Title:	**Susceptibility of La Crosse Municipal Wells to Enteric Virus Contamination from Surface Water Contributions**
Project I.D.:	WDNR Project # 165 and WDNR Project # 177
Principal Investigators:	Randy Hunt, US Geological Survey, and Mark Borchardt, Marshfield Clinic Research Foundation
Research Assistant:	Nathaniel Haas, University of Wisconsin – La Crosse
Period of Contract:	March 1, 2001 - June 30, 2002 (DNR Project #165) July 1, 2002 - June 30, 2003 (DNR Project #177)

Background/Need: Human gastrointestinal viruses are known to contaminate municipal drinking water wells. In one peer-reviewed study, 141 of 448 groundwater sites (31.5%) in 35 states were positive for viruses. It is further known that approximately half of all waterborne infectious disease outbreaks are due to the consumption of contaminated groundwater. However, few studies have examined the routes by which pathogenic viruses enter drinking water wells. One potential route of virus entry into wells is surface water infiltration. Large surface water bodies, like the Mississippi River, are significant sources for fecal-related pathogens; these pathogens can move with infiltrating surface water and potentially enter adjacent drinking water wells. Ideally, these vulnerable wells could be identified and additional water treatment steps taken as necessary.

Objectives: The primary objective of the present study was to monitor the municipal drinking water wells of La Crosse, Wisconsin, for human gastrointestinal viruses and relate the amount of Mississippi River water infiltrating the wells to the frequency of virus detection. A secondary objective was to relate microbial indicators of water sanitary quality with the occurrence of gastrointestinal viruses.

Methods: One river water site and four drinking water wells in La Crosse, WI were sampled monthly from March 2001 to February 2002. All samples were taken prior to chlorination at the wellhead. The wells selected for study were predicted to have different levels of surface water contributions based on previous hydrogeological modeling. The actual amount of surface water entering the wells was measured during the study based on $^{18}O/^{16}O$ and $^{2}H/^{1}H$ ratios. Water samples were analyzed by reverse-transcription polymerase chain reaction (RT-PCR) for five groups of viruses: enteroviruses, rotavirus, hepatitis A virus (HAV) and norovirus genogroups 1 and 2. This method detects the presence of viral RNA. Enteroviruses detected by RT-PCR were further analyzed by nucleotide sequencing to identify the type of enterovirus. In addition, samples were analyzed by cell culture for the presence of infectious enteroviruses and HAV. Tests for microbial indicators of sanitary quality included total coliforms, *E. coli*, fecal enterococci, and somatic and male-specific coliphages.

Results and Discussion: By RT-PCR, 24 of 48 municipal well water samples (50%) were positive for gastrointestinal viruses and 10 of 12 (83%) river samples were virus-positive. The viruses detected included enteroviruses, rotavirus, HAV, and noroviruses. All well water samples were negative for all microbial indicators of sanitary quality. Thus, there was no association between indicator occurrence and virus occurrence as measured by RT-PCR. The detection frequency of viruses in La Crosse wells is higher than previously published studies, probably because the La Crosse wells are located in a highly permeable clean sand/gravel aquifer. It is important to note that the study wells were in active service and properly constructed and maintained.

Contrary to expectations, viruses were found in all wells regardless of the level of surface water contributions. One well had no measurable surface water contributions and yet 6 of the 12 monthly samples were virus-positive. This suggests that there were other unidentified sources, in addition to

surface water, responsible for the contamination. The most likely other source was municipal sanitary sewer lines, which have been documented to leak. Three of the four municipal wells were located in densely populated residential areas underlain with a network of sanitary sewers. The capture zone of one well includes a wastewater lift station where the sewer line is pressurized, which may explain why this well had the highest frequency of virus detects.

Two wells had appreciable contributions of surface water with travel times from river to well of approximately 6 months and >1 year, respectively. Viruses are reported to survive in groundwater for times on the order of 6 to 9 months. Viruses moving with travel times greater than 1 year would likely be substantially degraded, therefore the viruses detected in the well with the travel time > 1 year were likely from leaking sewer lines. The well with the 6 month travel time, however, was located on French Island – an area without sewer lines in the well's capture zone. Therefore, viruses detected in this well likely originated from the river.

Enteroviruses detected in the wells by RT-PCR were sequenced and found to be a diverse assortment of echoviruses and group A and group B coxsackieviruses. These viruses can cause acute and severe chronic illnesses but their health effects via waterborne transmission are unknown. For this reason they are on the US Environmental Protection Agency Contaminant Candidate List as requiring additional research.

The public health significance of these findings is unknown. The RT-PCR assay cannot distinguish between infectious and inactivated viruses. The cell culture assay for infectious enteroviruses was negative for all the well samples, suggesting that the enteroviruses detected by RT-PCR were inactivated, although the different outcomes between the RT-PCR and cell culture methods may be due to their difference in detection sensitivity (the former is more sensitive). Of particular concern is that three well samples were positive for infectious HAV. HAV is a persistent virus in the environment, more so than enteroviruses, which may explain the difference in cell culture results for these two virus groups. It should be noted that the infectious HAV were detected in samples taken before the water was chlorinated. The City of La Crosse chlorinates with gaseous chlorine at each wellhead at a dose that, if the contact time with the virus is sufficient, should inactivate HAV.

Conclusions/Implications/Recommendations: There is frequent occurrences of viral RNA in the La Crosse drinking water wells included in this study, some of which are attributable to surface water infiltration and the rest must be derived from another unidentified fecal source. To ascertain the public health significance of these findings it would be necessary to conduct an epidemiological study relating virus occurrence to a defined health endpoint. It is likely given the chlorination dose at each wellhead and residence time within the system that any viruses present in the groundwater are inactivated before ingestion. However, evaluations of the effectiveness of the present disinfection system were outside the scope of this research.

Related Publications:

Hunt, R. J., T. B. Coplen, N. L. Haas, D. A. Saad, and M. A. Borchardt. (in review). Investigating surface water—well interaction using stable isotope ratios of water. Journal of Hydrology.

Haas, N. L. 2003. Susceptibility of La Crosse, Wisconsin, municipal wells to enteric virus contamination from surface water contributions. Masters Thesis, University of Wisconsin – La Crosse, La Crosse, Wisconsin, 88pp.

Borchardt, M.A., N.L. Haas, and R.J. Hunt. (in review) Vulnerability of Drinking Water Wells in La Crosse, Wisconsin, to Enteric Virus Contamination from Surface Water Contributions. Applied and Environmental Microbiology.

Key Words: groundwater, water isotopes, drinking water, gastrointestinal viruses, public health

Funding: WDNR and U.S. Geological Survey

Final Report: A final report containing more detailed information on this project is available for loan from Wisconsin's Water Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.