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3817 Mineral Point Road
Madison, WI 53705-5100
Tel 608/262.1705
Fax 608/262.8086
www.uwex.edu/wgnhs/

James M. Robertson
Director and State Geologist

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Kenneth R. Bradbury
Wisconsin Geological and Natural History Survey
University of Wisconsin–Extension
Mark A. Borchardt
Marshfield Clinic Research Foundation
(Present affiliation: USDA–Agricultural Research Service)
Madeline B. Gotkowitz
Wisconsin Geological and Natural History Survey
University of Wisconsin–Extension
Susan K. Spencer
Marshfield Clinic Research Foundation
(Present affiliation: USDA–Agricultural Research Service)

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Kenneth R. Bradbury
Wisconsin Geological and Natural History Survey
University of Wisconsin-Extension

Mark A. Borchardt[1]
Marshfield Clinic Research Foundation

Madeline Gotkowitz
Wisconsin Geological and Natural History Survey
University of Wisconsin-Extension

Susan K. Spencer[1]
Marshfield Clinic Research Foundation

[1] Present Affiliation: USDA-Agricultural Research Service

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Human Viruses as Tracers of Wastewater Pathways Into Deep Municipal Wells

Abstract

Enteric viruses, because of their small size, have a high potential to move deeply through the subsurface environment, penetrate aquitards, and reach confined aquifers. Until recently, few water utilities or researchers were aware of possible virus presence in deep aquifers and wells. Over the past several years, repeated detection of viruses in water from deep wells in Madison, Wisconsin, shows that viruses can be significant groundwater contaminants and potential threats to human health.

During 2008 and 2009 we collected a time series of 26 approximately monthly virus samples from six deep municipal water-supply wells in Madison. Sampling for viruses requires a time series approach because virus concentrations, and virus species, vary with time in individual wells. The wells range in depth from approximately 700 to 900 feet and draw water from a series of Cambrian sandstones. Three of these wells are reportedly cased and grouted through a regional aquitard thought to protect the wells from surface contamination, and three have shallow casings. We also sampled local lakes and untreated sewage as potential virus sources.

Viruses were detected up to 61 percent of the time in each well sampled, and many groundwater samples were positive for virus infectivity. Lake samples contained viruses over 75 percent of the time. Sewage samples were extremely high in viruses, with all samples positive. Virus concentrations varied significantly with time, and there was apparent temporal correlation between virus detections in sewage, lakes, and groundwater.

Correlation between viral serotypes found in sewage, lakes, and groundwater suggests very rapid transport, on the order of weeks, from the source(s) to wells. Water isotope analyses indicated surface water to be an unlikely source of viruses; the most likely source of the viruses in the wells is leakage of untreated sewage from the Madison sewer system, which contains a large number of clay pipes installed before 1950.

Potential pathways for virus transport from the surface to the wells include porous-media flow, rapid transport through fractures, transport down failed well casings, and flow through cross-connecting wells. Human enteric viruses might be excellent tracers of recently recharged groundwater in urban settings when virus sources exist.

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Introduction

Virus contamination of groundwater

Among the many waterborne pathogens of humans, enteric viruses have the greatest potential to move deeply through the subsurface environment, penetrate aquitards, and reach confined aquifers. Enteric viruses are extremely small (27-75 nm), readily passing through sediment pores that would trap much larger pathogenic bacteria and protozoa. Viruses have been found in groundwater at depths of 67 m (Keswick and Gerba 1980; Robertson and Edberg 1997) and 52 m (Borchardt and others 2003) and lateral transport has been reported as far as 408 m in glacial till and 1600 m in fractured limestone (Keswick and Gerba 1980). Several recent studies have demonstrated widespread occurrence of viruses in domestic and municipal wells in the United States (Abbaszadegan and others 2003; Borchardt and others 2003; Fout and others 2003; Borchardt and others 2004), and approximately half of waterborne disease outbreaks attributable to groundwater consumption in the United States have a viral etiology (National Primary Drinking Water Regulations, 2006). The US Environmental Protection Agency 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 rarely been explicitly investigated. In the most comprehensive groundwater-virus study to date, Abbaszadegan and others (2003) sampled 448 groundwater sites in 35 states and found 141 sites (31.5%) were positive for at least one virus type.

Previous virus sampling in the Madison area

The work reported here builds on previous virus sampling of deep groundwater in Madison, Wisconsin. During 2005 and 2006 we undertook initial virus sampling of three deep bedrock wells serving the city of Madison, Wisconsin (Borchardt and others 2007a). Each of these high-capacity wells is over 700 feet deep and cased to at least 220 feet below the surface. The vertical hydraulic gradient is downward due to a major cone of depression beneath Madison. Two of the wells (wells 7 and 24) are cased through the Eau Claire shale, a regional aquitard described by Bradbury and others (1999) and thought to provide excellent protection to the underlying sandstone aquifer. A third well (well 5, now abandoned) was open both above and below the shale. Conventional wisdom suggested that viruses would not be detected in any of the three wells due to the probable long travel times from the surface to the wells, the depths of the wells, and the assumed short (six months to two years) lifetime of the viruses. The surprising result of the study was that viruses were repeatedly detected in the two wells thought to have greatest protection due to their deep casings (wells 7 and 24). Viruses were detected in 4 of 10 samples from well 7 and 3 of 10 samples from well 24 (Borchardt and others 2007a). Moreover, five of the seven positive samples tested positive for infectivity, suggesting relatively rapid transport from the virus source to the wells. Replicate sampling and

careful laboratory procedures have ruled out laboratory contamination as a source for the viruses. The human enteric viruses detected include serogroups coxsackieviruses and echoviruses as wells as poliovirus vaccine strain Sabin 1. The Madison, Wisconsin wells are typical of wells now in use in many cities throughout Wisconsin and the United States. These high-capacity wells range in age from less than five to over 50 years and were constructed according to accepted well drilling practices, which include grouted well casing to depth. The wells produce water from one or both of two aquifers. The shallow bedrock aquifer is composed of sandstone and dolomite. The deeper bedrock aquifer is composed of sandstone. A regional aquitard, the Eau Claire aquitard, is composed of shale and siltstone, and separates the two aquifers, but may contain fractures or be absent beneath the nearby Madison lakes. Although the water utility samples the wells regularly for a long list of organic and inorganic contaminants, including bacteria, the wells are not tested for viruses, presumably because viruses have not been thought to be present in the subsurface. Our previous work in Madison shows that this assumption is false.

During 2007 and 2008 Bradbury and others (2008) sampled six municipal wells in Madison on an approximately monthly basis, with the goals of obtaining a time series of virus presence or absence in wells in a deep bedrock aquifer, understanding the sources of the viruses, and of assessing the transport pathways of viruses to the wells. Viruses were detected at least twice in every one of the six wells, but no well was virus-positive in every sampling round. Overall, 43 percent of the samples were virus-positive, and virus concentrations ranged from 0.00 to 6.15 genomic copies per liter (gc/l), with a mean of 0.47 gc/l. Samples from three wells were positive for virus infectivity. Lake samples were positive 78 percent of the time, and ranged from 0.00 to 27.6 gc/l, with a mean of 5.8 gc/l. Not surprisingly, Madison sewage was extremely high in viruses, with all samples positive, and concentrations ranging from about 50,000 to over two million gc/l, with a mean of 581,000 gc/l. Virus results varied significantly with time, and there was apparent correlation between virus levels in sewage, lakes, and groundwater.

Several different species (serotypes) of viruses were identified in wells, sewage, and lake water during the 2008 study, and in many cases wells and sewage contained identical virus serotypes. Detected viruses include the enterovirus serotypes echovirus 3, echovirus 6, echovirus 11, coxsackievirus A16 and B4, the adenovirus serotypes 2, 6, 7, 41, as well as GI norovirus and rotavirus. The apparent correlation between viral serotypes found in sewage, lakes, and groundwater suggests very rapid transport from the sources to wells. Viral serotypes vary seasonally and annually, and so correlation between surface and subsurface serotypes would be unexpected if transport times from the surface to groundwater exceed many months. The Madison Lakes are probably not the main source of the viruses found in the wells as lake water contained some but not all of the serotypes found in the wells, and wells without lake-derived water had viruses present. Furthermore, the $^{18}O/^{2}H$ signature of water produced by these wells is not consistent with a significant proportion of lake water in the recharge to most of the wells. Virus levels in lake water were much lower than in sewage, thus significant volumes of lake water would be required to produce the virus levels measured in the wells.

The most likely source of the viruses in the wells is the leakage of untreated sewage from the Madison sewer system. Given the high concentrations (millions of genomic copies per liter) of viruses in sewage, it would take very little sewage to produce the virus concentrations observed in the wells.

Understanding how the viruses moved from a near-surface source (sanitary sewers) to the deep bedrock wells is critical to assessing the magnitude of the virus problem, the human health risks, and to developing remedial actions. However, based on the limited sampling to date it was difficult to elucidate a pathway or mechanism that delivers the viruses to the wells. Given that the viruses originated near the land surface there are four conceptual models of virus transport to the confined aquifer: (1) transport through the aquitard by porous-media flow; (2) transport by porous-media flow around the edge of the aquitard or through nearby "windows" or breaches in the aquitard, including local lakes; (3) transport by rapid flow through fractures in the aquitard or through cross-connecting nearby wells; and (4) transport by rapid flow along the well annulus through damaged, deteriorated, or poorly installed grout or breaches in the well casing.

Knowledge about the local hydrogeologic system and virus survival time makes some of these conceptual models more probable than others. The only environmental source of human enteric viruses is human fecal waste, and within the city limits of Madison human fecal waste is presumably only present in sanitary sewers. From this presumed point of entry, viruses must travel downward over 200 feet though the upper sandstone aquifer, an additional 10 to 30 feet downward through the Eau Claire aquitard to reach the top of the Mount Simon aquifer. Once in the Mt Simon aquifer the viruses must move laterally some unknown distance to the production wells. Based on such a travel path, pathway 1 seems very unlikely because travel times would likely be far longer than the six months to two years these viruses can survive in the environment (Yates and others 1985, John and Rose 2005, Schijven and others 2006). Transport pathways 2 and 3, through breaches in the aquitard or through fracture pathways, are more probable, but one must still account for the long travel distance through the upper sandstone aquifer above the aquitard. Pathway 4, transport down the annulus of the well itself through deteriorated or poorly installed grout or through breaches in the well casing, seems the most likely mechanism for virus transport. This pathway could produce rapid downward movement of water with delivery directly to the well bore. Although the three wells tested in the previous study were drilled, cased, and grouted according to accepted practice it is impossible to confirm that the grout has remained intact over the entire length of the casing in wells that are now 27 years (Well 24) and 41 years old (Well 7).

During the previous virus study in Madison (Borchardt and others, 2007a) we collected limited samples for analysis of environmental isotopes. Tritium, deuterium, and oxygen-18 have long been used in hydrogeologic studies to help distinguish groundwater age and source areas (Clark and Fritz, 1997). Previous tritium data suggested that Madison wells 5 and 24 produce relatively "old" groundwater (little or no tritium content), while well 7 produces "younger" water (tritium near the levels in modern precipitation). We hoped that oxygen-18/deuterium data would be useful in confirming or discarding flow paths

that include surface water contributions from the nearby Madison lakes. However, the oxygen-18/deuterium data were not definitive, possibly due to subsurface mixing and or seasonal variations in the ^{18}O concentrations in precipitation. Hunt and others (2005) showed that a time series of ^{18}O/deuterium ratios is necessary to unambiguously distinguish surface-water inputs from terrestrial recharge; the previous study obtained only single isotope samples from each well.

In a population, like that of Madison, various viruses have a temporal pattern, arriving and disappearing from the population over the course of a year. Infected people in Madison shed enteroviruses, which are flushed through the sanitary sewers to the sewage treatment plant. There are 64 serotypes of enteroviruses and only a couple of serotypes are present in the population at any given time. One enterovirus strain will be dominant in Madison in August and a different strain dominant in October, which will differ from the strains present the following year. These temporal patterns and changes in the relative abundance of viruses and virus serotypes have been documented in wastewater for enteroviruses and adenoviruses (Sedmak and others 2003; Sedmak and others 2005; Carducci and others 2006). Add in all the other human enteric viruses that can be detected and sequenced, and the viruses in wastewater shed by that population become a "virus signature" for that point in time. The signatures can be used as a tracer of virus movement from source(s) (presumably leaking sanitary sewers or lake water) to the study wells. Using deuterium and O-18 as an isotope signature, Hunt and others (2005) used a similar conceptual approach for estimating the time of travel of river water through the riverbank to adjacent wells.

The virus signature has several information components: (1) the general type of virus (e.g., norovirus or enterovirus), which gives information on the size, charge, and "lifespan" of the virus particle; (2) the quantity of virus (e.g. genomic copies/liter), which provides a time-varying signal whose amplitude may be observed along the suspected transport route and well; and (3) the virus serotype or nucleic acid molecular fingerprint, which can be tracked over time in wastewater and well water and, in conjunction with virus quantity, gives information on transport time. For example, the presence of echovirus 18 in wastewater in October followed by its detection in a well in December might suggest a 2 month time of travel from the source(s) to the well, but could also suggest a 14-month travel time if echovirus 18 had been present the previous October. This is why obtaining a measure of virus variation in the source water is critical. Of course, one would want to base time estimates on multiple virus detections and samples. Working with these virus signature components as separate lines of evidence, or perhaps combining them using multivariate techniques such as cluster analysis or multidimensional scaling, and corroborated with isotope and chloride data, we believe will allow powerful inferences about virus transport routes to the drinking water wells.

One limitation of this approach is that for reasons not well understood among environmental virologists, there is substantial spatial and temporal variability in virus occurrence in groundwater. Unlike common contaminants such as chloride and nitrate, virus detections in water wells do not suggest that a plume of viruses is continually present between a source area and down-gradient receptor (that is, the well). Rather, virus

contamination occurs intermittently. One approach to compensate for spatial variability is to sample large volumes of well water (~ 1000 liters) as commonly practiced. An approach to compensate for temporal variability is to increase sampling frequency, which is now affordable. The benefit of collecting numerous large sample volumes is that, spatial and temporal variability notwithstanding, the underlying biological and hydrogeologic patterns begin to emerge. A similar approach was recently reported by Borchardt and others (2007b) where several hundred water samples for viruses allowed the study team to quantify virus intrusions into municipal drinking water distribution systems.

Project objectives and scope

The objectives of this project were to follow up the previous study by Bradbury and others (2008) and (1) continue into a second year virus sampling from the study wells and wastewater treatment plant and increase sampling frequency to biweekly, (2) continue into the second year isotope and major inorganic ion sampling from the study wells and in addition obtain data on chemical tracers of wastewater, (3) develop a time series of virus signatures (i.e., the set of virus types) and quantities in the wells and from these data infer the time of travel and transport pathway. Additional, possibly corroborating, information on virus transport pathways was to be collected from depth-discrete water samples under pumping conditions.

Acknowledgments

This project was funded by the Wisconsin Department of Natural Resources through the State of Wisconsin Groundwater Joint Solicitation Program. The Madison Water Utility participated in the project by providing information about wells and access to wells for sampling. WGNHS Geotechnician Peter Chase and assistant Andrew Aslesan spent many hours in sample collection. Matt Volenec and Phil Bertz of the Marshfield Clinic assisted with the virus analyses. The Madison Metropolitan Sewerage District provided samples of sewage influent. Jeff Helmuth of the Wisconsin Department of Natural Resources administered the grant.

Procedures and Methods

Selection of wells for sampling

The wells used in this project are referred to as "long-term wells" and were the same six wells used in the earlier study by Bradbury and others (2008) That report describes the rationale for well selection. This current report focuses on the results from long-term sampling of Madison Water Utility wells number 7, 11, 12, 13, 19, and 30. The Madison Water Utility currently operates 27 deep high-capacity wells completed in bedrock aquifers. The wells draw from a Cambrian-age sandstone aquifer underlying the city (Bradbury and others, 1999). This aquifer lies beneath 30 to 100 feet of glacially-deposited sand and gravel and lake sediment. Most of these high-capacity wells are over 700 feet deep and cased to about 200 feet below the surface. Water enters the wells through open boreholes in the rock below the casing. Although cement grout is emplaced to seal the annular space between well casings and surrounding geologic materials, the integrity of these grout seals is often suspect and nearly impossible to test. About one-third of the Water Utility's wells are cased through the Eau Claire shale, a regional aquitard described by Bradbury and others (1999) and thought to provide excellent protection to the underlying sandstone aquifer. The other two-thirds of the wells, most of which are the older wells, are "cross-connecting"; open both above and below the shale or drilled in places where the shale is thin or absent. These wells are presumably more vulnerable to contamination than the deeply cased wells.

The sampled wells include three wells (wells 7, 11, and 12) reported to be multi-aquifer wells (open both above and below the Eau Claire aquitard) and three wells (wells 13, 19, and 30) reported to be cased through the aquitard. We sampled surface water from Lakes Mendota, Monona, and Wingra as well as clarified sewage influent at the Madison Metropolitan Sewage District. Samples were also collected for inorganic chemistry and isotope analyses.

Figure 1 shows the spatial distribution of wells, and figure 2 shows the construction of the long-term sampled wells. Our previous report (Bradbury and others, 2008) includes construction data for other sampled wells. Figure 2 also shows the typical conceptualization of subsurface hydrostratigraphy in Madison. The complex geologic stratigraphy is simplified to consist of upper glacial materials (till, sand and gravel, or lake sediment) covering a shallow bedrock aquifer composed of sandstone and dolomite. Shale of the Eau Claire Formation forms a regional aquitard and separates the upper bedrock aquifer from a deep bedrock aquifer composed of sandstone. This aquitard is no more than about 11 feet thick over much of the Madison area, and is missing in some places. Crystalline Precambrian rock bounds the bottom of the system. Vertical hydraulic gradients in groundwater beneath the city are downward due to a regional cone of depression beneath the Madison metropolitan area (Bradbury and others, 1999). Figure 2 shows this diagrammatically – the potentiometric surface of the deep sandstone aquifer is lower than the water table in the shallow aquifer. In this situation water and any contaminants in the upper aquifer have the hydraulic potential to move vertically

downward and reach the underlying deep aquifer. Wells are typically cased and grouted through the upper geologic units and consist of open holes below the casing.

Construction diagrams of individual wells (figure 2) show the variation in well construction, thickness of layers, and presence and thickness of the aquitard. Wells 11, 12, and 13 are termed “cross-connected” wells because either the aquitard is missing completely (wells 11, 13) or the well casings do not extend through the aquitard (well 12) and the open hole provides a vertical conduit between the upper and lower aquifers. These cross-connecting wells are thought to be much more susceptible to contamination than “confined” wells (wells, 7, 19, and 30), in which the casing extends through the aquitard.

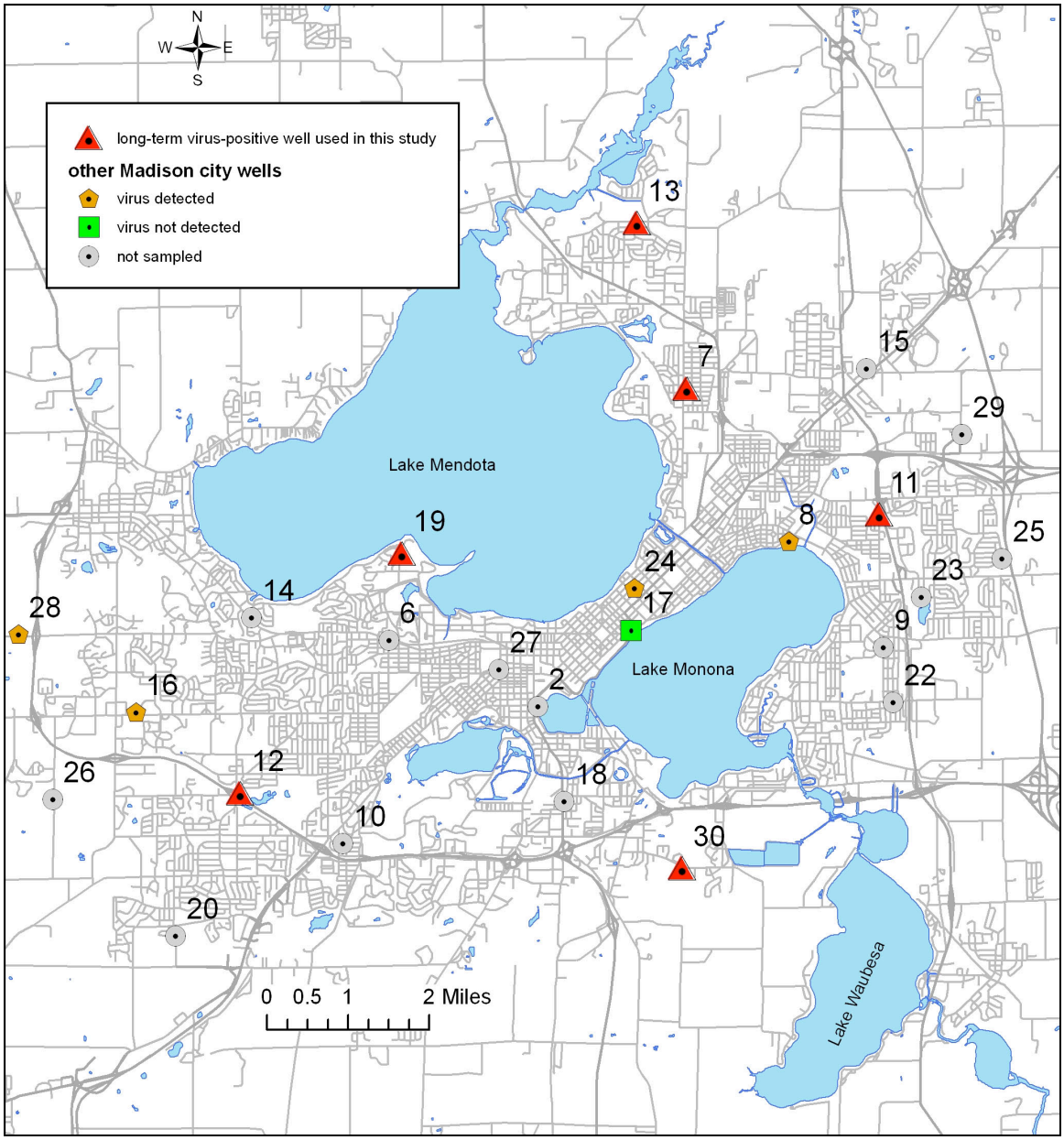

Figure 1. Location of sampled wells and virus detections. Wells 7, 11, 12, 13, and 19 are the long-term sampling sites used in this study. "Virus detected" indicates that the well tested positive for viruses on at least one date during previous projects. Numbers refer to Madison Water Utility well numbers.

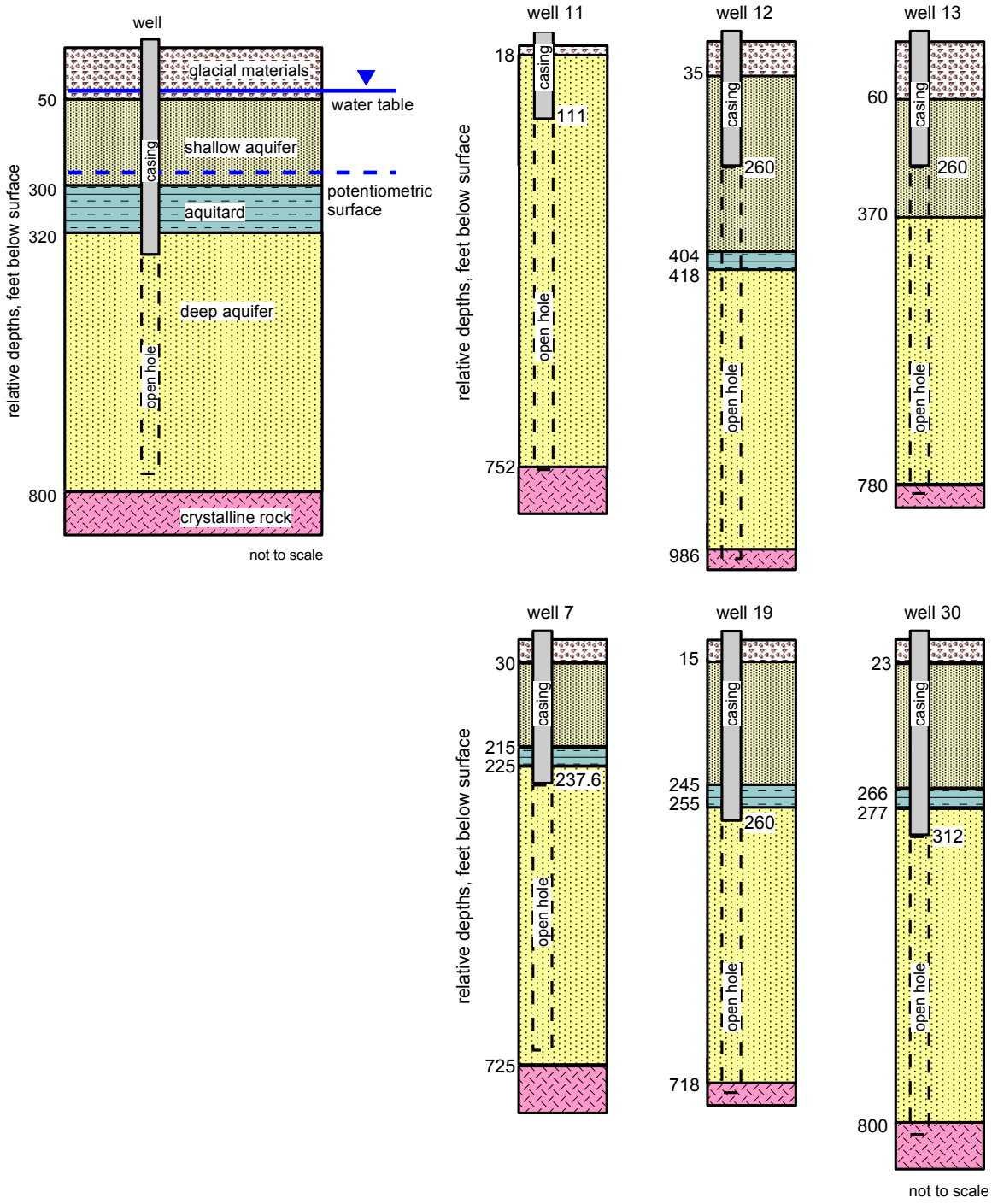

Figure 2. Construction details of the "long-term" municipal wells sampled throughout the project. Diagram at upper left shows typical hydrostratigraphy and well construction for the Madison area.

Sampling procedure at municipal wells

All well samples were collected at the wellhead while the high-capacity well pumps were running. Viruses were concentrated using glass wool filters, a method that has been fully validated (Lambertini and others 2008). Samples were obtained from a sampling tap on the well discharge line prior to treatment and discharge to the well reservoir. At wells where the pH exceeded 7.5, the pH was adjusted to between 6.5 and 7.0 using acid injection ahead of the filter. The Madison wells are plumbed so that there is zero back pressure between the reservoir and the well discharge line; this lack of pressure required the use of a booster pump to force sampled water through the glass wool filter. We used a portable heavy-duty peristaltic pump and food-grade tubing for this purpose; the pump and tubing were sterilized with a chlorine solution between each sample. Sampling each well required several hours of pumping; between 700 and 1000 liters of water were passed through the filter and the filtered volume was measured using a flow accumulator. A field blank was collected by pumping nineteen liters of reverse-osmosis water through a glass wool filter, using decontaminated field equipment. The filters were stored, transported and analyzed as described below.

Sampling procedure at lakes

The procedure for sampling lakes was similar to that for sampling the wells. A decontaminated pump and tubing were submerged in the lake, approximately 10 feet offshore. The water was pumped through a pre-filter to remove particulate matter. The sample stream was then acidified to a pH between 6.5 and 7.0, because the lake water was typically above pH 7.5. The acidified influent was split between two glass wool filters used in parallel. Filter effluent was directed onto the lake shore. Lake water was pumped at a rate of approximately 4 liters/minute until a total sample volume of about 1000 liters was passed through the filters. The pre-filter and two glass wool filters were transported on ice to Marshfield for analysis. The field equipment was decontaminated according to Marshfield standard procedures prior to re-use.

Sewage influent sampling

Clarified and settled sewage influent was collected and provided by the staff of the Madison Metropolitan Sewerage District at the Nine Springs sewage treatment plant. The influent was transferred to four sterile one-liter containers and shipped to Marshfield for analysis.

Virus analyses and sequencing

Pre-filters and glass wool filters were transported to the laboratory on ice and processed within 24 to 48 hours of sampling. Filters were eluted with beef extract/glycine and the eluate flocculated and concentrated with polyethylene glycol following the methods described in Borchardt and others (2004) and Lambertini and others (2008).

Samples were analyzed for six virus groups: enteroviruses, adenoviruses, rotavirus, hepatitis A virus (HAV), and norovirus genogroups I and II. Viruses were detected by real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and TaqMan probe using the LightCycler (Roche Inc.) platform. The procedures, primers, and probes are described in Lambertini and others (2008). Standard curves were established by treating stocks of each virus type with Benzonase (Novagen, Madison, WI) for 30 min at 37°C, followed by incubation for 2 days at 4°C, leaving only the nucleic acid contained within intact capsid-protected virions, and removing extraneous viral nucleic acid that would have inflated the estimate of genomic copy number. Viral RNA or DNA mass was measured fluorometrically using RiboGreen (Molecular Probes, Eugene, OR) or PicoGreen (Molecular Probes) and a CytoFluor Series 4000 fluorimeter (Applied Biosystems, Framingham, MA), then converted to genomic copies based on the nucleic acid molecular weight of that virus. Intact viruses were serially diluted, and each dilution was seeded into separate 0.14 ml volumes of negative final concentrated sample volume (FCSV) and extracted using the QIAamp DNA Blood Mini Extraction Kit (Qiagen). Therefore, the standard curves represent the entire quantitation process and include any matrix effects from the elution and flocculation procedures. Crossing points were calculated automatically by the LightCycler with the second derivative maximum method, and plotted against the decimal logarithm of viral RNA or DNA concentration.

qRT-PCR controls for each batch of reactions included an extraction negative control (unseeded FCSV), negative controls for the RT and PCR cocktails, and a positive control of known low viral concentration seeded into an FCSV matrix. This positive control also served as the LightCycler reference control, validating the use of the standard curves. qRT-PCR inhibition was evaluated by seeding 800 copies of hepatitis G virus (HGV) Armored RNA® (Asuragen Inc., Austin, TX) into the RT reaction of every sample. qRT-PCR was performed using HGV primers provided by the manufacturer and a laboratory-designed probe. Inhibition was considered absent when the crossing point of the HGV seeded samples was less than one cycle higher than the inhibition reference control (crossing point = 32).

Samples that were qRT-PCR-positive for enteroviruses were further evaluated for virus infectivity by cell culture using three cell lines (BGMK, RD, and Caco-2). Infectivity was gauged by two outcome measures: 1) Observation of cytopathic effect (CPE) in cultures held six weeks; 2) Integrated cell culture-PCR (ICC-PCR) in which a $\geq$ 10-fold increase in virus genomic copies in cell lysates from 2 week or 6 week cultures compared to the initial virus quantity in the FCSV cell culture inoculum was considered infectious.

All enterovirus and adenovirus positive samples were identified to serotype by sequencing using the ABI Prism 3100 Genetic Analyzer and previously described methods (Borchardt and others 2007a).

Isotopic and geochemical sampling and analysis

Samples for ions and isotopes were collected at the municipal wells from the sampling tap while the wells were running. Field collection followed standard procedures for collection of field parameters (pH, temperature, dissolved oxygen). Surface water samples were collected from open water along the shoreline with the exception of winter months, when lake water was pumped through a hole cut into the near-shore ice. Samples were analyzed for NO_3 and Cl^- at the Madison - Dane County Public Health Laboratory, a certified water analysis laboratory. Samples were analyzed for stable isotopes at the US Geological Survey Isotope Laboratory, where deuterium was determined by manganese reduction and oxygen-18 was determined by mass spectrometry on CO2 gas. Samples were analyzed for tritium at the University of Waterloo (Ontario) Environmental Isotope Laboratory by liquid scintillation counting on enriched samples. Samples for low-level tritium analyses, by enrichment and counting of H2-gas, were sent to the University of Miami Tritium Laboratory.

Attempts at depth-discrete sampling

One objective of this study was to gain insight into the subsurface transport pathways by which viruses reach deep municipal supply wells. Our approach focused on collecting depth-discrete water samples from several of the six study wells under pumping conditions. This proved challenging because submersible high-capacity pumps and associated discharge pipe installed in each supply well limit access for sampling equipment. We experimented with several methods over the course of this project, although none were satisfactory.

We were able to collect a depth-discrete sample for virus analyses from Madison municipal well 18, when it was taken offline for maintenance in early 2009. A Grundfos submersible electric pump was lowered to a depth of 252 feet, approximately one foot below the base of the well casing. Over 220 gallons were pumped through a glass-wool filter for virus analyses. Although this did not replicate production conditions, the sample likely represented water quality at the base of the casing. Virus results from the well 18 sampling are described in this report. In addition, samples were collected for stable isotope analysis and for analysis for various tracers of wastewater at the Wisconsin State Laboratory of Hygiene.

One of our attempts at downhole water sampling made use of a municipal supply well scheduled for abandonment in northeast Madison. We threaded a thin gas-displacement sampling pump down the well between the high-capacity pump shaft and the well casing. This attempt failed due to the difficulty of inserting the sampling pump tubing to sufficient depths. We concluded that this sampling method was not likely to succeed in the online wells and might pose unacceptable risks of equipment loss or well damage.

Another Madison well, well 15, came offline for maintenance in 2009. We were able to collect geophysical logs from this well but were unable to sufficiently purge the well to warrant sample collection. We had numerous conversations with the Madison Water Utility staff regarding well 15 and made some progress in design of an access tube to be installed inside the casing, alongside the high-capacity pump and discharge pipe. This

would have permitted in-well sampling at various depths in the borehole to help determine where viruses might be entering the well, and would have served as a prototype sampling installation for other deep wells. However, after careful examination of the well and wellhead we were forced to abandon this idea due to the expense and uncertainty of the project.

During 2009, we also worked with the Water Utility in the design and installation of a multilevel sampler (FLUTe system) inside a new observation well drilled near city well 29. The purpose of this well is to monitor water quality between well 29 and a nearby landfill, and the need for the well is related to the Utility's investment in a manganese filter for well 29. The initial FLUTe installation developed a leak and the replacement system was installed after this virus sampling project ended. There is potential to collect samples for virus analysis from the FLUTe, however there are some limitations caused by the flow rate and resulting sample volumes.

Although none of these initiatives ultimately proved successful, there are several positive outcomes from this effort. These include improved communication with the Water Utility staff and better mutual understanding of the potential benefits of vertical sampling and the physical constraints at the wells. Recently we learned our proposal to the US EPA STAR program on infrastructure sustainability will be funded. This will allow us to further advance our capability to collect depth-discrete samples under pumping conditions within the production wells and at depth-discrete sampling systems, such as the FLUTe.

Results

Precipitation, climate, and water levels during the study period

The Madison area received unusually high precipitation during the study period. Figure 3 shows the distribution of precipitation and air temperature between January 2007 and May 2009. Intense rainfall during August, 2007 caused minor flooding during that fall. Record snowfall (over 100 inches) occurred during the winter of 2007-2008. Finally, June 2008 was the second wettest month on record, with a rainfall of 10.9 inches in the Madison area (MMSD, 2008). Very intense rainfall between June 9 and 12, 2008 caused major flooding across southern Wisconsin. Following large storms in early July, the remainder of 2008 and the spring of 2009 had fewer major precipitation events.

Surface-water and groundwater levels and storm sewer flows responded to the precipitation events. Figure 4 summarizes storm sewer flows, the elevation of Lake Mendota, and groundwater levels in two local monitoring wells. Rapid increases in groundwater levels show that rapid recharge occurred after storm events. The Spring Harbor storm sewer drains street runoff from west Madison and discharges into Lake Mendota. It is one of several such storm sewers in the Madison area. Maximum storm flows occurred after the heavy rains in August 2007 and June 2008. A significant flow event also occurred during early January 2008 following an unusually warm "January thaw". Notable storm sewer flows were measured in March, April, and May 2009, after a seven month quiescent period of little storm water flow.

The June 2008 precipitation event is also important because it resulted in extremely high flows in the Madison sanitary sewers (MMSD, 2008). Sewage flows often increase during precipitation evens due to stormwater infiltration through leaky sewers and contributions from basement sump pumps. The average flow to the Nine Springs Wastewater Treatment Plant is about 41 million gallons per day (MGD). During the first significant rains on June 8, flows increased to 122 MGD, and then declined to about 80 MGD for several days. Several discharges of untreated sewage diluted with rainwater in the system occurred during this rain event, on June 9. The largest discharge was into the Cherokee Marsh and the Yahara River upstream of the Highway 113 bridge (1,080,000 gallons). There was a smaller discharge into the Cherokee Marsh on the south side on Golf Road (17,200 gallons). There were also two discharges to Starkweather Creek (245,000 gallons on the east side of the Dane County Regional Airport and 48,000 gallons near Milwaukee Street), a small discharge into Lake Mendota at Carroll Street, and two small discharges into Squaw Bay on Lake Monona; one on the south shore (50,000 gallons) and one on the east shore (4,000 gallons) (Jon Schellpfeffer, MMSD, written communication).

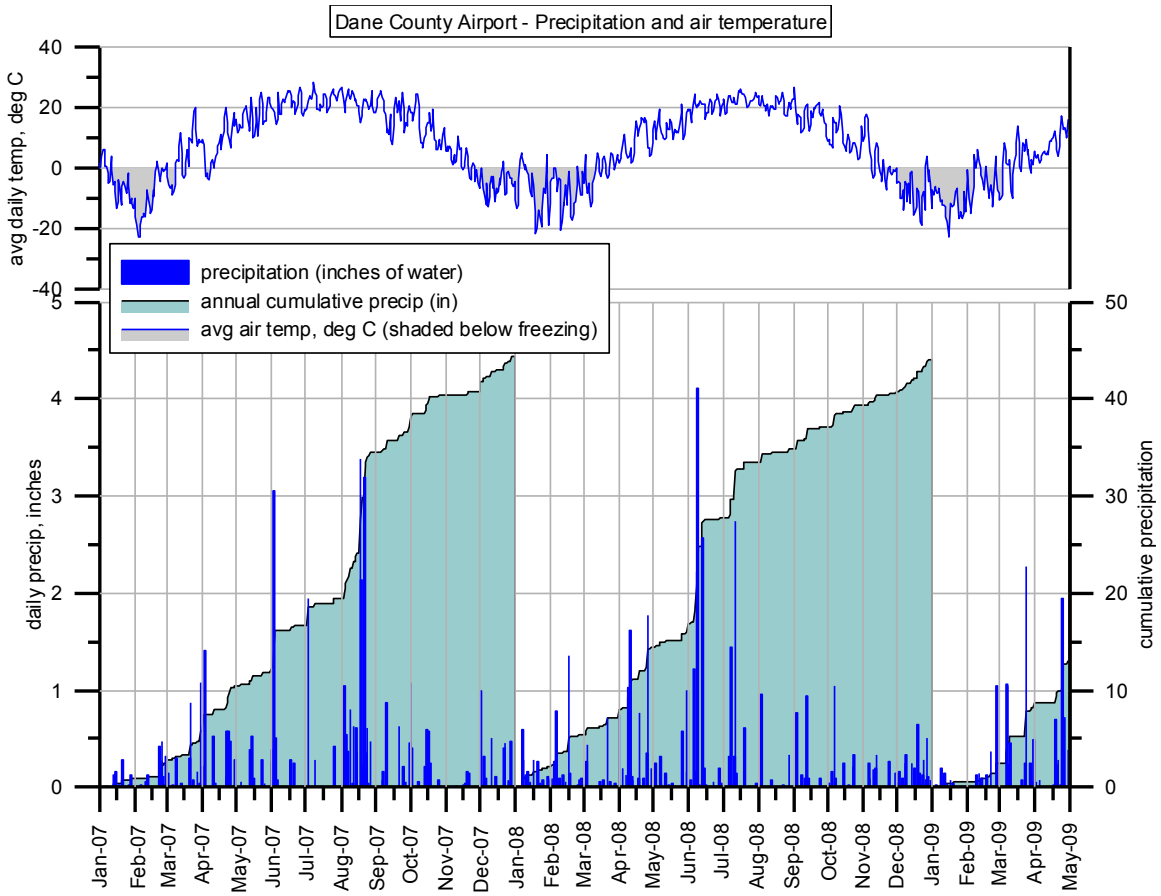

Figure 3. Precipitation and air temperature in the Madison area.

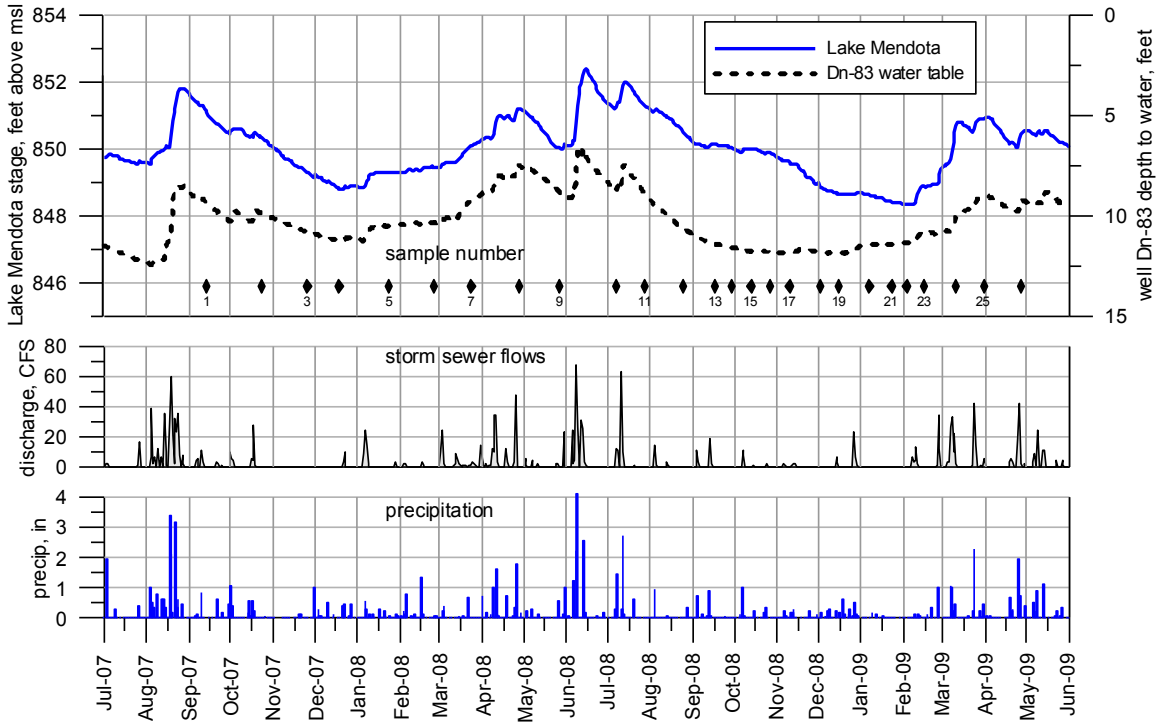

Figure 4. Sampling dates, stormwater flows, lake levels, and groundwater levels during the study period. Stormwater flows are from the Spring Harbor Storm Sewer (USGS site ID 05427965). Groundwater levels are from observation well DN-83 (water-table well; USGS). Sample numbers indicate times when samples were collected for virus analysis.

Viruses

The data reported here are limited to the six long-term wells (wells 7, 11, 12, 13, 19, and 30) sampled between September 2007 and May 2009, and lake and sewage influent sampling over the same period. Bradbury and others (2008) report virus results from earlier sampling. Well samples included 147 samples from the six different wells. Lake Mendota was sampled 18 times. Sewage influent was sampled on 26 different dates (Appendix A). The overall virus sampling consisted of 191 samples from wells, lakes, and sewage influent. Complete analytical data from the individual virus samples are available as Wisconsin Geological and Natural history Survey Open-File Report 2010-04B. This report is available online at http://www.uwex.edu/wgnhs/wofrs.htm.

Table 1 summarizes the overall virus results by sample source. Overall, water samples from wells were positive for viruses in 47 percent of the samples, and virus concentrations ranged from 0.00 to 6.27 gc/l (genomic copies per liter), with a mean of 0.65 gc/l. Lake Mendota samples were positive 82 percent of the time, and ranged from 0.00 to 532 gc/l, with a mean of 44 gc/l. This range does not include an outlier sample of 69,900 gc/l collected on March 31, 2009. Such an extremely high value suggests that the sample filter encountered a clump of fecal material in the lake. Not surprisingly, influent to the Madison sewage treatment plant was extremely high in viruses, with all samples positive, and concentrations ranging from about 13,000 to over thirty-six million gc/l, with a mean of 2,010,000 gc/l.

Virus results varied significantly with time, and, as with our previous report (Bradbury and others, 2008) there is some apparent correlation between virus levels in sewage, lakes, and groundwater. Figure 5 shows the percentage of virus detections in wells along with virus concentrations in sewage and Lake Mendota water and monthly precipitation totals. During the fall and winter of 2007, the wells were 40 to 80 percent virus-positive. The positive percentage declined to about 20 percent in early 2008, and to zero in late May 2008 before jumping to over 80 percent in July 2008. Similarly, in the fall of 2008 the wells were 50-80 percent positive, but viruses were absent from the wells through the winter of 2008-2009. Virus detections increased to 80-100 percent from March through May of 2009. It is interesting to note that the July 2008 increases in virus detections followed the extreme rainfall events the preceding June, and the March-May 2009 concentration peaks followed heavy rains during early 2009.

Virus concentrations in sewage, while always in the thousands of gc/l, peaked in November 2007, declined through May 2008, and then rose to a peak of over 10^7 gc/l in October 2008. Following this peak the sewage virus loads decreased steadily to 10^4 gc/l in May of 2009. The virus content of Lake Mendota followed a similar trend, with a peak of 103 gc/l in October 2008 and a decrease during the winter of 2008-2009.

Table 1. Summary of virus detections by water source, wells 7, 11, 12, 13, 19, 30 only.

		virus detection (gc/l)			
water source	number of samples	percent positive	min	max	mean
wells	147	46.6	0.00	6.27	0.65
Lake Mendota	18	82	0.00	532*	44*
sewage influent	26	100.0	12,900	36,310,000	2,010,000

*Maximum concentration detected in Lake Mendota was 69,900 gc/l on 3/1/09. This sample is an outlier that may indicate raw sewage in the lake; it was excluded from the statistical summary.

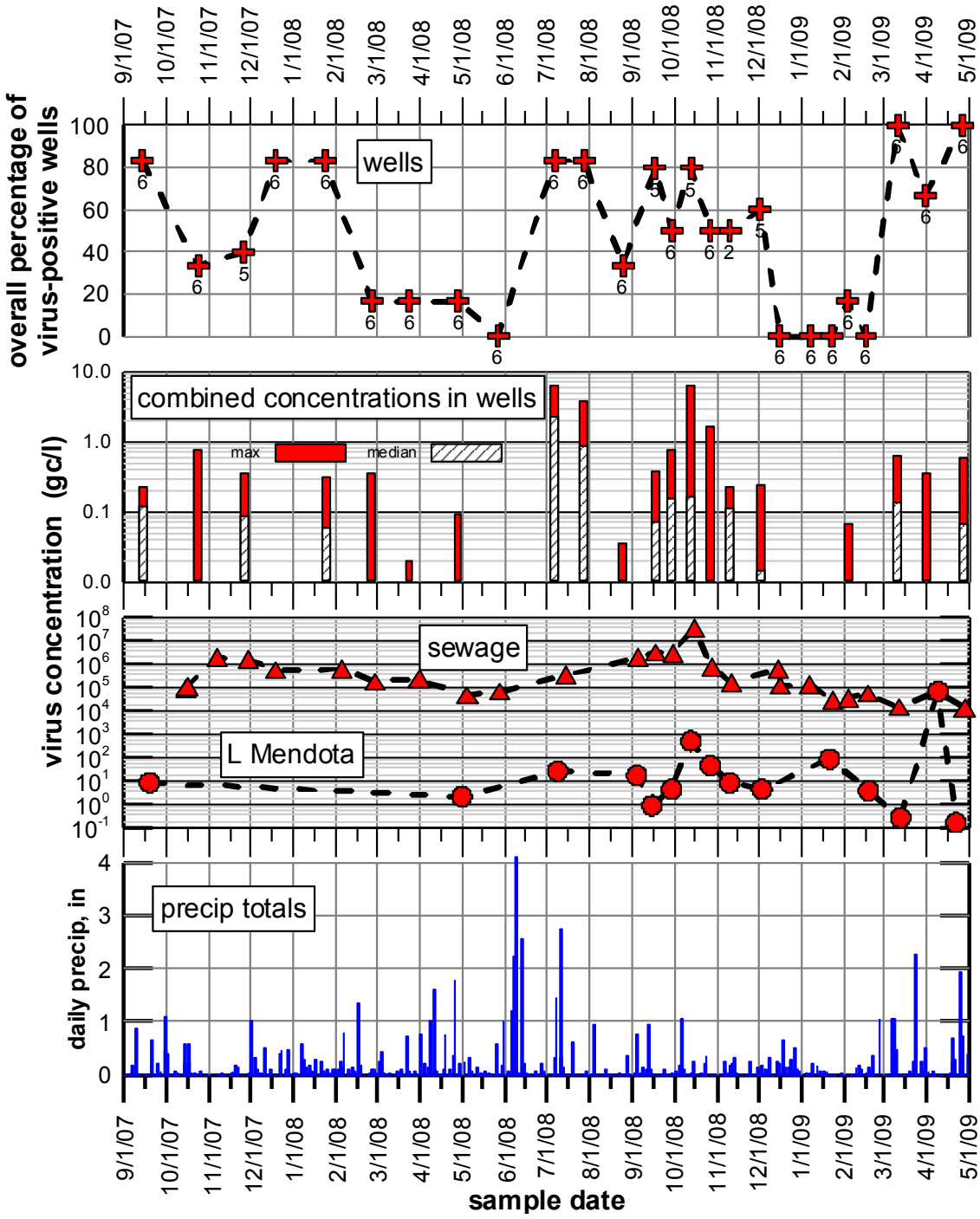

Figure 5. Overall virus detections in wells, monthly precipitation totals, and virus concentrations in Lake Mendota and sewage influent. Numbers next to well samples indicate number of wells sampled on that date.

Well-by-well virus results

Viruses were detected at least eight times in every one of the six wells repeatedly sampled for this study, but no well was virus-positive in every sampling round. Figure 6 shows virus concentrations through time for each well, along with the overall percentage of detections in each well. Note that each well had spikes in virus concentrations in January, June and July 2008, during the period of record rainfall or snowmelt in the Madison area.

Virus subtyping and Infectivity

Fifteen different serotypes of viruses were identified in wells, sewage, and lake water during this study, and in many cases wells, lakes, and sewage contained identical virus serotypes (table 2). Consistent with our previous study (Bradbury and others, 2008), the apparent correlation between viral serotypes found in sewage, lakes, and groundwater is important because it suggests very rapid transport from the surface to groundwater. Viral serotypes vary seasonally and annually, and so correlation between surface and subsurface serotypes would be unexpected if transport times from the surface to groundwater exceed many months. Although some viruses (A41, A2, echovirus 3, echovirus 11) were found in both lakes and wells, other viruses found in wells (A7, echovirus 6, CoxA16) were never found in lakes, suggesting that the lakes are not a source for these viruses in groundwater. All viruses detected in well water were also detected in sewage influent, with the exception of coxsackievirus B3, detected in wells 7, 11, and 30 at from .03 to 3.7 gc/L.

Adenovirus 41 (A41) was the virus serotype most frequently identified in this study, and ranks first in the viruses identified in all three sample sources (wells, sewage influent, and lakes). Table 2 summarizes the overall virus rankings. The Adenovirus 31 (A31) serotype was the second most common virus detected in wells and sewage, but was less common in lake water. Adenovirus 2 (A2) was the third most common virus in wells and sewage and the second most common in lake water. Following the top three ranks there is significant drop off in detection frequency of other viruses (table 2). For example, the third most common virus in wells, Adenovirus 2, was detected 11 times overall, but the fourth most common virus, Echovirus 3, was detected only 5 times. Table 3 presents all virus data collected for the six long-term wells.

Some, but not all, samples positive for viruses were shown to be infective by either cytopathic effect or ICC-PCR. Samples marked with “X” on figure 6 tested positive for infectivity. Sewage influent samples always tested positive for infectious enterovirus or adenovirus or both. No pattern of infectivity related to season or precipitation or temperature is apparent from these data.

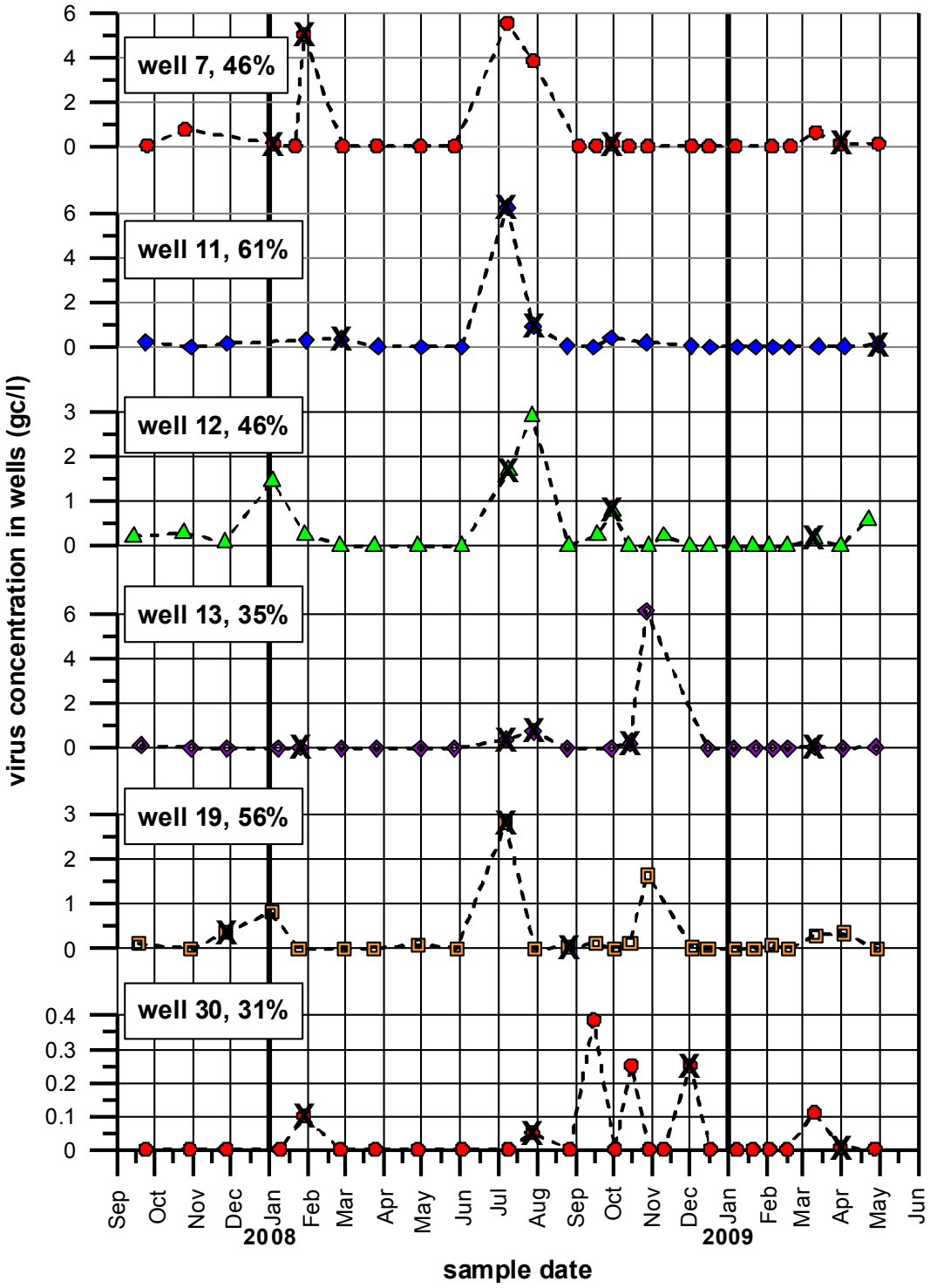

Figure 6. Virus concentrations through time for each of the six long-term wells. Percentages next to well labels show percent virus-positive samples. "X" over symbol indicates that sample was positive for infectious enterovirus or adenovirus or both. Wells 11, 12 and 13 are multi-aquifer; wells 7, 19 and 30 are cased into the deep sandstone.

Table 2. Most frequently detected enterovirus and adenovirus serotypes by rank. Number of detections in parentheses. Multiple viruses indicate a rank tie. Numbers and letters refer to virus serotypes; E6 (echovirus 6) A41 (Adenovirus 41), etc. A "?" after a letter means the isolate could not be subtyped.

Rank	wells	sewage	lakes
1	A41 (38)	A41 (18)	A41 (12)
2	A31 (12)	A31 (15)	A2, E3 (3)
3	A2 (11)	A2, E3 (5)	A31, E11, E30 (2)
4	E3 (5)	E9 (4)	--
5	E30, A7 (4)	A6, E11 (3)	--
6	Cox A16, Cox B3, Cox B4, E11 (3)	Cox A16, Cox B4 (2)	--
7	A5, E6, E9, E71 (1)	A5, A7, E6, E30, E71, E? (1)	--

Temporal coincidence of viral serotypes

There is temporal coincidence between serotypes present in sewage influent and serotypes present in groundwater. Our previous study (Bradbury and others, 2008) suggested correlation between the occurrence of specific serotypes present in sewage and serotypes present in samples from wells. The continued time-series sampling in the present study strengthens this observational relationship. Table 4 codes each detected serotype by color to make these correlations more visually apparent. The correlations are most obvious with those viruses that have an intermediate to low occurrence; a common virus like A41 is not informative for temporal relationships because it is nearly always present in sewage. In contrast, the temporal coincidence of rare viruses is striking. For example, coxsackievirus A16 appears in only two sewage samples in July and August 2008 and it appears in the wells only at the same time, July 2008. There are other instances of temporal coincidence of virus serotypes between sewage and wells. For example, A31 was not detected in either sewage or wells from September to November 2007, but appeared in sewage in December and wells in January. It was not detected in either source during January through March 2008, but reappeared in sewage in April 2008 and in wells in July 2008. Virus A2 was present in both sewage and wells in the fall of 2008 but was absent in both during the winter. Virus E30 was not detected in any samples until February 2009, when it appeared in sewage during February and March and in wells in both March samples. E11 appeared in both sewage and wells in the fall of 2007 and spring of 2008 and then was not detected in either sewage or wells for the remainder of the study. The temporal coincidences are not perfect, though. For example, coxsackievirus B3 was detected twice in the wells, but never in the sewage likely because its concentration in sewage was near the limit of detection. More frequent sewage sampling may have resulted in a coxB3 detect. Similarly, the results for Lake Mendota may not show the same degree of virus temporal coincidence because there were fewer lake samples and groundwater was sampled at six locations whereas the lake was sampled at only one.

Table 3. Summary of virus detection by sampling period. Virus notation as in Table 2 and in addition NoVGI and NoVGII = Norovirus genogroups I and II, respectively; ND= sampled but not detected; NS = no sample collected.

sample round	date	Project Year	Well samples						Sewage	Lake Mendota
			7	11	12	13	19	30		
1	9/14/2007	1	A7	A2	A41	A2, E?	A41	ND	NS	A41, A2
2	10/24/2007	1	E3	ND	A6, E11	ND	ND	ND	A6, A2, E11, E3	
3	11/26/2007	1	NS	E11	A41	ND	A2, E?	ND	E11, CoxB4, A41, NoVGI	
4	12/19/2007	1	ND	NS	A41	ND	A2	ND	A2, A41, A31	NS
5	1/24/2008	1	A7,A31,E6	A31	A31	A41	ND	A2	A2	NS
6	2/26/2008	1	ND	E?	ND	ND	ND	ND	A41, E6, E11, NoVGI	NS
7	3/24/2008	1	ND	A41	ND	ND	ND	ND	A2, NoVGI, R	NS
8	4/28/2008	1	ND	ND	ND	ND	E11	ND	A6, A31, E11, G1, R	A2
9	5/27/2008	1	ND	ND	ND	ND	ND	ND	A6, A41, A31	NS
10	7/7/2008	1	A7,A31,Cox A16	A41, A31, E3	A2, E3	Cox A16	A41, E3	ND	A7, A41, A31, E3, CoxA16, NoVGI	A41, E30, E3
11	7/28/2008	2	A7, A31, Cox B3	CoxB3	E3	A41, A31, CoxA16	ND	A41, E?	NS	NS
12	8/25/2008	2	ND	A31	ND	ND	A41	ND	A41, A31, CoxA16	A5
13	9/17/2008	2	A41	ND	A2	NS	A41	A41	A41, E?	A41, NoVGI
14	9/29/2008	2	A41	A41	A41	ND	ND	ND	A41, A31, E9	A41
15	10/13/2008	2	ND	NS	ND	A41	A41	A41, CoxB3	A12, A31, E25	A41, A31
16	10/27/2008	2	ND	A41	ND	A41	A41, A31	ND	A41, A31, E25	A41
17	11/10/2008	2	NS	NS	A41	NS	NS	ND	A41, A31, E71	A41, A31
18	12/2/2008	2	ND	A31	ND	NS	A41	A41, E71	A41, A31, E3	A41
19	12/16/2008	2	ND	ND	ND	ND	ND	ND	A41, A31, E3	ND
20	1/7/2009	2	ND	ND	ND	ND	ND	ND	A41, A31, E3, E9	ND
21	1/23/2009	2	ND	ND	ND	ND	ND	ND	A41, A31, E9	A41
22	2/3/2009	2	ND	ND	ND	ND	A31	ND	A41, A31	ND
23	2/16/2009	2	ND	ND	ND	ND	ND	ND	A41, E30, Cox B4, NoVGI, NoVGII	E11
24	3/11/2009	2	A41, A31	A41	A41, E30, CoxB4	A41	A41, CoxB4	A41, CoxB4	A41, A31, E30, E9, NoVGI	A41, E11
25	3/31/2009	2	A41, E30	A41, E30	ND	ND	A41, E30	A41	A2, A41, E9, NoVGI	E3
26	4/27/2009	2	A41	A2	A41, E9	A5	A2	A2	A5, A41, A31, CoxB2, E9	E3

Table 4. Virus subtypes detected by sample source and time. Viruses grouped in order of detection frequency. Numbers and letters refer to virus serotypes; E6 (echovirus 6) A41 Adenovirus 41, etc.

	Sample round	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
	median sample date	14-Sep-07	24-Oct-07	26-Nov-07	19-Dec-07	24-Jan-08	26-Feb-08	24-Mar-08	28-Apr-08	27-May-08	7-Jul-08	28-Jul-08	25-Aug-08	17-Sep-08	29-Sep-08	13-Oct-08	27-Oct-08	10-Nov-08	2-Dec-08	16-Dec-08	7-Jan-09	23-Jan-09	3-Feb-09	16-Feb-09	11-Mar-09	31-Mar-09	27-Apr-09
sewage	A41	x		x	x		x			x	x	NS	x	x			x	x	x	x	x	x	x	x	x	x
	A31				x				x	x	x	NS	x		x	x	x	x	x	x	x	x	x		x		x
	A2	x	x		x	x		x				NS														x	
	E3		x								x	NS							x	x	x						
	E30											NS												x	x		
	A7										x	NS															
	E11		x	x			x		x			NS															
	CoxA16											NS															
	CoxB3											NS															
	CoxB4			x								NS												x			
all wells	A41	x		x	x	x		x			x	x	x	x	x	x	x	x	x				x		x	x	x
	A31					x					x	x	x				x		x					x			
	A2	x		x	x	x					x			x													x
	E3		x								x	x															
	E30																							x	x		
	A7	x				x					x	x															
	E11		x	x					x																		
	CoxA16																										
	CoxB3											x				x											
	CoxB4																							x			
lakes	A41	x	NS	NS	NS	NS	NS	NS		NS	x	NS		x	x	x	x	x	x			x			x		
	A31		NS	NS	NS	NS	NS	NS		NS		NS				x		x									
	A2	x	NS	NS	NS	NS	NS	NS	x	NS	x	NS															
	E3		NS	NS	NS	NS	NS	NS		NS	x	NS														x	x
	E30		NS	NS	NS	NS	NS	NS		NS	x	NS															
	A7		NS	NS	NS	NS	NS	NS		NS		NS															
	E11		NS	NS	NS	NS	NS	NS		NS		NS												x	x		
	CoxA16		NS	NS	NS	NS	NS	NS		NS		NS															
	CoxB3		NS	NS	NS	NS	NS	NS		NS		NS															
	CoxB4		NS	NS	NS	NS	NS	NS		NS		NS															

Chemistry and environmental isotopes in virus study wells

During this second year of study, the six long-term wells were sampled once for tritium, five times for nitrate and chloride, and four times for the stable isotopes of water, O^{18} and deuterium (Appendix B). Measurements of pH, specific conductance and dissolved oxygen were collected during each sampling event (Appendix C). Results presented below include these samples and samples collected during the first year of study (previously reported in Bradbury and others 2008).

The geochemical and isotopic data were compiled to assess potential indicators of a well's vulnerability to virus contamination. Well vulnerability is assumed to increase with increasing proportion of lake water, shallow groundwater, and recently recharged groundwater that reaches the well. Each well's susceptibility to viruses may also be related to the physical condition of the well, including its age, depth of casing, and proximity to a sewer main.

Chloride and nitrate are naturally occurring constituents of groundwater, however elevated concentrations of these constituents may be attributed to contamination from septic systems, fertilizer, sewers or road salt. Background nitrate levels in Wisconsin aquifers are generally less than 2 mg/L, and average chloride concentrations in Dane County wells are about 8 mg/L (Kammerer 1981). Chloride and nitrate measurements from this study (Appendix B) are summarized in Table 5.

Nitrate levels are low in all wells, but the average chloride concentration varies from 2.1 mg/L to 44.2 mg/L in the study wells. Causes of the large variation in chloride at each well may include the proportion of shallow water reaching each well or the proximity of a well's capture zone to a heavily salted roadway.

Tritium (^{3}H) content of water indicates the age of well water and indicates which wells produce a large proportion of recent recharge. Interpretation of groundwater age from these tritium data is complicated by mixing of groundwater of various ages. This mixing of waters presumably occurs along the open-interval of each of these deep wells. Results from Year 2 of this study are presented in Appendix D; Table 5 reports the average tritium concentration from each study well over several sampling rounds. As illustrated in table 5, wells 12 and 30 produce water with very low tritium, suggesting that these wells produce predominantly old groundwater. This is consistent with the relatively low virus concentrations measured at these wells. The three wells with elevated maximum virus concentrations (wells 11, 13 and 7) have appreciably higher tritium than wells 12 and 30. Although well 19 produces a greater volume of recently recharged groundwater (that is, higher tritium) it does not appear vulnerable to high virus concentrations, but it did have the second highest virus detection rate, 56%.

Table 5. Summary of characteristics of the six long-term wells, including virus, tritium, chloride, and nitrate results.

Well	Aquifer	Year of construction	Well depth (feet)	Casing depth (feet)	Distance to surface water (feet)	Distance to a sewer main (feet)	Number of times sampled for viruses	Number of virus-positive samples	Percent virus-positive samples	Maximum virus concentration (gc/L)	Average tritium concentration (TUs)	Average chloride concentration (mg/L)	Average nitrate concentration (mg/L)
11	multi-aquifer	1959	752	111	4740	410	23	14	61	6.27	5.5	44.2	2.5
13	multi-aquifer	1959	780	128	2,510**	715	23	8	35	6.14	1.6	7.8	1.7
7	confined	1939	736	238	3410	4,250	24	11	46	5.53	5.1	11.6	<0.1
12	multi-aquifer	1957	529	260	1,115*	610	26	12	46	2.91	0.40	2.1	1.0
19	confined	1970	710	260	835	5,400	25	14	56	2.83	3.7	4.4	<0.1
30	confined	2003	800	312	4525	1,530	26	8	31	0.39	0.39	3.6	<0.1

* Well 12 distance reported is to storm water retention pond; well is about two miles from Lake Wingra.

** Well 13 distance reported is to Cherokee Marsh.

Surprisingly, the percentage of virus detections in deeply-cased wells is similar to that in cross-connected wells. For the wells reported to be cross-connected (wells 11, 12, and 13) samples were virus-positive in 34 out of 72 samples, for a detection rate of 47%. For wells reported to be deeply cased (wells 7, 19, and 30), the detection rate was 33 detects out of 75 samples, or 44%.

We also examined relationships between well construction (total depth, depth of casing) and virus detections. In general, shallow well casings and older age appear to correlate to higher virus concentrations. However, the sample size in this study was too small to support any robust statistical correlations. In addition, the precision of the qPCR method does not allow evaluating of differences of 1 to 3 genomic copies. The expectation that wells with a shallow total depth would have higher virus concentrations is not borne out by the data. Total depth of well does not have a clear relationship to the well's susceptibility to high virus concentrations. The elevated virus concentrations measured at wells 7, 11 and 13 might reflect variation in aquifer properties along the deep well bores, with these wells producing a greater proportion of groundwater from the uppermost portions of the open interval.

The proximity of a well to a sewer main is also evaluated with respect to maximum virus detections (Table 5). The lack of correlation apparent in this table suggests that the forced and gravity mains may not be the only, or the primary, source of viruses to the subsurface. Smaller sewer lines, such as laterals extending from mains to side streets and individual homes and businesses, are ubiquitous along city streets. These laterals may be a source of subsurface viral contamination to wells.

A second method employed to evaluate the impact of near-by sewers on well water quality was analysis of a single sample for a suite of compounds indicative of waste water, such as personal care products and detergents. Groundwater collected from municipal supply well 18 just below the base of casing was below detection limits for the 44 compounds analyzed for in this sample (Appendix E). As described previously in this report, attempts to collect additional depth-discrete samples in the study wells were not successful. Samples of whole water from these supply wells were not analyzed for these compounds due to the significant dilution expected to occur within the aquifer.

The deuterium (^{2}H) and oxygen-18 (^{18}O) contents of water can identify which wells, if any, produce some groundwater that recharged from surface-water bodies, such as the Madison lakes. Surface water systems are a potential source of viruses to near-by groundwater supply wells (e.g. Borchardt and others, 2004), and municipal wells were sampled for ^{2}H and ^{18}O about 12 times during this study (Appendix F). The oxygen isotope data collected during this study indicate that none of the sampled wells are dominated by recharge from surface water. Shown in Figure 7, samples from well 19 lie to the right of a local meteoric water line (LMWL) from Dane County (Swanson and others, 2006).

The meteoric water line represents the relationship between deuterium and oxygen-18 that is characteristic of precipitation anywhere in the world; the *local* meteoric water line is characteristic of precipitation in a given geographic area. Groundwater samples originating from terrestrial recharge should plot on or close too, the LMWL. Surface water samples usually plot to the right of the LMWL because water that has been exposed to open-water evaporation (lakes, wetlands) becomes depleted in lighter isotopes. Accordingly, surface water samples from the Yahara lakes plot significantly to the right of the LMWL (figure 7). Although well 19 is reportedly cased through the Eau Claire aquitard (Table 1), it is located very close to Lake Mendota (Fig. 1) and likely receives some groundwater recharged through the lake. Well 7 also plots slightly to the right of the LMWL and the other wells, and it could have limited contribution from surface water. In contrast, well 30 has an $\delta^{18}O$ composition lower than that reported for modern groundwater by Bradbury and others (1999), and is indicative of cooler climates; thus, it is likely that well 30 produces appreciable amounts of glacial melt water from the Pleistocene – amounts not seen in the other study wells. The oxygen isotope ratios from wells 11, 12 and 13 lie along the LMWL and indicate little to no contribution of surface water at these wells.

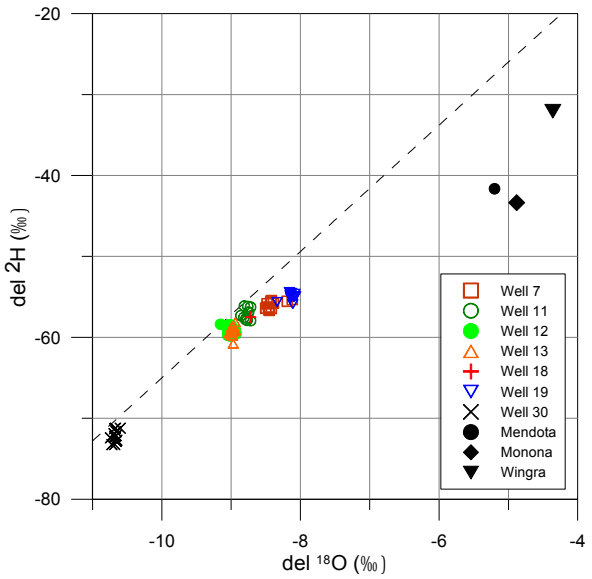

Figure 7. ^{18}O - ^{2}H composition of well and lake water. Dashed line is the local meteoric water line (Swanson and others, 2006).

The variability in a well's isotopic composition can also help identify wells with surface water contributions, because the fractionation of surface water varies seasonally. Thus, the isotopic composition of groundwater recharged from a surface water source is expected to be more variable than the isotopic composition of groundwater recharged by direct infiltration of precipitation (Hunt and others 2005). The median and standard deviation of $\delta^{18}O$ from samples collected in each study well over the project period are shown in Figure 8. The greater variability in $\delta^{18}O$ values in samples from wells 19 and 7 is consistent with the interpretation that these wells receive a small amount of recharge from lake water. Alternatively, Hunt and others (2005) identify variations in the pumping

pumping history – both in the well of interest as well as nearby wells as being a mechanism that can affect the variability in water isotope composition.

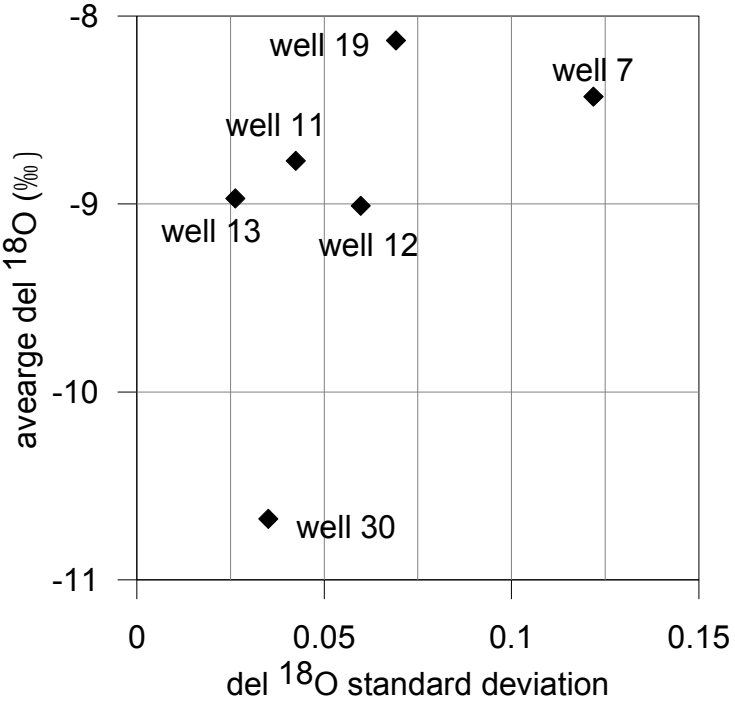

Figure 8. Variation of ^{18}O in well water samples. Each well was sampled 11 or 12 times (see Appendix A).

Discussion

Significance of virus detections

Viruses were detected in at least eight samples from each of the six municipal wells chosen for long-term sampling in this project, and the percentages of samples positive for viruses ranged from 31 % in well 30 to 61% in well 11. These findings are consistent with our previous work (Borchardt and others, 2007a; Bradbury and others, 2008) and show that even deeply cased municipal wells in confined aquifer settings can be susceptible to pathogen contamination. It is clear from these results that casing these deep wells across a regional aquitard (the Eau Claire aquitard) does not prevent virus contamination, or even significantly reduce the percentage of virus detections. However, the absolute concentrations of viruses (in gc/l) were appreciably lower in two of the deeply cased wells (wells 13 and 30) than in the other wells sampled, and, as shown above, larger casing depth appears to be correlated with lower virus concentrations. Multiple samples from each well tested positive for infectivity, showing that these viruses can represent a public health threat if the water is not disinfected by chlorination or other means.

These results also show that time-series sampling is absolutely necessary to detect and quantify virus presence in these wells. Each well produced several consecutive virus-negative samples followed by one or more consecutive positive samples. Single, or even quarterly samples from wells will not provide an accurate measure of virus presence in deep groundwater.

Correlations between viral serotypes in sewage and groundwater- implications for transport time

The apparent correlation between serotypes present in sewage influent and those present in groundwater samples (table 4) continues to be the most intriguing aspect of our virus research in Madison. The presence or absence of identical serotypes in these two reservoirs at roughly the same time, and the similar variability in serotypes over time suggests very rapid transport (days or weeks) between the surface and the groundwater system.

We acknowledge that to date the apparent correlations in table 4 have not been tested statistically, and detailed statistical analysis was beyond the scope of the present study. In future work, such as our STAR infrastructure proposal recently funded by USEPA, we intend to explore the significance of these correlations.

Potential virus pathways to wells

As stated in the introduction to this report, the four conceptual models of virus transport to the confined aquifer include (1) transport through the aquitard by porous-media flow; (2) transport by porous-media flow around the edge of the aquitard or through nearby "windows" or breaches in the aquitard, including local lakes; (3) transport by rapid flow through fractures in the aquitard or through cross-connecting nearby wells; and (4) transport by rapid flow along the well annulus through damaged, deteriorated, or poorly installed grout or breaches in the well casing. This current project has not been able to definitively confirm or discount any of these potential flow paths, although the simultaneous detection of viruses in multiple wells miles apart suggests that pathway 4, failure of the well casings, is unlikely because it would require that numerous deep well casings are failing at once. During the course of this project we also learned that many unabandoned private wells still exist within the city of Madison. The Madison Water Utility has an active program for locating and properly abandoning (plugging) these old and currently unused wells. Although these wells might serve a conduits for transport of contaminants from the surface to the shallow aquifer such wells are, with few exceptions, quite shallow, and do not explain the movement of viruses to the deep aquifer.

The experience gained in this project will focus future research efforts in two areas. The experience gained in sampling at discrete depths from production wells under pumping conditions underscores the need to secure adequate funding to succeed in this task. The installation and operation of depth-discrete monitoring systems, such as the FLUTe,

suggest that laboratory methods or experimental design that allows for sampling a smaller volume of groundwater may prove useful.

Lakes as a source of viruses

Although at first glance infiltrating lake water seems a plausible source for the viruses found in the municipal wells, two lines of evidence show that the lakes are probably not the primary virus source. First, the deuterium/oxygen-18 relationships (figure 9) suggest that only two wells (7 and 19) receive some limited proportion of lake-derived water, while all wells contained viruses. Second, with the exception of the July 2008 levels in Lake Mendota, virus concentrations in the lakes are generally as low as or lower than virus concentrations in the wells. Assuming significant mixing and dilution with virus-free water in the aquifer, the lake virus contents are likely too low to account for the virus levels in the wells.

Sanitary sewers as a source of groundwater contamination

The high rates of detection of human enteric viruses in groundwater sampled during this project suggests that exfiltration from sanitary sewers has a significant impact on groundwater quality. Sanitary sewers are a major part of civic infrastructure in urban settings and represent a significant potential source of groundwater contamination. Sewer exfiltration, or outward leakage of sewage wastes, represents a potential source of pathogens, toxic chemicals, pharmaceutical compounds and other materials to the subsurface environment (Bishop and others 1998). There have been two schools of thought on the significance of sewer exfiltration (Rutsch and others 2008). Some investigators argue that the overall impact of sewer exfiltration is insignificant due to the small volumes of leakage and to biodegradation and sorption of contaminants in the soil zone. Others (e.g. Leif Wolf 2004; Osenbrück and others 2007) believe that exfiltration can be a major source of groundwater contamination. Most studies conclude that the impact of sewage exfiltration on groundwater is quite variable in time and space and there is currently a lack of knowledge about both the quantity of leakage and its consequences for the environment (Rutsch and others 2008). Hunt and others (2010) recently documented the presence of sewage effluent in shallow groundwater at a community in Wisconsin.

Conclusions and Recommendations

Conclusions

This study confirms the frequent occurrence of human enteric viruses in groundwater pumped from deep municipal water-supply wells in Madison, Wisconsin. Viruses were found in all wells sampled, with 15 different serotypes of viruses identified. Some, but not all, samples positive for viruses were shown to be infective by either cytopathic effect or ICC-PCR. The percentage of virus-positive samples ranged from 61% in wells know to have multi-aquifer construction or shallow casings to 30 % in well 30, a new, deep well deeply cased across a regional aquitard. Detection percentages in cross-connected and deeply cased wells were similar. The simultaneous detection of viruses in multiple wells miles apart shows that virus presence cannot be attributed to a single surface source or a single defective well. Instead, these detections suggest widely distributed or multiple virus sources and multiple pathways from the virus source to the wells.

Virus sampling of municipal wells requires multiple samples and a time-series approach. Although viruses were found in every well sampled, no well contained detectable viruses on every sample date. Furthermore, on some sample dates no viruses were found in any well, and on other dates every well contained viruses. This study included 26 sample events over 20 months.

Virus detections in municipal wells are correlated with recharge events. Significant increases in virus detections and concentrations followed rainfall and snowmelt events in August 2007; January, June, and July 2008; and March and April 2009. During these times the sewers are often surcharged with water and increased leakage from the sewers is very likely.

Leakage from urban sewers beneath Madison is the most likely source of the viruses detected in the municipal wells, as supported by several lines of evidence. First, the raw sewage carries a very high (10^4-10^7 gc/l) virus load, and both the physical characteristics of the sewers (age, location) and visual inspections (video logs showing breaks and root invasions) suggest that they leak. Second, with one exception, all viruses detected in well water were also detected in untreated sewage. Third, variations in virus serotypes identified in the sewage also appear in well water, with significant temporal correlation. Fourth, the hydraulic gradients beneath Madison are strongly downward, which would transport viruses downward from the near-surface sewers toward the deep aquifer.

One of the most intriguing findings of this work is the temporal variation and correlation between virus serotypes in sewage and groundwater. In several instances an occurrence of a "new" virus in sewage is followed within weeks by detection of the same virus in water produced from municipal wells. The implied transport from the sewers to the wells occurs much more rapidly than previous porous-media calculations or modeling have suggested. Transport along preferential pathways such as fractures or poorly-grouted

well casings is required to explain the virus occurrence. If such rapid transport exists, then deeply-cased municipal wells may be much more vulnerable to shallow contamination than previously assumed.

This work also supports the concept of enteric viruses as potentially excellent groundwater tracers. Viruses have very desirable tracer properties of mobility, unique identification, and most importantly, quantification over a broad concentration range, from millions to a fraction of genomic copies per liter. Further research on viruses as tracers is needed.

Recommendations

This time-series study confirms earlier work by our research group (Borchardt and others, 2007a; Bradbury and others, 2008) showing that human enteric viruses are present in water produced by deep high-capacity municipal wells in Madison, WI. While similar studies have not been conducted in deep wells in other Wisconsin or Midwestern cities it seems likely that other municipalities might have similar virus occurrences. Many of the viruses detected in this study were shown to be infective. Therefore it is important that municipal water systems using groundwater as a source disinfect the produced water in a manner known to deactivate viruses.

The work reported here suggests several avenues for additional research, as follows:

- additional investigations of potential virus pathways. Our research group will be attempting to identify virus pathways and transport mechanisms under a recently-received STAR grant from the USEPA;
- small-scale and theoretical research into virus transport mechanisms, such a colloid-facilitated transport;
- a better understanding of virus presence inside aquifers. Our current work has sampled only water produced by high-capacity wells, which represents a mixture of water entering the well bore from various depths and directions. We do not yet have a good picture of the distribution of viruses in the geologic materials outside of the well.

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Appendices

Note: These appendices contain basic field and laboratory analytical data collected during the project, with the exception of the virus analyses. Analytical virus data are contained in WGNHS Open-File Report 2010-04B, available at http://www.uwex.edu/wgnhs/wofrs.htm

Appendix A: Sample collection dates and parameters

Round number	Date	Project Year	Stable Isotopes	Tritium	Major Ions	Chloride and Nitrate
1	9/14/2007	1	X	X	X	
2	10/24/2007	1	X			
3	11/26/2007	1	X			
4	12/19/2007	1	X		X	
5	1/24/2008	1	X			
6	2/26/2008	1	X			
7	3/24/2008	1	X			
8	4/28/2008	1		X		
9	5/27/2008	1				X
10	7/7/2008	1	X			
11	7/28/2008	2				
12	8/25/2008	2	X			X
13	9/17/2008	2		X		X
14	9/29/2008	2				
15	10/13/2008	2				X
16	10/27/2008	2				
17	11/10/2008	2				X
18	12/2/2008	2				
19	12/16/2008	2				X
20	1/7/2009	2				
21	1/23/2009	2	X			
22	2/3/2009	2				
23	2/16/2009	2	X			
24	3/11/2009	2				
25	3/31/2009	2	X			
26	4/27/2009	2				

Project Year 1 results are reported in Bradbury and others 2008

Appendix B: Inorganic Ions

Date Collected	Field ID	Well or lake	Chloride, mg/L	Nitrate+ Nitrite, mg/L
5/27/2008	7-9	WELL 7	11.77	<0.18
5/27/2008	11-9	WELL 11	45.31	2.526
5/27/2008	12-9	WELL 12	2.509	1.408
5/27/2008	13-9	WELL 13	7.688	1.778
5/27/2008	19-9	WELL19	4.112	<0.18
5/27/2008	30-9	WELL 30	4.089	<0.18
8/25/2008	Mendota-12	MENDOTA	40.20	0.348
8/25/2008	7-12	WELL 7	11.11	378.4
8/25/2008	11-12	WELL 11	48.62	3.340
8/25/2008	12-12	WELL 12	1.892	0.722
8/25/2008	13-12	WELL 13	8.000	1.761
8/25/2008	19-12	WELL19	4.214	<0.18
8/25/2008	30-12	WELL 30	4.048	<0.18
9/17/2008	Mendota-13	MENDOTA	40.59	0.570
9/17/2008	7-13	WELL 7	13.47	<0.18
9/17/2008	11-13	WELL 11	49.86	2.738
9/29/2008	12-14	WELL 12	2.598	1.472
9/17/2008	13-13	WELL 13	2.303	1.260
9/17/2008	19-13	WELL19	4.129	<0.18
9/17/2008	30-13	WELL 30	3.035	<0.18
10/13/2008	Mendota-15	MENDOTA	41.26	0.404
10/13/2008	7-15	WELL 7	13.41	<0.18
9/29/2008	11-14	WELL 11	45.92	2.557
10/13/2008	12-15	WELL 12	1.844	0.696
10/13/2008	13-15	WELL 13	8.095	1.818
10/13/2008	19-15	WELL19	4.029	<0.18
10/13/2008	30-15	WELL 30	3.361	<0.18
11/10/2008	Mendota-17	MENDOTA	82.83	0.844
11/10/2008	7-17	WELL 7	10.68	<0.18
11/10/2008	11-17	WELL 11	50.32	2.709
11/10/2008	12-17	WELL 12	2.502	1.526
11/10/2008	13-17	WELL 13	7.730	1.788
11/10/2008	19-17	WELL19	4.051	<0.18
11/10/2008	30-17	WELL 30	3.259	<0.18
12/16/2008	Mendota-19	MENDOTA	46.67	1.053
12/16/2008	7-19	WELL 7	16.5	<0.18
12/16/2008	11-19	WELL 11	49.13	2.727
12/16/2008	12-19	WELL 12	2.132	0.770
12/16/2008	13-19	WELL 13	11.58	2.197
12/16/2008	19-19	WELL19	4.202	<0.18
12/16/2008	30-19	WELL 30	3.284	<0.18

Appendix C: Field parameters

Sample ID	Well or lake	Date Sampled	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
7-12	7	9/3/2008	6.92	0.1	776
7-13	7	9/17/2008	7.29		727
7-14	7	9/30/2008	7.14	0.9	792
7-15	7	10/13/2008	7.34	0.1	700
7-16	7	10/28/2008	7.4		671
7-18	7	12/2/2008	7.4	0.1	656
7-19	7	12/16/2008	7.47		694
7-20	7	1/6/2009	7.17		701
7-21	7	1/21/2009	7.45		688
7-22	7	2/4/2009	7.39		640
7-23	7	2/19/2009	7.29		
7-24	7	3/11/2009	7.43	1	648
7-25	7	3/31/2020	7.47		506
7-26	7	4/30/2009	7.42	1	646
11-12	11	8/25/2008	7.21	3.5	913
11-13	11	9/15/2008	7.14		855
11-14	11	9/29/2008	7.28	2	820
11-16	11	10/27/2008	7.38	2	833
11-18	11	12/2/2008	7.39	2.5	779
11-19	11	12/17/2008	7.27		780
11-20	11	1/7/2009	7.23		785
11-21	11	1/22/2009	7.37		770
11-22	11	2/5/2009	7.36		763
11-23	11	2/18/2009	7.39		760
11-24	11	3/13/2009			
11-25	11	4/3/2009	7.39	2.5	811
11-26	11	4/30/2009	7.37	3.5	717
12-12	12	8/26/2008	6.92	6	537
12-13	12	9/18/2008	7.3		540
12-14	12	9/30/2008	7.49	5	570
12-15	12	10/13/2008	7.44	5	433
12-16	12	10/29/2008	7.44	2	533
12-17	12	11/10/2008	7.48	5	523
12-18	12	12/1/2008	7.43		563
12-19	12	12/16/2008	7.44		515
12-20	12	1/5/2009	7.45		496
12-21	12	1/20/2009	7.42		504
12-22	12	2/2/2009	7.35		514
12-23	12	2/16/2009	7.43	2.5	520
12-24	12	3/10/2009	7.4	2	509

12-25	12	3/31/2009	7.47	2.5	485
Sample ID	Well or lake	Date Sampled	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
12-26	12	4/22/2009	7.48		540
13-12	13	8/25/2008	7.07	3	718
13-14	13	9/29/2008	7.1	2	645
13-15	13	10/14/2008	7.33	4	574
13-16	13	10/27/2008	7.39	1	596
13-19	13	12/15/2008	7.42		585
13-20	13	1/5/2009	7.4		617
13-21	13	1/22/2009	7.29		589
13-22	13	2/5/2009	7.46		564
13-23	13	2/17/2009	7.4	2.5	566
13-24	13	3/10/2009			
13-25	13	4/2/2009	7.39		652
13-26	13	4/28/2009	7.42	2	596
18-23	18	2/19/2009	7.26		630
19-12	19	8/26/2008	6.94	0.8	622
19-13	19	9/17/2008	7.33		577
19-14	19	10/2/2008	7.23	0.8	544
19-15	19	10/14/2008	7.09	1	570
19-16	19	10/28/2008	7.45		538
19-18	19	12/3/2008	7.41		551
19-19	19	12/15/2008	7.39		533
19-20	19	1/6/2009	7.39		522
19-21	19	1/21/2009	7.45		547
19-22	19	2/4/2009	7.47		523
19-23	19	2/17/2009	7.44		530
19-24	19	3/11/2009	7.46	1	524
19-25	19	4/2/2009	7.44	2.5	569
19-26	19	4/29/2009	7.46	1	519
30-12	30	8/27/2008	6.95	0.2	590
30-13	30	9/15/2008	7.07		680
30-14	30	10/2/2008	7.37	0.1	537
30-15	30	10/15/2008	7.4	0.1	497
30-16	30	10/29/2008	7.45		516
30-17	30	11/10/2008	7.45	0.1	506
30-18	30	12/1/2008	7.49	0.1	516
30-19	30	12/17/2008	7.17		508
30-20	30	1/7/2009	7.23		506
30-21	30	1/20/2009	7.45		486
30-22	30	2/2/2009	7.36		500
30-23	30	2/16/2009	7.42	1	497
30-24	30	3/10/2009	7.37	1	827
30-25	30	3/31/2009	7.47	1	506

30-26	30	4/27/2009	7.45	1	496
Sample ID	Well or lake	Date Sampled	pH	Dissolved oxygen (mg/L)	Specific conductance (µhos/cm)
Monona-12	Monona	8/28/2008	7.72	6	563
Mendota-12	Mendota	9/4/2008	8.18	7.5	502
Mendota-13	Mendota	9/15/2008	8.8		463
Mendota-14	Mendota	9/29/2008	8.54		483
Mendota-15	Mendota	10/13/2008	8.15		554
Mendota-16	Mendota	10/27/2008	7.36		5.75
Mendota-17	Mendota	11/10/2008	8.21		575
Mendota-18	Mendota	12/3/2008	8.33		
Mendota-19	Mendota	12/17/2008	8.27		
Mendota-20	Mendota	1/7/2009	8.36		573
Mendota-21	Mendota	1/21/2009	8.29		581
Mendota -22	Mendota	2/2/2009	8.34		226
Mendota-23	Mendota	2/18/2009	8.13		475
Mendota-24	Mendota	3/13/2009	7.76		204
Mendota-25	Mendota	4/9/2009	7.74		571
Mendota-26	Mendota	4/22/2009	8.7		599

Appendix D: Tritium concentrations

well or lake	Tritium, September 2007[3]		Tritium April, 2008[3]		Tritium September, 2008		Tritium, previous studies
		± 1σ		± 1σ		± 1σ	
7	4.6	0.5	5.40	0.6	5.4	0.6	8.9[1], 9.9[1], 19.6[2]
30	<0.8	0.3	<0.8	0.4	0.37	0.09	
19	4.4	0.5	3.70	0.5	3.1	0.5	
12	<0.8	0.6	<0.8	0.4	0.41	0.09	
11	6.3	0.9	5.40	0.6	4.7	0.6	
13	2.5	0.7	1.30	0.4	1.1	0.4	
Monona	8.7	0.7					15.1[2]
Wingra	9.2	0.8					13.7[2]
Mendota	8.5	0.7					11.4[2]

[1] Tritium reported in Borchardt and others 2007; samples collected in June 2003 and May 2004

[2] Tritium reported in Bradbury and others 1999; samples collected in June 1995

[3] Tritium reported in Bradbury and others 2008

Appendix E: PCP compounds

Results summary for Well 18 sample

Compound	ng/LSample
acetaminophen	ND<1.0 ng/L
ampicillin	(*C,F)ND<5 ng/L
azithromycin	(*B,C,F)<5 ng/L
caffeine	(*C,F)<5 ng/L
carbadox	(*C,F)ND<5 ng/L
carbamazepine	(*B)<1.0 ng/L
cefotaxime	(*D,F)ND<500 ng/L
ciprofloxacin	(*D,F)ND<5 ng/L
clarithromycin	(*B,D,F)ND<5 ng/L
cloxacillin	(*F)ND<5 ng/L
codeine	(*C,F)<5 ng/L
cotinine	(*D,F)ND<5 ng/L
digoxigenin	(*F)ND<5 ng/L
digoxin	(*D,F)ND<250 ng/L
diltiazem	(*B,F)<5 ng/L
paraxanthine	(*B,F)<5 ng/L
diphenhydramine	(*B,F)<5 ng/L
enrofloxacin	(*D,F)ND<5 ng/L
erythromycin	(*F)ND<5 ng/L
fluoxetine	ND<1.0 ng/L
lincomycin	(*C,F)ND<5 ng/L
lomefloxacin	(*D,F)ND<5 ng/L
miconazole	(*B,F)<5 ng/L
norfloxacin	(*D,F)ND<5 ng/L
ofloxacin	(*B,D,F)<5 ng/L
oxacillin	(*F)ND<5 ng/L
oxolinic acid	(*C,F)<5 ng/L
penicillin G1	(*C,F)ND<5 ng/L
penicillin V1	(*C,F)ND<5 ng/L
roxithromycin	(*B,D,F)<5 ng/L
sarafloxacin	(*D,F)ND<5 ng/L
sulfachloropyridazine	(*F)ND<5 ng/L
sulfadiazine	(*F)ND<5 ng/L
sulfadimethoxine	(*F)ND<5 ng/L
sulfamerazine	(*F)ND<5 ng/L
sulfamethazine	(*B,F)<5 ng/L
sulfamethizole	(*F)ND<5 ng/L
sulfamethoxazole	(*B,F)<5 ng/L
sulfanilamide	(*C,F)ND<5 ng/L

sulfathiazole	(*F)ND<5 ng/L
thiabendazole	(*F)ND<5 ng/L
trimethoprim	(*F)ND<5 ng/L
tylosin	(*D,F)ND<5 ng/L
virginiamycin	(*C,F)ND<5 ng/L

NOTES

(*A)- Elevated RL due to chromatographic baseline noise at compound's retention time

(*B) - Background signal noted in blank sample.

(*C) - Spike recovery was below 70% for this compound; result could contain low bias

(*D) - Spike recovery was above 130% for this compound; result could contain high bias

(*E) - Analyte not reported due to lack of chromatographic response.

(*F) - RL based upon lowest standard analyzed with acceptable back calculated recovery.

(*G) - RL adjusted to account for amount of sample extracted.

Appendix F: Stable isotope results

Date Collected	Field ID	Well or lake	Delta 2H x 1000	Delta 18O x 1000
7/7/2008	7-10	WELL 7	-56.68	-8.45
7/7/2008	11-10	WELL 11	-57.6	-8.78
7/7/2008	12-10	WELL 12	-59.08	-8.94
7/7/2008	13-10	WELL 13	-59.8	-8.98
7/7/2008	19-10	WELL19	-55.9	-8.11
7/7/2008	30-10	WELL 30	-72.73	-10.65
8/25/2008	7-12	WELL 7	-56.26	-8.46
8/25/2008	11-12	WELL 11	-57.32	-8.86
8/25/2008	12-12	WELL 12	-59.56	-8.93
8/25/2008	13-12	WELL 13	-59.26	-8.96
8/25/2008	19-12	WELL19	-55.01	-8.1
8/25/2008	30-12	WELL 30	-72.41	-10.74
8/25/2008	Mendota-12	MENDOTA	-42.38	-5.86
1/23/2009	7-21	WELL 7	-55.54	-8.19
1/23/2009	11-21	WELL 11	-57.8	-8.79
1/23/2009	12-21	WELL 12	-59.66	-8.96
1/23/2009	13-21	WELL 13	-58.54	-8.97
1/23/2009	19-21	WELL19	-54.39	-8.16
1/23/2009	30-21	WELL 30	-72.15	-10.66
1/23/2009	Mendota-21	MENDOTA	-43.45	-6.13
2/16/2009	18-23	WELL 18	-57.51	-8.72
2/16/2009	7-23	WELL 7	-55.33	-8.12
2/16/2009	11-23	WELL 11	-57.94	-8.72
2/16/2009	12-23	WELL 12	-59.64	-9.03
2/16/2009	13-23	WELL 13	-59.12	-8.94
2/16/2009	19-23	WELL19	-54.8	-8.15
2/16/2009	30-23	WELL 30	-71.23	-10.6
3/31/2009	7-25	WELL 7	-56.61	-8.45
3/31/2009	11-25	WELL 11	-57.89	-8.77
3/31/2009	12-25	WELL 12	-59.82	-9.02
3/31/2009	13-25	WELL 13	-59.33	-9
3/31/2009	19-25	WELL19	-55.01	-8.09
3/31/2009	30-25	WELL 30	-72.85	-10.66