ground water

Case Study/

Norovirus Outbreak Caused by a New Septic System in a Dolomite Aquifer

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Abstract

Septic systems that are built in compliance with regulations are generally not expected to be the cause of groundwater borne disease outbreaks, especially in areas with thick vadose zones. However, this case study demonstrates that a disease outbreak can occur in such a setting and outlines the combination of epidemiological, microbiological, and hydrogeological methods used to confirm the source of the outbreak. In early June 2007, 229 patrons and employees of a new restaurant in northeastern Wisconsin were affected by acute gastroenteritis; 6 people were hospitalized. Epidemiological case-control analysis indicated that drinking the restaurant's well water was associated with illness (odds ratio = 3.2, 95% confidence interval = 0.9 to 11.4, $P = 0.06$). Microbiological analysis (quantitative reverse transcription-polymerase chain reaction) measured 50 genomic copies per liter of norovirus genogroup I in the well water. Nucleotide sequencing determined the genotype as GI.2 and further showed the identical virus was present in patrons' stool specimens and in the septic tank. Tracer tests using dyes injected at two points in the septic system showed that effluent was traveling from the tanks (through a leaking fitting) and infiltration field to the well in 6 and 15 d, respectively. The restaurant septic system and well (85-m deep, in a fractured dolomite aquifer) both conformed to state building codes. The early arrival of dye in the well, which was 188 m from the septic field and located beneath a 35-m thick vadose zone, demonstrates that in highly vulnerable hydrogeological settings, compliance with regulations may not provide adequate protection from fecal pathogens.

Introduction

Despite significant improvements in the sanitary quality of drinking water in the United States since passage of the Safe Drinking Water Act in 1974, disease outbreaks continue to result from pathogen-contaminated groundwater. There were 50 water borne disease outbreaks associated with drinking water in the United States between 2003 and 2006 of which 16 were caused by pathogen contamination at the drinking water source. The source in the majority of these outbreaks, 14 (88%), was contaminated groundwater, resulting in 2288 illnesses (Liang et al. 2006; Yoder et al. 2008). Reporting of water borne outbreaks by each state is voluntary, and the actual number of outbreaks is likely greatly underreported (Craun et al. 2006).

Pathogen-contaminated groundwater is often attributed to septic systems failing from age or neglect, although only a handful of outbreak investigations have, in fact, implicated this source. Unequivocal evidence linking a specific septic system with an outbreak is rare. A large

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outbreak at a restaurant in the Yukon Territory of Canada was clearly linked to well water contamination by a wood-lined septic pit (Beller et al. 1997). On the other hand, new septic systems that are properly constructed according to the building code and approved by inspection are assumed to prevent pathogen transport to groundwater, casting them as improbable pathogen sources for an outbreak. Whether this is a sound assumption for all hydrogeological settings has not been fully evaluated.

Water supply wells in fractured rock and karst settings are particularly vulnerable to contamination from surface sources because groundwater flow rates in such media can be very rapid and contaminant attenuation is often minimal. Major disease outbreaks have been documented in such settings in the past (Worthington et al. 2002; O'Reilly et al. 2007). Even though such vulnerability is well established, onsite septic systems are commonly approved for such areas as long as overlying soils meet minimum thickness and grain-size requirements (e.g., Wisconsin Department of Commerce 2001). Often suspected of contaminating nearby wells, septic systems with direct links to disease outbreaks are usually difficult to prove because such proof requires parallel and simultaneous data on source and receptor concentrations, epidemiology, and groundwater flow. To the authors' knowledge, Beller et al. (1997) is the only previous study to have used epidemiological data, pathogen molecular fingerprinting, and dye tracers to clearly link a groundwater borne outbreak with a septic system. However, the hydrogeological setting and groundwater flow were not characterized in this investigation.

Noroviruses are responsible for approximately 50% of all gastroenteritis outbreaks worldwide (Patel et al. 2009). Symptoms include acute vomiting and diarrhea usually lasting 2 to 3 d. In developed countries like the United States, approximately 900,000 children less than 5 years old are norovirus infected annually, of which 64,000 (12%) are hospitalized (Patel et al. 2008). Deaths from norovirus infections in developed countries are not uncommon, particularly in the elderly (Harris et al. 2008). The infectious dose is low, less than 10 virions (Teunis et al. 2008), and immunity is short lived (Patel et al. 2009), resulting in outbreaks that spread rapidly and involve large numbers of individuals. The viral particle is small, 30 to 38 nm, and stable in the environment (Farkus and Jiang 2007; Charles et al. 2009), attributes that favor norovirus transport and survival in groundwater. Consumption of fecal-contaminated groundwater has resulted in a number of norovirus outbreaks (Wilson et al. 1982; Lawson et al. 1991; Beller et al. 1997; Parshionikar et al. 2003; Kim et al. 2005; Gallay et al. 2006; O'Reilly et al. 2007), although in many of these the contamination source was never identified.

We describe the investigation of a large norovirus outbreak at a newly opened restaurant with a private well located in the fractured rock setting of northeastern Wisconsin. As the unsaturated zone was 35 m thick and the restaurant septic system was new and complied with state codes, it was dismissed as a possible source of the outbreak when the investigation commenced. However, data provided by a suite of interdisciplinary methods all converged to indicate the new septic system was indeed to blame. Many groundwater investigations focus on processes of, or risks associated with, groundwater contamination, but only infer impacts on public health. The virus study reported here demonstrates the combined use of hydrogeological, microbiological, and epidemiological lines of evidence to prove a direct link between a groundwater contamination source and a human disease outbreak.

Hydrogeological Setting

The restaurant and study area are located in the Door Peninsula of northeast Wisconsin (Figure 1). The Door Peninsula is about 100 km in length and 10 km wide and separates Lake Michigan on the east from Green Bay on the west. Silurian-age dolomites form the peninsula and dip gently to the east. The dolomite sequence ranges in thickness from 0 to more than 150 m (Sherrill 1978). These rocks form an important regional carbonate aquifer, bounded on the east and west by Lake Michigan and Green Bay and bounded below by shale of the Maquoketa Formation, a regional aquitard. The local climate is generally cool and moist, with an average annual precipitation of 80 cm and average annual temperature of 6.4 °C.

The Door Peninsula has long been known as an area having high vulnerability to groundwater contamination (Sherrill 1978; Bradbury 2003). Over broad areas of the peninsula soils are thin or absent, and groundwater recharge can be rapid, with little attenuation of surface contaminants. Much of the region is rural, and local residents rely on groundwater as a source for water supply.

Numerous past studies have characterized the dolomite aquifer in the Door Peninsula (Sherrill 1978; Muldoon et al. 2001; Rayne et al. 2001). The dolomite is densely fractured, and secondary dissolution has enlarged both fracture apertures and secondary porosity. Groundwater flow is characterized by recharge through vertical fractures and rapid lateral movement along near-horizontal high-permeability zones. These high-permeability zones appear to be continuous on the scale of kilometers and usually form the contributing zones for local water wells. Lateral groundwater velocities in the region can be rapid. Muldoon and Bradbury (2005) measured velocities up to 35 m/d in natural-gradient tracer experiments, and Rayne et al. (2001) predicted velocities up to 65 m/d using a regional numerical model. Using temperature, electrical conductivity, and environmental isotopes, Bradbury et al. (2002) documented very rapid (4 to 35 m/d) vertical movement of water immediately following recharge events. Although numerous small (centimeter-scale) solution features occur in the region, classic karst features such as large sinkholes and caves are relatively rare. Accordingly, an operable conceptual model for groundwater flow in the region consists of rapid recharge through a thin soil zone, rapid vertical movement along vertical fractures, and rapid horizontal movement along regional-scale near-horizontal fractures.

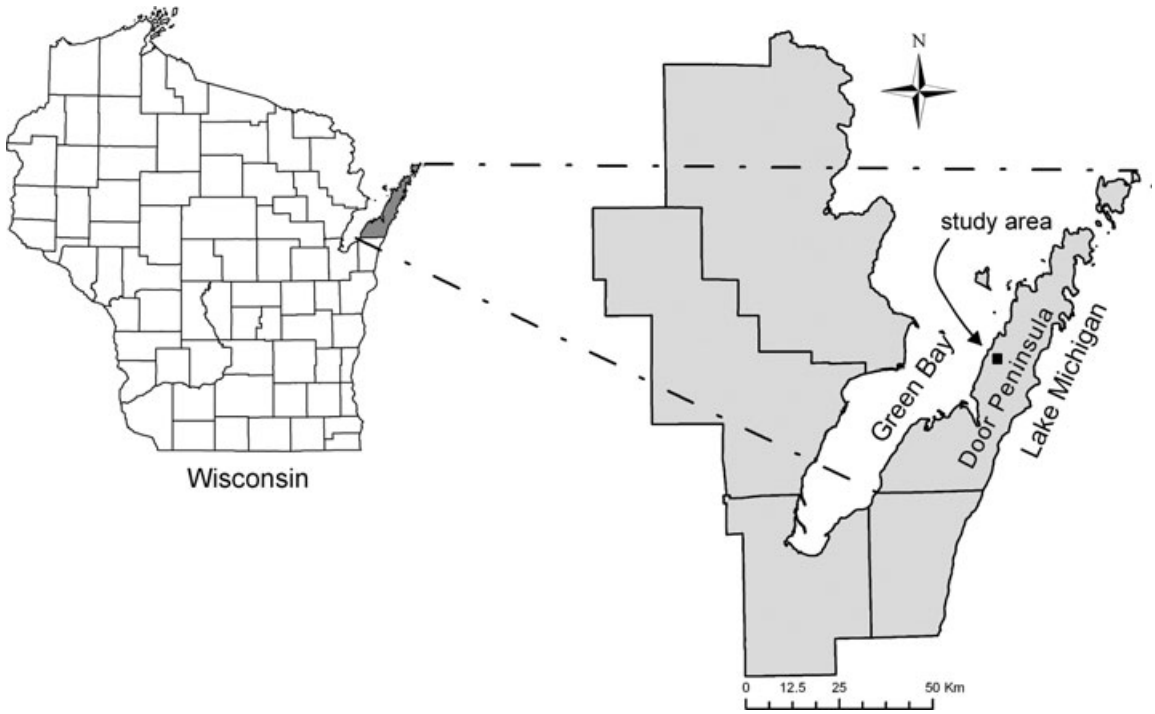

Figure 1. Location of the restaurant study area in northeast Wisconsin.

Outbreak Background

On June 1, 2007, the Door County Public Health Department (DCPHD) in the state of Wisconsin received an initial report that four employees at a new large (300-seat) restaurant were ill with acute gastroenteritis. The restaurant had first opened to the public for business 3 weeks earlier, on May 10, 2007. Both the private well and septic system servicing the restaurant were also newly constructed and conformed to Wisconsin State Code. The restaurant closed voluntarily on June 1 as the outbreak investigation commenced.

The restaurant septic system is a conventional design consisting of two large concrete settling tanks in which effluent moves by gravity to a dosing tank. Effluent in the dosing tank is pumped to a subsurface infiltration field, buried approximately 1 ft below the land surface, where effluent moves through the infiltration tubing by gravity. Kitchen wastewater is piped separately to two grease interceptor tanks upstream of the settling tanks; the second grease interceptor tank also serves as a flow equalization tank.

The restaurant well is 85.3-m deep and cased to 51.8 m. It is 188 m from the septic leach field. Typical of a groundwater source, noncommunity, drinking water system in the United States, the water was not treated prior to consumption.

Methods

Epidemiological Investigation

Cases of acute gastrointestinal illness were defined as individuals who experienced diarrhea or vomiting within 2 to 5 d after consuming food or beverages at the restaurant between the opening date (May 10, 2007) and the last day of service before closing (May 31, 2007). Cases were identified from phone calls made by ill patrons to DCPHD and the local hospital; controls were family members or friends of cases who visited the restaurant on the same dates but were not ill. Interviews were conducted by DCPHD nurses using a standardized outbreak questionnaire from the Wisconsin Division of Public Health that was modified to include specific menu items. Stool specimens were requested from symptomatic patrons and employees and submitted to the Wisconsin State Laboratory of Hygiene for enteric pathogen analysis. Odds ratios (OR) to identify food and drink exposures related to illness were calculated using Epi Info version 3.3.2 (Su and Yoon 2003).

Microbiological Investigation

Tap water samples were collected from the restaurant and nearby homes by trained technicians on various dates during the investigation. Samples for coliform and *Escherichia coli* bacteria were collected aseptically in 1-L sterile bottles and analyzed by the Wisconsin State Laboratory of Hygiene using Colilert® (IDEXX Laboratories Inc., Westbrook, Maine) as described in section 9223B in Standard Methods for the Examination of Water and Wastewater (American Public Health Association 2005). Human enteric viruses were concentrated from tap water by glass wool filtration (Lambertini et al. 2008); mean sample volumes from the restaurant and homes was 932 L (range 655 to 1666 L, $n = 4$) and 655 L (range 492 to 803 L, $n = 11$), respectively. Viruses were eluted from

glass wool filters and further concentrated by flocculation with polyethylene glycol (PEG) following previously described procedures (Lambertini et al. 2008). The equipment blank control for virus concentration and elution was confirmed to be negative. The final concentrated sample volumes from which viruses were quantified were approximately 2 mL. One 3 L sample for virus analyses was collected from the effluent in the septic tank dosing chamber just prior to the infiltration field. Viruses in this sample were concentrated by direct PEG flocculation without glass wool filtration.

Quantitative polymerase chain reaction (qPCR) was performed for six groups of human enteric viruses: adenovirus, enterovirus, hepatitis A virus, norovirus genogroup I (GI) and genogroup II, and rotavirus. Reverse transcription (RT) of the RNA viruses (all except adenovirus) was conducted in a separate reaction. All qPCR assays were performed in a 96-well plate format on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) using PCR mixes prepared with the LightCycler DNA master hybridization probe kit (Roche Diagnostics) with fluorescence generated by TaqMan probes (TIB Molbiol, Berlin, Germany). Procedures for nucleic acid extraction, RT reagents, primers and probes, PCR inhibition controls, and standard curves are described in detail in Lambertini et al. (2008). However, the norovirus analyses in the present study had several changes. Norovirus cDNA was created using random hexamers (Promega, Madison, Wisconsin) and the qPCR thermal conditions were a hot-start polymerase activation step for 10 min at 95 °C, followed by 45 cycles of 15 s at 94 °C and 1 min at 60 °C. The primers and probes for norovirus qPCR were the same as those described in Jothikumar et al. (2005).

Norovirus genotyping was performed by sequencing a product from a separate PCR targeting a 327 base pair region of the polymerase gene using primers JV12 and JV13 (Vinje and Koopman 1996). Amplified DNA detected by gel electrophoresis was gel purified (QIAquick PCR Purification Kit; Qiagen, Valencia, California) and sequenced in both directions using the same primers as those for the PCR with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and the ABI Prism 3100 Gene Analyzer. Consensus sequences were constructed with DNAStar (Madison, Wisconsin), and database searches were run using the BLAST (Basic Local Alignment Search Tool) service provided by the National Center for Biotechnology Information (NCBI, Bethesda, Maryland). Multiple sequence alignments were carried out with the MegAlign program in DNAStar.

Hydrological Investigation

A natural-gradient tracing experiment using fluorescent dyes (Alexander et al. 2008) was conducted to evaluate potential subsurface connections and travel times from the restaurant septic system to the restaurant well and several local domestic wells. Based on a hypothesis that the septic system plumbing might be leaking, two different dyes were introduced at two different points in the system (Figure 2). Injection point 1 was a toilet inside the restaurant. Injection point 2 was the dosing chamber at the end of the chain of septic tanks and adjacent to the pipe leading out to the septic infiltration field. The dosing chamber contains a pump that moves (or "doses") effluent from the septic system to the infiltration field. Inside the septic system, point 2 was hydraulically downgradient of point 1, and dye injected at point 2 was expected to reach the infiltration field ahead of dye injected at point 1. Eosin dye was added at point 1 and fluorescein dye was added at point 2 on September 21, 2007. The dosing chamber pumped a dose of dye and effluent toward the infiltration field immediately after the fluorescein was added.

Fluorescein and eosin were purchased as dye concentrates from Chromatech Inc. (Canton, Michigan). Chromatint Uranine HS Liquid (fluorescein) stock number D11006, lot number 082207C (35% dye by weight), and

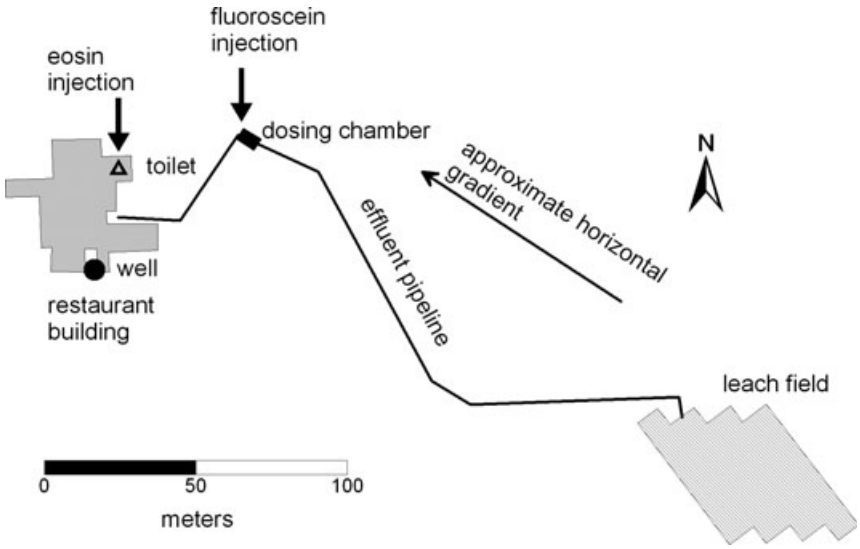

Figure 2. Site layout of the restaurant, well, and septic system.

Chromatint Red 0143 (eosin) stock number D13802, lot number 020706 (33% dye by weight), were repacked into appropriately sized HDPE containers for transport to the dye input site. The injection at point 1 consisted of 4 kg eosin dye (10.9 L liquid dye concentrate). The injection at point 2 consisted of 2 kg fluorescein dye (4.77 L liquid dye concentrate). Split samples of each dye were diluted and injection dye content was confirmed by spectrofluorophotometric analysis.

Two types of samples were collected to monitor the movement of the fluorescent dyes through this system. Monitoring for the dye tracing test included a time series of direct water samples from the restaurant well and a series of activated charcoal detectors installed in the water systems at several upgradient and downgradient domestic wells. The detectors were installed in the water reservoir on the back of the most used toilet in each house. Charcoal detectors are integrating samplers that recorded the passage of dye at any time during the interval they were in the water but did not yield quantitative data on the dye concentration. Direct water samples allowed quantification of dye concentration but only at the time the sample was collected. Water samples were collected in 4-mL borosilicate glass vials with polyethylene closures. The activated carbon detectors, also known as "bugs," were constructed with 2 g of 6 to 12 mesh, type AC coconut charcoal enclosed in a 2 × 4 in section of milk filter sock material sealed at both ends with stainless steel closures.

Dye concentrations were analyzed using a Shimadzu RF-5000 scanning spectrofluorophotometer at the University of Minnesota, Department of Geology & Geophysics Dilute Solutions Laboratory. Direct water samples were run in their 4-mL glass vials. A portion of the carbon samples were removed from the milk filter sock packet and placed into a 16 × 125 mm disposable test tube for elution. The eluent solution was 70% HPLC/ACS grade isopropyl alcohol and 30% high-purity deionized water saturated with 10 g/L ACS grade sodium hydroxide. Ten milliliters of eluent were poured over each sample and allowed 1 h to extract any fluorescent dye present on the carbon. The resulting eluate was transferred with a disposable pipette to a 13 × 100 mm disposable borosilicate test tube for analysis. The resulting spectra were emission referenced with $\Delta\lambda$ of 15 nm, 5 nm bandwidths, a scan rate of 30 nm/s, and 0.02-s response time. Samples were scanned from 400 to 650 nm easily covering the expected peak fluorescence ranges of eosin and fluorescein dye. All spectra were fitted using PeakFit™ version 4.0 nonlinear curve fitting software (Systat Software Inc. 2004). Alexander et al. (2008) present additional details about the tracer experiment.

Results

Epidemiological Investigation

Of the individuals reporting illness to DCPHD, 211 patrons and 18 food workers met the case definition. In addition, there were 15 probable cases and 8 secondary cases (Table 1); 4 ill patrons were excluded from the analysis because they ate at the restaurant after the defined exposure period. Illness onset peaked on June 1 and by June 6, 6 d after the restaurant closed; no more illnesses were reported (Figure 3). Illness duration was between 1 and 7 d and included symptoms of nausea, vomiting, diarrhea, and abdominal cramping (Table 1). The majority of patron cases were adult and female. Six primary cases and one secondary case were hospitalized. Of 18 stool specimens analyzed for enteric pathogens, 4 were positive for norovirus, 1 was positive for *Campylobacter* spp., and 1 was positive for *Salmonella enterica* subspecies *enterica*.

ORs were calculated for 45 restaurant menu items of which 5, when consumed by patrons, were associated with illness (Table 2). Four of the items were related to water exposures: a glass of drinking water, ice, house salad washed at the restaurant, and alcoholic beverages. The latter was the only item negatively associated with illness; patrons who consumed alcohol were less likely to have become ill. Consuming steak was the only food exposure that was strongly associated with illness that

Table 1
Illness and Demographic Characteristics of People Reporting Acute Gastrointestinal Illness to Door County Health Department, Wisconsin, May 10 to June 5, 2007

Group	Number of People (%)[1]
Patrons	211 (84)
Staff	18 (7)
Secondary cases[2]	8 (3)
Probable cases[3]	15 (6)
Total	252
Patrons characteristic ($n = 211$)	
Female	124 (59)
Age (years)	
Median	60
Range	5–92
Incubation period (h)	
Median	36
Range	8–72
Illness duration (d)	
Median	2
Range	1–7
Symptoms	
Nausea	170 (81)
Vomiting	133 (63)
Diarrhea	181 (86)
Bloody diarrhea	3 (1)
Abdominal cramps	142 (67)
Fever	82 (39)
Hospitalized	6 (3)
Deaths	0 (0)

[1]Number of people unless otherwise noted.
[2]Defined as members of the same household who met the case definition but did not eat at the restaurant.
[3]Defined as people who ate at the restaurant and reported illness but without diarrhea or vomiting.

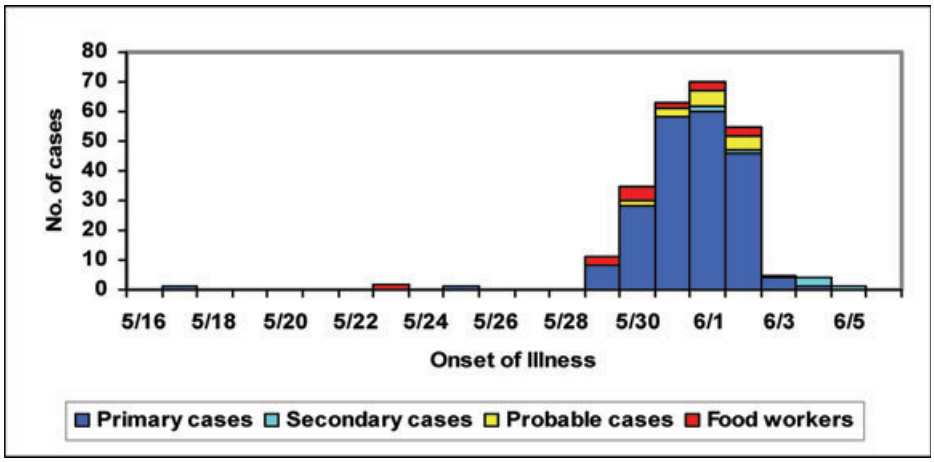

Figure 3. Epidemic curve of the outbreak, May 17 to June 6, 2007.

did not have a clear connection with the restaurant's water. Drinking soda was not found to be an illness risk factor (OR = 1.7, 95% confidence interval = 0.4 to 6.4, $P = 0.34$).

Microbiological Investigation

The restaurant well water was sampled on eight dates for microbiological analyses during the outbreak investigation and these results are reported in Table 3. Prior to opening to the public, the Health Department on April 27, 2007 tested the restaurant's water for bacterial indicators and four samples were negative. On June 1, 2007, the date the outbreak was reported and the restaurant closed, water and ice samples were indicator negative, likely because the well had been chlorinated earlier that day and ice is not an ideal medium for recovering bacteria. Seven water samples collected on June 5 and 6, 2007, from various restaurant taps (kitchen, basement, and outdoor) were all positive for coliform and *E. coli* bacteria; three samples collected on the latter date proved positive for norovirus GI at an average concentration of 50 genomic copies per liter. These samples were negative for the five other virus groups tested. The well was chlorinated again on June 7, 2007, eliminating bacterial indicators in one subsequent sample until a positive sample on June 12, 2007.

Ultraviolet (UV) disinfection delivering a dose of 40 mJ/cm^2 was installed on the restaurant's main water line from the well on June 15, 2007. All subsequent bacterial indicator samples collected immediately downstream of the UV unit were negative, although raw well water samples remained coliform positive through July (Table 3) and until the end of October 2007 (data not shown).

A second round of virus sampling was undertaken on June 28, 2007, and norovirus GI was still present in the raw well water, in the clarified septic effluent just before the septic infiltration field, and in a UV-treated sample (Table 3). This latter sample was likely positive because UV irradiation will inactivate a pathogen while leaving its nucleic acid amplifiable by PCR (Simonet and Gantzer 2006).

The private wells of three homes located upgradient from the groundwater flow path to the restaurant well and one home downgradient were sampled for viruses on June 15, 2007. One well was virus negative, one well was positive for enteroviruses, and two wells were positive for both enteroviruses and adenoviruses (Table 3). None of the private wells were positive for norovirus GI, suggesting that the virus source was restricted to the restaurant site.

Table 2
Associations Between Illness and Restaurant Menu Items as Determined from Cases and Controls Completing the Investigation Interview

Food or Drink Item Consumed[1]	Number of Cases (%), $n = 32$	Number of Controls (%), $n = 24$	OR (95% CI)	P
House salad	14 (44)	3 (13)	5.4 (1.3–22.0)	0.012
Steak	9 (28)	0 (0)	Undefined	0.004
Water	27 (84)	15 (63)	3.2 (0.9–11.4)	0.06
Ice	25 (78)	14 (58)	2.6 (0.8–8.2)	0.097
Alcohol beverages	9 (28)	13 (54)	0.3 (0.1–1.0)	0.045

[1]Only food items that were associated ($P \leq 0.1$) with illness are listed. Forty other food items were questioned and found to be unassociated with illness. CI = confidence interval.

Table 3
History and Results of Virus and Bacterial Indicator Testing of Well Water Samples Collected During the Outbreak Investigation

Sample Date	Sample Location	Virus Type Detected	Virus Concentration (genomic copies per liter)	Total Coliform	*E. coli*	Number of Indicator Samples Tested
April 27, 2007	Restaurant tap	ND[1]	—	Absent	Absent	4
June 1, 2007	Restaurant tap	ND	—	Absent	Absent	2
	Restaurant melted ice	ND	—	Absent	Absent	1
June 5, 2007	Restaurant tap	ND	—	Present	Present	4
June 6, 2007	Restaurant tap 1	Norovirus GI	34	Present	Present	3
	Restaurant tap 2	Norovirus GI	45			
	Restaurant tap 3	Norovirus GI	70			
June 11, 2007	Restaurant tap	ND	—	Absent	Absent	1
June 12, 2007	Restaurant tap	ND	—	Present	Present	1
June 15, 2007	Home 1	Adenovirus	0.02	ND	ND	
		Enterovirus	0.1			
	Home 2	Enterovirus	0.5	ND	ND	
	Home 3	Negative	—	ND	ND	
	Home 4	Adenovirus	0.06	ND	ND	
		Enterovirus	0.2			
	Restaurant post-UV	ND	—	Absent	Absent	5
June 28, 2007	Restaurant pre-UV	Norovirus GI	8	ND	ND	
	Restaurant post-UV	Norovirus GI	0.3	ND	ND	
	Restaurant septic tank	Norovirus GI	79,600			
July 30, 2007	Restaurant pre-UV	ND	—	Present	Absent	1
	Restaurant post-UV	ND	—	Absent	Absent	1
July 31, 2007	Restaurant pre-UV	ND	—	Present	Absent	1
	Restaurant post-UV	ND	—	Absent	Absent	4

[1]ND = not done.

Norovirus genotyping was conducted on three restaurant tap water samples, three stool samples from symptomatic adults, and the single sample from the restaurant's septic system. All seven norovirus isolates had identical 327 base pair sequences of the polymerase gene, indicating connected movement of the virus among well water, septic system, and ill patrons. Compared to other noroviruses listed in the NCBI GenBank database, the norovirus from this outbreak was most closely related to the GI.2 Southampton strain (91% identity, with the primers removed for comparison). The nucleotide sequences of the noroviruses from the three sources, well water, stool specimens, and septic system, have been submitted to GenBank and assigned accession numbers GQ330906, GQ330907, and GQ330908.

Hydrogeological Investigations

The restaurant supply well (Wisconsin unique well number OV691) is typical of low-capacity water wells in northeastern Wisconsin (Figure 4). The well is 85.3 m deep, with a nominal diameter of 15.2 cm. A steel casing extends from the ground surface to 51.8 m, and the annular space outside the casing is filled with neat cement grout. Below the casing, the hole is open to the surrounding rock. According to the well construction report, there is no surface soil at the well location, with bedrock extending to just below the surface. The well conforms to Wisconsin Administrative Code for proper well construction.

During geophysical logging in June 2007, the static water level was 34.8 m below the ground surface. A previous measurement taken just after the well was drilled, in November 2001, showed that the static water in the well was 48.9 m. Comparison of these two measurements indicates the water level in the well fluctuates over a range of at least 14 m. This range of fluctuation is typical of wells of similar construction in central Door County and demonstrates the dynamic nature of the local groundwater flow system, rapid recharge, and less effective porosity.

The restaurant well encounters at least one significant horizontal fracture feature that correlates with known regional fracture zones in the county. Muldoon et al. (2001) correlated stratigraphic and hydrogeological features in the subsurface across Door County using a combination of geophysical logging and stratigraphic analysis. The natural gamma log (Figure 4) obtained from the restaurant well correlates to an earlier gamma log over the depth interval between 50 and 132 m in a nearby core hole (Dr-394) described in detail by Muldoon et al. (2001). Using this log as a key, the restaurant well is open to the Mayville and Byron dolomites, both of Lower Silurian age. The caliper log from the restaurant well shows a significant anomaly at 75.5-m depth, where the hole diameter increases from the nominal

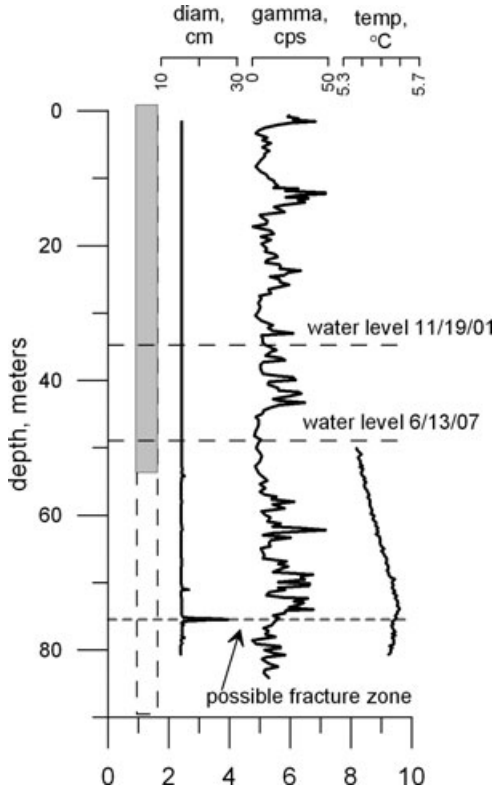

Figure 4. Construction details of the restaurant well, showing borehole geophysical logs.

15.2 to more than 27 cm. A change in the slope of the borehole fluid temperature log at the same elevation (Figure 4) shows that groundwater enters or leaves the borehole preferentially at this point. In Door County, such caliper and/or temperature "kicks" usually occur where major bedding-plane fractures intersect boreholes (Sherrill 1978), and these bedding-plane fractures or fracture zones can be traced laterally from many kilometers (Muldoon et al. 2001).

The hydraulic conductivity of the fracture zone at 75.5 m is estimated to be about 4×10^{-4} cm/s using the program TGUESS (Bradbury and Rothschild 1985) to predict hydraulic conductivity based on reported specific capacity. For comparison, Muldoon et al. measured hydraulic conductivity of about 1×10^{-4} cm/s in the same stratigraphic interval using short-interval straddle packers.

Both the eosin and fluorescein tracers arrived at the restaurant supply well in relatively short time periods and were easily distinguished from background values (Figure 5). The breakthrough of eosin occurred 6 d after tracer injection, and the eosin peak arrived 34 d after injection. The first breakthrough of fluorescein occurred 15 d after injection, and the fluorescein peak arrived 38 d after injection. Continued detection of both dyes occurred until sampling at the restaurant ended on December 20, 90 d into the test. The continued presence of dye in the restaurant well following chlorination of the restaurant well (which destroyed all dye in the well bore) on December 11 shows that dye was still being delivered to the well by the aquifer at that time.

After water sampling at the restaurant ended, the charcoal sampler monitoring of the wells and spring continued into the first half of 2008. Dye was detected on the charcoal samplers at four nearby domestic wells and an ephemeral spring (Figure 6). At well B, fluorescein was detected on the sampler 113 d after injection and eosin was detected later. At well D, fluorescein was detected 106 d after injection, and both fluorescein and eosin were detected 113 d after injection. At well A, both dyes were detected starting on February 19, 2008, 151 d after injection. At well K, both dyes were detected starting on April 1, 2008, 193 d after injection. The ephemeral spring was dry at the start of the dye traces but began flowing during March 2008 and both dyes were then detected at the spring. The detection of fluorescein at downgradient wells B and D shows that effluent from the septic system infiltration field reached these wells in about 110 d.

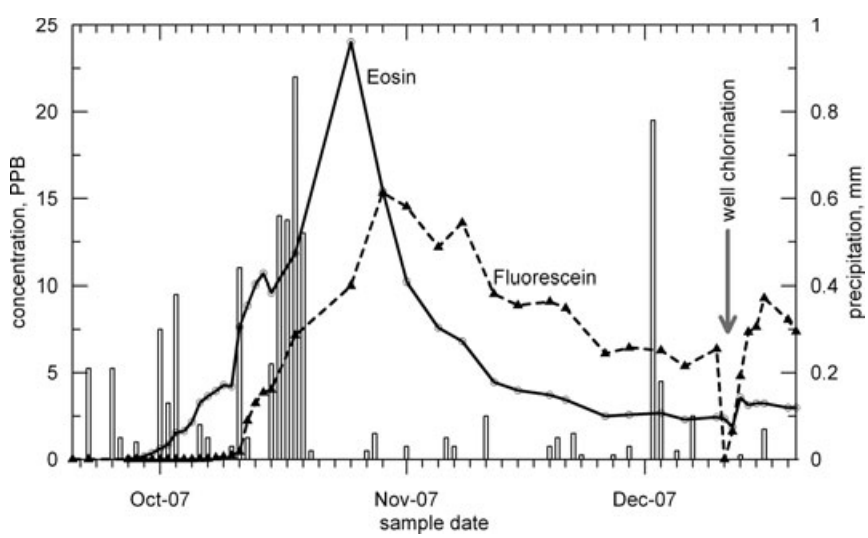

Figure 5. Tracer concentrations in the restaurant supply well.

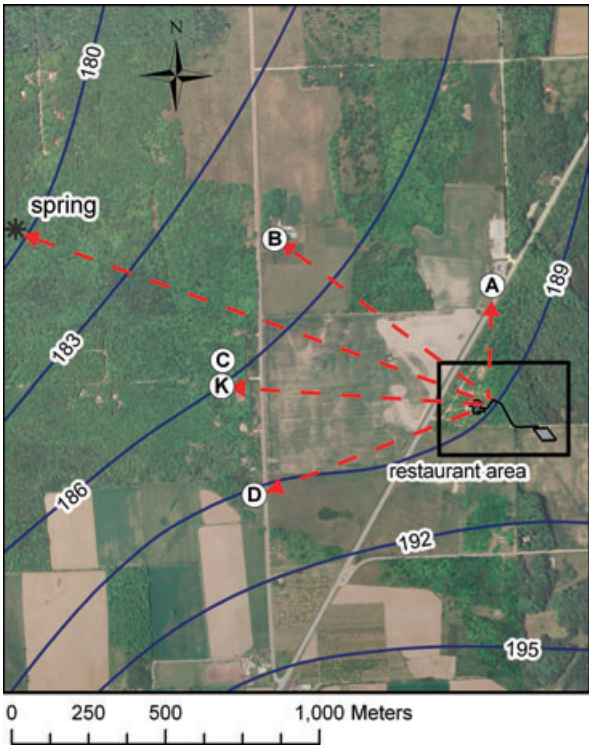

Figure 6. Dye trace detections at offsite wells. Points A, B, C, D, and K represent domestic wells sampled for dyes. Contours represent average potentiometric levels in meters above sea level. Red lines show point-to-point tracer pathways. Restaurant is shown in detail in Figure 2.

Discussion

When the outbreak was first reported and possible sources of the illness were discussed among the investigative team, the restaurant septic system seemed an unlikely source. It was new, installed by a licensed plumber, County sanitarians had reviewed the construction plans and inspected the installation, and the system was fully conforming to Wisconsin Administrative Code (Chapter Comm 83 2000). Once it was learned the restaurant well was positive for *E. coli*, all attention turned to neighboring houses. Using potentiometric surface data (Sherrill 1978), the Door County Soil and Water Conservation Department delineated the restaurant's zone of contribution and downgradient discharge area and selected parcels for well testing and sanitary surveys. These activities did not identify a nearby contamination source. Piece by piece, as data became available from methods in epidemiology, microbiology, and hydrogeology did the weight of evidence point toward the restaurant's own septic system.

Without this interdisciplinary approach, it is unlikely the source of the outbreak would have been uncovered. The positive *E. coli* tests showed the well had fecal contamination, but a positive indicator result does not consequently mean an enteric pathogen is also present (Borchardt et al. 2003a; Locas et al. 2007). The epidemiological case-control study strongly suggested pathogen exposure occurred via the restaurant water; however, as powerful as case-control designs are in identifying relevant exposures, they are not capable of determining how contamination occurred in the first place. The microbiological investigation identified the etiologic agent as norovirus GI in the well and discovered the same virus strain present in the septic system and in the diarrheic stool of ill patrons. Whether the restaurant septic system and well were truly hydraulically connected or if another upgradient source was contaminating the restaurant well remained indeterminate. The final piece of the puzzle was the dye tracer test showing effluent traveling from both the septic tanks and infiltration field was somehow reaching the well. Each method alone would not have provided sufficient evidence to prove the new septic system was the cause of the outbreak. Even if the dye trace had been the only test conducted, key information necessary for showing a definitive link would have been unknown such as whether the septic system and well water indeed contained an enteric pathogen or whether the ill patrons had, in fact, consumed the well water and were exposed. Groundwater professionals asked to lend their expertise in future outbreak investigations need to understand the strengths and limitations of these approaches.

A forensic investigation in March 2008 showed that the second settling tank just upstream from the dosing tank was leaking. Excavation revealed a torn reducer fitting on a pipe near the top of the settling tank that connected to the flow equalization tank (Ayres Associates 2008). The stone aggregate surrounding the pipe was stained with effluent and fluorescing eosin was observed on the aggregate when it was placed under UV light. The eosin flushed down the toilet must have escaped through the leak, moved through the gravel bedding between the tank and bedrock, and once in the fractured dolomite was able to move in 6 d to the well, ahead of the fluorescein injected at the dosing tank. Effluent contaminated with GI norovirus likely followed the same pathway.

But how could fluorescein have reached the well when the dye was introduced into the dosing tank downstream from the leak? The dosing tank and pipe to the infiltration field were not leaking, and the dosing pumps were delivering effluent to the field at the correct design specifications (Ayres Associates 2008). The answer is the soil conditions under the infiltration field must be inadequate for preventing fluorescein from rapidly (in 15 d) moving to the well. The infiltration field is located on a slight ridge above the restaurant's elevation. Test pits dug on the ridge as part of the site approval process, and after the outbreak as part of the forensic investigation, all showed sandy loam topsoil underlain with sandy clay loam subsoil. Beneath the subsoil beginning at 0.76 to 1.37 m depth, there was glacial till composed of sandy loam, gravel, and large cobbles; bedrock was not reached at 3.05 m, the deepest pit depth. The site was deemed conforming to the state's guidance document, "In-Ground Soil Absorption Component Manual for Private Onsite Wastewater Treatment Systems" (Wisconsin Department of Commerce 2001), and the forensic investigation concluded that the 15-d travel time of the dye only indicated

that the septic system was recharging the groundwater as it was designed to do (Ayres Associates 2008).

However, 15 d is a too short time period to inactivate viruses below the level necessary to protect public health. In groundwater with temperatures less than 20 °C, like in Wisconsin, virus inactivation rates may be as low as 0.02 $\log_{10}$ per day (John and Rose 2005), meaning it would take 200 d for a 4-log reduction in virus concentration, the U.S. treatment goal for drinking groundwater (USEPA 2006). In a novel study, Charles et al. (2009) tracked human virus survival in groundwater incubated at 12 °C for 2 years. Infectious adenovirus 2 and poliovirus 3 were detected by cell culture for up to 364 and 140 d, respectively. Some groundwater incubations were still quantitative RT-PCR positive for norovirus GI, the same genogroup responsible for the present outbreak, up to 651 d. Modeling virus transport and removal in sandy unconfined aquifers, Schijven et al. (2002, 2006) determined for the infection rate to be less than the acceptable limit of 10^{-4}/year, the protection zones around Dutch wells should be sized to give a virus travel time of 1 to 2 years. The current protection zone guideline in the Netherlands is 60-d travel time.

The tracer test allowed estimation of relative groundwater velocities in the study area. Because the actual tracer paths are unknown, the estimated velocities represent straight-line point-to-point travel through the groundwater system and should not be interpreted as "true" groundwater velocities. In addition, we neglected vertical movement of the tracer from the ground surface to the aquifer and treated the entire tracer path as horizontal. Given these simplifying assumptions, tracer velocities range from 0.8 to 12.5 m/d (Table 4). The rates to the offsite wells (wells B and D) should be the most representative of true regional groundwater velocities due to the longer travel paths, and these are in the range of 7 to 8 m/d. Such velocities are reasonable for the fractured dolomite of the Door Peninsula. For comparison, Muldoon and Bradbury (2005) reported velocities of 0.5 to 32 m/d from natural-gradient tracer tests at a quarry about 20 km south of the restaurant site. A Darcy calculation at the restaurant site using an average horizontal gradient of 0.0046, hydraulic conductivity of 0.35 m/d, and an effective porosity of 0.0005 (Rayne et al. 2001) yields an average linear velocity of 3.2 m/d.

Thick unsaturated zones are assumed to protect groundwater from contamination by pathogens. In this case, however, the tracer test clearly showed that, at this site, the 35-m thick unsaturated zone beneath the septic system did not significantly attenuate either the dyes or the viruses. Understanding the physical processes of rapid pathogen transport through the unsaturated fractured rock at this site is beyond the scope of this paper, but it is likely that saturated flow along preferential pathways within the fractures occurs, while the matrix blocks themselves remain unsaturated. Such a process has been described by Pruess (1999) and other investigators, and is consistent with the rapid recharge and well responses observed by Bradbury et al. (2002) at nearby field sites.

Travel time for the outbreak norovirus from the infiltration field to the well was likely less than 15 d. Similar to the mechanism operating in pore-exclusion gel chromatography, virus particles or the colloid-sized particles with which viruses are associated are unable to diffuse into the matrix and are forced to enter preferential flow pathways. The result is a virus transport velocity that is faster than a dissolved nonreactive tracer velocity, which is retarded by diffusion into and out of the matrix. This mechanism has been measured in field settings. For example, McKay et al. (1993) found that the transport velocity of bacteriophages to be 30 to 500 times faster than bromide in clay-rich (25% to 45%) glacial deposits. And in a floodplain aquifer composed of cobble, gravel, and sand, DeBorde et al. (1999) measured bacteriophage and poliovirus transport velocities that were as much as twofold greater than that for bromide. It cannot be determined which of the two routes, infiltration field or the leaking pipe fitting, contributed the most noroviruses to the well. Regardless, the 15-d fluorescein travel time suggests that, even without the leak, the infiltration field presents an ongoing substantial risk of contamination for the restaurant well.

The investigative methods used in the present study made clear that the septic system was the contamination

Table 4
Determination of Apparent Tracer Velocities

Injection Point and/or Dye Type[1]	Detection Point	Horizontal Distance (m)	First Arrival (d)	Peak Arrival (d)	Apparent Horizontal Velocity (m/d)
Toilet (E)	Restaurant well	29	6	34	0.8–4.8
Dosing chamber (F)	Restaurant well	68	15	38	1.8–4.5
Leach field[2] (F or E)	Restaurant well	188	15	38	4.9–12.5
F	Well B	885	113	—	7.8
E	Well D	800	113	—	7.1
F	Well D	800	106	—	7.5

[1]E = eosin; F = fluorescein.
[2]Although dye was not injected at the leach field, both the toilet and the dosing chamber discharge to the leach field.

source but did not answer how the norovirus entered the septic system in the first place. GI noroviruses are specific to human hosts (Patel et al. 2009); therefore, the source must be human. Two restaurant employees became ill on May 23, 2007, and one, the dishwasher, reported in his interview with the County Health Department that he had multiple episodes of vomiting and diarrhea in the restaurant bathroom during work. During illness, GI noroviruses are shed in diarrheic stool at concentrations between 10^4 and 10^{10} per gram (Chan et al. 2006). Assuming the employees released 1000 mL of vomitus and stool into the toilet, and given the total volume of the two settling tanks and dose tank is 10^5 L, the norovirus concentration in fully mixed tanks would have been between 10^2 and 10^8 per liter. The measured concentration in the dose tank was nearly 10^5 noroviruses per liter (Table 3), and this was measured 4 weeks after the outbreak began and the restaurant closed and 13 days after it reopened and diluting waste water again entering the septic system. A norovirus concentration of 10^8/L in the septic tanks could have been diluted by groundwater by a factor of 2×10^6 and still the norovirus concentration in the well would have been in the range of the measured value, 50 genomic copies per liter. Thus, it is conceivable that the noroviruses shed from only one ill employee were sufficient to contaminate the septic system and subsequently the well.

The vulnerability of fractured limestone aquifers to microbial contamination has been long understood, even among the lay public. On November 22, 1955, the *Door County Advocate*, still the local newspaper today, headlined a story "Local Geology is Illness Factor, 'Summer Flu' Study Is Made by Scientists, Limestone Fissures Let Wastes Get into Wells Say the Researchers." Given this history, local residents handle the poor sanitary quality of their groundwater by drilling wells with casings much deeper than required by state code, drinking bottled water, or installing household treatment systems such as UV disinfection. In the case of the restaurant, the well water now passes through an onsite treatment train consisting of polypropylene dual gradient sediment filters, UV light disinfection (40 mJ/cm^2), three retention tanks dosed with chlorine (3 mg/L), and finishing with activated carbon filtration.

In contrast, the role septic systems have in contributing microbial contaminants to these aquifers and the public health threat this poses is not widely understood or accepted, in part because there are few outbreak investigations that undertake the steps described here to link definitively illnesses with a specific septic system. Moreover, it is tacitly accepted that the effluent from onsite septic systems reaches groundwater, with the assumption that wastes will be biodegraded in the soil zone and attenuation and dilution in the groundwater system will remove pathogens or reduce them to noninfectious levels. This assumption clearly failed in the outbreak described here and likely fails elsewhere. Even in nonfractured aquifers, viruses have been observed in groundwater downgradient of septic systems (Alhajjar et al. 1988; DeBorde et al. 1998), and sporadic childhood diarrheal illness with a viral etiology has been associated with the geographic density of conventional septic systems in central Wisconsin (Borchardt et al. 2003b). Previous research (Borchardt et al. 2007) has demonstrated the ability of viruses to travel hundreds of meters in the subsurface through porous media and remain viable. Transport in fractured carbonate and karst terrains is more rapid with less potential attenuation. Accordingly, permitting conventional septic systems to be constructed above vulnerable fractured limestone aquifers, particularly for facilities like restaurants that generate a large volume of waste water, should be reexamined if public health is to be adequately protected.

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