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Article

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Human Enteric Viruses in Groundwater from a Confined Bedrock Aquifer

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Confined aquifers are overlain by low-permeability aquitards that are commonly assumed to protect underlying aquifers from microbial contaminants. However, empirical data on microbial contamination beneath aquitards is limited. This study determined the occurrence of human pathogenic viruses in well water from a deep sandstone aquifer confined by a regionally extensive shale aquitard. Three public water-supply wells were each sampled 10 times over 15 months. Samples were analyzed by reverse transcription−polymerase chain reaction (RT-PCR) for several virus groups and by cell culture for infectious enteroviruses. Seven of 30 samples were positive by RT-PCR for enteroviruses; one of these was positive for infectious echovirus 18. The virus-positive samples were collected from two wells cased through the aquitard, indicating the viruses were present in the confined aquifer. Samples from the same wells showed atmospheric tritium, indicating water recharged within the past few decades. Hydrogeologic conditions support rapid porous media transport of viruses through the upper sandstone aquifer to the top of the aquitard 61 m below ground surface. Natural fractures in the shale aquitard are one possible virus transport pathway through the aquitard; however, windows, cross-connecting well bores, or imperfect grout seals along well casings also may be involved. Deep confined aquifers can be more vulnerable to contamination by human viruses than commonly believed.

Introduction

Confined aquifers are permeable water-bearing geologic formations (i.e., sand, gravel, fractured rock) that are bounded by lower-permeability geologic formations called aquitards. Two broad categories of aquitards exist: unlithified (non-rock) aquitards composed of clay or silt-rich deposits and indurated (rock) aquitards such as shale, siltstone, quartzite, carbonates, and igneous rocks. Confined aquifers are the primary source of water for many municipalities throughout the world. Municipalities often assume low-permeability aquitards provide barriers to flow, limiting the migration of contaminants into the aquifer. However, aquitard integrity can be compromised by features such as fractures, erosional or depositional windows, and incomplete lateral extent, all of which can provide avenues for aquifer contamination. There may also be anthropogenic pathways such as improperly abandoned or cross-connecting wells. Cherry et al. (*1*) provide a review of aquitard science and point out that the capability of an aquitard to limit the transmission of contaminants depends strongly on the type of contaminant. For example, solutes have much less propensity for transmission than dense nonaqueous phase liquids (DNAPLs). Little is known about virus migration through aquitards.

Among the many waterborne pathogens of humans, enteric viruses have the greatest potential to move deep into the subsurface environment, penetrate an aquitard, and reach a confined aquifer. Enteric viruses are extremely small (27−75 nm), readily passing through sediment pores that would trap much larger pathogenic bacteria and protozoa. Adsorption to sediment grains is the primary virus removal mechanism, although the strength of the adsorptive forces depends on sediment and water chemistries, and viruses may still be transported some distance. Several recent studies have demonstrated widespread occurrence of viruses in domestic and municipal wells in the United States (*2*−*5*). Approximately half of waterborne disease outbreaks attributable to groundwater consumption in the United States are presumed to have a viral etiology (*6*, *7*). Disease outbreaks related to groundwater contaminated by viruses have also been documented in other parts of the world (*8*, *9*). The U.S. Environmental Protection Agency (EPA) has listed several viruses on its drinking water Contaminant Candidate List, emphasizing that waterborne viruses are a research priority (http://www.epa.gov/safewater/ccl/index.html).

Although the vulnerability of groundwater to virus contamination is now recognized, the occurrence of viruses in confined aquifers has not been explicitly investigated. In the most geographically extensive survey of groundwater virus contamination in the United States, Abbaszadegan et al. (*2*) sampled 448 groundwater sites in 35 states and found that 141 sites (31.5%) were positive for at least one virus type. Whether any of these samples were from confined aquifers is not noted in the study. Powell et al. (*10*) used multilevel piezometers to take depth-specific samples from five deep sandstone aquifers in the U.K., one of which was overlain by thin siltstone and mudstone strata. In this aquifer, samples from a depth of 91 m were positive for coliphages, coliform bacteria, fecal streptococci, and clostridia spores, but human viruses were not present.

The objective of the present study was to evaluate the occurrence of human viruses in the confined Mount Simon sandstone aquifer. In much of south central Wisconsin, the Mount Simon aquifer is approximately 75 m deep and overlain by regionally extensive shale known as the Eau Claire aquitard. Local water utilities routinely case municipal supply wells through the Eau Claire aquitard, assuming that it protects water in the underlying Mount Simon aquifer from pathogens. Although pathways allowing pathogen movement through aquitards have been suspected in aquitard assessments, strong evidence is rare in groundwater studies.

Experimental Section

Site Geology and Hydrogeology. Groundwater was sampled from municipal wells drawing water from the Mount Simon aquifer in Madison, WI, population 220 000 (Figure 1). The

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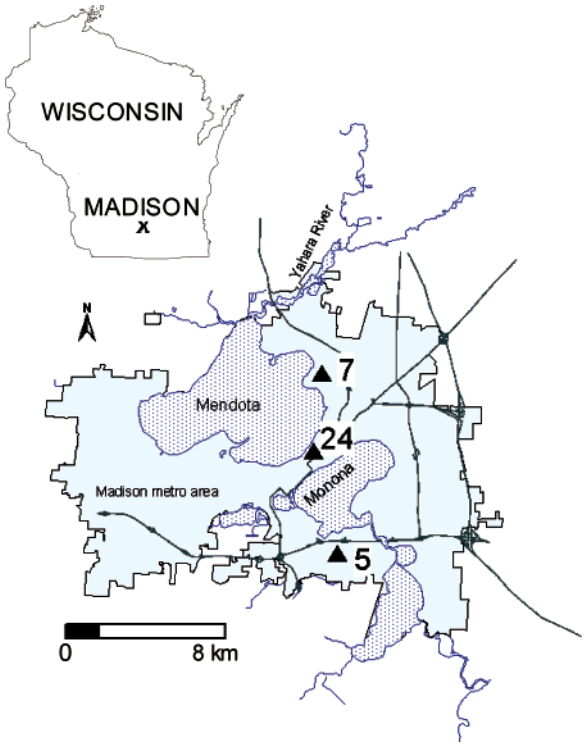

FIGURE 1. Location of study area in south-central Wisconsin. Inset shows the three wells tested in the Madison metropolitan area.

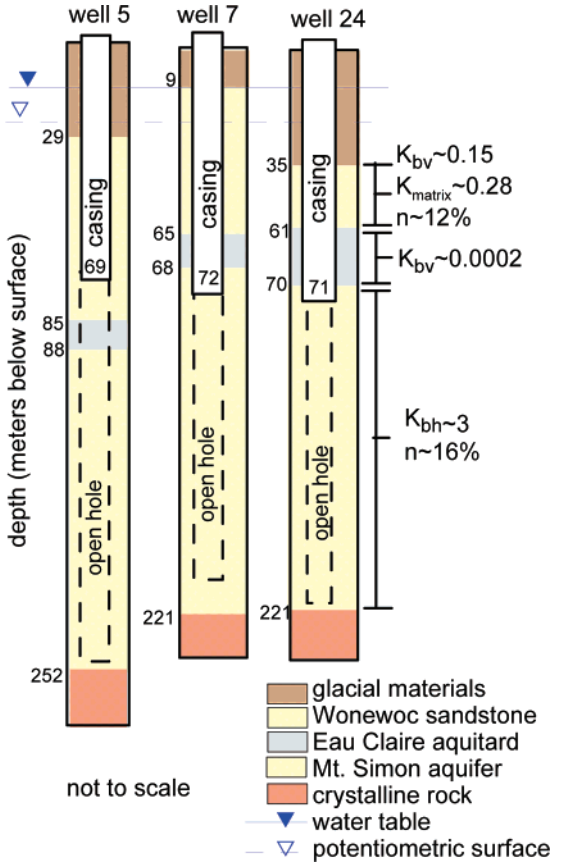

FIGURE 2. General hydrostratigraphy and construction of the three study wells. Reported bulk-vertical, matrix, and bulk-horizontal conductivities (K_{bv}, K_{matrix}, and K_{bh}, respectively, in m/day) and porosity values (n) are from other studies of the regional aquifer (see text).

Eau Claire aquitard occurs about 65 m below ground surface and consists of clayey to sandy siltstone with thin laminae of fine-grained siltstone and shale units. It separates sandstone of the underlying Mount Simon aquifer from an upper unconfined aquifer consisting of glacial deposits underlain by the Wonewoc sandstone (*11*) (Figure 2).

Madison has 19 production wells of which three wells, 5, 7, and 24, were selected for this study. Wells 7 and 24 are located in highly urbanized areas in the center of the city, 970 and 480 m away from the shore of Lake Mendota, respectively (Figure 1). Well 5 is located in a suburban area on the southern boundary of Madison and is adjacent to the municipal sewage treatment plant. Each well produces 3700−7500 m^3 per day. Aquitard thickness is approximately 3 m in wells 5 and 7 and nearly 9 m in well 24. Wells 7 and 24 are cased below the Eau Claire aquitard and draw water only from the Mount Simon aquifer. The casing for the third well, well 5, does not reach the depth of the aquitard and groundwater pumped from this well is likely a mix of waters from both aquifers.

There is substantial hydraulic potential for groundwater to move downward from the glacial deposits toward the deep aquifer. Regional groundwater pumping has caused a 20 m decline in the Mount Simon aquifer's potentiometric surface in the Madison area (*12*). Bradbury et al. (*13*) measured downward vertical hydraulic gradients of 1.8 across the Eau Claire aquitard between the surficial sandstone aquifer and the Mount Simon aquifer.

Virus Sampling and Analyses. Water samples for viruses were collected monthly from each of the three wells for 10 months, from June 2003 through November 2003 and May 2004 through August 2004, for a total of 30 samples. All samples were untreated groundwater collected at the wellheads prior to chlorination. Viruses were concentrated with a 1MDS filter (CUNO, Meriden, CT) following standard virus filtration methods (*14*). The mean sample volume was 1448 L (n = 30, range 844−1889). Filters were eluted with beef extract, and the eluate was flocculated and concentrated with polyethylene glycol following the methods described in Borchardt et al. (*4*).

Samples were analyzed for five virus groups: enteroviruses, rotavirus, hepatitis A virus (HAV), and norovirus genogroups I and II. All viruses were detected by reverse-transcription−polymerase chain reaction (RT-PCR), followed by Southern hybridization to confirm virus identity. Borchardt et al. (*3*, *4*) describe the procedures, primers, and probes. RT-PCR inhibition, which could result in false negatives, was evaluated for all samples by seeding the RNA extraction concentrates with a Norwalk virus RNA internal standard. Inhibition was mitigated by diluting the extraction concentrate 1:5 or 1:10 with nuclease-free water. False positives from virus or amplicon contamination of the samples were avoided by including negative controls for the filter eluent, RNA extraction step, and RT-PCR and hybridization reagents (*3*, *4*).

Samples that were enterovirus positive by RT-PCR were further evaluated for enterovirus infectivity by cell culture following methods previously described (*4*). Cultures were observed for 14 days for viral cytopathic effect (CPE), then aliquoted into new cultures for another 14 day observation period to confirm the first passage results. In addition, after the first 14 day passage, an aliquot of each of the removed cell sheets was analyzed for viral RNA (positive strand) following the same RT-PCR procedures used for the water samples. The same aliquot was also analyzed for negative-strand RNA, following the method of Cromeans et al. (*15*), because it is diagnostic for replicating enteroviruses. Enteroviruses were identified to serotype by nucleotide sequencing following the method of Ishiko (*16*), a method used previously to identify enterovirus isolates from groundwater (*4*).

TABLE 1. Enterovirus Detection Results for Three Drinking Water Wells in Madison, Wisconsin

well	positive/ total	positive sample date	enterovirus identity	BLAST search[a] % identity[b]	BLAST search[a] E-score[c]
5	0/10				
7	4/10	Sept 24, 2003	coxsackievirus B3	100	0.0
		June 30, 2004	echovirus 18	100	0.0
		July 28, 2004	echovirus 18	100	0.0
		Aug 25, 2004	poliovirus sabin 1	100	0.0
24	3/10	Sept 24, 2003	echovirus 9	98	2×10^{-95}
		Nov 19, 2003	coxsackievirus B3	99	0.0
		July 28, 2004	coxsackievirus B1	98	6×10^{-99}

[a] Sequences were submitted for a BLAST search of the GenBank sequence database at the National Center for Biotechnology Information web site. [b] Percent identity is the degree of invariance between the query sequence and the most closely matching sequence posted in the database. [c] E-score is the expected number of better matches with the query sequence that occur in the database by chance. The lower the E-score the better the match.

Isotope Sampling and Analyses. Wells were sampled twice for the isotopes deuterium (^{2}H), tritium (^{3}H), and oxygen-18 (^{18}O). Isotope samples were analyzed at the University of Waterloo, Ontario, Environmental Isotope Laboratory. In addition, one sample from well 24 was tested for low-levels of tritium at the University of Miami, Florida, Tritium Laboratory. Deuterium was determined by manganese reduction; oxygen-18 was determined by mass spectrometry on CO_2 gas. Tritium was determined by liquid scintillation counting on enriched samples (University of Waterloo) and distillation followed by electrolytic enrichment and low-level counting (University of Miami). Tritium results are reported in tritium units (TU; 1 TU equals 1 tritium atom in 10^{18} atoms of hydrogen). Deuterium and oxygen-18 results are reported as per mil (‰) differences from the concentrations in Vienna Standard Mean Ocean Water.

Results and Discussion

Virus Analyses. Of the 30 well water samples collected for virus analyses, seven (23%) were positive for enteroviruses (Table 1). Other enteric viruses tested (rotavirus, HAV, and noroviruses) were absent in all samples. The seven enterovirus-positive samples were taken from wells 7 and 24, which are both cased through the Eau Claire aquitard and draw water from the confined Mount Simon aquifer. The well water samples were enterovirus positive in the summer and autumn months, the same time of year when the incidence of enterovirus infections and their occurrence in wastewater peaks in Wisconsin (*17*). Well 5 was virus-negative throughout the 10 month sampling period, even though this well bore is open to both the upper, unconfined aquifer and the Mount Simon aquifer. Viruses may have been absent because there was not a nearby source of human fecal waste released into the environment.

There are numerous reports of enteroviruses identified in groundwater in the United States, primarily using cell culture detection methods (*7*, *18*). Three recent studies used the RT-PCR method similar to the present study. Abbaszadegan et al. (*2*) investigated 448 wells in 35 states and found that 68 wells (15.2%) were positive for enteroviruses. Six of these positive wells drew water from sandstone aquifers. Fout et al. (*5*) analyzed 321 monthly samples taken over a year from 29 wells located in the continental United States, Puerto Rico, and the Virgin Islands and found 15 samples (5%) and 11 wells (38%) were enterovirus-positive. Borchardt et al. (*4*) tested 48 samples taken monthly over a 1 year period from six shallow wells (<49 m) providing drinking water to a single municipality in Wisconsin. Twenty samples (42%) and six wells (86%) were positive for enteroviruses. In the present study, 23% of the well water samples were enterovirus-positive, a proportion similar to that in the previously published work.

Enteroviruses are common human pathogens estimated to cause 30−50 million infections annually in the United States (*19*). The prefix "entero" is descriptive of their host infection route from entry via ingestion, passage through the enteric system, and excretion in the host's feces. The viruses are small (30 nm diameter) and icosahedral shaped. There are 64 enterovirus serotypes. The diseases associated with enterovirus infections are wide-ranging and include the common cold, nonspecific fever, diarrhea, and a variety of severe illnesses involving respiratory, cardiovascular, or neurologic systems. The most common neurologic disorder is aseptic meningitis, which is estimated to result in 50 000 hospitalizations each year in the U.S. (*19*). How many enterovirus infections are attributable to waterborne transmission is unknown, and for this reason, enteroviruses have been placed on the EPA's drinking water Contaminant Candidate List.

Five enterovirus serotypes were identified in the seven positive samples (Table 1). Two of the identified serogroups, coxsackieviruses and echoviruses, are ubiquitous in the U.S. population and have been previously reported in groundwater (*7*). However, finding the poliovirus vaccine strain Sabin 1 in groundwater was unexpected. Oral administration of the vaccine was discontinued in the U.S. in 2000 to reduce the amount of poliovirus released into the environment via fecal shedding and the possible reversion to wildtype virulent strains. Of the three oral poliovirus vaccine strains, the Sabin type 1 is considered the most stable (*20*). Circulation of vaccine-derived strains in population subgroups has been reported (*21*). A large population of foreign students and their families live in the Madison area to attend the University of Wisconsin. Many of the foreign students' home countries still use the oral vaccine, making them a conceivable source for the poliovirus found in the groundwater. Laboratory contamination of the groundwater samples is highly unlikely because this vaccine strain has never been used in the laboratory where the samples were analyzed.

The seven enterovirus-positive samples were analyzed by cell culture for infectivity. All seven were negative for cytopathic effect. However, five of the CPE-negative cultures were positive by RT-PCR despite being incubated for 14 days at 37 °C and being diluted by washing and refeeding the cell sheets on day 7. This suggests the viruses were replicating but not producing CPE, which is not uncommon for environmental isolates of enteroviruses (*22*, *23*). Another explanation is the viruses did not replicate, but there were enough virions present in the initial cell culture inoculum that they remained adsorbed and intact on the cells until detected by RT-PCR 14 days later.

One of the five RT-PCR positive cultures was positive for the replicating negative RNA strand. Enteroviruses are positive-sense single-stranded RNA viruses. The comple-

TABLE 2. Isotope Results for Three Drinking Water Wells in Madison, WI

well	sample date	TU value	tritium method[a]	δ^2H ‰[b]	δ^{18}O ‰[b]	chloride range[c] (mg/L)
5	June 18, 2003	1.4	enriched	−72.24	−10.65	3−4
	May 12, 2004	<6	standard	−70.65	−10.53	
7	June 18, 2003	8.9	enriched	−57.18	−8.56	9−24
	May 12, 2004	9.9	low-level counting	−57.78	−8.36	
24	June 18, 2003	<0.8	enriched	−60.21	−8.88	4−5
	May 12, 2004	<0.8	enriched	−60.20	−8.80	
	May 12, 2004[d]	1.03	low-level counting			

[a] Enriched method analyzed at University of Waterloo, error ± 0.8 TU; standard method analyzed at University of Waterloo, error ± 0.8 TU; low-level method analyzed at University of Miami, error ± 0.09 TU. [b] Precision of ^{2}H analysis is ± 0.8‰; precision of ^{18}O analysis is ± 0.2‰ [c] Chloride samples collected between 1993 and 1999 and reported by the WI DNR (*26*) [d] Duplicate tritium samples were collected on May 12, 2004.

mentary negative strand is only produced when the virus is replicating. Detecting the negative strand is definitive evidence that the virus in this sample, an echovirus 18 collected from well 7, was infectious.

Isotope and Chloride Analyses. Isotopic compositions and chloride concentrations indicate that groundwater in the sampled wells is relatively young and probably did not originate as recharge from the nearby lakes. The isotopes tritium, oxygen-18, and deuterium have long been used as natural tracers in hydrologic studies to gain insights about groundwater age and origin (*24*). Atmospheric tritium increased dramatically following atomic weapons testing in the 1950s and 1960s. Radioactive decay has reduced the tritium content of groundwater (half-life 12.4 years) recharged over 40 years ago to generally no more than about 0.1 TU today. The tritium concentrations found in well 7 are substantial (Table 2) and in the range of recent precipitation, indicating that most or all of the water from this well entered the groundwater system since the mid-1950s, and possibly much more recently. The other two wells, 5 and 24, contained low but detectable tritium values (Table 2), likely indicating a mixture of post- and pre-1950s water or strong influence of diffusion driven mass transfer of tritium from fractures into the rock matrix (*25*).

Chloride concentrations in the three wells parallel the tritium results (Table 2). Background nonanthropogenic chloride values for the Mount Simon aquifer are less than 1 mg/L, but the concentrations in the three wells sampled are greater than 1 mg/L, with well 7 being the highest (*26*), consistent with tritium. The chloride is likely derived from road salting; substantial road salting in Madison began in the 1950s, and chloride concentrations in the lakes and in some Madison wells have gradually increased since then.

The presence of tritium and chloride in the Mount Simon aquifer is consistent with what is known about the hydrogeologic conditions beneath Madison. Long-term pumping from the Mount Simon aquifer has caused downward flow of recharge water from the surface through the glacial deposits and then through the upper Wonewoc sandstone to the top of the Eau Claire aquitard. Simple one-dimensional advective flow calculations based on Darcy's Law and characteristics of the Wonewoc sandstone indicate that substantial tritium and chloride arrival at the top of the Eau Claire aquitard sometime between a few years and a few decades ago is hydraulically reasonable. Penetration of tritium and chloride across the Eau Claire aquitard is also reasonable. Aquitards commonly contain fractures that provide preferential groundwater flow pathways (*1*). Taken together, the tritium and chloride data show that the bulk of the groundwater pumped from well 7, and some of the water from wells 5 and 24, recharged the aquifer and penetrated beneath the Eau Claire aquitard anytime within the last 30 or 40 years, even possibly within a year or two, and such rapid bulk water travel times are consistent with simple Darcy's Law estimates of advective travel times. However, these travel times are too long to explain the presence of relatively ephemeral viruses detected below the aquitard in wells 7 and 24.

Although on a regional scale, significant recharge probably occurs as downward leakage from the Madison Lakes, the stable isotope compositions show no evidence that water in the three wells originated in the lakes. Stable isotope ratios of water are conservative in aquifers at low temperature, but surface water becomes isotopically fractionated when the humidity is less than 100%. Evaporation preferentially enriches surface water in ^{18}O relative to ^{2}H. As a result, δ^{18}O and δ^2H ratios can be used to identify groundwater sources and understand surface water interaction with wells (*27*). The ratios of oxygen-18 to deuterium for all three study wells did not deviate substantially from the ratio defined by the meteoric water line established for Madison by Swanson et al. (*28*). Water samples from the Madison lakes Mendota, Monona, and Wingra, collected in late June 1995, gave values that were much different than those from wells 7 and 24 (i.e., δ^{18}O ranges between −5.57 and −6.29 ‰ for the lakes). These values, characteristic of water having undergone free-surface evaporation, did not appear in the sampled wells.

Viruses in the Mount Simon Aquifer. The detection of viruses in the confined Mount Simon aquifer beneath the shale aquitard breaks with conventional wisdom and is considered surprising. Or is it? For viruses to be present, there must be pathways allowing rapid transport into the deep aquifer. Transport times must be rapid because virus survival time in the subsurface is on the order of a few weeks to a few years, depending on virus type, water chemistry, microbial interactions, and groundwater temperature (*29*, *30*). The aquifers beneath Madison have an annual mean temperature of 10 °C, favoring longer virus survival times. Nevertheless, the matrix permeability of the shale is too small to allow virus transport within even the upper time limit of virus survival, and therefore, the possibilities for virus transport through the aquitard must involve preferential pathways.

There are four conceptual pathways into the Mount Simon aquifer. Transport vertically through the upper sandstone aquifer followed by (1) transport through fractures in the Eau Claire aquitard, (2) transport through depositional or erosional stratigraphic windows in the Eau Claire aquitard, or the anthropogenic pathways, (3) transport down open wells or boreholes that cross-connect the upper aquifer with the Mount Simon aquifer across the Eau Claire aquitard and (4) transport across the Eau Claire aquitard along the pumping well annulus that is damaged, deteriorated, or has poorly installed grout or breaches in the well casing.

All four pathways must begin with a virus source, and within the Madison city limits, significant human fecal waste is only present in sanitary sewers. Wastewater influent in Wisconsin can contain hundreds to thousands of culturable enteroviruses per liter (*17*). Sanitary sewers may leak, depending on age and pipe material (*31*). Two of the three study wells, wells 7 and 24, are located in densely developed urban areas with numerous sewer lines in proximity. The sewer lines are buried 2−9 m below the surface, a depth not far above the water table which is at 10 m.

Viruses following pathways 1 or 2 would need to traverse four segments in the natural hydrogeological setting: (1) through the glacial deposits to the top of the upper Wonewoc sandstone, (2) through 26−56 m of Wonewoc sandstone to the top of the Eau Claire aquitard, (3) through 3−9 m of shale aquitard to the top of the Mount Simon sandstone, and (4) through the Mount Simon sandstone to the well. The first segment is likely rapid because the glacial deposits include permeable coarse sand and gravel beds, and moreover, in the area surrounding well 7 the top of the Wonewoc sandstone is only 9 m below ground surface. Bounding calculations were performed using Darcy's Law with available hydrogeologic parameters (Figure 2) to assess the reasonableness of the next three transport segments.

The second transport segment is the Wonewoc Formation, a clean, poorly cemented, fine- to medium-grained sandstone. This sandstone's estimated bulk vertical hydraulic conductivity (K_{bv}) (*12*) is similar to its measured matrix hydraulic conductivity (B.L. Parker, written communication), which in conjunction with the poor cementation, suggests that the Wonewoc Formation is dominated by matrix flow at wells 5, 7, and 24. The mean matrix effective porosity of the Wonewoc sandstone is 12% (range 8−17%, $n = 7$) (Parker, written communication). On the basis of these parameters and the vertical gradient induced by pumping (0.02−0.15) (*13*), the estimated travel time through the Wonewoc sandstone is a few months to a few years. Measured pore throat diameters in the Wonewoc are on the order of tens of microns (Parker, written communication), hundreds of times larger than enterovirus diameters, suggesting viruses could easily physically pass through the sandstone matrix. Cores of the Wonewoc appear as loose sand when removed from the drilling rig, and field experiments have shown virus transport in sand can be rapid (*32*).

For viruses to cross the Eau Claire aquitard, the third transport segment, preferential pathways such as fractures or windows must be present. Although fractures in the Eau Claire could not be directly observed, fractures commonly occur in shaley geologic materials (*1*). Field measurements of the bulk K_v of the Eau Claire aquitard have not been conducted; however, Krohelski et al. (*12*) estimated it to be 0.0002 m/day and found that a regional scale numerical model for groundwater flow calibrated well with this value. This value for bulk K_v is orders of magnitude larger than typical matrix values for shale (*33*), and the presence of vertical fractures is one reasonable explanation for this much larger value. Hart et al. (*34*) showed that relatively widely spaced vertical fractures of moderate aperture (50 μm) could account for a two order-of-magnitude increase in bulk over matrix K_v for a shale aquitard in eastern Wisconsin.

There are many examples of rapid transport of colloid particles through fractured materials. McKay et al. (*35*) demonstrated that the viruses PRD-1 and MS-2 move through a fractured clay with apertures ranging between 5 and 30 μm and bulk horizontal hydraulic conductivity ranging between 2×10^{-10} and 2×10^{-6} m/s. The virus velocity was 2−5 m/day, 100−200 times faster than the conservative tracer bromide. McKay et al. (*36*) found that transport velocities of bacteriophage through a column of fractured shale saprolite were similar to velocities calculated using aperture estimates derived from the cubic law. In a field-scale experiment in the same material, McKay et al. (*37*) measured transport velocities for colloids of 5−200 m/day under normal gradient conditions.

Depositional or erosional stratigraphic windows also could provide a route through the Eau Claire aquitard. Recent studies show that the hydraulically resistive part of the Eau Claire formation underneath Madison actually has aquitard characteristics much thinner (0.5−7 m) than previously thought (*13*) and may be entirely absent in some areas. The deep Madison lakes, Mendota and Monona, are depressions formed by erosion through the aquitard during the Pleistocene glaciation, and smaller erosional windows could exist elsewhere in the shale.

Once across the Eau Claire aquitard, virus transport through the Mount Simon sandstone into the pumping wells is feasible. Flow in the Mount Simon likely occurs in the matrix in poorly cemented sections and in bedding parallel fractures in the firmly cemented sections. Vertical and horizontal fractures in the Mount Simon sandstone are visible in optical borehole logs from several Madison wells. The bulk horizontal hydraulic conductivity of the Mount Simon aquifer is approximately 3 m/day (*12*), and the mean effective porosity is 16% (range 8−23%, $n = 4$) (B.L. Parker, written communication). Therefore, velocities for matrix flow through the Mount Simon aquifer range from 69 to 690 m/year for horizontal gradients of 0.01 and 0.1, respectively. Transport velocities in fractures could theoretically be 10−1000 times faster than matrix flow.

The third and fourth conceptual pathways are anthropogenic and specific to historical well construction and abandonment procedures in Madison. Whether any abandoned open well or boreholes remain in Madison is unknown, although they are believed to exist. Modeling studies have shown that entire contaminant plumes can be transported from an upper aquifer, through an aquitard, and into the underlying aquifer by cross-connecting wells or boreholes (*38*). In addition, Hart et al. (*34*) showed that a relatively small number of cross-connecting wells or boreholes could create an order of magnitude or more difference between the vertical matrix and vertical bulk hydraulic conductivities in a shale aquitard.

Last, faulty annular well seals could be responsible for cross-connecting the upper aquifer with the Mount Simon aquifer. Drilling records for virus-positive wells 7 and 24 show they were constructed according to accepted practice, although aging (well 7, 68 years; well 24, 27 years) may have deteriorated the well grout or casing. Meiri (*39*) described where a faulty well seal was responsible for contaminant transport across a clayey aquitard. These anthropogenic pathways could transport viruses to the wells, but the elevated tritium and chloride levels suggest there must be larger inputs of recent recharge to the Mount Simon aquifer that cannot be accounted for by leaky well seals.

The vertical travel distance from the sewers down to the Mount Simon aquifer is 60−65 m, which is not an unrealistic transport depth. Viruses readily move to depths of 30 m, and a depth as great as 67 m has been reported (*40*). A private domestic well cased 52 m in fractured dolomite was positive for enterovirus, rotavirus, and norovirus (*3*). In an urban area in the United Kingdom, Powell et al. (*10*) collected depth-specific samples from an aquifer overlain with several mudstone bands. Beneath these bands at a depth of 47 m, samples were positive for coliphages, coliforms, fecal streptococci, and culturable enterovirusues. The study investigators suggest this microbial contamination resulted from leaky sewers with microbial transport along natural fissures (fractures) and bedding planes.

Determination of the exact transport pathway for viruses to reach the study wells is beyond the scope of the present

study. We have shown there are several plausible pathways. On the basis of current knowledge of the local hydrogeological setting, it is not necessary to invoke anthropogenic pathways to account for the viruses in the Mount Simon aquifer. Detecting viruses in wells 7 and 24 is, perhaps, not so surprising after all.

Implications for the Drinking Water Industry. Hydrogeologists and water utility managers often assume that deep municipal wells, such as those sampled in this study, are protected from microbial contaminants originating at or near the land surface. This is particularly true for wells cased through laterally extensive aquitards composed of clay or shale into deep aquifers. It has been believed that vertical transport times through aquitards were too long and microbial survival time too short for microbial contaminants to reach these confined aquifers (*41*). In the present study, the presence of human enteric viruses in the confined Mount Simon aquifer indicates that viruses are able to penetrate through or otherwise bypass the overlying Eau Claire aquitard. The understanding of hydrology and solute contaminant transport in fractured rock is in its infancy and even less is known about virus transport in fractured rock. The most robust microbial transport models based on colloid filtration theory cannot yet reliably predict virus occurrence in a field setting (*42*). The safest assumption from a public health perspective is that drinking water drawn from a confined aquifer is as vulnerable to microbial contamination as an unconfined aquifer and requires a similar level of disinfection.

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