

2016 Project Abstract

For the Period Ending June 30, 2019

PROJECT TITLE: Bacterial Assessment of Groundwater Supplies Used for Drinking Water

PROJECT MANAGER: Raymond M. Hozalski

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04f

APPROPRIATION AMOUNT: \$ 299,000

AMOUNT SPENT: \$299,000

AMOUNT REMAINING: \$0

Sound bite of Project Outcomes and Results

Sixteen groundwater systems throughout Minnesota were sampled and found to be largely free of bacteria that cause gastrointestinal infections but may contain “opportunistic” bacteria (i.e., *Legionella*) that can cause lung infections. Deep wells and/or chlorine disinfection are recommended for minimizing the risk of exposure to disease-causing bacteria via groundwater supplies.

Overall Project Outcome and Results

Recent reports out of the neighboring states of Wisconsin and Iowa have suggested that drinking water supplies obtained from groundwater wells can be contaminated with disease-causing microorganisms (i.e., pathogens), including viruses and bacteria. The main goal of this study was to assess the microbiological quality of groundwater supplies in Minnesota with an emphasis on disease-causing bacteria. In this study, microorganisms were collected from large volumes of water (100 to 400 gallons) using a filtration system from 16 public groundwater systems throughout the State of Minnesota. The groundwater wells varied in depth from 37 to 1,204 feet and in the relative age of the groundwater. The groundwater systems were sampled at the wellhead or source, after treatment if any (i.e., two systems did not disinfect before distribution), and from one location in the distribution system. With one exception, the groundwater supplies were free of enteric pathogens (i.e., pathogens with a conventional fecal-oral exposure route that cause gastrointestinal infections). *Escherichia coli* (i.e., *E. coli*) was only detected in water from a non-potable well with a documented history of contamination. *Legionella*, bacteria that are considered opportunistic pathogens, were detected in four of the 16 public groundwater supplies. *Legionella* can cause potentially fatal lung infections in people with weakened immune systems such as the elderly and chemotherapy patients. *Legionella* levels decreased substantially in a system employing chlorine disinfection. The results from this study suggest that groundwaters supplying public water systems in Minnesota are largely free of enteric pathogens but may contain opportunistic pathogens. This research also suggests that lower bacteria concentrations in groundwater is generally associated with deeper wells (i.e., > 150 ft) extracting older groundwater. Disinfection should be considered for systems with shallow wells (i.e., < 150 ft) due to the potential for high bacteria concentrations and to control opportunistic pathogens when present.

Project Results Use and Dissemination

Dissemination activities included two poster presentations and one oral presentation by graduate student John Galt. John presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the annual Minnesota Section AWWA Conference in Duluth, MN on September 14, 2017. John also presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the AWWA Water Quality Technology

Conference in Portland, OR on November 12, 2017. An oral presentation on the work was delivered by John at the Minnesota Section of the American Water Works Association meeting in Duluth, MN in Fall 2018. The MN Section AWWA meeting is an important audience for the work as it includes representatives from water utilities around the state, consulting engineers that work in the state and region, and representatives from state agencies (e.g., MDH). Tim LaPara also participated in outreach activities. Tim gave an oral presentation on the project findings at the Minnesota Section of the American Water Works Association meeting in Duluth, MN in Fall 2019. Tim also spoke to the City of Riverton's City Council and to the water quality manager at the City of Eagan about the results. Finally, Tim also participated in an open house concerning the water system in the City of Hastings.



Environment and Natural Resources Trust Fund (ENRTF)

M.L. 2016 Work Plan

Date of Status Update: December 19, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Bacterial Assessment of Groundwater Supplies Used for Drinking Water

Project Manager: Raymond M. Hozalski

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Location: Statewide

Total ENRTF Project Budget:

ENRTF Appropriation: \$299,000

Amount Spent: \$299,000

Balance: \$0

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04f

Appropriation Language:

\$299,000 is from the trust fund to the Board of Regents of the University of Minnesota to characterize and analyze bacterial communities in Minnesota groundwater used as drinking water supplies and link the microbiological data to other water quality indicators for drinking water supply safety. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Bacterial Assessment of Groundwater Supplies Used for Drinking Water

II. PROJECT STATEMENT:

Use of quantitative PCR (qPCR) targeting DNA or RNA provides a rapid, powerful, and specific screening tool that can be used to directly enumerate pathogenic organisms of concern. Investigations of the microbiological safety of groundwater supplies in the neighboring state of Wisconsin using such a technique found many contaminated wells. Unfortunately, we know virtually nothing about the microbiological quality of Minnesota's groundwater supplies. This is particularly problematic because groundwater is used as the source for more than 97% of Minnesota's public water supplies serving about 75% of the state's population; often that water is provided without any disinfection. Thus, the goal of this study is to characterize and quantify all of the bacteria, including the direct quantification of several disease-causing organisms, in selected drinking water systems supplying groundwater throughout the state of Minnesota. We plan to sample approximately 15-20 water systems overall including systems in the Karst region in the southeast, the agricultural areas to the west and southwest, forested areas to the north, as well as a few systems in the Twin Cities Metro area. We predict that the majority of Minnesota's groundwater supplies from deep aquifers are free of bacterial pathogens but that supplies from shallow aquifers and those in Karst regions are likely to be impacted by agriculture, septic systems, etc. and contain unsafe levels of pathogens. Our secondary hypothesis is that the microbiological safety of Minnesota's groundwater will correlate to other chemical measures of drinking water quality, such as nitrate levels (an indication of agricultural impacts) and elevated tritium levels (tritium is measured to assess well vulnerability). The specific goals of this work are to:

1. Quantify and characterize the microorganisms in groundwaters around the state
2. Attempt to link the microbiological data to conventional or chemical water quality indicators, including nitrate and tritium
3. Disseminate the information gained from this work to stakeholders by working with the Minnesota Department of Health (MDH), Minnesota Rural Water Association (MRWA), and by presenting at the Minnesota section of the American Water Works Association (AWWA) Annual Conference.

The selection and sampling of the water systems will occur first (Activity 1). The collected samples will then be analyzed for bacterial community composition and to quantify specific bacterial pathogens using state-of-the-art DNA-based technologies (Activity 2). Water samples will also be evaluated for chemical indicators of contamination and for microbial contamination using conventional culture-based assays (Activity 3). Finally, we will disseminate the research results to sampled utilities directly, as well as to the general public and to interested water professionals. We will preserve the anonymity of participating utilities in our press releases and publications upon their request and work with them and the MDH to determine how best to communicate the results from their individual systems to their customers.

This research would compliment on-going efforts by the Minnesota Department of Health to evaluate the safety of Minnesota's public water supplies with respect to pathogenic viruses and a recent state report showing increased nitrate contamination of water supplies.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:

Work was done on all three of the main activities of the project as described below. We identified and are currently planning to proceed with sampling 23 water systems in four different regions of the state: Metro area (4), North central (i.e., Brainerd area; 7), Southwest (i.e., Marshall area; 5), and Southeast (i.e., Rochester area; 7). This list of systems includes many that disinfect (chlorine) and several that do not. DNA extractions have been done for all water samples collected to date. Selected water quality parameters were also measured on site during sampling or soon after returning to the laboratory. There have been no significant problems or delays in our progress thus far.

Project Status as of October 25, 2017:

Work was done on all three of the main activities of the project as described below. We have proceeded with sampling 16 water systems in four different regions of the state: Metro area (4), North central (i.e., Brainerd area); 4), Southwest (i.e., Marshall area); 4), and Southeast (i.e., Rochester area); 4). This list of systems includes many that disinfect (chlorine) and several that do not. DNA extractions/purifications have been done for all water samples collected to date. Selected water quality parameters were also measured on site during sampling or soon after returning to the laboratory. There have been no significant problems or delays in our progress thus far. In fact, we are ahead of schedule.

Project Status as of January 1, 2018:

Most of the sampling for microbial and chemical data has been completed. Several well water samples remain to be collected in the spring of 2018 for chemical analysis (carbamazepine and tritium). Quantification of various pathogens by the polymerase chain reaction (PCR) are to be completed during the remainder of winter and spring of 2018. All collected DNA samples are to be submitted for sequencing analysis during this time period. Overall, progress is again ahead of schedule.

Project Status as of July 1, 2018:

All sample collection is complete with the exception of one round of well water samples for carbamazepine analysis for one of the 16 cities under investigation. Again, carbamazepine is a pharmaceutical compound (i.e., anti-seizure medication) that serves as an indicator of human wastewater contamination. Quantification of pathogens, DNA sequencing, and tritium (i.e., water age) analyses are complete, and data are being processed for clarity and dissemination. After collection and processing of the remaining well water samples, well water from all cities will have been analyzed for carbamazepine, concluding all sample collection and lab work. The remaining work will be comprised of data analysis and interpretation of results.

Project Status as of January 1, 2019:

The final water sample for carbamazepine analysis was collected and all samples were analyzed for this compound, which serves as an indicator of wastewater contamination. The results from the analyses of the groundwater samples for tritium, an indicator of water age, were also completed. The results from the sequencing of the bacterial DNA extracted from the raw, treated, and distributed groundwater samples have been analyzed using appropriate software (QIIME and R) and supercomputing as well as conventional computing resources. Graphs and tables of the results have also been prepared. A presentation on the work was delivered by M.S. student John Galt at the annual MN Section of the American Water Works Association meeting in Duluth, MN in Fall 2018. This meeting is an important audience for the work as it includes representatives from water utilities around the state, consulting engineers that work in the state and region, and representatives from state agencies (e.g., MDH).

Overall Project Outcomes and Results:

Recent reports out of the neighboring states of Wisconsin and Iowa have suggested that drinking water supplies obtained from groundwater wells can be contaminated with disease-causing microorganisms (i.e., pathogens), including viruses and bacteria. The main goal of this study was to assess the microbiological quality of groundwater supplies in Minnesota with an emphasis on disease-causing bacteria. In this study, microorganisms were collected from large volumes of water (100 to 400 gallons) using a filtration system from 16 public groundwater systems throughout the State of Minnesota. The groundwater wells varied in depth from 37 to 1,204 ft and in the relative age of the groundwater. The groundwater systems were sampled at the wellhead or source, after treatment if any (i.e., two systems did not disinfect before distribution), and from one location in the distribution system. With one exception, the groundwater supplies were free of enteric pathogens (i.e., pathogens with a conventional fecal-oral exposure route that cause gastrointestinal infections). *Escherichia coli* (i.e., *E. coli*) was only detected in water from a non-potable well with a documented history of contamination. *Legionella*, bacteria that are considered opportunistic pathogens, were detected in four of the 16 public groundwater supplies. *Legionella* can cause potentially fatal lung infections in people with weakened immune systems such as the elderly and chemotherapy patients. *Legionella* levels decreased substantially in a system

employing chlorine disinfection. The results from this study suggest that groundwaters supplying public water systems in Minnesota are largely free of enteric pathogens but may contain opportunistic pathogens. This research also suggests that lower bacteria concentrations in groundwater is generally associated with deeper wells (i.e., > 150 ft) extracting older groundwater. Disinfection should be considered for systems with shallow wells (i.e., < 150 ft) due to the potential for high bacteria concentrations and to control opportunistic pathogens when present.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Select and sample 15 to 20 groundwater utilities across the state of Minnesota.

Description:

Approximately 15-20 water groundwater utilities across the state of Minnesota will be selected for sampling that represent a variety of aquifer types (e.g., fractured bedrock, shallow unconfined, deep confined) and overlying land uses (e.g., agricultural, residential, forested). Each of the water systems will be sampled three times throughout a 1-year period to evaluate temporal (i.e., seasonal) variations in water quality. We will also sample from the source, after any treatment (if present), and from taps in the distribution system in order to evaluate whether the groundwater supply is contaminated, the effectiveness of the treatment (if any), and whether any contamination occurs during distribution.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 51,181
Amount Spent: \$ 51,181
Balance: \$ 0

Outcome	Completion Date
1. Work with MDH personnel to select and contact water utilities	September 1, 2016
2. Collect samples from 15-20 water utilities across MN 3 times each	August 1, 2017

Activity Status as of January 1, 2017:

We have successfully identified numerous public water supplies from which to collect water samples. We identified and are currently planning to proceed with sampling 23 water systems in four different regions of the state: Metro area (4), North central (i.e., Brainerd area; 7), Southwest (i.e., Marshall area; 5), and Southeast (i.e., Rochester area; 7). This list of systems includes many that disinfect (chlorine) and several that do not. We have successfully sampled the water from eight of those water utilities on two occasions (each).

Activity Status as of October 25, 2017:

We have proceeded with sampling 16 water systems in four different regions of the state: Metro area (4), North central (i.e., Brainerd area; 4), Southwest (i.e., Marshall area; 4), and Southeast (i.e., Rochester area; 4). Seven of the original 23 water systems were removed from the study because of failure of utility personnel to respond to our inquiries and/or recognition that we could accommodate only 16 water systems. The final list of systems includes many that disinfect (chlorine) and two that intentionally do not disinfect. It is important to note that some systems attempt to disinfect but our measurements suggest poor chlorination control. The total sampling effort will involve 16 water systems × 3 samples/system × 4 times each ≅ 200 samples in total. We have successfully sampled the water from all of those water utilities on at least two occasions each.

Activity Status as of January 1, 2018:

A total of 116 samples for microbial analyses have been collected across all regions. Nearly all sample locations at all water utilities in the Metro area and the Brainerd area have been sampled three times or more. Water utilities in the Southwest and Southeast regions have been sampled twice, except for one utility in the Rochester area which has been sampled three times. Further sampling may be conducted during the spring/summer of 2018.

Activity Status as of July 1, 2018:

Sample collection for microbial analysis is complete, totaling 116 samples. The well water from one of the 16 cities under investigation remains to be collected for carbamazepine analysis, after which all sampling will be concluded.

Activity Status as of January 1, 2019:

The last well water for carbamazepine analysis from one of the 16 cities under investigation was collected, concluding the sampling activities.

Final Report Summary:

A total of 116 samples were collected for microbial analyses from 17 wells at 16 groundwater systems around the state. These samples consisted of microorganisms recovered from large volumes of water (300 L to 1,500 L) using an ultrafiltration method. The systems were sampled from 2 to 4 times each and at multiple locations including the raw groundwater, after treatment (if any), and from a service tap in the distribution system. Additional raw groundwater samples were collected once from each well for analysis of chemical water quality indicators including tritium and carbamazepine.

ACTIVITY 2: Characterize the bacterial communities in Minnesota’s groundwaters that are used as drinking water supplies.

Description:

We will make detailed characterizations of the microorganisms in the sampled waters using state-of-the-art, next-generation DNA sequencing technology to generate between 50,000 and 100,000 sequences per sample. We will also directly (as opposed to indirectly, like the fecal coliform assay) quantify the presence of about a dozen organisms (e.g., *Salmonella*, *Legionella*, etc.) known to cause disease in humans. Finally, we will use software that is freely available to University researchers at the Minnesota Supercomputing Institute to statistically analyze the data and correlate our microbiological data to other water quality data (see Activity 2).

The composition of the bacterial communities in the sampled waters will be tracked by using the polymerase chain reaction (PCR) to amplify the V3-region of the 16S rRNA gene, purifying these PCR products using a commercially-available kit, and then directly sequencing these PCR products using an Illumina MiSeq instrument. The Illumina MiSeq data will be processed using Mothur to trim for sequence quality and to determine the diversity of the bacterial communities (Chao estimator, Shannon Index, etc.) and to identify the individual members of the bacterial community. The structure of the bacterial communities will be statistically compared by weighted Unifrac. We can multiplex as many as 256 samples simultaneously.

Quantitative PCR (qPCR) will be used to quantify the 16S rRNA gene (a measure of bacterial biomass) as well as for genes that are specific to pathogens of interest (*Campylobacter*, *Legionella*, etc). We anticipate performing qPCR analyses on about 200 DNA extracts in total. There are 19 specific gene targets so we will perform nearly 4,000 qPCR reactions.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 173,074
Amount Spent: \$ 173,074
Balance: \$ 0

Outcome	Completion Date
1. Extraction and purification of DNA from water samples	September 1, 2017
2. Sequencing of 16S rRNA genes for bacterial community analysis	March 1, 2018
3. Quantify total bacteria and pathogens in water samples via qPCR	June 1, 2018
4. Statistical analysis of data	December 1, 2018

Activity Status as of January 1, 2017:

We have begun to extract and purify DNA from the filters used to isolate microorganisms from the water that we have sampled so far. We anticipate quantifying 16S rRNA genes (as a measure of total bacterial biomass) from these samples to ensure that our sample collection approach is successful.

Activity Status as of October 25, 2017:

We have continued to extract and purify DNA from the 109 filters used to concentrate microorganisms from the water that we have sampled so far. We also have processed 7 clean filters as negative controls. To date, we have completed DNA extractions for 93 out of the 116 filters (80%). We have quantified 16S rRNA genes (as a measure of total bacterial biomass) in 70 of these DNA extracts and have started to quantify pathogens, beginning with *Legionella*, in the DNA extracts (30 to date). We have also sent samples to the University of Minnesota Genomics Center (UMGC) for sequencing of PCR-amplified 16S rRNA gene fragments; this will allow us to identify/characterize the bacteria in these water supplies.

Activity Status as of January 1, 2018:

Quantification of selected pathogens (16 bacterial and 1 viral) and total bacteria has begun via the polymerase chain reaction (PCR). All collected DNA samples have been analyzed for 2 of the selected 17 pathogen gene targets. The two gene targets for which all samples have been analyzed are Shiga toxins 1 and 2 for quantification of Enterohemorrhagic *E. coli*. All samples were non-detect for Shiga toxins 1 and 2. More than half of the samples have been analyzed for the 16S rRNA gene for total bacteria. Total bacteria concentrations ranged widely from 10^3 to 10^8 gene copies per liter. The samples (96 out of 116) sent to the University of Minnesota Genomics Center (UMGC) have been sequenced and the output data are ready to be processed and analyzed. Work continues on quantifying the remaining 15 of 17 pathogen gene targets in the DNA extracts, and by submitting the remaining DNA extracts for DNA sequencing through the UMGc.

Activity Status as of July 1, 2018:

Quantification of the 16S rRNA gene (total biomass) and selected pathogens is complete. Total biomass concentrations in the water samples ranged from below detection ($\sim 10^4$ gene copies per liter) to 10^8 gene copies per liter, depending on the city and sampling location within each drinking water system (i.e., raw well water vs. treated water vs. tap water). The main takeaway message from the investigation that is of relevance to public health is that the majority of water samples were negative for all pathogen-related gene targets we tested for. In other words, most water samples, including untreated groundwater samples, appear to be free of potential disease-causing microorganisms. Gene targets that were detected sporadically include the *ssrA* gene targeting *Legionella* spp., and the *atpE* gene targeting *Mycobacterium* spp. *Legionella* and *Mycobacteria* are two genera that contain opportunistic pathogens that can impact the health of immuno-compromised people including the elderly and chemotherapy patients. Most samples tested positive for the 23S rRNA gene targeting *Enterococcus* spp. Follow-up investigations of this assay, however, suggest a high probability that these are false-positive detections. All samples were negative for the one viral gene target (*Adenovirus*).

DNA extracts from all samples have been sequenced by the UMGc and analyzed by our team. Preliminary interpretation of the sequencing results suggest that we MN groundwaters primarily contain very rare bacteria, most of which are not currently well characterized. Sequences have been processed for diversity analysis and the results are being prepared for interpretation.

Activity Status as of January 1, 2019:

DNA was extracted from the microbes recovered from the water samples using membrane filtration. The extracted DNA was used as template for quantitative PCR analyses targeting 14 genes corresponding to pathogenic bacteria, one gene for a DNA virus, and the 16S rRNA gene as a marker for total bacteria. Raw water 16S rRNA gene concentrations, indicating the numbers of total bacteria in the water, ranged from 10^5 – 10^8 gene copies L^{-1} , decreased to background levels after disinfection, then rebounded at the tap in the majority of cities.

There was no significant difference in 16S rRNA gene concentrations from source-to-tap in the two cities that do not employ chlorine disinfection. Regarding potential pathogens, all samples were negative for the targeted genes from *Campylobacter jejuni*, *Shigella* spp., and Adenovirus; *Escherichia coli*-specific genes were only detected in water from a non-potable well with a documented history of contamination. Genes markers for two genera, *Legionella* and *Mycobacteria*, that include species that are opportunistic pathogens, were detected in four of the 16 public groundwater supplies, with *Legionella* spp. levels decreasing in disinfected systems while *Mycobacteria* spp. levels tended to increase. *Mycobacteria* spp. are known to be very hardy bacteria that are resistant to disinfection via chlorine, which explains why they often dominate the bacterial communities in water systems containing free chlorine or combined chlorine (i.e., chloramines). While a few species of mycobacteria are known opportunistic pathogens, most species have not been associated with disease in humans. Hence, the presence of mycobacteria is not, in and of itself, a cause for concern. In fact, no samples tested positive for *Mycobacterium avium* Complex (MAC), which includes the two species of mycobacteria that are most often associated with human disease. Raw water samples contained diverse and previously uncharacterized organisms as revealed by DNA sequencing analyses, and beta diversity analyses suggest that community composition is driven by source water and/or disinfection. The results from this study suggest that groundwaters supplying public water systems in Minnesota are largely free of enteric pathogens but may contain opportunistic pathogens. Opportunistic pathogens are called “opportunistic” because they rarely infect healthy people; rather, they infect people with weak or weakened immune systems such as the very young, elderly, and chemotherapy patients.

Final Report Summary:

DNA extracted from the microorganisms recovered from the water samples was used as template for quantitative PCR analyses targeting 14 genes corresponding to pathogenic bacteria, one gene for a DNA virus, and the 16S rRNA gene as a marker for total bacteria. Raw water 16S rRNA gene concentrations, indicating the numbers of total bacteria in the water, ranged from 10^5 – 10^8 gene copies L^{-1} , decreased to background levels after disinfection, then rebounded at the tap in the majority of cities. There was no significant difference in 16S rRNA gene concentrations from source-to-tap in the two cities that do not employ chlorine disinfection. Regarding potential pathogens, all samples were negative for the targeted genes from *Campylobacter jejuni*, *Shigella* spp., and Adenovirus; *Escherichia coli*-specific genes were only detected in water from a non-potable well with a documented history of contamination. Genes markers for two genera, *Legionella* and *Mycobacteria*, that include species that are opportunistic pathogens, were detected in four of the 16 public groundwater supplies, with *Legionella* spp. levels decreasing in disinfected systems while *Mycobacteria* spp. levels tended to increase. Opportunistic pathogens are called “opportunistic” because they rarely infect healthy people; rather, they infect people with weak or weakened immune systems such as the very young, elderly, and chemotherapy patients. *Mycobacteria* spp. are known to be very hardy bacteria that are resistant to disinfection via chlorine, which explains why they often dominate the bacterial communities in water systems containing free chlorine or combined chlorine (i.e., chloramines). While a few species of mycobacteria are known opportunistic pathogens, most species have not been associated with disease in humans. Hence, the presence of mycobacteria is not, in and of itself, a cause for concern. In fact, no samples tested positive for *Mycobacterium avium* Complex (MAC), which includes the two species of mycobacteria that are most often associated with disease in humans. Raw water samples contained diverse and previously uncharacterized organisms as revealed by DNA sequencing analyses, and beta diversity analyses suggest that community composition is driven by source water and/or disinfection. The results from this study suggest that groundwaters supplying public water systems in Minnesota are largely free of enteric pathogens but may contain opportunistic pathogens.

ACTIVITY 3: Analysis of Conventional Water Quality Indicators.

Description:

At the same time that we collect samples for microbiological analysis, we will also collect samples for analysis of conventional water quality parameters. Of these conventional parameters, we will measure temperature, pH, chlorine, chloride, sulfate, nitrate, and carbamazepine (a conservative chemical tracer for

sewage). We will also measure tritium (a radioactive form of hydrogen), as this is used to assess aquifer vulnerability during the development of wellhead protection plans. We will also quantify fecal coliforms, *Escherichia coli*, and *Enterococcus* spp. using cultivation-based methods.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 74,746
Amount Spent: \$ 74,746
Balance: \$ 0

Outcome	Completion Date
1. Perform “conventional” culture-based microbiological assays for comparison with qPCR results obtained in Activity 2	September 1, 2017
2. Quantify chemical parameters and correlate with microbiological results obtained in Activity 2	December 1, 2017

Activity Status as of January 1, 2017:

We have quantified total coliforms and *E. coli* concentrations using a culture-based assay (Colilert) for all of the water supplies from which we have collected water. All of the samples have been negative for total coliforms, except for one tap water sample (Most probable number of 12.2/100 mL). All of the samples have been negative for *E. coli*. Tap water total chlorine levels have ranged from non-detect to 4.02 mg/L as Cl₂.

Activity Status as of October 25, 2017:

We have quantified total coliforms and *E. coli* concentrations using a culture-based assay (Colilert) for the 116 water samples collected to date. Most of the samples were negative for total coliforms. Ten out of the 116 samples tested positive for total coliforms (ranging from 1 to 727 most probable number (MPN) per 100 mL). The sample with 727 MPN/100 mL was taken from a non-potable water tap at a state park that is not used for drinking. This sample was also the only one that tested positive for *E. coli* (249.5 MPN/100mL). Samples that tested positive for total coliforms included raw groundwater samples, finished water leaving treatment plants, and tap water samples. Tap water total chlorine levels ranged from non-detect to 4.02 mg/L as Cl₂. Well water samples from 12 out of 16 of the sites have been sent to a laboratory in Waterloo, Ontario, Canada to quantify tritium levels. Tritium results can be used to determine the approximate age of the groundwater. We have completed the sample preparation (solid phase extraction) for the carbamazepine analyses for well water samples from 9/16 of the sampling sites.

Activity Status as of January 1, 2018:

Results from the 12 samples submitted to the University of Waterloo have been analyzed for tritium content. Tritium levels ranged from <0.8 to 7.2 tritium units (TU). Lower tritium levels are associated with older water, and higher tritium levels are associated with younger water. The tritium results correlate strongly with well depth. Deeper wells tend to have older water while shallower wells have younger water. Well water samples for 9 of the 16 sampling sites are currently undergoing analysis for carbamazepine. The remaining samples which have not been collected for tritium and carbamazepine analysis are to be collected in the spring of 2018. Of the 116 collected samples, 52 have been analyzed for nitrite and nitrate concentrations, and 59 have been analyzed for chloride and sulfate concentrations. Nitrite concentrations ranged from non-detect to 0.038 mg/L as N and nitrate concentrations ranged from non-detect to 6.60 mg/L as N.

Activity Status as of July 1, 2018:

All samples have been analyzed for all water quality parameters except carbamazepine. One city’s well water remains to be collected for carbamazepine analysis, after which all previously processed samples designated for carbamazepine analysis will be evaluated simultaneously. Tritium content has been assessed for all wells and ranged from below detection (<0.8 tritium units, indicating “old” water) to 7.2 tritium units (“young” water).

Activity Status as of January 1, 2019:

The analysis of all groundwater samples for carbamazepine and tritium was completed. This research suggests that total bacteria (as 16S rRNA genes) concentrations are lower in deeper wells that draw older water (tritium content < 0.8 TU), and greater in shallower wells that draw younger water (tritium content > 0.8 TU). In general, tritium content and well depth were good predictors of total bacteria (i.e., 16S rRNA gene) concentrations in raw water samples. Raw water samples collected from the metro and southeast regions (regions with the deepest wells and lowest tritium content) contained 16S rRNA gene concentrations between $10^6 - 10^7$ gene copies L^{-1} , while raw water samples collected from the central and southwest regions (regions with shallower wells and higher tritium content) contained gene concentrations above 10^7 gene copies L^{-1} with several samples exceeding 10^8 gene copies L^{-1} (systems C1 and SW3). Carbamazepine was only detected in the water from 4 wells and those tended to be shallower wells (115 feet or less) with younger water according to the tritium results, with one exception (a 298 ft deep well in southwestern Minnesota). The carbamazepine results suggest that very few wells supplying public water systems show any evidence of wastewater impacts.

Final Report Summary:

Well depths, tritium content, and carbamazepine concentration at the raw water sampling locations varied from region to region. The central and southwest regions contained the shallowest wells; more than half of the wells in these two regions were less than 100 ft deep. Deeper wells were found in the metro region (range 219 – 414 ft), but the deepest wells were located in the southeast region (range 334 – 1204 ft). All wells in the southeast region penetrated through the karst geology, circumventing the possibility of drawing groundwater that may be susceptible to surface contamination. Tritium content varied significantly from region to region and correlated with well depth. All samples from the central region and the majority of samples collected from the southwest region (4/5) contained young water (i.e., tritium content was detectable, median 5.1 and 5.2 TU for the central and southwest regions, respectively). Two of the four samples from the metro region and all samples from the southeast region did not contain detectable levels of tritium (< 0.8 TU), indicating older water. Carbamazepine was detected in 3/5 wells in the southwest region and one well in the central region. Most of these were shallow wells (37 to 115 ft deep) with one exception, a 298 ft deep well in the southwest region.

V. DISSEMINATION:

Description:

Findings will be disseminated directly to each of the sampled utilities as a written report and an in-person presentation. Findings will also be disseminated and archived via reports to LCCMR, peer-reviewed publications, presentations at conferences, and city council meetings (if appropriate). We will also, when appropriate, disseminate results via press releases to the media and via the MDH website. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the medical community and we will seek avenues to share the results with this community. We will preserve the anonymity of participating utilities in our press releases and publications upon their request and work with them and the MDH to determine how best to communicate the results from their individual systems to their customers.

Status as of January 1, 2017:

There has been no dissemination activity during this project period.

Status as of October 25, 2017:

Graduate student John Galt presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the Minnesota Section AWWA Conference in Duluth, MN on September 14, 2017.

Status as of January 1, 2018:

Graduate student John Galt presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the annual Water Quality and Technology Conference in Portland, OR on November 12, 2017.

Status as of July 1, 2018:

There has been no dissemination activity during this project period. Results are currently in the process of interpretation and dissemination in the form of John Galt’s research thesis.

Status as of January 1, 2019:

An oral presentation on the work was delivered by M.S. student John Galt at the annual MN Section of the American Water Works Association meeting in Duluth, MN in Fall 2018. This meeting is an important audience for the work as it includes representatives from water utilities around the state, consulting engineers that work in the state and region, and representatives from state agencies (e.g., MDH). John Galt completed and submitted his M.S. thesis to the University of Minnesota. We are currently working on a manuscript that will be submitted to a peer-reviewed journal as well as the final report.

Status as of July 1, 2019:

Tim LaPara spoke to the City of Riverton's City Council and to the water quality manager at the City of Eagan about the results. Finally, Tim also participated in an open house concerning the water system in the City of Hastings.

Final Report Summary:

Dissemination activities included two poster presentations and one oral presentation by graduate student John Galt. John presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the annual Minnesota Section AWWA Conference in Duluth, MN on September 14, 2017. John also presented a poster entitled “Are Waterborne Pathogens in Minnesota’s Groundwater?” at the AWWA Water Quality Technology Conference in Portland, OR on November 12, 2017. An oral presentation on the work was delivered by John at the Minnesota Section of the American Water Works Association meeting in Duluth, MN in Fall 2018. The MN Section AWWA meeting is an important audience for the work as it includes representatives from water utilities around the state, consulting engineers that work in the state and region, and representatives from state agencies (e.g., MDH). Tim LaPara also participated in outreach activities. Tim gave an oral presentation on the project findings at the Minnesota Section of the American Water Works Association meeting in Duluth, MN in Fall 2019. Tim also spoke to the City of Riverton's City Council and to the water quality manager at the City of Eagan about the results. Finally, Tim also participated in an open house concerning the water system in the City of Hastings.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Explanation
Personnel:	\$ 251,500	For Drs. Hozalski (\$52,899) and LaPara (\$48,809) for directing the project and for a graduate (\$139,494) and an undergraduate student (\$10,298) at the University of Minnesota.
Professional/Technical/Service Contracts:	\$20,000	University of Minnesota Genomics Center for DNA sequencing (~300 samples) and other services
Equipment/Tools/Supplies:	\$22,500	General laboratory supplies (\$12,000) qPCR reagents (\$5,000) DNA extraction kits, purification kits (\$5,000)
Travel Expenses in MN:	\$5,000	Miscellaneous travel within MN. This will include travel to 15-20 treatment facilities across Minnesota 3 times each for sampling (\$3,000) and travel to water utilities and MN-

		section AWWA conference in Duluth in 2017 and 2018 for dissemination of results (\$2,000).
TOTAL ENRTF BUDGET:	\$299,000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.75

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
		\$	
State			
U of MN	\$127,807	\$127,807	In-kind contribution; indirect costs not charged to this project
TOTAL OTHER FUNDS:	\$127,807	\$127,807	

VII. PROJECT STRATEGY:

A. Project Partners:

The Minnesota Department of Health will aid in selecting water utilities to be sampled and facilitate access to the systems for sampling.

B. Project Impact and Long-term Strategy:

The long-term goal of the proposed research is to gain a better understanding of the microbiological quality of groundwater in Minnesota. This research is especially important because more than 90% of Minnesota’s public water supplies use groundwater as their source, often providing this water without disinfection. Numerous new laboratory techniques have been developed over the last decade to enable microbiologists to analyze the microbes in drinking water with astounding detail and precision. The proposed study, therefore, will generate novel and critically important knowledge on the microbiological quality of Minnesota’s public water supplies and will provide sound guidance for protecting the public health of Minnesotans.

There is increasing evidence that groundwater supplies can be contaminated by pathogenic microorganisms including viruses. Groundwater has typically been considered a safe water source that can be consumed with little or no treatment because the water is naturally filtered by the porous media it passes through. Some groundwaters, such as those in unconfined aquifers or in Karst regions, can become contaminated with pathogens from anthropogenic activities on the overlying land surface including septic systems and agricultural activities. Further, contamination of water supplies can also occur during water distribution. For example, negative pressure events resulting from valve or pumping changes can draw water into the water mains from the surrounding soil. Also, bacteria present in biofilms that have colonized the interior of the water mains can be shed into the water and end up at the taps of water consumers.

Sampling the source, post-treatment, and distribution system followed by DNA-based quantification and characterization of the microbial communities present will allow us to determine the extent of contamination and contaminant origin (i.e., groundwater or distribution system). With this information, we can recommend

changes to systems with evidence of contamination such as installing or improving water treatment (e.g., UV irradiation) or changing distribution system operation and maintenance protocols (e.g., increase residual disinfectant concentration or add/increase distribution system flushing program). Such changes are expected to improve the quality and microbiological safety of Minnesota's groundwater systems that have shown evidence of contamination. This research project will sample 15 out of the 947 community groundwater supplies (1.6%) and provide a preliminary assessment of the safety of these representative supplies throughout the state. Based on the results of this investigation, an expanded investigation might be warranted to sample a broader array of systems to fully document the extent of the problem. Future efforts might also be directed at sampling and evaluating the quality of water supplied by private wells, as 20% of Minnesotans receive their water from private wells. Finally, in systems that implement changes to address contamination issues, a follow-up study could be done to determine the effectiveness of those changes.

C. Funding History: N/A

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached visual

X. RESEARCH ADDENDUM: See attached

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2017, July 1, 2017, January 1, 2018, July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

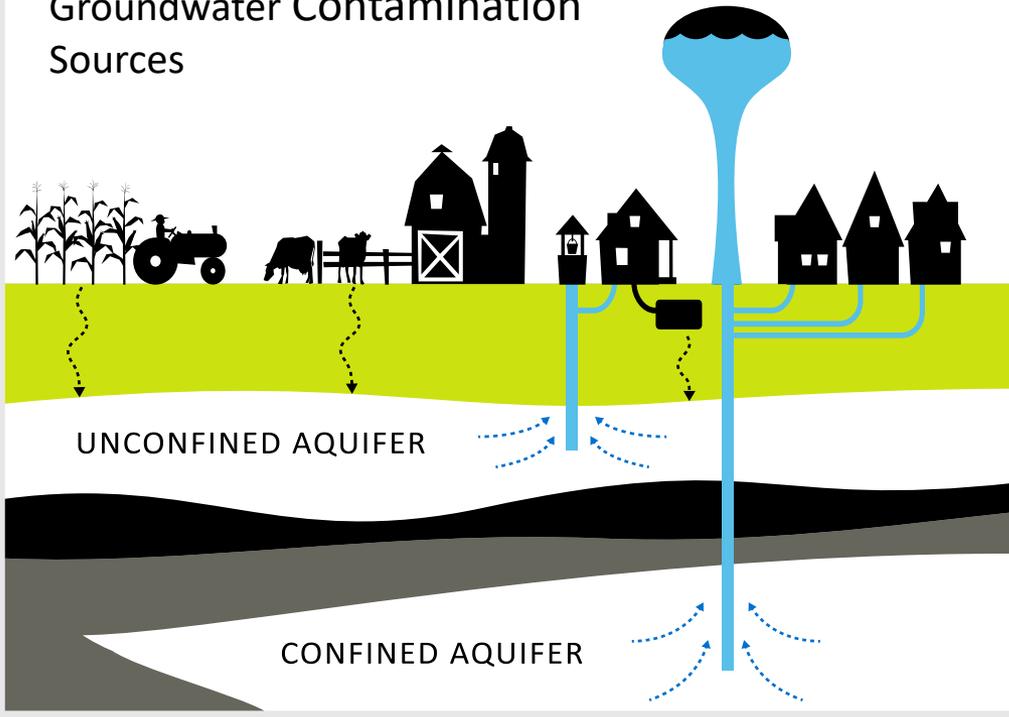
Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget



Project Title: Bacterial Assessment of Groundwater Supplies Used for Drinking Water
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04f
Project Manager: Raymond M. Hozalski
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: \$299,000
Project Length and Completion Date: 3 Years, June 30, 2019
Date of Report: Expenditures Through 6/30/2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	Water Utility Selection and Sampling			Groundwater microbiome analysis			Analysis of Conventional Water Quality				
Personnel (Wages and Benefits)	\$41,216	\$41,216	\$0	\$147,199	\$147,199	\$0	\$63,085	\$63,085	\$0	\$251,500	\$0
<i>Project Management, Raymond M. Hozalski (\$52,899; 8% of time; 75% to salary, 25% to fringe benefits)</i>											
<i>Project Management, Timothy LaPara (\$48,809; 8% of time; 75% to salary, 25% to fringe benefits)</i>											
<i>Graduate Student at U of M (\$139,494; 50% of time; 59% to salary, 41% to benefits)</i>											
<i>Undergraduate Student at U of M (\$10,298; 25% of time during academic year; 100% to salary, 0% to benefits)</i>											
Equipment/Tools/Supplies											
<i>General lab supplies (\$12,500), reagents for qPCR (\$5,000), use of UMG's facilities for qPCR and Illumina sequencing (\$20,000), DNA extraction kits (\$3,000), PCR purification kits (\$2,000)</i>	\$6,965	\$6,965	\$0	\$24,875	\$24,875	\$0	\$10,661	\$10,661	\$0	\$42,501	\$0
Travel expenses in Minnesota											
<i>Travel to approximately 15-20 water utilities across Minnesota three times each to collect water samples; travel to annual AWWA water conference in Duluth to disseminate results; travel to the sampled water utilities to discuss results</i>	\$3,000	\$3,000	\$0	\$1,000	\$1,000	\$0	\$1,000	\$1,000	\$0	\$5,000	\$0
COLUMN TOTAL	\$51,181	\$51,181	\$0	\$173,074	\$173,074	\$0	\$74,746	\$74,746	\$0	\$299,001	\$0.00

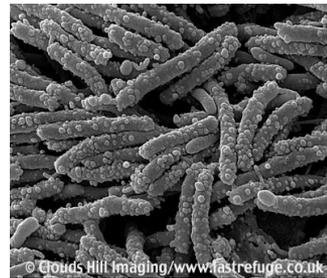
Groundwater Contamination Sources



Adapted from: www.mn.gov/governor/images/nitrate_sources_infographic.pdf



Shallow wells



Deep wells



<http://pixshark.com/happy-person.htm>



<http://pixshark.com/happy-person.htm>