VIRUSES IN GROUND WATER

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Abstract. Waterborne disease outbreaks are often associated with non-porous media aquifers, such as fractured bedrock (metamorphic rock) or karst (limestone) aquifers e.g. South Bass Island Ohio USA (Fong et al., 2007). In these aquifers, ground water flow is fast and direct with little opportunity for inactivation or attachment to the aquifer matrix. In addition, coarse, glacial flood, gravel-cobble aquifers also appear to have fast and direct ground water flow. In the United States, public water supplies may be either ground water or ground water under the direct influence of surface water, as determined by the State. For ground water (only) wells, fifteen public water supply wells in the United State representing several aquifer types are found to be enteric virus positive using BGM cell culture methods. Eight of these wells are located in fractured bedrock, karst or gravel-cobble aquifers and were enteric virus positive using BGM cell culture methods.

Keywords: Enteric virus, enterovirus, reovirus, BGM cell culture, ground water, public water supply well, karst, fractured bedrock, gravel, cobble, Ground Water Rule, US EPA

1. Introduction and Background

Public health authorities, in both developed and undeveloped countries, rely on the use of sanitary setback distances between human (and animal) waste disposal sites and drinking water wells to protect human health. Unfortunately, most authorities do not vary setback distances based on hydrogeologic setting despite the knowledge that fecal contamination can have very differing subsurface

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mobility depending on aquifer type (Taylor et al., 2004). The purpose of this paper is to describe some of the data that documents the variable hazard from viruses (and bacteria) pathogenic to humans as a function of aquifer type.

Viruses (as used herein) include all enteric viruses pathogenic to humans as long as they are shed via the gut. This includes enteric viruses such as most adenoviruses serotypes that may primarily infect organs other than the gut but are shed via the gut. Viruses that infect only bacteria are identified specifically as bacteriophage or coliphage. Enteric virus contamination is recognized only through cell culture methods that identify infectious virions. Analyses using molecular methods are not considered herein because these methods are incapable of discriminating infectious from non-infectious agents.

Public water supplies may transmit fecal contamination if they are subject to one or more of the following risk factors: 1) sensitive aquifers; 2) aquifers in which viruses may travel faster and further than bacteria (e.g. alluvial or coastal plain sand aquifers; 3) shallow unconfined aquifers; 4) aquifers with thin or absent soil cover; 5) wells previously identified as having been fecally contaminated; and 6) high population density combined with on-site wastewater treatment systems, particularly those in aquifers with restricted geographic extent, such as barrier island sand aquifers. Other risk factors also may allow or facilitate fecal contamination transmission to PWS wells.

This paper emphasizes identifying important risk factors at PWS wells where 4-log virus inactivation requirement, as required by the Ground Water Rule (GWR) (USEPA, 2006) is not achieved. However, the risk factors apply to all wells because any PWS well may cause waterborne disease when there is any interruption in treatment. However, because waterborne disease outbreaks are not random, but rather are associated with (some) sensitive aquifers (e.g. the recent large Walkerton, Ontario and South Bass Island, Ohio outbreaks both occurred in the Upper Silurian Bass Island Formation), identifying sensitive aquifers is very important.

1.1. AQUIFER SENSITIVITY

The GWR (USEPA, 2006) recommends that aquifer type, which is usually well-correlated with lithology, be established in order to determine if a specific aquifer is sensitive. The GWR suggests that all limestone aquifers, igneous and metamorphic aquifers, and gravel aquifers be regarded as sensitive. This is because limestone is the lithology most likely to be karst; igneous and metamorphic aquifers are likely to be highly fractured; and gravel aquifers are likely to have direct flow paths and rapid ground water velocities due to the shape and large size of their pores. Pumping wells increase the natural flow velocities in sensitive aquifers to a greater degree than they would in a fine-grained, unconsolidated aquifer, for example. States may designate additional sensitive aquifer types if they feel it is necessary to do so to protect public health (e.g. a state may designate sand and gravel aquifers as sensitive).

1.1.1. Karst Aquifer

Karst is defined as a type of geologic terrane within which flowing ground water has dissolved significant portions of the area=s soluble (usually carbonate) rocks (Fetter 2001). Where karst regions occur, infiltrating precipitation and ground water create a permeability structure characterized by numerous and often large, interconnected conduits. Through time, these conduits continue to enlarge, creating unique surface and subsurface drainage networks and characteristic surface landforms. Ground water velocities are usually rapid, and flow paths very direct in karst environments, especially in the vicinity of pumping wells. Microbial pathogens released into karst aquifers from sources such as septic systems or livestock operations are likely to reach drinking water consumers in an infective state. For example, the Walkerton, Ontario *E. coli* outbreak in May, 2000 is believed to have been caused by fecal pollution of a karst aquifer system (Worthington et al., 2002). Table 1 summarizes information about well-known waterborne disease outbreaks that have been reported in karst geologic settings in North America (USEPA, 2002c).

Waterborne disease outbreaks are not randomly distributed. Rather they are more likely to occur in some aquifers, especially karst limestone aquifers. The two outbreaks in New Braun, TX, the outbreak in Georgetown, TX and the outbreak in Brushy Creek, TX all resulted from contaminated wells located in the Edwards Aquifer, a sensitive karst limestone aquifer. Similarly, the outbreaks in South Bass Island, OH, Walkerton, Ontario and Drummond Island, MI all resulted from contaminated wells located in the Upper Silurian Bass Island Formation, a sensitive karst limestone aquifer.

The outbreak in Cabool, Missouri is commonly attributed to replacement of water meters without disinfection follow-up and by broken water mains (e.g. Gelderich et al., 1992). However the outbreak data show that several bloody diarrhea cases preceeded the water main breaks. It is most likely that outbreak was due to intermittent *E. coli* O157:H7 contamination of karst limestone ground water, similar to events in Walkerton Ontario a decade later.

Karst regions are typically characterized by the following: underground drainage networks with solution openings that range in size from enlarged fractures to large caves; closed surface depressions, known as sinkholes, where the dissolution of the underlying bedrock has caused the collapse of overlying rock and sediment; and discontinuous surface water drainage networks that are

TABLE 1. Waterborne Disease Outbreaks Reported in Karst Hydrogeologic Settings in North America

Location	Reference	Number Illnesses/Agent
Richmond Heights, FL, USA	Weissman et al., 1976	1,200 cases/*Shigella*
Cabool, MO, USA	Swerdlow et al., 1992; Gelderich et al., 1992	243 cases/*E. coli* O157:H7; 4 deaths
Georgetown, TX, USA	Hejkal et al., 1982	8,000 cases/Coxsackievirus; 36 cases/Hepatitis A Virus (HAV)
Braun Station, TX, USA (two separate outbreaks)	D=Antonio et al., 1985	251 cases/Norwalk virus; 2000 cases/*Cryptosporidium*
Henderson County, IL, USA	Parsonnet et al., 1989	72 cases/unknown
Lancaster, PA, USA	Bowen and McCarthy 1983	49 cases/HAV
Racine, MO, USA	MO Department of Health, unpublished report 1992	28 cases/HAV
Walkerton, Ontario, Canada	Golder Associates 2000; Health Canada 2000	1,346 cases/*E. coli* O157:H7 (+ *Campylobacter*); 6 deaths
Reading, PA, USA	Moore et al., 1993	551 cases/*Cryptosporidium* (not recognized as GWUDI until after the outbreak)
Brushy Creek, TX, USA	Bergmire-Sweat et al., 1999; Lee et al., 2001	1,300 – 1,500 cases/*Cryptosporidium* (not recognized as GWUDI until after the outbreak)
South Bass Island, OH, USA	Ohio EPA, 2005; CDC, 2005; O'Reilly et al., 2007; Fong et al., 2007	1,450 cases of Norovirus, *Campylobacter*, *Salmonella*
Drummond Island, MI, USA	Ground Water Education in Michigan, 1992; Chippewa County Health Department, unpublished report, 1992	39 cases/unknown
Buttermilk Falls spring, Meade County, KY, USA	Bergeisen et al., 1985	73 cases/HAV

related to the unique subsurface hydrology (Winter et al., 1998). In other mature karst landscapes, characterized by relatively pure limestone in areas of high precipitation, caves and caverns are formed in the subsurface. Conduits in carbonates and gypsum can be quite large with some exceeding 100 feet in diameter (i.e., caves) and several miles in length. Mammoth Cave, Kentucky has a mapped length of more than 340 miles of interconnected conduits distributed over five horizontal levels. Ground water velocities have been measured there at more than 1,000 feet per hour (USEPA, 1997).

Indeed, it is the rapid ground water velocities in karst aquifers that necessitate their characterization as sensitive aquifers. In the karst region of Slovenia, an indicator of fecal contamination injected into a karst aquifer reportedly traveled approximately 24 miles in less than 4 months (Bricelj, 1999). Using conservative ground water tracers, ground water velocities measured in karst aquifers are as high as approximately 0.3 miles per hour (USEPA, 1997). In Florida, ground water velocities surrounding a well have been measured at several hundred feet per hour (USEPA, 1997). In a confined karst aquifer in Germany which was breached by monitoring wells, ground water traveled approximately 650 feet in less than 4 days (Orth et al., 1997). In the Edwards Aquifer, Texas, Slade et al. (1986) reported that dye traveled 200 feet in 10 minutes. This data all indicates that ground water flows extremely rapidly through karst aquifers. Worthington et al. (2002) compares tracer test ground water travel times with predicted capture zone model travel times using the porous media assumption for ground water flow to the PWS wells at Walkerton, Ontario. The measured travel time is substantially faster than the predicted travel time.

Well-developed karst systems may have underground streams because of the large size of interconnected openings in the rock. Underground streams can have flow rates as great as those of surface streams. It is also not unusual in karst terrains for surface streams of considerable size to disappear into solution cavities (swallow holes) intersecting a streambed, creating a discontinuous surface drainage system. These same streams may reappear at the surface at other locations (Winter et al., 1998). Seeps and springs are thus common in karst regions.

Sinkholes in karst regions can play a particularly devastating role in the potential for microbial contamination of ground water supplies. For example, sewage treatment lagoons have been known to leak and eventually collapse over sinkholes. This phenomenon has been documented in West Plain, Missouri in 1978 (Craun, 1984); in Lewiston, Minnesota in 1991; and in Altura, Minnesota in 1974 and 1976 (Jannik et al., 1991). In Missouri, 759 illnesses resulted from the contamination of domestic wells due to this 1978 sinkhole collapse (Craun, 1984).

Even in the absence of sinkhole collapse, the potential for rapid infiltration of fecal contamination through overlying soils into karst aquifers is great. Residual soils, formed by bedrock dissolution, are characteristic of well-developed karst regions. These soils are typically clay-rich, but can have great variation in thickness and hydraulic conductivity. Soil macropores transmit water rapidly, and are caused by channels formed by decayed roots, insect and animal burrows, dessication cracks, soil failure surfaces, and soil piping (EPA, 1997). Rapid flow in the overlying soil may also occur via vertical fissures, even when there is substantial residual soil cover (Smart and Frederich 1986, cited in EPA 1997). Where a thin mantle of glacial till or outwash deposits are present, infiltration velocities may also be high (Crowther 1989, cited in EPA 1997).

The actual transport of fecal bacteria within karst aquifers has been studied at a variety of localities (Malard et al., 1994; Orth et al., 1997; Tranter et al., 1997; Gunn et al., 1997). Malard et al. (1994) suggested that both fractures (discussed in section 2.1.2) and karstification contribute to rapid bacterial transport in limestone. For this reason, Malard et al. (1994) consider the risk of bacterial contamination greater in limestone than in any other type of aquifer.

It is important to note that concentrations of bacteria within karst environments often vary significantly with rainfall. Personne et al. (1998) found that high aquifer water levels, induced by high rainfall, correlated with high bacteria levels in the aquifer. The water level in one Edwards Aquifer well (582 feet deep with a water table 240 feet deep) began rising within 1 hour after a rainfall event (Slade et al., 1986). Mahler et al. (2000) studied fecal coliform and enterococci bacteria near a wastewater irrigation site, and found the presence of bacteria in ground water directly followed rainfall events. Mahler=s data suggests that small sampling intervals of 3 to 4 hours are necessary to describe the breakthrough of bacteria at a monitoring well screened in a karst aquifer.

The potential for rapid transport of bacteria and viruses through karst aquifers necessitates that they be monitored carefully for contamination. Bacteria can rapidly percolate into the unsaturated zone of karst aquifers, as well as be further transported to the saturated zone during periods of intensive rainfall. In fact, Malard et al. (1994) found high occurrence rates for bacteria in a karst aquifer as long as a year after surface pollution had essentially ceased. This data demonstrates that sensitive aquifers can be contaminated even when surface pollution sources are difficult to identify. Furthermore, research shows that surface water and ground water drainage divides generally do not coincide in karst regions due to complex patterns of surface water and ground water flow. For example, a stream may disappear in one surface water basin and reappear in another basin. This situation makes it even more difficult to successfully inventory sources of fecal contamination in the recharge area of a karst well (Winter et al., 1998). Such situations are part of the motivation behind the focus on monitoring sensitive aquifers, rather than merely looking for potential sources of bacterial contamination. In summary, bacterial contamination of karst aquifers is both fairly likely and highly unpredictable, although correlations with rainfall events are common.

1.1.2. Fractured Bedrock Aquifers

Any solid block of igneous or metamorphic rock that is surrounded by fractures is considered essentially impermeable. Thus, all flow is forced to take place within the fractures. A detailed understanding of flow in a fractured bedrock aquifer requires knowledge of fracture widths, orientations, the degree to which individual fractures are mineral-filled, and the degree of fracture interconnection and spacing (Freeze and Cherry, 1979). Most fracture widths are smaller than one millimeter (mm), and a fracture=s capability to transmit ground water (i.e., hydraulic conductivity) is roughly proportional to the cube of the fracture width (National Research Council, 1990). Thus, small changes in fracture width result in very large changes in hydraulic conductivity. For example, a 1 mm fracture can transmit 1000 times more water than a 0.1 mm fracture, provided that other factors are constant (e.g. hydraulic gradient).

Freeze and Cherry (1979) report void space as high as 10 percent of total volume in igneous and metamorphic rock. Other data presented in Freeze and Cherry (1979) suggest that the first 200 feet beneath the ground surface produces the highest water yields to wells because fractures at shallow depths are wider, more numerous, and more interconnected. Nevertheless, municipalities sometimes derive high volumes of water from wells located in fault zones that extend to greater depths.

Tracer tests have been used in several studies to estimate ground water flow rates in fractured bedrock. Malard et al. (1994) report that dye traveled approximately 140 feet in a fractured bedrock aquifer in 2 hours. Becker et al. (1998) reports that water traveled approximately 118 feet in about 30 minutes. Ground water velocities in fractured bedrock aquifers are comparable to velocities in karst aquifers. Thus, fractured bedrock aquifers are vulnerable to contamination by waterborne pathogens. Table 2 (from USEPA, 2002c), summarizes some of the most recent cases of waterborne disease outbreaks due to contamination of wells screened in fractured bedrock aquifers in North America.

TABLE 2. Waterborne Disease Outbreaks Reported inFractured Bedrock Aquifers in North America

Location	Reference	Number Illnesses/ Agent
Couer d=Alene, ID, USA	Rice et al., 1999	117/*Arcobacter butzleri*
Island Park, ID, USA	CDC, 1996	82 cases/*Shigella*
Big Horn Lodge, WY, USA	Anderson et al., 2003	35/Norovirus
Northern AZ, USA	Lawson et al., 1991	900 cases/Norovirus
Atlantic City, WY, USA	Parsionikar et al., 2003	84/Norovirus

1.1.3. Gravel Aquifers

Gravel aquifers, as defined here, are unconsolidated water-bearing deposits of well-sorted pebbles, cobbles, and boulders. Gravel aquifers consist primarily of coarse grains larger than approximately 4 mm or approximately 0.16 inches in diameter, although they may have minor amounts of smaller diameter material as well. Gravel aquifers are often limited in area and are generally produced by high energy events such as catastrophic glacial outburst floods or flash-floods at the periphery of mountainous terrain. They can also sometimes be found at fault-basin boundaries or in glacio-fluvial deposits such as crevasse fillings, eskers, kame terraces, and outwash/valley trains. Typically, these are small, relatively localized aquifers.

2. Virus Occurrence in Public Water Supply Wells

Outbreaks occur as a result of a treatment failure, inadequate treatment or as the result of source water fecal contamination in untreated wells. This paper focuses on virus occurrence and outbreaks in untreated PWS wells in the United States. However, it is often difficult for researchers to obtain consent to sample untreated wells for viruses. Thus, most virus occurrence data reported here were obtained from samples collected prior to treatment in treated wells. If well treatment data are available, untreated wells with virus occurrence are noted.

In the US, PWS wells are classified as either ground water or ground water under the direct influence (GWUDI) of surface water because the ground water is closely connected to surface water. Surface water systems are at risk due to occurrence of *Cryptosporidium* (and *Giardia*) an organism highly resistant to inactivation by chlorination. Thus, in the US, GWUDI wells are often required to have conventional filtration or UV inactivation to protect against *Cryptosporidium* (or are able to demonstrate 2-log Cryptosporidium removal by bank filtration).

Ground water PWS wells are regulated by the Total Coliform Rule (TCR) and the Ground Water Rule. GWUDI wells are regulated by the TCR and the surface water treatment rules (e.g. Long-Term 2 Surface Water Treatment Rule [LT2SWTR]). This paper focuses only on virus occurrence and outbreaks in PWS wells regulated by the TCR and GWR. It is sometimes the case that PWS are retrospectively recognized as GWUDI wells, often after recognition of a cryptosporidiosis or giardiasis outbreak associated with well water (e.g. South Bass Island, OH; Brushy Creek, TX). In these retrospective misclassification examples, any virus occurrence or viral illness data are herein considered to be ground water rather than GWUDI PWS systems because the PWS wells were operating as ground water systems at the time of the outbreak.

Virus occurrence data are limited. High quality virus-positive samples using cell culture methods are available, in the absence of an outbreak, from only 15 PWS wells (Pedley et al., 2006). With such a small sample, it is difficult to confirm suspected risk factors based on infectious enteric virus occurrence. Thus, the conclusions herein are provisional, subject to additional data collection.

Cell culture methods, although capable of identifying infectious viruses, have very limited capability to discriminate among the fecal viruses. All cell cultures reported herein used Buffalo (African) Green Monkey (BGM) kidney cells as the host monolayer. Continuous lines of BGM cells are optimized to recover poliovirus but also favor recovery of many (but not all) of the enteroviruses. For example, some coxsackie A viruses are not recoverable using BGM cells. Also, BGM cells favor recovery of reoviruses. Although most reoviruses have not been shown to cause illness in humans, at least one reovirus serotype (Type 3) is a known human pathogen (Tyler et al., 2004).

In the US (USEPA, 2006), about 114 million people receive ground water from PWS wells. The total number of wells is unknown but the wells are operated by 147,000 PWS systems, each regulated by the TCR and GWR. Most of the population receives treated water but 20 million (18%) consume untreated ground water (USEPA, 2006).

For the population consuming untreated ground water, few data are available to evaluate risk factors associated with their PWS wells. In an unpublished analysis based on available PWS well latitude and longitude data (of unknown quality) and a digital karst limestone map of the United States (Tobin and Weary, 2006), Anzollin (unpublished personal communication, 2006) estimates that about 45,000 PWS wells (29,000 systems) in the US are located in karst limestone terrane and these wells serve a population of about 48 million people.

It is reasonable to assume that eighteen percent of that population (about 8 million people) receive untreated ground water from PWS wells in karst limestone terrane. At present, similar data and analyses are not available for PWS wells fractured bedrock aquifers.

2.1. VIRUSES IN KARST LIMESTONE PWS WELLS

As listed in Table 1, numerous outbreaks have resulted due to fecal (both viral and bacterial) contamination of PWS wells in karst limestone aquifers. Some wells were completely untreated, others were inadequately treated. Because viral outbreaks have occurred in untreated ground water systems in the United States, it is expected that enteric viruses would be identified in raw source ground water from PWS wells, in the absence of an outbreak. Examples of viral outbreaks in untreated ground water supply wells include outbreaks in Ohio, Missouri, and Illinois.

In the South Bass Island, Ohio PWS outbreak, the karst limestone aquifer was contaminated by norovirus via septic tanks and land disposal of septage. Untreated PWS wells (prior to the outbreak) were determined to be positive for fecal bacteria, coliphage and adenovirus (by PCR) (Fong et al., 2007). Other untreated groundwater PWS wells in karst limestone where outbreaks occurred include Racine, Missouri (Missouri Dept. of Health, 1992), Henderson County, Illinois (Parsonnet et al., 1989), Drummond Island, Chippewa County, Michigan (Chippewa County Health Department, Unpublished Report, 1991, GEM, 1992). One large norovirus outbreak in Switzerland (La Newuveville, Bern Canton) also occurred in an untreated karst limestone ground water system (Hafliger et al., 2000, Maurer and Sturchler, 2000; Hrudey and Hrudey, 2004).

Infectious enteric viruses have been identified in four karst limestone PWS wells in the United States. Available data indicate that all four wells were treated before distribution and no health effects are known. At S_'s Market well, in Juniata County, Pennsylvania, Lindsey et al. (2002) identified enteric virus contamination with a measured concentration of 52 PFU/100 liters. These viruses were subsequently shown to be reovirus (Pedley et al., 2006). In a Centre County Pennsylvania fraternal organization building well, Lindsey et al. (2002) identified enteric virus concentration of 18 PFU/100 liters. As described in Pedley et al. (2006), these viruses were identified using serotyping to be echovirus Type 13 and Type 20 and poliovirus Type 3. Because poliovirus Type 3 was used as a laboratory control, it is likely that the poliovirus occurrence was an artifact. In Ash Grove Well #3, Greene County, Missouri, Davis and Witt (2000) report the presence of a non-polio enterovirus with a concentration of 2.1 MPN/100 liters. Poliovirus overgrowth occurred in this sample so additional analysis was undertaken to ensure the presence of non-polio enteroviruses (Jim Vaughan, personal communication to Philip Berger). In Tennesseee well W-1, Johnson (2005) identified (presumed) enterovirus at a concentration of 7 MPN/100 liters.

2.1.1. Indicators of Viruses in Karst Limestone PWS wells

It is well established (e.g. Lieberman et al., 2003) that no single bacterium or bacteriophage indicates (with high likelihood) enteric virus occurrence, although many of these organisms are indicators of fecal contamination. For virus occurrence in karst limestone wells, two of the four wells (Ash Grove #3 and W-1) were enteric virus positive but negative for any bacterial or coliphage indicator organism. However, it is impossible to determine whether additional sampling at these two wells might, in the future, identify coliform bacteria or another indicator when viruses and their indicators are both intermittently present. In contrast, the other two wells (S_'s Market and the fraternal organization) were both positive for a suite of indicators, including *E. coli*, *Clostridium perfringens* spores, enterococci, somatic and male-specific coliphage. Given the small number of karst limestone wells with enteric virus occurrence, it is impossible to make conclusions about the likelihood of enteric virus and fecal indicator co-occurrence and the optimum indicator organism.

Ground water turbidity is sometimes proposed as an indicator for pathogen occurrence or removal efficiency. Unlike surface water, interpreting turbidity measurements in ground water supplies is problematic because pumping wells can create turbidity as a result of on/off pump cycling. Most importantly, pathogen occurrence data collected during and after the South Bass Island, OH outbreak (Fong et al., 2007) shows no relationship between occurrence and turbidity. The wells sampled are located in the karst limestone Bass Island Formation. At the time of the outbreak, the wells were regulated as ground water rather than as surface water (GWUDI). The airport well was positive for TC, *E. coli*, enterococci, coliphage, *Arcobacter*, and human adenovirus (by PCR). Field turbidity at the time of sampling was 4.14 NTU. In contrast, the Skyway Lounge well was positive for the same organisms but the field turbidity was 0.25 NTU.

2.2. VIRUSES IN FRACTURED BEDROCK PWS WELLS

Outbreaks associated with PWS wells in fractured bedrock aquifers are listed in Table 2. Of these outbreaks, only the outbreak at Big Horn Lodge, WY (Anderson et al., 2003) occurred due to virus occurrence in an untreated PWS well. Viruses have been found in three PWS wells drilled into fractured bedrock aquifers. Of these three wells, at least one (in North Carolina) and possibly two wells were untreated. However, no health effects were observed as the result of virus contamination at an untreated well.

At a PWS well in a Chester County, Pennsylvania campground, Lindsey et al. (2002) identified enteric virus with a concentration of 0.21 PFU/100 L. These viruses were subsequently shown to be coxsackievirus type B5 (Pedley et al., 2006). At a subdivision PWS well in Speedwell, North Carolina, echovirus type 11 was recovered at a concentration of 212 MPN/100 L (Lieberman et al., 2002; Dahling, 2002). At a trailer park in Mountain Home, Idaho, coxsackievirus type B4 was recovered at a concentration of 9.5 MPN/100 L (Lieberman et al., 2002; Dahling, 2002). Poliovirus type 1 was used as the laboratory control by Dahling (2002).

2.2.1. Viruses at the PWS Well in Speedwell, North Carolina

The PWS well in Speedwell, North Carolina is located at the foot of a steep incline. About six residences, each with individual septic tanks, are located uphill from the PWS well. The well was untreated at the time of sampling but had a history of total and fecal coliform occurrence. The well was protected by a concrete pad poured around the surface casing but the well was located in a small topographic depression.

The PWS well was constructed in 1987, drilled to 550 feet total depth, with surface casing installed to 57 feet depth. The uppermost 20 feet was grouted with concrete. The well has a measured yield of 15 gallons per minute.The PWS well is constructed in Precambrian gneiss, metagreywacke, schist and amphibolite. The soils (Figure 1) are mapped as the Cowee and Evard soils which are clay loam, loam and sandy loam with 5–30% clay and thick saprolite formation. Measured soil permeability is reported to be 0.6 to 2.0 inches per hour (Goldston et al., 1948).

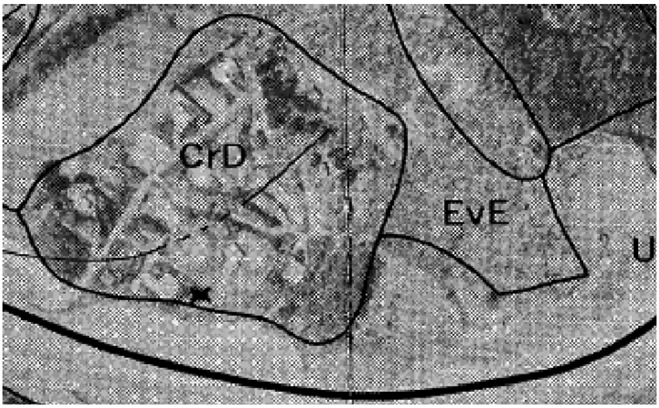

Figure 1. Aerial photograph showing soil mapping units for the subdivision at Speedwell, North Carolina (Goldston et al., 1948)

The PWS well was sampled monthly between March, 1993 and February, 1994. Only one sample, collected on June 22, 1993 was virus positive (at a concentration of 212 MPN/100 L). Weekly rainfall amounts for June, 1993 were measured at Cullowhee, North Carolina, about 3 miles from the well location. Precipitation was 1.08 inches for June 1–7, 2.06 inches for June 8–14, 1.08 inches for June 15–21, and 1.18 inches for June 21–28. Average precipitation in Cullowohee in June is 4.5 inches so the precipitation was slightly higher than normal.

Of the twelve monthly samples, 2 samples were positive for Total Coliform but negative for *E. coli*. One sample was positive for enterococci and one sample was positive for male-specific coliphage. All monthly samples were negative for *Clostridium perfringens* spores, somatic coliphage and *Bacteroides* bacteriophage. Most importantly, the June 23, 2003 sample was positive for echovirus type 11 and negative for all fecal or other indicators. No health effects were identified due to echovirus type 11 occurrence at high concentration.

2.3. VIRUSES IN COARSE GRAVEL GLACIO-FLUVATILE PWS WELLS

Only one or possibly two PWS wells have even been sampled for viruses in coarse gravel glacio-fluvatile deposits. One site was negative for enteric virus (DeBorde and Ward, 1995). The other site, in Milton-Freewater, Oregon (Figure 2) was sampled monthly for a year between December, 1992 and November, 1993.

Four samples (February 9, March 9, May 11, and August 10) were enteric virus positive with concentrations ranging between 10 and 20 MPN/100L (20, 15, 11 and 10 respectively) (Lieberman et al., 2002; Dahling, 2002). In the February sample the viruses were unidentified. The March sample recovered reovirus, the May sample recovered coxsackievirus type A7, coxsackievirus type B1, echovirus type 15 and echovirus type 24. The August sample recovered coxsackeivirus type B1 and echovirus type 15 (Dahling, 2002).

The Milton-Freewater PWS well was constructed in 1962 to serve a motel located in the alluvial valley adjacent to the Walla Walla river. The well is 96 feet deep with (probably) a bentonite slurry grout seal. This well and others in the valley have a history of Total and Fecal Coliform occurrence. Geologic investigations by the Oregon Department of Health (D. Nelson, personal communication to Philip Berger) suggest that the valley has coarse gravel at least to 165 feet depth, is located in a region where river water naturally discharges to ground water but may have discontinuous, partial confining layers.

In addition to the enteric virus occurrence data, the Milton-Freewater PWS well had multiple occurrences of fecal indicators (Lieberman et al., 2002;

Figure 2. Coarse glacio-fluvatile gravel deposits in Umatilla, County, Oregon (Johnson and Makinson, 1988)

Dahling, 2002). All twelve monthly samples were Total coliform positive and half were *E. coli* positive. Five samples were positive for enterococci and nine samples were somatic coliphage positive. Three samples were positive for male-specific coliphage and one sample each was positive for Clostridium *perfringens* spores or *Bacteroides* bacteriophage.

In keeping with the gross fecal contamination at the Milton-Freewater PWS well, enteric virus positive samples were also positive for fecal indicator or total coliform organisms (Lieberman et al., 2002; Dahling, 2002). The February positive sample was also positive for Total Coliform. The March positive sample was also positive for Total coliform, somatic and male-specific coliphage. The May positive sample was also positive for *E. coli,* enterococci, and somatic coliphage. The August positive sample was also positive for *E. coli*, enterococci, somatic coliphage and *Clostridium perfringens* spores. Oregon.

2.4. VIRUSES IN (OTHER) POROUS MEDIA PWS WELLS

Enteric virus has been recovered from two PWS wells in glacial outwash deposits (interbedded sand, gravel and clay) in Southern Michigan (Francy et al.,

2004) and one PWS well in unconsolidated deposits in Bradford County, Pennsylvania (Lindsey et al., 2002). The viruses recovered in Michigan were not serotyped. The Bradford County Pennsylvania well viruses were identified as reovirus. Enteric viruses were also recovered in two of eight samples from a PWS well in a sand and gravel aquifer in Wisconsin (Wisconsin Department of Health, unpublished data, 2000). Banks et al. (2001) report enteric virus in a PWS well in a coastal plain aquifer in Wicomico County, Maryland that was subsequently determined to be rotavirus.

2.5. VIRUSES IN KARST WELLS DESIGNATED (BY THE STATE) TO BE GROUND WATER UNDER THE DIRECT INFLUENCE OF SURFACE WATER

Three Alabama karst wells sampled monthly for one year (Lieberman et al., 2002; Dahling, 2002) are regulated by the State as if they are surface, rather than ground water. These wells are regulated by the Long Term 2 Surface Water Treatment Rule rather than by the Ground Water Rule. As ground water under the direct influence of surface water, they may be required to have coagulation and filtration in addition to disinfection. Because of the close connection between surface water and ground water in these wells, it is not surprising that multiple samples were enteric virus positive. For one well, seven of twelve samples were virus positive. The other two wells each were enteric virus positive in four of twelve samples. As discussed above, a greater hazard results when karst wells are miss-classified as ground water rather than as ground water under the direct influence of surface water because ground water receives less treatment than surface water. Ground water supplies, even in karst limestone, are not required to disinfect unless fecal contamination is identified. Although rare (perhaps not more than 10% of wells or samples), it is possible that enteric viruses can be present in the absence of fecal indicator organisms.

In the absence of treatment, public health protection relies upon pathogen natural attenuation processes such as straining, wedging (microstraining), attachment or inactivation. The efficiency of natural attenuation processes is summarized in Schijven et al. (2002) and Berger (2003).

3. Conclusions

Waterborne disease outbreaks in untreated (and inadequately treated) PWS wells tapping karst limestone and fractured bedrock continue to be recognized. Also, enteric viruses have been recovered from treated (but also untreated) PWS wells from these same aquifers. Because ground water flow is fast and direct with little opportunity for pathogen attenuation in karst limestone and fractured bedrock aquifers, PWS and residential wells in these aquifers should be surveilled with extra vigilance. Treatment and supplemental monitoring should be carefully considered as part of a multibarrier approach to protecting public health.

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