Final Abstract

Final Report Approved on January 10, 2025

M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

Project Title: Monitoring Emerging Viruses in Minnesota's Urban Water Cycles

Project Manager: Sebastian Behrens

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Funding Source:

Fiscal Year:

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

Appropriation Amount: $416,000

Amount Spent: $393,075

Amount Remaining: $22,925

Sound bite of Project Outcomes and Results

We studied the longevity of viruses in wastewater and found that enveloped viruses were more rapidly degraded in wastewater than non-enveloped viruses. Degradation rates depended on virus sorption to biosolids. Sorption behavior varies as water chemistry changes during treatment. Some viruses might be released with water effluent to the environment.

Overall Project Outcome and Results

One critical role of wastewater treatment is to remove or neutralize infectious biological agents. Coliform bacteria and bacteriophages are commonly used as indicators for fecal contamination in the environment. However, the presence of coliform units may not reflect the distribution of infectious human enteric viruses in the environment. Some viruses are considered transmittable from wastewater and receiving surface waters (e.g. rotavirus) because they have low infectious doses and are sources of waterborne illness with outbreaks potentially occurring far from the source of contamination. The onset of the SARS-CoV-2 pandemic renewed interest in understanding the fate of human enteric viruses in our sanitary infrastructure.

This project was designed to characterize the fate of viral particles during aerobic activated sludge treatment in

wastewater treatment bioreactors. The molecular test developed, and the viral decay data generated enabled us to quantify viral decomposition and removal dynamics in the context of viral interactions with biosolids and other sorbent surfaces in complex biological matrixes.

Our results shed light on the different behavior and degradation rates of enveloped and non-enveloped viruses in activates sludge. We found that enveloped viruses were more rapidly degraded in wastewater, however they were present long enough to be of concern for wastewater treatment facilities, stormwater overflow events, and wastewater intrusion in drinking water. Outcomes from this project might become particularly relevant during potential future avian influenza or corona virus outbreaks in humans, because some strains of these viruses are excreted in feces. The project also shed light onto how viral particles are protected from degradation when they aggregate in bacterial biofilms and how this process impacts the removal of viruses during water and wastewater treatment. We found that viral particle surface attachment and aggregation is controlled by solution pH and therefore varies during different stages of wastewater treatment.

Project Results Use and Dissemination

The outcomes of this project are summarized in the PhD Thesis of an Environmental Engineering graduate student from the University of Minnesota. The thesis is available through University Libraries. Results will also be available soon as research article in a scientific journal on water treatment and human health.

Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

General Information

Date: January 13, 2025

ID Number: 2021-121

Staff Lead: Noah Fribley

Project Title: Monitoring Emerging Viruses in Minnesota's Urban Water Cycles

Project Budget: $416,000

Project Manager Information

Name: Sebastian Behrens

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Project Reporting

Final Report Approved: January 10, 2025

Reporting Status: Project Completed

Date of Last Action: January 10, 2025

Project Completion: June 30, 2024

Legal Information

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

Appropriation Language: $416,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to develop rapid testing, quantification, and human exposure risk assessment models for enveloped viruses such as coronaviruses in urban wastewater and drinking water treatment processes.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: This project will address the presence and fate of enveloped viruses (e.g. coronaviruses) and their survivability in aqueous environments with emphasis on wastewater and drinking water treatment processes.

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

Over the last month MPCA and Minnesota Department of Health have been receiving several questions related to the risk that COVID-19 poses to wastewater professionals. This project will develop new detection methods and quantitative risk assessment (QRA) models that MPCA and the Department of Health can use to better quantify future risks related to the role of the urban water cycle in the spread of enveloped viruses. Past research efforts have focused mainly on nonenveloped human enteric viruses such as human noroviruses. However, avian influenzas, SARS, MERS, and the ongoing COVID-19 pandemic, have been caused by enveloped viruses. These viruses have direct connection to wastewater and drinking water purification when they are excreted in feces or urine. Recent reports show that SARS-CoV-2 has been detected in stool samples of COVID-19 cases. Increasing circulation of viruses such as SARS-CoV-2 in a population will increase virus loads in to sewer systems of our cities. It is important to collect information about the occurrence and fate of enveloped viruses in sewage to understand if there is a risk to sewage workers, but also to determine if sewage surveillance could be used to monitor the circulation of enveloped viruses in our communities.

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.

SARS-CoV-2 (the virus that causes COVID-19), as well as any other disease-causing viruses in wastewater, are currently impossible to detect by wastewater professionals as they conduct their day-to-day work. The currently unknown role of the environment, specifically the urban water cycle, in the spread of enveloped viruses highlights the need for the development of rapid testing methods and risk assessment model proposed in this project. In order to effectively control outbreaks and pandemics of novel enveloped viruses in the future we need to understand what conditions influence their environmental persistence. In this project we will study the fate of enveloped viruses in the urban water cycle and identify locations of potential human exposure. In order to achieve this goal, we will develop new, cost-efficient, molecular screening methods that will allow the rapid detection and quantification of enveloped viruses in environmental water samples. The new testing methods will be useful to municipalities and state agencies, and health departments to estimate the risk of infection and illness when a population is exposed to enveloped viruses in the environment. Sewage surveillance can also serve as early warning of (re-)emergence of COVID-19 in the Twin Cities and local communities connected to central sewage treatment.

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?

The proposed project will have the following specific project outcomes:

(1) Development and optimization of methods for extracting, purifying, and quantifying enveloped and non-enveloped viruses and their nucleic acids from complex sample matrices such as wastewater, residual biosolids, and surface waters.

(2) Quantification of presence and survivability of enveloped and non-enveloped viruses during wastewater and drinking water treatment processes, as well as when they are released into the environment via wastewater effluent or land-applied biosolids.

(3) Quantitative risk assessment will be conducted to better characterize exposure and transmission pathways for enveloped viruses through the urban water cycle environment.

Project Location

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

Region(s): Metro

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

Statewide

When will the work impact occur?

During the Project

Activities and Milestones

Activity 1: Development of molecular assays for extracting, purifying, and quantifying enveloped viruses in complex aquatic samples.

Activity Budget: $157,187

Activity Description:
We will sample five of the nine wastewater treatment plants operated by Metropolitan Council Environmental Services in the Twin Cities metropolitan area and one wastewater treatment plant operated by Brainerd Public Utilities in Brainerd, Minnesota. Detecting and quantifying viruses in environmental samples requires concentrating the virus in the samples into a smaller volume to improve detection limits. Standard concentration methods will be carefully evaluated with respect to their efficiency for enveloped viruses because their outer lipid layer makes them more sensitive to temperature, pH, and organic solvents. We will evaluate if flow cytometry can be used for viral particle enrichment from wastewater and surface waters. In this activity we will also develop assays for the reliable and fast quantification of enveloped coronaviruses and SARS-CoV-2 using reverse transcription quantitative PCR. Strict process and quality control protocols for RNA extraction, recovery, and quantification using appropriate virus surrogates will be performed to ensure assay reliability and to quantify the efficiency of virus detection in water and wastewater samples. The process controls will include inoculating viral surrogates into a wastewater samples before virus concentration, adding viral surrogate concentrate before RNA extraction, and adding known amounts of a viral RNA/cDNA standard before RT-qPCR.

Activity Milestones:

Description	Approximate Completion Date
Comparative metagenomic sequence analysis of virus populations in collected water and wastewater samples	June 30, 2022
Quality control and quality assurance of extraction and detection methods using appropriate virus surrogates	June 30, 2022
Development of RT-PCR methods for the detection of enveloped viruses in environmental samples	July 31, 2023

Activity 2: Quantification of presence and survivability of enveloped viruses during wastewater treatment processes.

Activity Budget: $162,174

Activity Description:
In this part of the project we will use the new tests developed in Activity 1 to answer specific questions regarding the fate of viruses in different compartments of the urban water cycle. The study will characterize how different stages of wastewater treatment will impact virus particle partitioning between solids, liquid, and air and how engineering design of water treatment plants will impact viral persistence in wastewater, in wastewater effluent, biosolids, and aerosols. We plan to analyze the partitioning of viral particles in sludge settling experiments quantifying viral particles in the supernatant vs. the settle sludge. Non-Infectious, intact viral particles of SARS-Cov-2 will be used in sorption experiments with wastewater to determine their partitioning between solids and liquid phases. Modeling of viral decay and wastewater flow rates through the samples urban sewage systems will be performed to accurately relate viral concentration at the point of sampling to the presence of virus within the catchment population. Positive RT-qPCR signals for SARS-CoV-2 RNA in wastewater will be confirmed by sequencing analysis to validate our assay specificity for the tested environmental samples. Sequence analysis will be performed using the Gopher-Pipeline of the University of Minnesota Supercomputing Institute.

Activity Milestones:

Description	Approximate Completion Date
Quantification of enveloped viruses in raw sewage, wastewater effluent, biosolids, and surface waters	July 31, 2023
Quantification of virus particle partitioning between solids, liquid, and aerosols during different water treatment processes	December 31, 2023
Comparison of different water treatment plant designs (continuous flow, sequencing batch reactors, anaerobic digestion	December 31, 2023

Activity 3: Quantitative risk assessment to characterize exposure and transmission pathways for enveloped viruses in the urban water cycle environment.

Activity Budget: $96,639

Activity Description:

In this part of the project we will characterize risks associated with occupational exposures to wastewater during operation and maintenance of sewage treatment infrastructure. Results from this project will inform QRA models to understand how much an impact viruses in the environment will have on the health of wastewater professionals. The QRA framework will consist of four steps, including hazard identification, dose−response, exposure assessment, and risk characterization. The quantified virus gene copy numbers (Activity 2) will be analyzed to quantify their statistical signatures. The estimated risk will be evaluated using available EPA health benchmarks for microbial pathogen exposure scenarios. Monte Carlo simulations will provide a range of uncertainty in infection risks to human health from exposure to wastewater and other virus containing environmental water samples. We plan to set up membrane air filters at the Brainerd wastewater treatment plant to quantify the concentration of enveloped coronaviruses and SARS-CoV-2 in different indoor facilities of the plant. Filter sampling is an easy-to-use method, relatively inexpensive, and the samples are suitable for many types of downstream analyses such as nucleic acids-based analysis. Samples will be taken over long periods of time to increase total aerosol mass collected.

Activity Milestones:

Description	Approximate Completion Date
Statistical data analysis of virus concentrations in aqueous samples	December 31, 2023
Characterization of occupational and public exposure scenarios	June 30, 2024
Quantitative risk assessment and development of guidelines to manage virus exposure risks through wastewater	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Leisa Thompson & George Sprouse	Metropolitan Council, Environmental Services, Wastewater & Water	Facilitation and support of sample collection efforts. Data discussion and communication with policy-makers, planning agencies, and water service providers in the Twin Cities metropolitan region. Please also see attached letter of support from MCES.	No
Prof. Dr. Timothy LaPara	University of Minnesota	Dr. LaPara is a full professor in the Department of Civil, Environmental, and Geo-Engineering. His area of expertise are environmental engineering and environmental microbiology. Dr. LaPara will serve as co-PI on the proposed project. He will co-advise the graduate student and postdoc, and support data analysis and discussion.	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 target audience for research results from this project are professionals in all areas of water treatment including employees of public water works and distribution system. The quantitative test to detect enveloped viruses in environmental water samples that will be developed in this project is critical to ensure the health and safety of workers servicing sewage system and employees at wastewater and drinking water treatment plants.
We will work closely with Leisa Thompson and George Sprouse from the Metropolitan Council Environmental Services (MCES, Department of Wastewater and Water, see letter of support), to ensure knowledge transfer and communication with policy-makers, planning agencies, and water service providers in the Twin Cities metropolitan region. Results will be shared with state agencies and health departments as soon as they become available to ensure instant public access to new testing methods and recorded water quality data. Upon completion of the project results will be disseminated through scholarly publications in peer-reviewed scientific journals. Highlights and project updates will be published on the Behrens lab and University websites. Talks and poster presentations will be given at local water conferences. The Environment and Natural Resources Trust Fund will be acknowledged 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 research questions addressed in this project are critical to prepare environmental engineers and public health workers in Minnesota in the event that enveloped viruses causing a deadly outbreak or pandemic enter the urban water cycle. Results will be published and shared with state agencies and health departments as soon as they become available to ensure instant public access to new testing methods and recorded water contamination data. Upon completion of the project publications and outcomes will be summarized in a final report downloadable from our University website. Talk and poster presentations will be given at local conferences.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded

Wastewater Treatment Process Improvements	M.L. 2016, Chp. 186, Sec. 2, Subd. 04k	$398,000
Engineered Biofilter for Sulfate and Metal Removal from Mine Waters	M.L. 2016, Chp. 186, Sec. 2, Subd. 04p	$440,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount	$ Amount Spent	$ Amount Remaining
Personnel										
Post Doctoral Researcher		TBD			20.25%	2		$123,257	-	-
1 Graduate Student		tbd			43.61%	1.5		$152,155	-	-
Faculty		CO-PI			26.7%	0.09		$14,000	-	-
Faculty		PI			26.74%	0.33		$54,588	-	-
							Sub Total	**$344,000**	**$344,000**	**-**
Contracts and Services										
							Sub Total	**-**	**-**	**-**
Equipment, Tools, and Supplies										
	Tools and Supplies	Consumable and supplies: $18K for years 1 & 2 and $15K for year 3. Cost for consumable and supplies include $ 3K for flow cytometry supplies (dyes, standard beads, buffer, filters); $ 5K per year for water chemistry analysis (nutrients, DOC, ions, metals); $11K for each year for general lab supplies and reagents for DNA/RNA extraction, cDNA synthesis, primer and quantitative PCR. Lab services: $ 5K for each year to run the flow sorter and use of the UMGC DNA sequencing services.	for DNA/RNA extraction, cDNA synthesis, chemical and reagents for flow cytometry and quantitative PCR, water chemistry analysis, lab services to use UMGC DNA sequencing services, lab services to run the flow sorter					$66,000	$49,075	$16,925
							Sub Total	**$66,000**	**$49,075**	**$16,925**
Capital Expenditures										
							Sub Total	**-**	**-**	**-**

Acquisitions and Stewardship										
							Sub Total	**-**	**-**	**-**
Travel In Minnesota										
	Miles/ Meals/ Lodging	Mileage in Minnesota	sample collection from wastewater treatment plants and drinking water facilities around the state					$2,000	-	$2,000
	Conference Registration Miles/ Meals/ Lodging	meals and lodging for students	meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association).	X				$1,000	-	$1,000
							Sub Total	**$3,000**	**-**	**$3,000**
Travel Outside Minnesota										
							Sub Total	**-**	**-**	**-**
Printing and Publication										
	Publication	Sponsored Publications $1500 per year based on current rates	Publication costs for research results on this project					$3,000	-	$3,000
							Sub Total	**$3,000**	**-**	**$3,000**
Other Expenses										
							Sub Total	**-**	**-**	**-**
							Grand Total	**$416,000**	**$393,075**	**$22,925**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel In Minnesota	Conference Registration Miles/Meals/Lodging	meals and lodging for students	meals and lodging to allow students to participate in local conferences and meetings to present research on this grant. Travel would be to formally present of project findings(e.g. Minnesota Wastewater Operators Association or the Minnesota Section of the American Water Works Association).

Non ENRTF Funds

Category	Specific Source	Use	Status	$ Amount	$ Amount Spent	$ Amount Remaining
State						
			State Sub Total	**-**	**-**	**-**
Non-State						
In-Kind	Indirect costs for this proposal, though not allowed, are listed as in-kind contribution of 55% MTDC which is the Federally Negotiated rate with the U of MN. The indirect is proportionate to the awarded funds at a rate 55% so if the award is reduced the F&A would be reduced.	To pay for administrative and facility expenses for this project	Secured	$201,696	$201,696	-
			Non State Sub Total	**$201,696**	**$201,696**	**-**
			Funds Total	**$201,696**	**$201,696**	**-**

Attachments

Required Attachments

Visual Component

File: 2c30b554-09f.pdf

Alternate Text for Visual Component

The fate of infective viruses in the urban water cycle and locations of potential human exposure. Viruses that are excreted in feces, urine, and vomit enter the sewage system. Toilet flushing or problems with indoor plumbing systems may form virus-laden aerosols that could result in human exposure. Viruses are transported through the municipal sewage system to the wastewater treatment plant (WWTP). Workers servicing sewage systems could be exposed to infective viruses. Combined sewage overflo...

Supplemental Attachments

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

Title	File
Letter of support MCES	3d489c91-23e.pdf
Research_Addendum_2021-121_Behrens_approved	7a3532b9-198.docx
Background Check Certification Form ENRTF 2021	55838697-619.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Revisions 07/072021: Proposal title corrected as suggested. Background check certification form uploaded. No further changes made.

Revisions 03/04/2021: Thank you for pointing out that there was a word missing in a sentence in the description of Activity 3. The missing word has been added. The sentence now reads: "Results from this project will inform QRA models to understand how much an impact viruses in the environment will have on the health of wastewater professionals."

Revision 02/15/2021: Approved research addendum has been uploaded and is now attached to this work plan.

Revisions 02/13/2021: Work plan activity descriptions have been updated to reflect approved research addendum revisions following peer review. Activities 1-3 have been reordered to match the approved timeline of the project.

Revisions 01/30/2021: A statement has been added to the Dissemination section about how the Environment and Natural Resources Trust Fund will be acknowledged on any publications resulting from this project.

The main changes between the work plan and the initial proposal are pertaining to the project budget. The LCCMR recommended funding for this project is $416,000, while the initial proposal request for funding was for $489,000. In order to lower the overall project cost by $73,000 the following adjustments have been made to the budget:

1) 3rd-year funding for the postdoctoral research fellow has been cut
2) Co-PI summer salary has been cut in half
3) Expenses for consumable and supplies has been slightly increased in order to match the recommended total funding amount of $416,000

As consequence of the personnel budget reduction we will have to limit the number of sampling locations and overall

number of samples that can be processes during the funding period. We will therefore have to focus our work on water treatment facilities in the metro region. However, project outcomes and impact of the proposed research from this project will continue to be applicable statewide.
Dissemination efforts and publication of the project outcomes are now described in detailed in a dissemination plan.

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?

N/A

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

No Amendments Entered

Status Update Reporting

Final Status Update August 14, 2024

Date Submitted: January 5, 2025

Date Approved: January 10, 2025

Overall Update

Our results shed light on the different behavior and degradation rates of enveloped and non-enveloped viruses in wastewater and provide guidance on how to efficiently recover viral genomes (single stranded RNA, double stranded RNA, and double stranded DNA) from wastewater and biosolids. We found that enveloped viruses were more rapidly degraded in wastewater, however they were present long enough to be of concern for wastewater treatment facilities, stormwater overflow events, and wastewater intrusion in drinking water. Outcomes from this project might become particularly relevant during potential future avian influenza or corona virus outbreaks in humans, because some strains of these viruses are excreted in feces. The project also shed light onto how viral particles are protected from degradation when they aggregate iwith suspended solids in biological treatment systems and how these physiochemical particle interactions impacts the removal of viruses during wastewater treatment. We found that viral particle surface attachment and aggregation is controlled by solution pH which can vary strongly during different stages of wastewater treatment.

Activity 1

This project was designed to characterize the fate of viral particles during aerobic activated sludge treatment in wastewater treatment bioreactors. The molecular test developed, and the viral decay data generated helped to characterize viral decomposition and removal dynamics in the context of viral interactions with biosolids and other sorbent surfaces in complex biological systems such as wastewater.

We developed new molecular detection assays for two enveloped and two non-enveloped viruses in wastewater and biosolids. Including different strains of respiratory and gastroenteric viruses allowed us to compare how different viral particle surface properties and genome characteristics affect their biodegradation during wastewater treatment. Virus groups included in the study were adenovirus, respiratory coronavirus, respiratory syncytial virus, and rotavirus.

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

Activity 2

The decomposition rates of the four virus strains were assessed by performing batch experiments and sampling sequencing batch reactors

containing activated sludge. These experiments provided data to calculate decay rates of the different virus particles. We found that the stability of viruses in wastewater is largely influenced by electrostatic interactions between viruses and charged surfaces, where the interaction of viral particles with biosolids, metal/mineral complexes, and sorbents depends on the pH, ionic strength, and the polarities and charges of the virus and solid surface. Efficiency of viral decomposition and retention during the activated sludge process is therefore different for enveloped and non-enveloped virus strains and will change with wastewater composition as the water moves through the plant.

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

Activity 3

The viral removal data was assessed in terms of decomposition rates (determined in preliminary viral decomposition batch experiments) and hydraulic retention in reactors modeled as continuous flow mixed reactors. The virus decay rates were analyzed using mass balance equations for a reaction occurring in a continuous flow mixed reactor. The impact of virus partitioning to solids and aqueous fractions of activated sludge was analyzed assuming steady state conditions (achieved in viral decomposition batch experiments and virus-dosed reactor experiments) compared to maximum sorption capacities of sludge biosolids as determined in sodium azide ('killed' no biological activity) virus

sorption experiments.
Future work should examine additional enveloped viruses to elucidate how specific virus characteristics contribute to their aggregation and sorption behavior in in activated sludge in response to pH and also seasonal temperature changes during water treatment. There is also a need for more viral concentration data across all respiratory virus and excretion types. Such data would allow to more comprehensively assess and quantitatively linking virus wastewater concentrations to human exposure risks during water treatment.
(This activity marked as complete as of this status update)

Dissemination
Data from Activity 1 and 2 have been analyzed and a manuscript for publication was prepared. The manuscript has been submitted to a scientific journal for publication and is currently under review. Funding support from this project has been acknowledged in the respective section of the manuscript as per author guidelines of the journal. Once the review process is complete and the manuscript is accepted for publication the citation can be added to this final report or the final project abstract.
We are currently still analyzing sets of data collected as part of Activity 2 and 3. We anticipate a second manuscript to be published from this part of the project before the end of the year.

Status Update Reporting

Status Update December 1, 2023

Date Submitted: December 4, 2023

Date Approved: January 31, 2024

Overall Update
We completed our work to develop sensitive molecular detection and quantification assays for potentially infectious gastrointestinal and respiratory viruses in environmental water samples and activated sludge. Standard curves and detection limits of all viral gene quantification assays have been determined. The first set of reactor data has been analyzed and the results are currently prepared for publication.

Activity 1
We developed new molecular detection and quantification assays for a selected set of enteric viruses with different biophysical virus structures. Assays for the following enteric viruses have been developed: bovine coronavirus (BOV), bovine respiratory syncytial virus (BRSV), canine adenovirus (CADV), and porcine rotavirus (PRTV). This will allow us to normalize SARS-CoV-2 concentrations and build a reference database for viral degradation rates in environmental water samples and sewage. This work completes Activity 1.
(This activity marked as complete as of this status update)

Activity 2
Using the molecular viral detection and quantification assays developed in Activity 1 we quantified viral gene copy numbers by sampling bioreactors that were seeded with activated sludge from the wastewater treatment plant in Brainerd, MN. The reactors received viral surrogates and we collect wastewater and biosolids for viral gene quantification from various steps along the water treatment process (influent “raw” sewage, activated sludge, and effluent water). We expect to complete these experiments in the last 6 month of the project. The data will allow us to quantify specific viral decay rates for various enteric viruses and compare them to plant operational parameters.

Activity 3
We optimized a modified sample preparation protocol based on propidium monoazide (PMA) pre-treatment of wastewater and biosolids which allows us to distinguish free viral DNA from intact viral particles. The new protocol will be used in conjunction with the bioreactor sampling experiments in Activity 2 and permit to differentiate between viral particle degradation rates (and the decline in infectious load) and the presumably slower decomposition of free viral DNA (with no associated infection risk).

Dissemination
We began analyzing and summarizing data from Activity 1 for publication in a scientific journal. Methods and results sections have been written und the first draft of the discussion is currently prepared. We anticipate a second publication from the project once data collection for Activity 2 has been completed. End of June an initial set of data was presented on a poster at a conference of the Association of Environmental Engineering and Science Professors (AEESP) at Northeastern University in Boston.

Status Update Reporting

Status Update June 1, 2023

Date Submitted: May 31, 2023

Date Approved: June 2, 2023

Overall Update

We continued our efforts to develop sensitive molecular detection and quantification assays for potentially infectious gastrointestinal and respiratory viruses in environmental water samples and activated sludge. Progress was delayed because we received the wrong synthetic gene fragments for our standard curves. Only after extensive lab testing and communication with the company that synthesized the standard DNA fragments for us, were we able to identify the problem and solve the issues. Since then, we received new gene fragments for our standard dilution series and continued our work to determine detection range and limits of all viral gene quantification assays in the environmental water samples.

Activity 1

We developed new molecular detection and quantification assays for a selected set of enteric viruses with different biophysical virus structures. Because of our labs biosafety level 2 restriction and the need for continued dosing of viruses to our lab bench sequencing batch reactors at detectable concentrations for assay development, we required high concentration viral particle stock solutions of non-infectious viruses. We therefore focused on enteric viruses for which modified-live virus vaccines for animals are commercially available. Selected viruses for study now include bovine coronavirus (BOV), bovine respiratory syncytial virus (BRSV), canine adenovirus (CADV), and porcine rotavirus (PRTV) which cover the full spectrum of enveloped/non-enveloped, single-strand RNA/DNA, and double-strand DNA viruses found in wastewater. This will allow us to normalize SARS-CoV-2 concentrations and build a reference database for viral degradation rates in environmental water samples and sewage. This work is part of milestone three of Activity 1 and will be completed together with the reactor sampling and quantification work of Activity 2 in the second half of 2023.

Activity 2

Since viral particles adsorb to particulate matter in environmental waters (especially wastewater) their extraction and reliable quantification is often impaired by matrix effects. However, previous studies have reported detection of viral genes in the range of 10-100 gene copies per reaction, which often rely on several liters of filtered water. Considering the detection limits based on current methods, we decided to add an additional pre-processing step to concentrate virus particles from sludge samples prior to DNA/RNA extraction. Methods we are currently evaluating include the use of Nanotrap Magnetic virus particles and the sequential treatment of samples with sodium pyrophosphate and polyethylene glycol to elute viruses from sludge and precipitate viruses into a smaller volume. Using the new viral detection and quantification assays developed in Activity 1 we continued our work of sampling our bioreactors that were seeded with activated sludge from the wastewater treatment plant in Brainerd, MN. The reactors previously received viral surrogates (Activity 1) and we collect samples for viral gene quantification in different types of samples (influent “raw” sewage, activated sludge, and effluent water). These ongoing experiments will allow to determine viral decay rates for the selected groups of enteric viruses in correlation to plant parameters.

Activity 3

One of the limitations of wastewater-based epidemiology is that the detection of viral gene copies in any environmental matrix (liquid, solids, air) is not directly correlated to the number of infectious viral particles. Free viral DNA (and to some extend RNA) from damaged viral particles may still be present in activated sludge even after a virus is structurally compromised and no longer virulent. We therefore are evaluating a new sample preparation method based on Propidium monoazide (PMA) pre-treatment. PMA is a dye that intercalates with and covalently crosslinks with nucleic acids when exposed to strong visible light, preventing amplification of bound DNA/RNA. PMA only permeates damaged

membrane barriers and will thus only bind genetic material from structurally compromised viral particles and genetic material floating freely in solution. By extracting DNA and RNA from each sample both with and without pre-incubation with PMA we will be able to distinguish between free viral nucleic acids and intact, virulent particles using the same, quick, and sensitive molecular quantification assays developed in Activity 1.

Dissemination

The molecular assay development under Activity 1 and the viral decay rates quantified under Activity 2 will be summarized for publication in a scientific journal once data collection and analysis are complete. The graduate student working on the project will present the first set of reactor data the Association of Environmental Engineering and Science Professors (AEESP) Research & Education Conference from June 20-23, 2023, at Northeastern University in Boston.

Status Update Reporting

Status Update December 1, 2022

Date Submitted: December 6, 2022

Date Approved: December 14, 2022

Overall Update
In the last half year, we continued our efforts to develop and optimize our protocols for extracting, purifying, and quantifying enveloped viruses from environmental water samples including activated sludge. While initial efforts were focus on the detection and quantification of SARS-CoV-2 we now broadened our spectrum of analytical techniques to other viruses often detected in the urban water cycle and surface water environments receiving the effluents from animal feeding operations and wastewater treatment.

Activity 1
Viruses are highly diluted in wastewater, and validated methods for their reliable quantification, and suitable reference viruses, are the main needs to be established before wastewater-based epidemiology can reliably be established as an early warning system for the pandemic spread of viral diseases. Strict process and quality control protocols for RNA extraction, recovery, and quantification using appropriate virus surrogates need to be developed to ensure assay reliability and to quantify the efficiency of virus detection in water and wastewater samples.
Over the last 6 months we identified viruses that can serve as external and internal process controls. We hope that quantifying these viruses alongside SARS-CoV-2 will enable us to normalize SARS-CoV-2 concentrations, so that, the amount of human waste found in the wastewater can be taken into consideration. We are in the process of developing molecular assay for the detection of two external (bovine corona virus and bovine respiratory syncytial virus) and two internal (human adenovirus and human rotavirus) reference viruses that will allow to normalize SARS-CoV-2 concentrations and build a reference database for viral degradation rates in sewage. This work is part of milestone three of Activity 1 and will continue into the first half of 2023.

Activity 2
All viruses are susceptible to natural degradation determined by factors such as temperature, water chemistry, exposure to UV light, and the microbial community. The kinetic decay rate of a virus dependents on the characteristics of the individual virus but also on the environmental conditions within a sewage system, which varies from location to location. Viral particles adsorb to particulate matter in wastewater which affects their environmental persistence and degradation. To overcome limitations imposed by spatial and temporal variations in environmental conditions and viral concentrations on our ability to reliably quantify and model viral decay rates we setup six lab-bench sequencing batch reactors. The reactors were seeded with activated sludge from the wastewater treatment plant in Brainerd, MN and operated under controlled conditions that resemble full-scale plant operational practices. The reactor will be seeded with viral surrogates (Activity 1) and we will quantify viral gene concentrations in different types of samples (influent “raw” sewage, activated sludge, and effluent water). The experiment will enable us to quantify viral particle partitioning between solid and liquid phases and allow for the reliable quantification of viral decay rates in correlation to plant operational parameters.

Activity 3
The data we are collecting as part of the experiments performed under Activity 2 will provide the basis to better understand viral partitioning between solids and liquid phase and as well as the conditions that drive viral degradation rates and persistence in various environmental compartments. The data will inform the statistical analysis and exposure risk assessment of this Activity which will begin as we generate more data as part of the experiments performed under Activity 2.

Dissemination

The molecular assay development under Activity 1 and the viral decay rates quantified under Activity 2 will be summarized for publication in a scientific journal once data collection and analysis are complete. We plan to share the published results with state agencies and the Department of Health to inform them about the new viral quantification methods and their application to monitor viral biomarkers in environmental water samples.

Status Update Reporting

Status Update June 1, 2022

Date Submitted: May 30, 2022

Date Approved: June 2, 2022

Overall Update

Our focus in the first year of this project was on Activity 1. We developed and optimized quantitative polymerase chain reaction assays to detect in quantify the RNA genomes of enveloped viruses in environmental water samples. We advanced the sensitivity of the assays and were able to lower the detection limit compared to current standard assays used by other labs in the state. This will allow us to quantify the fate of enveloped virus (including the virus that causes COVID-19) in a variety of environmental water samples (e.g. raw wastewater) with high precision and accuracy even at concentrations of only 1-2 viral gene copies per liter.

Activity 1

We completed the first two milestones of Activity 1 in the first year of this project. We compared different viral target genes and optimized primer selection based on selectivity and specificity for SARS-CoV-2 viral nucleocapsid genes. The tested different viral lysis and RNA extraction protocols on environmental water samples and optimized assay conditions for the reliable detection and quantification of viral RNA by quantitative PCR after reverse transcription of the RNA into cDNA (copy DNA). Current standard methods collect composite water samples over 24 hours and extract RNA from 100 mL to 1 Liter of samples to be able to detect viral RNA genes copies. Using a magnetic bead enrichment protocol and careful assay optimization we are now able to extract RNA from just 5 mL of water (e.g. wastewater) without the need of composite sample collection for 24 hour. The is an improvement over current standard methods as it will allow for faster sampling and quantification of viral RNA in lower samples volumes. We will continue to test our new assay by quantifying viral decay rates in lab-bench sequencing batch reactor before we use the new assays to quantify viral load in environmental water samples (Activity 2).

Activity 2

We started working on Activity 2 of the project by testing the new assay developed in Activity 1 on different types of environmental samples (raw sewage, activated sludge, effluent) that we collected from wastewater treatment plants across the state (Metropolitan, Brainerd, Mankato, Blue Lake). This part of the project will be our main focus in the second year of the project.

Activity 3

We will need to collect and analyze more samples (as part of Activity 2) to be able to better understand environmental distribution and occurrences in various environmental compartments as part of an exposure risk analysis. This part of the project will start in year 2 and continue into year 3.

Dissemination

Once the method development of Activity 1 is complete we plan on publishing the result of the new assay in a scientific journal. We will then share the published results with state agencies and the Department of Health to inform them about the new viral testing method and the collected water quality data.