

Upper Peninsula Groundwater Sampling
Menominee County, Michigan

May 8, 2009

Prepared for:
Public Health, Delta and Menominee Counties

Prepared by:
Marc P. Verhougstraete
Research Assistant,

Asli Aslan Yilmaz, Ph. D.
Post Doctoral Research Assistant,

And

Joan B. Rose, Ph. D.
Homer Nowlin Chair in Water Research

The Water Quality, Environmental, and Molecular Microbiology Laboratory
Department of Fisheries and Wildlife
13 Natural Resources Building
Michigan State University
East Lansing, MI 48824

Phone: (517) 432-4412

Fax: (517) 432-1699

rosejo@msu.edu

INTRODUCTION

Menominee County is located in the southern part of Michigan's Upper Peninsula. 24,000 residents inhabit Menominee's 1,340 miles². This county touches four watersheds (Menominee, Cedar-Ford, Escanaba, and Lake Michigan) and the land use consists primarily of agriculture, industrial, and residential.

In February 2009, a well water sample tested positive for coliform bacteria and nitrates. The homeowner of the said well noted the water had turned color and had a "manure" taste which was not typical for the drinking water. Subsequent testing of nearby wells, identified 4 out of 5 wells had coliform bacteria in the water and nitrate levels above 10 mg/L (the Maximum Contaminant Level Goal established by the United States Environmental Protection Agency (US EPA)). The results initiated a response from the health department to send out letters to property owners in the surrounding area informing them of the recent results, and risks associated with nitrates, nitrites, and bacteria in water. The health department offered to test wells for bacteria and nutrients and evaluate well construction. Ten homeowners responded to the letter asking for further analysis of well water.

The Water Quality and Environmental Microbiology Laboratory of Michigan State University (East Lansing, Michigan) was asked to apply source tracking markers to the ten wells of Menominee County, Michigan. Our laboratory assayed for fecal indicator bacteria, human and bovine *Bacteroides* markers, and viruses to: identify the level of bacteria present in the groundwater, identify the source(s) of pollution that may be entering the drinking water wells, and identify the potential viruses that humans may be exposed to from drinking the groundwater in the area of concern.

METHODS

The Water Quality and Environmental Microbiology Laboratory of Michigan State University (East Lansing, Michigan) applied a multifaceted approach to investigating the groundwater of Menominee County. Our analysis included fecal indicator bacteria (total coliforms, *E. coli*, enterococci, and coliphage), molecular source tracking markers (human and bovine *Bacteroides*), and viruses (Adenovirus serotypes 40/41).

Sample Collection

Ten households in Menominee County were tested for the aforementioned analysis. One sample (9.5 L) was collected at the tap or outdoor spigot from each house on May 8th, 2009 (Table 1). All samples were placed on ice, taken to St. Ignace, Michigan, and transferred to Michigan State University personnel. Samples were delivered to the Water Quality and Environmental Microbiology Laboratory of Michigan State University.

Table 1: Sample location

Sample ID	Well Owner	Well Address	Date Collected
1	Todd LaFave	N15560 Co. Rd. 557	5/8/2009
2	Bob Oliver	W5437 #28 Lane	5/8/2009
3	John Adams	W5225 Co. Rd. 360	5/8/2009
4	Mike VanCourt	N10694 0-1 Lane	5/8/2009
5	Lori Freis	W5256 Co. Rd. 366	5/8/2009
6	Sherry Smith	W5134 Co. Rd. 366	5/8/2009
7	Robert Kuntze	W5125 Co. Rd. 366	5/8/2009
8	Tim Kuntze	W5149 Co. Rd. 366	5/8/2009
9	Crystal Vanltese	W5270 Co. Rd. 366	5/8/2009
10	Paul Anderson	W5333 Co. Rd. 366	5/8/2009

Fecal Indicator Analysis

Each sample was analyzed for total coliforms and *E. coli* using IDEXX Colilert®, enterococci using IDEXX Enterolert®, and Coliphage (CN-13) using a modification of U.S. EPA Methods 1601 and 1602 (US EPA 2001). Samples were processed for the fecal indicators immediately upon receiving them at the laboratory. 100 ml of water were used for each total coliform and *E. coli*, and enterococci assay. The coliphage assay was performed using 10 ml of sample.

Molecular Analysis

Two liters of groundwater from each well were filtered through a 0.45 um mixed cellulose esters filter (Pall Corporation, Ann Arbor, Michigan). The filters were placed in 10 ml of a buffered solution, centrifuged at 4000 x g for 15 minutes, and then removed. DNA was extracted from the sample using MagNa Pure Compact Nucleic Acid isolation kit-large volume (Roche Applied Science, Indianapolis, IN).

PCR *Bacteroides* (human and bovine)

Polymerase chain reaction (PCR) amplification was performed on the extracted DNA. Established primers for both human and cow *Bacteroides* sequences were used (Bernhard 2000). Gel electrophoresis was performed on the PCR product and run on a 1.2% w/v agarose gel at 95 V for approximately one hour.

qPCR *Bacteroides* (human)

Quantitative PCR amplification was performed targeting established primers (Yampara et al., 2008) and an in-house developed probe for *Bacteroides thetaoethiomicron* alpha mannanase gene.

Viruses

Eight liters of groundwater were filtered through HA filters and processed for nucleic acid extraction (Haramoto et al, 2005). The extracts were then analyzed for Adenovirus (serotypes 40 and 41) by qPCR using established primers and probes (Xagorarakis et al., 2007).

RESULTS

Ten samples were analyzed for fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci, and coliphage), molecular source tracking markers (human and bovine Bacteroides), and viruses (Adenovirus 40/41). A summary of the bacterial indicators, molecular source tracking markers, and virus results is described in Appendix 1.

Wells 4, 5, 6, 9, and 10 had detectable levels of total coliforms with concentrations ranging from 1.0 to 108.1 colonies/ 100 ml. Enterococci was detected in wells 1 and 4 (1.0 and 4.0 colonies/ 100 ml, respectively). *E. coli* and coliphage (CN-13) were not detected above method detection limits in any wells. The lower detection limits for total coliforms, *E. coli*, and Enterococci was 1 colony/ 100 ml and 10 plaque forming units/ 100 ml for coliphage.

Each well was tested for bovine and human Bacteroides using conventional PCR method. Neither of the organisms was detected in any of the wells. Each well was further assessed using a quantitative PCR method for human Bacteroides that also failed to detect the marker in any of the wells.

Virus analysis was performed on each well. No Adenovirus 40/41 serotypes were detected in any of the wells.

DISCUSSION

The US EPA has established a Maximum Contaminant Level (MCL) for total coliforms and *E. coli* (the highest level of contamination allowed in drinking water) at zero cells. No wells had levels of *E. coli* or Coliphage above method detection limits (1 CFU/100 ml or 10 PFU/100 ml, respectively). Five wells tested positive for total coliforms, and two wells tested positive for Enterococci. Only well 4 tested positive for both indicators. Well 3 was the only well reported to be chlorinated. Coliforms and Enterococci may occur naturally in the environment and are not necessarily in and of themselves a threat to human health rather they represent a potential for the presence of harmful bacteria.

Bacteroides (human and bovine) source markers or Adenoviruses were not detected in any of the samples. The non-detection of Adenoviruses may be attributed to the low

filtration volume (<10 L). For adequate drinking water analysis, 100-500 L of water has been suggested as a target volume for virus detection. Further investigations should focus on assaying much larger volumes of water for virus analysis.

E. coli, Coliphage, Bacteroides, and Adenoviruses 40/41 were not detected in the wells, indicating the total coliform levels detected in wells 4, 5, 6, 9, and 10 may be part of the naturally occurring bacteria. There was a two month span between initial drinking water complaints and our analysis. Thus, the low detection of indicator bacteria and viruses may be a result of this time delay and may be representative of decreased contamination or bacteria die-off in the groundwater after 60 days.

We recommend sampling be conducted over a longer temporal period to better identify potential year round influences of the groundwater and that water samples be collected and analyzed at the time of the problems. Groundwater is susceptible to contamination during high rainfall events, and flooding. Thus during the spring thaws and rains may be a good time to sample.

REFERENCES

Bernhard, A.E. and K.G. Field. (2000). A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA, *Appl. Environ. Microbiology*. 66: 4571–4574.

Haramoto, E. Katayama, H., Oguma, K. and Ohgaki, S. (2005). Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa River in Japan. *Applied and Environmental Microbiology* 71:2403-2411.

United States EPA. (2001). Method 1601: Male specific (F+) and somatic coliphage in water by two-step enrichment procedure. EPA 821-R-01-030.

United States EPA. (2001). Method 1602: Male specific (F+) and somatic coliphage in water by single agar layer (SAL) procedure. EPA 821-R-01-029.

Xagorarakis, I., D. H. W. Kuo, K. Wong, M. Wong, and J. B. Rose. (2007). Occurrence of Human Adenoviruses at Two Recreational Beaches of the Great Lakes. *Appl. Environ. Microbiology* 73: 7874-7881.

Appendix 1. Summary of fecal indicators, molecular source tracking markers, and viruses results from groundwater samples

Sample ID	Total coliforms	<i>E. coli</i>	Enterococci	Coliphage (CN-13)	PCR		qPCR	qPCR
					Human Bacteroides	Bovine Bacteroides	Human Bacteroides	HAdV (F40,41)
1	<1.0	<1.0	1.0	<10	-	-	-	-, -
2	<1.0	<1.0	<1.0	<10	-	-	-	-, -
3	<1.0	<1.0	<1.0	<10	-	-	-	-, -
4	33.1	<1.0	4.0	<10	-	-	-	-, -
5	108.1	<1.0	<1.0	<10	-	-	-	-, -
6	2.0	<1.0	<1.0	<10	-	-	-	-, -
7	<1.0	<1.0	<1.0	<10	-	-	-	-, -
8	<1.0	<1.0	<1.0	<10	-	-	-	-, -
9	21.6	<1.0	<1.0	<10	-	-	-	-, -
10	1.0	<1.0	<1.0	<10	-	-	-	-, -