manure lagoons in Illinois found that antibiotic resistant genes, associated with leakage from the manure lagoons, were present in groundwater (Krapac et al. 2004). In a study of manure at a Wisconsin dairy farm, *E. coli* bacteria resistant to four different antibiotics were detected (Walczak et al. 2011).

Occurrence in Wisconsin

Many factors influence microbial transport in the subsurface, both vertically through the unsaturated zone, and with groundwater flow through an aquifer. Processes such as filtration, adsorption and "die-off" can all affect the fate and transport of microbial pathogens (Bradford et al. 2013). These microbial removal and attenuation mechanisms can be complex, with a number of factors influencing how effective they may be at reducing the number of pathogens in groundwater. Factors such as soil depth, presence of preferential flow paths, soil saturation, microbial biofilms, temperature, pH, flow rate, soil microbial flora and soil organic matrix can all influence microbial pathogen transport and survival.

Fecal waste from humans, domesticated animals, wildlife, and insects can all be sources of pathogenic microorganisms in the environment. Discharges of human and domesticated animal fecal waste to the environment include wastewater effluent discharge and infiltration, and the land application of animal manure, septage and municipal wastewater biosolids. The land application discharge of human waste, and some animal waste, are regulated activities in Wisconsin (per administrative codes: SPS 383, NR 206, NR 110, NR 204, NR 113, NR 214, NR 243). For these regulated activities, pathogen reduction, including soil treatment in the unsaturated zone, is required to remove and attenuate microbial pathogens that might be present in the waste. Soil treatment requirements in state administrative rules include minimum vertical separation distances between land disposal/application and groundwater. State rules also place limitations on waste discharge loading and application rates based on discharge site soil conditions.

The Wisconsin State Laboratory of Hygiene conducted a laboratory column study in 2001, investigating the transport of microorganisms through unsaturated soil (Standridge et al. 2001). The soil used for the study was ASTM C-33 standard sand soil, used in Wisconsin for mound septic systems. This soil is a "general" textured filter media soil, that contains a minimum of both fine and coarse grained material. Both bacterial and viral pathogen indicator organisms were used in the study. Results showed that, under "normal" loading rates, all 5 of the pathogen indicators used for the study were removed or attenuated with infiltration through 2 feet of unsaturated soil.

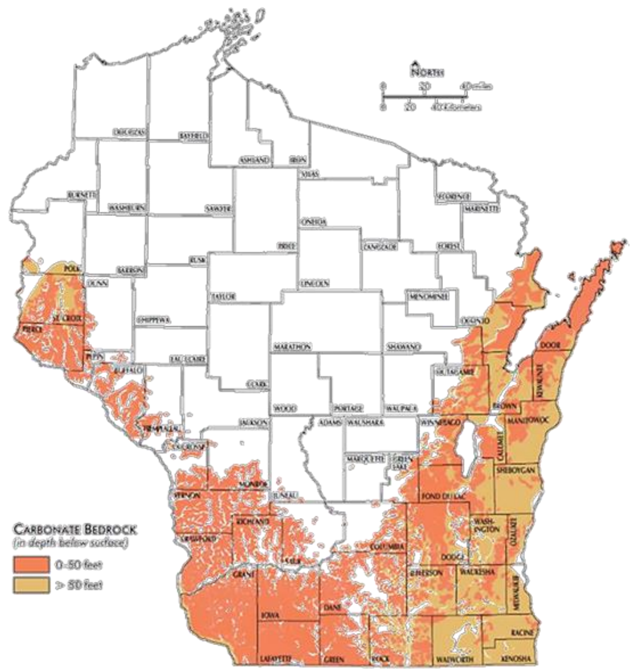
Most bacteria entering the ground surface along with rainwater or snowmelt are filtered out or attenuated as water seeps downward through the unsaturated soil zone to groundwater, however, some strains of bacteria can survive a long time and may find their way into the groundwater by moving through coarse grained soils, shallow fractured bedrock, quarries, sinkholes, inadequately grouted wells or cracks in well casing. Water supply wells may also be contaminated by insects or small rodents that can carry microbial pathogens into wells with inadequate caps or seals.

In Wisconsin, it is well known that groundwater in areas with karst geology is vulnerable to microbial contamination and needs special consideration and protection. Karst geology includes areas with soluble carbonate bedrock that may have relatively large fractures through which water flows rapidly and where sometimes karst surficial features, such as sinkholes, caves and disappearing streams are present. 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 microbial pathogens. 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, including Door County, parts of Kewaunee County and in southwestern WI. Some of these areas are especially vulnerable since, in addition to karst geology, they have very thin soil cover.

Analysis of statewide sampling results show that approximately 17% to 23% of private water supply wells in Wisconsin test positive for total coliform bacteria, and approximately 3% of private wells test positive for *E. coli* bacteria (Knobeloch et al. 2013; US GAO 1997).

A study that sampled private water supply wells in Kewaunee County (Kewaunee Co. 2014) showed that, county wide, private wells tested positive for total coliform bacteria at about the same percentage as statewide averages. Wells located in areas with shallower depths to bedrock, however, did test positive for total coliform and *E. coli* bacteria at percentages greater than state averages. Waste source and pathogen genetic markers were detected, using polymerase chain reaction (PCR) molecular methods, in some of the study wells that were tested for those markers.

A recent study in Wisconsin, designated the Southwest Wisconsin Groundwater and Geology (SWIGG) study, looked at the presence of total coliform bacteria and waste source and pathogen genetic markers in private water supply wells in Grant, Iowa and Lafayette Counties (Stokdyk et al. 2022). The study area, in southwestern Wisconsin, has karst geology and relatively thin soil cover. Sampling found total coliform bacteria in private wells in the study counties at percentages greater than, or similar to, statewide averages. Waste source and pathogen genetic markers were detected, using PCR molecular methods, in some of the study wells that were tested for those markers. The study also found possible correlations between a number of well construction, geologic and land use factors and potential sources of well contamination.



Karst potential in Wisconsin. Areas with carbonate bedrock within 50 feet of the land surface are particularly vulnerable to groundwater contamination. Figure: [WGNHS]

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. More than 60 private wells in Wisconsin 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).

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

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 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 dead, inactivated or 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 performed, 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 as municipal wells are generally completed in areas with sanitary sewer systems.

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 supply systems.

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.

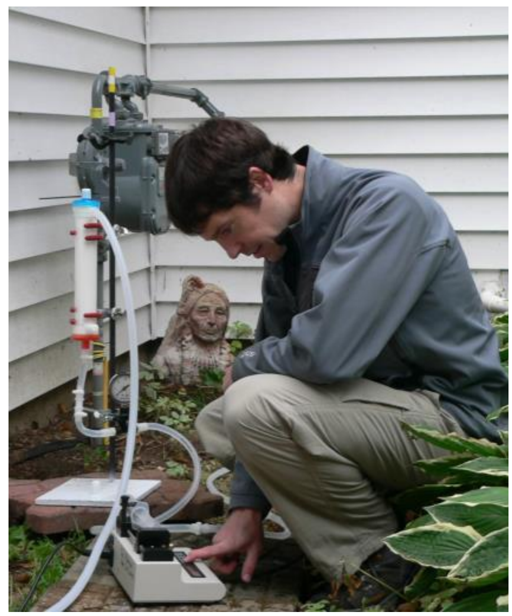
Funding from the Wisconsin Groundwater Research and Monitoring Program (WGRMP) has supported the development of laboratory techniques that have made it possible to discern whether bacteria are from human, animal or other sources (Pedersen et al. 2008; Long and Stietz 2009). These microbial source tracking (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). Analysis can successfully detect bovine adenoviruses an indicator of bovine fecal contamination of groundwater (Sibley et al. 2011).

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 culturable enteroviruses, or enterovirus or norovirus genetic material, was reported detected in Wisconsin during 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.



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*

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.

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.

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*

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

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