Final Abstract

Final Report Approved on March 27, 2025

M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

Project Title: Evaluating Coronavirus and Other Microbiological Contamination of Drinking Water Sources from Wastewater

Project Manager: Timothy LaPara

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04g

Appropriation Amount: $594,000

Amount Spent: $587,848

Amount Remaining: $6,152

Sound bite of Project Outcomes and Results

This research project investigated the chemical and microbiological water quality of private wells throughout the State of Minnesota. Although many wells showed indications of anthropogenic contamination, very few wells had detectable levels of microorganisms or chemicals that were directly of concern.

Overall Project Outcome and Results

This project investigated the chemical and microbiological quality of private wells serving Minnesotans. As such, we collected 85 private well water samples for microbiological analyses as well as 49 samples for chemical analyses.

Approximately 30% of private well water samples tested positive for total coliforms, an indicator of fecal contamination. In contrast, genetic testing revealed very few positive detections that were directly connected to a microbial pathogen of concern. Specifically, no well water samples tested positive for either SARS-CoV-II (the virus that caused the COVID-19 pandemic) or Cryptosporidium parvum. This latter result represented a significant difference between our study and a recent study by the Minnesota Department of Health of wells serving public water systems, in which Cryptosporidium

was commonly detected. Further research suggested that the assay used in the MDH study was likely to produce false-positive results.

We also developed sensitive methods to detect 13 organic compounds and 22 inorganic ions that could be indicators of contamination. We used the molar ratio of chloride to bromide to identify 10 sites with potential sewage contamination, although these results did not correlate well with the presence of total coliforms. Nitrate levels greater than 1 part per million were found at 7 sites, suggesting human influence on the groundwater. Furthermore, we found 9 sites with the molar ratio of sodium to potassium below 2, indicating pollution by agricultural runoff; and 10 sites had a molar ratio above 5, indicating pollution by sewage. Here again, these potentially impacted groundwater samples did not have high levels of total coliforms.

Finally, we demonstrated that the point-of-use filters from Aquamedix were able to reduce the total number of bacteria in private well water. This technology offered a potentially viable alternative for private well owners who are concerned about the microbiological quality of their water.

Project Results Use and Dissemination

We presented preliminary results of our work at the 2023 meeting of the Minnesota section of the American Water Works Association and at a monthly meeting of a City Council who was interested in our research. Although this project is now complete, we will continue to disseminate our research results via the publication of peer-reviewed manuscripts and an additional presentation at the 2025 meeting of the Minnesota section of the American Water Works Association (at no cost to LCCMR). We have also scheduled an additional presentation at a City Council meeting in April 2025.

Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

General Information

Date: March 31, 2025

ID Number: 2021-364

Staff Lead: Mike Campana

Project Title: Evaluating Coronavirus and Other Microbiological Contamination of Drinking Water Sources from Wastewater

Project Budget: $594,000

Project Manager Information

Name: Timothy LaPara

Organization: U of MN - College of Science and Engineering

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Project Reporting

Final Report Approved: March 27, 2025

Reporting Status: Project Completed

Date of Last Action: March 27, 2025

Project Completion: June 30, 2024

Legal Information

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04g

Appropriation Language: $594,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to survey public and private wells to identify sources of and evaluate solutions to microbiological contamination of drinking water sources by wastewater, including from the virus that causes COVID-19.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: With detection of coronavirus in human feces, there are urgent concerns about microbiological contamination of drinking water sources by wastewater. We will investigate this contamination, identify sources, and evaluate solutions.

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

A previous LCCMR project allowed us to investigate the microbiological quality of twenty public water systems (PWSs) supplying groundwater to Minnesota residents. In an ongoing project, we are investigating the microbiological quality of PWSs supplying treated surface water. The research proposed herein expands on those projects by including a state-wide investigation of private wells and by sampling surface and groundwater PWSs to analyze for SARS-CoV-2 (a.k.a. COVID-19). We will also attempt to identify the contamination sources and to offer potential solutions to this contamination by making well placement recommendations and by partnering with a small Minnesota business (AquaMedix; Eden Prairie, MN) that produces point-of-use treatment filters.

Groundwater is generally believed to be an abundant source of safe and high-quality water. In recent years, however, research performed by the Minnesota Department of Health and others has shown that groundwater can be contaminated with disease-causing microorganisms, including numerous viruses (e.g., norovirus) and protozoa (e.g., Cryptosporidium parvum). The presence of these microorganisms indicates that some groundwaters are being contaminated by wastewater, possibly from septic systems and leaky sanitary sewers. This raises serious concerns that SARS-CoV-2, known to be present in human feces of infected persons, could potentially enter groundwater.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

Our proposed solution to the problem is three-pronged. First, we will further document the microbiological contamination of groundwater by collecting water samples from both public and private wells. This work is necessary to confirm the limited work done to date by the Minnesota Department of Health (i.e., their work needs to be validated by an independent laboratory); our work also will expand on the previous work by considering private wells (which have yet to be studied and are more likely to be susceptible to contamination) and by looking for SARS-CoV-2. Second, we will attempt to identify the various sources of microbiological contamination by analyzing a range of inorganic and organic chemical fingerprints that have origins of septic tanks, animal wastes, and hospital wastes. With information on potential sources and their locations relative to the wells, we plan to develop recommendations for future well placements to minimize the risk of contamination. And third, we will test a potential treatment solution for the problem of microbiological contamination of groundwater by partnering with AquaMedix (Eden Prairie, MN), who will provide state-of-the-art in-home water filtration units that are designed to remove the microbiological contaminants of concern.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

Groundwater is undeniably one of Minnesota's most precious environmental resources. It is an abundant and high-quality source of water for drinking, for bathing, and for numerous other uses. Groundwater, however, can be contaminated with microorganisms that can make people sick (especially viruses, like SARS-CoV-2 and norovirus). This project will first focus on investigating the extent of contamination in both public and private water supplies. We will then identify the likely sources of this contamination, allowing us to make recommendations regarding well placement to minimize the risk of contamination in the future. Finally, we will investigate in-home treatment technologies by partnering with a Minnesota company (AquaMedix), such that we will offer a potential treatment solution to Minnesotans when the source of contamination cannot be identified and ameliorated or the costs of amelioration (e.g., drill a new well) are prohibitive.

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Activities and Milestones

Activity 1: Investigate public and private well water for microbiological contamination by viruses, bacteria, and protozoa.

Activity Budget: $198,000

Activity Description:
University of Minnesota personnel will identify volunteer partners (i.e., public water supplies and private well owners, to remain anonymous) from whom we will collect large-volume (> 200 gallons) water samples. We will concentrate the microorganisms in these samples and then analyze for harmful microorganisms using state-of-the-art assays that target genes (RNA and DNA) specific to the dangerous microbes. We will spend the first two years of the study period collecting, processing, and analyzing well water samples. Much of the sample collection, which is a labor-intensive process, will be performed by undergraduate students to help reduce project costs (while simultaneously providing a stellar research experience for our students). The genetic analyses for harmful microorganisms will be performed by either a graduate student or a post-doctoral research associate. We hope to partner with ~20 public water supplier and ~60 private well owners. Our plan is to collect 3-6 replicate samples from each volunteer participant (240-480 total samples). We will test for more than 20 different microbial pathogens, including adenovirus, norovirus, SARS-CoV-2, Legionella, Giardia lamblia, Cryptosporidium parvum, E. coli, and Mycobacterium avian. Results will be immediately shared with well owners (public or private) in an informal manner.

Activity Milestones:

Description	Approximate Completion Date
Sample collection and processing	June 30, 2022
Quantification of different microorganisms from samples	December 31, 2022
Data Analysis and Report Preparation	June 30, 2023

Activity 2: Identify potential sources of microbiological contamination of groundwater

Activity Budget: $198,000

Activity Description:
We will identify the source or sources of contamination at 5 sites identified in Activity 1 as "contaminated". We will generate multiple lines of evidence by analyzing organic and inorganic chemicals that are source fingerprints and conservative (i.e., do not degrade). First, we will measure the concentrations of inorganic ions (e.g., chloride, bromide, sodium, and potassium) as well as the concentrations of nitrate and organic carbon. These data will be used to differentiate among different waste sources. For example, we will use the sodium to potassium ratio to differentiate waste from septic tanks (i.e., human) from animal-derived sources. Second, we will analyze for specific organic chemicals, including a stilbene-type fluorescent whitening agent widely used in household laundry detergents, the artificial sweeteners sucralose and acesulfame, and an iodinated X-ray contrast media compound widely used in hospital CT scans. These data will allow us to further refine our identification of likely waste sources. Lastly, we will measure tritium as an indicator water age and aquifer vulnerability. Results will be immediately shared with well owners (public or private) in an informal manner as well as formally as part of our dissemination plan.

Activity Milestones:

Description	Approximate Completion Date
Sample collection and quantification of organic and inorganic chemicals from samples	December 31, 2022
Data Analysis and Report Preparation	June 30, 2023

Statistical analysis of correlating pathogen occurrence with potential sources	June 30, 2023

Activity 3: Validate that in-home water filtration systems can protect against microbiological contamination of groundwater

Activity Budget: $198,000

Activity Description:

This activity will build upon the results of the first project Activity. In the first year of the project, we will identify numerous water systems (both public water supplies and private wells) that are contaminated with harmful microorganisms. At one of the public water systems, we will recruit 5 volunteer residences where we will install in-home water filtration units from AquaMedix. Similarly, we will recruit 5 private wells with microbiological contamination and install in-home water filtration units in these homes. We will then collect water samples before and after the filters to investigate whether in-home filtration can resolve the health risks posed by microbiological contamination of groundwater.

Activity Milestones:

Description	Approximate Completion Date
Install in-home water filtration units	May 31, 2022
Collected and analyze water samples after intervention	December 31, 2022
Data Analysis and Report Preparation	June 30, 2023

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Brady Benson	Aquamedix	Aquamedix will provide some guidance on in-home water treatment technologies. We will purchase and install these technologies to test their effectiveness.	No
Boya Xiong	University of Minnesota	co-Project Manager	Yes
Raymond M. Hozalski	University of Minnesota	co-Project Manager	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
The results of this project will be disseminated directly to the participating public water supplies and private well owners. We will also disseminate our research results broadly through presentations at local (e.g., the annual conference of the Minnesota-section of the American Water Works Association), national, and international conferences. We also will publish our results in the peer-reviewed literature in the open-access format; this will enable LCCMR staff to publicize our research without any concerns with respect to copyright. We also will ask the University of Minnesota and/or MDH to produce press releases of our results, as appropriate. We will appropriately acknowledge the Minnesota Environment and Natural Resources Trust Fund during all dissemination activities through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?
The results of this project will be disseminated directly to participating public water supplies and private well owners. We also will disseminate our research results broadly through presentations at local (e.g., the annual conference Minnesota-section of the American Water Works Association), national, and international conferences. We also will publish our results in the open-access, peer-reviewed literature; this will enable LCCMR staff to publicize our research without any concerns with respect to copyright. We also will ask the University of Minnesota and/or MDH to produce press releases of our results, as appropriate.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Triclosan Impacts on Wastewater Treatment	M.L. 2014, Chp. 226, Sec. 2, Subd. 03c	$380,000
Bacterial Assessment of Groundwater Supplies Used for Drinking Water	M.L. 2016, Chp. 186, Sec. 2, Subd. 04f	$299,000
Wastewater Treatment Process Improvements	M.L. 2016, Chp. 186, Sec. 2, Subd. 04k	$398,000
Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04f	$325,000
Improving Drinking Water for Minnesotans through Pollution Prevention	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04f	$345,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
Undergraduate research assistant		Undergraduate students will be used to help collect samples, process these samples, etc. These are labor-intensive activities.			0%	1.5		$36,540	-	-
Graduate Research Assistant		Perform analyses, analyze data			44%	1.26		$100,221	-	-
Post-doctoral research associate		Perform experiments, analyze data			20%	2		$63,526	-	-
Boya Xiong		Co-project manager			27%	0.12		$19,562	-	-
Raymond Hozalski		Co-Project Manager			27%	0.24		$62,608	-	-
Timothy LaPara		Project Manager			27%	0.54		$134,048	-	-
Summertime Research assistant		Perform analyses and collect samples like an undergraduate student or post-doc			0%	0.25		$8,640	-	-
Researcher 3		Perform experiments; analyze data			26.9%	0.88		$38,067	-	-
Researcher 1		Perform chemical analyses of tracers of groundwater contamination			25%	0.15		$11,534	-	-
							Sub Total	**$474,746**	**$474,015**	**$731**
Contracts and Services										
University of Minnesota Genomics Center	Internal services or fees (uncommon)	UMGC provides at-cost access to state-of-the-art molecular/genetic equipment (e.g., next-gen DNA sequencing, droplet digital PCR), supplies, and technical expertise.				0		-	-	-
Masonic Cancer Center	Internal services or fees (uncommon)	This is an analytical biochemistry laboratory that provides access to numerous pieces of analytical equipment (e.g., GC-MS-MS) at-cost to University researchers. This facility				0		-	-	-

		will help us analyze specific chemicals of concern in groundwater.								
Department of Earth and Environmental Sciences	Internal services or fees (uncommon)	This laboratory provides access to analytical equipment at-cost to University researchers. This facility would be used to quantify cations and anions in groundwater.				0		-	-	-
The University of Waterloo-Environmental Isotope Laboratory	Professional or Technical Service Contract	We will need to send samples to a commercial laboratory to quantify the levels of tritium in the collected water. These tritium levels are used to determine the "age" of the water.		X		0		$5,286	$5,286	-
							Sub Total	**$5,286**	**$5,286**	**-**
Equipment, Tools, and Supplies										
	Tools and Supplies	In-home treatment units	To test the ability of in-home treatment units to improve the microbiological quality of drinking water. The cost will include the price of the filter ($50-$100 each) as well as the cost of installation (~$400-$450 each). We will install 10 of these systems.					$5,000	$5,000	-
	Tools and Supplies	Miscellaneous chemicals, laboratory supplies (e.g., glassware)	Numerous chemical reagents will need to be purchased to collect samples, process these samples, analyze the samples, and preserve the samples.					$29,462	$29,462	-
	Tools and Supplies	Expendable reagents for quantitative polymerase chain reaction	These reagents are needed to quantify genes linked to specific organisms					$29,014	$29,014	-
	Tools and Supplies	DNA and RNA extraction kits	Extract and purify DNA and RNA from groundwater samples					$19,714	$19,714	-
	Tools and Supplies	Membrane filtration units	Used to collect high volume water samples					$10,000	$10,000	-
							Sub Total	**$93,190**	**$93,190**	**-**

Capital Expenditures										
		CFX Connect Real Time PCR detection system	This instrument is critical for quantifying microorganisms via their DNA or RNA in water systems					$2,500	$2,500	-
		Steam generator for the autoclave	To complete this work, we need to use a sterilizer (autoclave). Our autoclave has broken and needs a new stream generator (cost > $20,000). We will use this project's funds ($1,126) to cover a fraction of the cost of repair (i.e., appropriate given how much this project uses the autoclave)	X				$1,126	$1,125	$1
							Sub Total	**$3,626**	**$3,625**	**$1**
Acquisitions and Stewardship										
							Sub Total	**-**	**-**	**-**
Travel In Minnesota										
	Miles/ Meals/ Lodging	Use of personal vehicles and/or vehicles rented from the University's fleet services	We will need to travel to different locations to collect groundwater samples.					$8,778	$7,858	$920
	Conference Registration Miles/ Meals/ Lodging	MN-section of the American Water Works Association	We will try to present our results at the MN-section of the American Water Works Association.					$2,000	$2,000	-
							Sub Total	**$10,778**	**$9,858**	**$920**
Travel Outside Minnesota										
							Sub Total	**-**	**-**	**-**
Printing and Publication										

	Publication	Page charges for publication	Most journals now allow "open access" publication, which effectively means that the authors retain the copyright of their published work. This would allow LCCMR staff to circulate our research manuscripts without concern or restrictions imposed by copyright laws.					$4,500	-	$4,500
	Printing	Poster printing	We will likely present our research in poster form, at local/University conferences and at the MN-section of the American Water Works Association.					$500	$500	-
							Sub Total	**$5,000**	**$500**	**$4,500**
Other Expenses										
		Equipment repair	We will heavily use pre-existing laboratory equipment to perform this project. This equipment routinely needs repair and maintenance.	X				$1,374	$1,374	-
							Sub Total	**$1,374**	**$1,374**	**-**
							Grand Total	**$594,000**	**$587,848**	**$6,152**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Contracts and Services - The University of Waterloo-Environmental Isotope Laboratory	Professional or Technical Service Contract	We will need to send samples to a commercial laboratory to quantify the levels of tritium in the collected water. These tritium levels are used to determine the "age" of the water.	We will use a commercial laboratory in Waterloo Canada for these analyses. To our knowledge, this is the only commercial laboratory in North America that performs these analyses. We have no knowledge of any laboratory in the State of Minnesota that can performed these analyses (commercial or university).
Capital Expenditures		Steam generator for the autoclave	This budget line is for a small fraction of the total cost of the equipment. This fraction was selected based on the how much this project will use this piece of equipment. **Additional Explanation :** This budget line is for a small fraction of the total cost of the equipment. This fraction was selected based on the how much this project will use this piece of equipment.
Other Expenses		Equipment repair	We are reducing the amount of funds budgeted for equipment repair to compensate for the $2500 in funds that will be used to purchase the CFX Connect Real Time PCR System. We are also reducing the funds budgeted by $1126 for equipment repair to fix our autoclave. Both amounts reflect the use of these pieces of equipment for this project.

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount	$ Amount Spent	$ Amount Remaining
State						
			State Sub Total	**-**	**-**	**-**
Non-State						
In-Kind	The University of Minnesota incurs indirect costs equal to 55% of the modified total direct costs. These costs are not charged to LCCMR.	Indirect costs (lights, overhead, support personnel, etc).	Secured	$302,241	$302,241	-
			Non State Sub Total	**$302,241**	**$302,241**	**-**
			Funds Total	**$302,241**	**$302,241**	**-**

Attachments

Required Attachments

Visual Component

File: 608d475d-6b5.pdf

Alternate Text for Visual Component

Groundwater is a precious resource that is often used as a supply for drinking water (both public and private water supplies). Although groundwater is typically assumed to be free of microbiological contamination, it often contains microorganisms that can make people sick. Our drinking water infrastructure is very robust and it properly protects public health, but the consumption of untreated groundwater could lead to illnesses among Minnesotans....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Peer review addendum (Final)	4a513a6e-dca.docx
Background Check Form	bb8757ae-67a.pdf
Presentation of Preliminary Results to a City Council in Minnesota	e62f6a2d-5ca.pptx
Presentation to the MN Section of the American Water Works Association	853177a8-947.pptx

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

I have modified the budget by reducing some of funds requested for faculty salary (esp. for Prof. Hozalski), for the graduate research assistant (cutting this appointment from 50% to 25% in the first year), and for undergraduate researchers. I also reduced our travel budget slightly. MARCH 22, 2021: I updated the work plan per the suggestions of LCCMR staff to include additional details.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

Yes

Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I understand the UMN Policy on travel applies.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	• Budget - Capital, Equipment, Tools, and Supplies • Budget - Other	A piece of our equipment broke; the cost of repair was more than cost of a new machine. We are requesting to re-allocate "repair funds" to "capital equipment" funds. The cost charged to this project reflects the usage of this project in the next two years. Additional funds will come from other projects and equipment users (i.e., this project will contribute $2500 to a $15000 piece of equipment).	April 7, 2022	Yes	April 11, 2022
2	Amendment Request	• Budget - Personnel	I would like to hire a former undergraduate student (graduation date = May 2022). The University requires that I hire her as a separate category because she is no longer a student. I am therefore requesting a shift of $9288 from the "undergraduate student" category in personnel to "summer researcher". The budget and work plan are otherwise unchanged.	May 4, 2022	Yes	May 9, 2022
3	Amendment Request	• Budget - Personnel	We would like to hire a person who is effectively a post-doc for this work but university rules require that this person be classified as a "Researcher 3" rather than a post-doc. Hence, we are requesting an amendment to hire a Researcher 3 for one year. The funds for this position come from the line item that was formerly for a "post doctoral research associate."	July 25, 2022	Yes	July 29, 2022
4	Amendment Request	• Budget • Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies	Tritium analysis is more costly ($20000) than originally requested; other laboratory costs are not needed ($0). Equipment repair funds ($1126) are needed but require the purchase of capital equipment (autoclave) so they need to be reclassified.	June 14, 2023	Yes	June 15, 2023

		• Budget - Travel and Conferences • Budget - Other	Our travel costs were over-estimated but our personnel costs (for undergraduates) were underestimated, so we would like to shift $8000 to personnel from travel. The scope and total cost of the project do not change.			
5	Amendment Request	• Budget - Personnel • Budget - Capital, Equipment, Tools, and Supplies	We over-budgeted for grad student salary so we reduced this amount (-$29034). We would like to hire a "Researcher 1" to perform some chemical analyses (+$11534). We also under-budgeted for materials/supplies, so we are asking for additional funds for miscellaneous supplies (+$5000), PCR supplies (+$7500), and DNA extraction kits (+$5000). These changes do not affect the scope or overall project cost.	September 20, 2023	Yes	September 25, 2023
6	Amendment Request	• Other • Budget - Personnel • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies • Budget - Non-ENRTF Funds Contributed	Our post-doc unexpectedly left UMN in June, so we need to shift salary/fringe from post-doc to faculty. We also under-budgeted for expendable materials and supplies; we request an additional $14462 for these activities. Our under-budgeting was caused by significant increases material costs, a poor original estimation of costs, and by substantial errors made by project personnel during the first year of the project. These changes should not significantly affect project goals or outcomes.	February 1, 2024	Yes	February 2, 2024
7	Amendment Request	• Other • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences	We substantially over-budgeted our need for "Services/Subawards - University of Waterloo" and for "Travel". In contrast, we substantially underbudgeted for "Tools and Supples - Expendable PCR Reagents" and for "Tools and Supplies - DNA Extraction kits". Our substantial under-budgeting was due to errors in processing samples (i.e., necessitating repeated	March 5, 2025	Yes	March 6, 2025

			analyses) and an underestimation of supply costs (i.e., caused by inflation). These budget changes do not significantly affect the project goals			

Status Update Reporting

Final Status Update August 14, 2024

Date Submitted: February 28, 2025

Date Approved: March 6, 2025

Overall Update

We collected microbial biomass from 85 high-volume (> 500 liters) water samples from private wells using ultrafiltration membranes. From these samples, we obtained genetic material (RNA and DNA) for subsequent analysis (Activity 1). We also collected 49 samples (all private wells) for chemical analyses (Activity 2) to identify sources of contamination. We also installed point-of-use water filters at 6 private homes to evaluate the benefits of these filters on the microbiological quality of well water (Activity 3). Genetic testing suggested that private wells frequently had populations of Escherichia coli and Pseudomonas aeruginosa; in contrast, no water samples tested positive for the SARS-CoV-2 or Cryptosporidium parvum. We developed sensitive methods to detect 13 organic compounds and 22 inorganic ions that could be indicators of contamination. Finally, we demonstrated that the point-of-use filters from Aquamedix were able to reduce the total number of bacteria in private well water. We presented preliminary results of our work at the 2023 meeting of the Minnesota section of the American Water Works Association. This project is now complete, although we will continue to disseminate our research results via the publication of peer-reviewed manuscripts (at no cost to LCCMR).

Activity 1

We collected microbial biomass from 110 high volume (> 500 liters) water samples from private residences (85 supplied by private wells; 25 supplied by public water systems) from which we extracted, purified, and preserved genetic material (RNA and DNA). We have also processed an additional 12 “blank” water samples that serve as negative controls for our analyses. These water samples were also analyzed by the conventional microbiological analyses; 24 of the private well samples tested positive for total coliforms, the standard microbiological assay for determining the microbiological quality of drinking water. We have also queried these samples for numerous bacterial, viral, and protozoan pathogens. We frequently detected Escherichia coli, Pseudomonas aeruginosa, and Legionella spp.; we occasionally detected human-specific Bacteroides spp., an indicator of fecal contamination. We did not detect either the SARS-CoV-2 virus or Cryptosporidium parvum. Our results, therefore, are substantially different from recent results generated by the Minnesota Department of Health in which Cryptosporidium parvum was frequently detected in wells used to supply public water systems. Our results, however, suggest that some private wells contain bacterial pathogens that could adversely affect the health of the water users.

(This activity marked as complete as of this status update)

Activity 2

We have collected 49 water samples from private wells to assess the quality of groundwater via the analysis of trace organic and inorganic compounds. These data were then used to help determine the source of pathogen pollution by correlation analyses. We used the molar ratio of chloride to bromide to indicate sewage contamination of groundwater; we identified 10 sites with potential sewage contamination, although this did not correlate well with the presence of fecal coliforms. Nitrate levels greater than 1 part per million were found at 7 sites, suggesting human influence on the groundwater. Furthermore, we found 9 sites with the molar ratio of sodium to potassium below 2, indicating pollution by agricultural runoff; and 10 sites had a molar ratio above 5, indicating pollution by sewage. Here again, these potentially impacted groundwater samples did not have high levels of fecal coliforms. These indicators overall did not correlate with pathogen data or each other, suggesting no systematic contamination of pathogens in the groundwater, but rather contamination is more likely incidental.

(This activity marked as complete as of this status update)

Activity 3

We installed point-of-use water filters from Aquamedix in 6 private homes (3 connected to public water supplies; 3 connected with private wells). This number is smaller than our intended goal (10 private residences) because we struggled to find willing participants given the intrusiveness and difficulties of installing the in-home filters (for example, several willing participants were disqualified because we were unable to install filters on their preexisting bathroom/kitchen faucets). We collected approximately 1-liter samples prior to, during, and after the installation of these filters. One of these residences exhibited low-level contamination with coliforms without filtration, but no detection of coliforms while filtration was on-going. None of these samples contained detectable levels of bacterial or protozoan pathogens with or without filtration, likely due to the relatively small sample volume, which led to higher limits of detection compared to the large-volume sample collection method that we used in Activity 1. Our results did confirm, nonetheless, that the filters reduced the total concentration of bacteria by at least 90%.
(This activity marked as complete as of this status update)

Dissemination

Our dissemination activities have focused on updating the private homeowners who volunteered to participate in our study (i.e., some of these private wells tested positive for coliform). We have also presented our results at a city council meeting that previously participated in a similar study performed by the Minnesota Department of Health; of particular interest, our results are substantially different because we have not detected Cryptosporidium parvum in any of our groundwater samples. We also presented our results to the Minnesota Section of the American Water Works Association in September 2023 (Duluth, MN). We anticipate authoring multiple research manuscripts (these are currently being written) in the next year that will be published in the peer-reviewed literature. We anticipate that the first papers should be submitted by June 2025; all publications will be attached to this report once they are in print. We also anticipate presenting our results at the annual meeting of the Minnesota Section of the American Water Works Association in September 2025. All future dissemination activities will be at no cost to LCCMR.

Status Update Reporting

Status Update January 1, 2024

Date Submitted: February 1, 2024

Date Approved: February 2, 2024

Overall Update

We have collected 105 high-volume (> 500 liters) water samples from private residences (90 private wells; 15 public water supplies). From these samples, we have obtained genetic material (RNA and DNA) for subsequent analysis (Activity 1). We have also collected 50 samples (all private wells) for chemical analyses (Activity 2) to identify sources of contamination. To date, we have not detected the SARS-CoV-2 virus or Cryptosporidium parvum in any sample. We have developed sensitive methods to detect 20 organic and inorganic compounds that could be indicators of contamination. We have also installed and collected point-of-use filters from Aquamedix at 6 private homes to evaluate the benefits of these filters on the microbiological quality of well water (Activity 3). Finally, we have presented preliminary results of our work at the 2023 meeting of the Minnesota section of the American Water Works Association. We have now completed all samples for this project and the remaining time will be spent of sample analysis, data interpretation, and dissemination of our research findings. This project will be completed by June 30, 2024, although the publication of peer-reviewed publications will have to occur after the project period is complete (at no cost to LCCMR).

Activity 1

To date, we have collected 105 high volume (> 500 liters) water samples from private residences (90 supplied by private wells; 15 supplied by public water systems) from which we extracted, purified, and preserved genetic material (RNA and DNA); we have also processed an additional 9 “blank” water samples that serve as negative controls for our analyses. These water samples were also analyzed by the conventional microbiological analyses; 33 of these samples tested positive for total coliforms although all samples were negative for Escherichia coli. The genetic analyses for these samples are in-progress. We have thus far queried these samples for the SARS-CoV-2 virus, all of which were negative. We have analyzed 85 samples for Cryptosporidium parvum, all of which showed very level background signals that were statistically similar to the results obtained from our filter “blanks.” From these results, we have no evidence to suggest the presence of Cryptosporidium parvum is common or significant in private wells, which is substantially different from recent results reported by investigators from the Minnesota Department of Health. We are presently performing the genetic analyses.

Activity 2

To date, we have collected around 50 water samples from private residences served by private wells for trace organic and inorganic analyses. The analyses have been and will be used to indicate whether the water has been impacted by three waste sources: human waste (e.g., septic tanks), animal waste (e.g., farm), and hospital waste. We have finished developing and validating analytical methods with sufficiently low detection limits (for groundwater) to analyze 20 organic pollutants in the water samples. We have begun to use the developed analytical method to concentrate and measure pollutants from groundwater samples. We have almost finished analyzing both inorganic ions for all the water samples. We have shipped our samples for tritium analysis. We anticipate finishing all pollutant analyses by the end of May 2024.

Activity 3

In Summer 2023, we installed point-of-use water filters from Aquamedix in 6 private homes (3 connected to public water supplies; 3 connected with private wells). This number is smaller than our intended goal (10 private residences) because we struggled to find willing participants given the intrusiveness and difficulties of installing the in-home filters (for example, several willing participants were disqualified because we were unable to install filters on their preexisting bathroom/kitchen faucets). The fewer number of participants should not affect the overall conclusions as there is

plenty of replication (i.e., 6 homes) to reach robust conclusions. We collected samples prior to, during, and subsequent to the installation of these filters. Preliminary results showed some contamination with coliforms without filtration, but no detection of coliforms while filtration was on-going. Water samples were also collected for DNA analysis, which will be performed in Winter/Spring 2024.

Dissemination

To date, our dissemination activities have focused on updating the private homeowners who volunteered to participate in our study (i.e., some of these private wells tested positive for coliform). We have also presented our results at a city council meeting that previously participated in a similar study performed by the Minnesota Department of Health; our results are substantially different because we have not detected Cryptosporidium parvum in any of our groundwater samples. We also presented our results to the Minnesota Section of the American Water Works Association in September 2023 (Duluth, MN). We anticipate authoring multiple research manuscripts in the next year that will be published in the peer-reviewed literature, and we also anticipate presenting our results at the annual meeting of the Minnesota Section of the American Water Works Association in September 2024.

Status Update Reporting

Status Update July 1, 2023

Date Submitted: June 14, 2023

Date Approved: June 15, 2023

Overall Update

We have made substantial progress on the first two of the proposed Activities thus far. We have collected 92 high-volume (> 500 liters) drinking water samples from private residences, nearly all supplied by private wells. From these samples, we have obtained genetic material (RNA and DNA) for subsequent analysis via quantitative polymerase chain reaction (qPCR). We have also collected 37 samples for chemical analyses. Both chemical and microbiological analyses of these samples is on-going. To date, we have not detected the SARS-CoV-2 virus in any drinking water sample, which suggests that this virus is not currently being transmitted via ground water. In addition, we have not detected Cryptosporidium parvum, a protozoan pathogen, which has been implicated by other researchers as being a common groundwater contaminant. We have been developing appropriate and sensitive methods to detect 20 organic and inorganic compounds that could be indicators of contamination. We are currently collecting additional samples for both Activities 1 and 2; in addition, we will partner with Aquamedix to perform in-home experiments to determine if water that is contaminated with harmful microbes can be improved with simple point-of-use treatment technologies.

Activity 1

To date, we have collected 92 high volume (> 500 liters) water samples from private residences from which we extracted, purified, and preserved genetic material (RNA and DNA); we have also processed an additional 9 “blank” water samples that serve as negative controls for our analyses. These water samples were also analyzed by the conventional microbiological analyses; 20 of these samples tested positive for total coliforms and 4 tests are currently pending (all samples were negative for Escherichia coli). The genetic analyses for these samples are in-progress. We have thus far queried these samples for the SARS-CoV-2 virus, all of which were negative. We have analyzed 85 samples for Cryptosporidium parvum, all of which showed very level background signals that were statistically similar to the results obtained from our filter “blanks.” From these results, we have no evidence to suggest the presence of Cryptosporidium parvum is common or significant in private wells, which is substantially different from recent results reported by investigators from the Minnesota Department of Health. We plan to continue collecting more samples during the Summer of 2023 and we anticipate finishing all genetic analyses by the end of December 2023.

Activity 2

To date, we have collected 37 water samples from private residences for trace organic and inorganic analyses. The analyses have been and will be used to indicate whether the water has been impacted by three waste sources: human waste (e.g., septic tanks), animal waste (e.g., farm), and hospital waste. We have finished the initial review of methods to analyze organic and inorganic pollutants, and we have begun to experiment methods of concentrating and measuring pollutants from groundwater samples. We plan to measure 9 inorganic ions and 6 organic compounds and may expand our scope further by including additional pollutants. We plan to continue collecting more samples during the Summer of 2023 and we anticipate finishing all pollutant analyses by the end of December 2023. We will also include tritium analyses that will indicate the age of groundwater in the new samples that we collect.

Activity 3

There has been minimal progress on Activity 3 so far, which is consistent with our original work plan. We have recently spoken with Aquamedix and we should be purchasing their in-home water treatment systems soon. We anticipate completing these experiments by mid-August 2023 with results available by December 2023.

Dissemination

To date, our dissemination activities have focused on updating the private homeowners who volunteered to participate in our study (i.e., some of these private wells tested positive for coliform). We have also presented our results at a city council meeting that previously participated in a similar study performed by the Minnesota Department of Health; our results are substantially different because we have not detected Cryptosporidium parvum in any of our groundwater samples. We will be presenting our results to the Minnesota Section of the American Water Works Association in September 2023 (Duluth, MN).

Status Update Reporting

Status Update January 1, 2023

Date Submitted: February 13, 2023

Date Approved: February 21, 2023

Overall Update

We have made substantial progress on the first two of the proposed Activities thus far. We have collected 85 high-volume (> 500 liters) drinking water samples from private residences, nearly all supplied by private wells. From these samples, we have obtained genetic material (RNA and DNA) for subsequent analysis via quantitative polymerase chain reaction (qPCR). We have also collected 29 samples for chemical analyses. Both chemical and microbiological analyses of these samples is on-going. To date, we have not detected the SARS-CoV-2 virus in any drinking water sample, which suggests that this virus is not currently being transmitted via ground water. In addition, we have not detected Cryptosporidium parvum, a protozoan pathogen, which has been implicated by other researchers as being a common groundwater contaminant. We have been developing appropriate and sensitive methods to detect 20 organic and inorganic compounds that could be indicators of contamination. We anticipate collecting and analyzing additional samples during the calendar year 2023 for both Activities 1 and 2; in addition, we will partner with Aquamedix to perform in-home experiments to determine if water that is contaminated with harmful microbes can be improved with simple point-of-use treatment technologies.

Activity 1

To date, we have collected 85 high volume (> 500 liters) water samples from private residences from which we extracted, purified, and preserved genetic material (RNA and DNA); we have also processed an additional 8 “blank” water samples that serve as negative controls for our analyses. These water samples were also analyzed by the conventional microbiological analyses; 20 of these samples tested positive for total coliforms (all samples were negative for Escherichia coli). The genetic analyses for these samples are in-progress. We have thus far queried 30 of these samples for the SARS-CoV-2 virus, all of which were negative. We have analyzed all 85 samples for Cryptosporidium parvum, all of which showed very level background signals that were statistically similar to the results obtained from our filter “blanks.” From these results, we have no evidence to suggest the presence of Cryptosporidium parvum is common or significant in private wells, which is substantially different from recent results reported by investigators from the Minnesota Department of Health. We plan to continue collecting more samples during the Summer of 2023 and we anticipate finishing all genetic analyses by the end of December 2023.

Activity 2

To date, we have collected 29 water samples from private residences for trace organic and inorganic analyses. The analyses have been and will be used to indicate whether the water has been impacted by three waste sources: human waste (e.g., septic tanks), animal waste (e.g., farm), and hospital waste. We have finished the initial review of methods to analyze organic and inorganic pollutants, and we have begun to experiment methods of concentrating and measuring pollutants from groundwater samples. We plan to measure 9 inorganic ions and 6 organic compounds and may expand our scope further by including additional pollutants. We plan to continue collecting more samples during the Summer of 2023 and we anticipate finishing all pollutant analyses by the end of December 2023. We will also include tritium analyses that will indicate the age of groundwater in the new samples that we collect.

Activity 3

There has been no progress on Activity 3 so far, which is consistent with our original work plan. We intend to perform experiments on the impact of point-of-use water treatment technologies in private residences during the Summer of 2023.

Dissemination

We have not publicly disseminated any research results to date. We have, however, updated the private homeowners who volunteered to participate in our study. We have also remained in communication with a public water system that has volunteered to participate in both our study and similar studies performed by the Minnesota Department of Health.

Status Update Reporting

Status Update July 1, 2022

Date Submitted: July 25, 2022

Date Approved: July 29, 2022

Overall Update
Our efforts to date have focused on identifying private homeowners willing to allow us to collect samples from the private wells (Activity 1). We have made substantial progress in this area. While we are continuing to collect samples for microbiological analysis, we are also now collecting water samples for performing chemical analyses to discern the source of any microbiological contamination (Activity 2).

Activity 1
Our efforts related to Activity 1 have focused on finding homeowners and public water supplies willing to provide samples for us to test. In 2021, we visited more than 45 locations to collect groundwater samples. In 2022, we have collected more than 24 samples as of June 30th. We have extracted and purified RNA and DNA from all of these samples and PCR-based detection of viruses, bacteria, and protozoa are on-going.

Activity 2
Per our original plan, work on Activity 2 did not begin until June 2022. At this point, therefore, minimal progress has been made on this Activity.

Activity 3
Per our original plan, work on Activity 3 did not begin until June 2022. At this point, therefore, minimal progress has been made on this Activity.

Dissemination
We have not made any efforts with project dissemination because we do not have results to share at this time.