

# Final Abstract

Final Report Approved on November 8, 2024

## M.L. 2020 Project Abstract

For the Period Ending June 30, 2024

**Project Title:** CWD Prion Research in Soils

**Project Manager:** Tiffany Wolf

**Affiliation:** U of MN - College of Veterinary Medicine

**Mailing Address:** 1988 Fitch Ave 495 AnSci/Vet Med

**City/State/Zip:** Saint Paul, MN 55108

**Phone:** (612) 625-0492

**E-mail:** wolfx305@umn.edu

**Website:** www.cvm.umn.edu

**Funding Source:**

**Fiscal Year:**

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 20a5

**Appropriation Amount:** \$336,000

**Amount Spent:** \$335,054

**Amount Remaining:** \$946

### Sound bite of Project Outcomes and Results

This project successfully initiated a groundbreaking line of environmental prion research at MNPRO, University of Minnesota, validating RT-QuIC for detecting chronic wasting disease prions in soils. Notably, it marked the first testing of prion contamination in Minnesota soils from Beltrami County, stemming from infected carcass materials.

### Overall Project Outcome and Results

Chronic wasting disease (CWD) is a contagious, 100% fatal neurological disease affecting deer, caused by prion proteins (a.k.a prions). Prions are resistant to degradation and can remain infectious in the environment for years. Following the identification of an area of Beltrami county public land where CWD-positive carcasses had been dumped, the contamination of the local environment by prions was a major concern. However, detection of prions in environmental samples, such as soils, is complex and few laboratories possess the capacity and expertise to analyze such samples. Therefore, a primary goal of this project was to establish the Minnesota Center for Prion Research and Outreach (MNPRO) as a leader in this emerging area of research. Specific objectives were to optimize methods for prion detection in Minnesota soils (Activity 1), and to conduct a baseline survey of prion contamination at the Beltrami County carcass dumpsite (Activity 2). Early soil profiles conducted by MNPRO demonstrated compounds known to contribute both to

prion binding (e.g., clay minerals) and RT-QuIC interference (e.g., organic matter, such as humic acids). Consequently, a key focus was to optimize soil extraction and analytical protocols to minimize false positives in RT-QuIC. These efforts allowed us to conduct testing of soil samples collected in association with carcass materials at the dumpsite in 2021. Using RT-QuIC, we detected prion activity in 20% of the soils samples from within the dumpsite. No soil samples from outside the dumpsite perimeter tested positive. While we are confident that our research demonstrates prion contamination of dumpsite soil, we remain committed to validating our findings through alternative testing methodologies. As we refine our detection methods for environmental prion detection, we will integrate these advancements into our ongoing research to deepen our understanding of prion persistence and remediation in the environment.

### **Project Results Use and Dissemination**

Project activities and results have been shared with the Minnesota Department of Natural Resources, local landowners in Beltrami Co., and both scientific and public audiences. Two manuscripts detailing this work are currently in preparation for scientific review and publication. These efforts have laid a strong foundation for ongoing and long term research at the Beltrami Co. dumpsite, paving the way for new proposals expected to be submitted for funding opportunities in 2025.



## Environment and Natural Resources Trust Fund

M.L. 2020 Approved Final Report

### General Information

**Date:** November 18, 2024

**ID Number:** 2020-087

**Staff Lead:** Michael Varien

**Project Title:** CWD Prion Research in Soils

**Project Budget:** \$336,000

### Project Manager Information

**Name:** Tiffany Wolf

**Organization:** U of MN - College of Veterinary Medicine

**Office Telephone:** (612) 625-0492

**Email:** wolfx305@umn.edu

**Web Address:** www.cvm.umn.edu

### Project Reporting

**Final Report Approved:** November 8, 2024

**Reporting Status:** Project Completed

**Date of Last Action:** November 8, 2024

**Project Completion:** June 30, 2024

### Legal Information

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 20a5

**Appropriation Language:** The appropriation in Laws 2019, First Special Session chapter 4, article 2, section 2, subdivision 8, paragraph (c), Sauk River Dam Removal and Rock Rapids Replacement, in the amount of \$2,768,000, no longer needed for its original purpose is transferred as follows:

(5) \$336,000 is transferred to the Board of Regents of the University of Minnesota to study chronic wasting disease prions in soils, including the assessment of sites where carcasses with chronic wasting disease have been disposed.

(d) Transfers and Availability

The transfers under this subdivision are effective June 30, 2021, and the transferred amounts are available until June 30, 2023.

M.L. 2022, Chp. 94, Sec. 2, Subd. 19 Carryforward; Extensions, (b) The availability of the transfers for the following projects is extended to June 30, 2024: (5) Laws of 2021 First Special Session, chapter 6, article 5, section 2, subdivision 20, paragraph (a), clause (5), CWD Prion Research in Soils

**Appropriation End Date:** June 30, 2024

## Narrative

**Project Summary:** The goal of this project is to advance research related to the contamination, persistence, and risk of transmission related to CWD prions in soil.

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

Chronic Wasting Disease is a contagious, 100% fatal neurological disease affecting deer. On 28 April 2021 our team was notified by the MN Department of Natural Resources (DNR) that deer carcasses originating from a CWD-positive deer farm in Beltrami County had been dumped on public land. Following a request from the DNR, we secured many samples, including bones, hides, soil cores, and plants from the location. Our Minnesota Center for Prion Research and Outreach (MNPRO) laboratory has RT-QuIC testing functionality, a highly advanced and sensitive prion detection assay that can be used for forensics research and environmental samples. We have completed an initial RT-QuIC analysis of select bone marrow and nervous tissues collected from the site and have identified at least two carcasses that are CWD positive. These results indicate CWD prions are at the dump site and have the potential to be transmitted to wild deer in the region through contact with the contaminated environment. An outbreak of CWD in wild white-tailed deer herds in northern Minnesota would negatively impact all deer related activities in the region, especially culturally and traditionally important recreational and subsistence hunting.

**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.**

CWD prions are resistant to degradation and can remain infectious in the environment for years. Therefore, it is critical that we determine the extent of CWD prion contamination across the Beltrami Co. carcass dump site to inform remediation and exclusion processes. In light of the positive carcass results, our team proposes to conduct soil sampling research to optimize RT-QuIC for prion detection in soil under different conditions with direct application to the Beltrami County farm associated dump site. These efforts will help: 1) to inform the DNR as to the distribution of CWD-causing prions at the site; 2) with devising key mitigation strategies aimed at preventing CWD from spreading to wild white-tailed deer herds within the region; and 3) to understand the ecology of CWD

**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?**

Specific outcomes of this project include: 1) the optimization of methods for prion detection in soils, under different conditions and of different compositions, which is critical to advancing future environmental research around CWD prion persistence, degradation, and remediation in Minnesota; and 2) a baseline seasonal survey of prion contamination in the soils of the Beltrami Co. carcass dumpsite to characterize the extent of prion contamination.

## Project Location

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

County(s): Beltrami

**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: Research and development for the detection of CWD prions in soil

**Activity Budget:** \$168,000

**Activity Description:**

RT-QulC is a CWD screening test that is capable of detecting prions in live and dead animals (including carcass remains), as well as the environment - samples such as plants, soil, and water. Yet environmental detection of prions using RT-QulC technology is still early in its development. Given the complex matrices of soils, there is much to learn in regard to the optimization of RT-QulC detection across soils with different mineral and organic content. In this project the MNPRO team will partner with the Pedersen Lab of University of Wisconsin - Madison, a leading laboratory in the study of prion binding, persistence, and transmission in soils, to study prion binding, detection, and transmission in a variety of different soil types and under different environmental conditions. This work will enhance our understanding of the persistence and transmission of CWD in the soil environment.

**Activity Milestones:**

Description	Approximate Completion Date
Development and optimization for CWD prion detection in soil by RT-QulC in the MNPRO	January 31, 2022
Experimental study of CWD prion binding and detection across different soil types and compositions.	December 31, 2022
Development and submission of scientific manuscript summarizing project findings.	June 30, 2023

### Activity 2: Characterize prions in soil at the Beltrami County farm dump site.

**Activity Budget:** \$168,000

**Activity Description:**

CWD prions can remain infectious in the environment for years, binding to soil. Given that at least one CWD-positive was determined to have been dumped there, the Beltrami Co. dump site represents a clear risk as a possible source for CWD transmission from the environment into wild deer, even after the removal of carcass material. During our initial investigation of the Beltrami Farm dump site, our team secured soil samples from below carcasses and will continue sampling soil in and around these point locations of high risk seasonally over the next year. These samples incorporate both upland and lowland soils, which are important soil types relevant to northern MN. Experiments in Activity 1, will be informed by the soil composition of the Beltrami Co. dump site, such that following closely on the success of Activity 1, we will begin screening the Beltrami soil samples. The results of the soil screening from around the dump site will be added to an overall assessment of ecological risk, informing DNR efforts for ongoing environmental and biosurveillance as well as future remediation.

**Activity Milestones:**

Description	Approximate Completion Date
Characterize soil composition of the Beltrami Co. farm dump site to prioritize additional prion sampling	January 31, 2022
Screen soil samples from the Beltrami Co. farm dump site for CWD prions using RT-QulC.	December 31, 2022
Prepare a risk assessment report of soil contamination of Beltrami dump site and future recommendations.	June 30, 2023

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Joel Pederson	Johns Hopkins University	Collaborator	No
Stuart Lichtenberg	University of Wisconsin	Collaborator	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 environmental dimension of CWD transmission is a critical area for CWD research. This unique project, combining forensics and environmental assessments, is a first of its kind. The methods and approach taken will be of broad interest to those tasked with characterizing the potential risks associated with CWD-positive carcasses left on the landscape, and the findings critical to the work of our own state and tribal agencies in protecting our wild deer herds and the surrounding ecosystem. Findings will be shared directly with state and tribal agencies (MN DNR, MN Board of Animal Health, MN Pollution Control Agency, Leech Lake Band of Ojibwe, Red Lake Band of Chippewa, White Earth Nation) through project reports. We will also use the following opportunities to share methods and findings more broadly:

- 1) dissemination via the MNPRO website: <https://mnpro.umn.edu/>
- 2) presentation at local, regional, and national scientific, management, and public/stakeholder meetings
- 3) publication of findings in peer-reviewed scientific (e.g. Science of the Total Environment) and professional journals (e.g. The Wildlife Professional)
- 4) dissemination to the media via press releases and UMN Research Briefs
- 5) testimonials to LCCMR and other policy platforms

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) 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 ENRTF Acknowledgement 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 environmental dimension of CWD transmission is a critical area for CWD research. This unique project, combining forensics and environmental assessments, is a first of its kind. The methods and approach taken will be of broad interest and lay a strong foundation for future hypothesis-driven research related to prion contamination, persistence, degradation and remediation. The Beltrami Co. dump site itself, depending on results produced from this project, has the potential to become a long-term environmental prion research demonstration site. Thus, the results from this project will be leveraged for funding from federal agencies, including USDA, USFWS, and NSF.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>Personnel</b>										
Assistant Professor		Two assistant professors will serve in the role of Primary Investigator (Project manager) and Co-PI to manage the project and guide research.			33.5%	0.34		\$59,304	-	-
MNPRO Researchers		Protein scientist will perform all lab analyses of soil for prion detection.			33.5%	2.24		\$205,408	-	-
Student researchers		Student researchers will facilitate data collection and analysis			0%	0.26		\$8,400	-	-
							<b>Sub Total</b>	<b>\$273,112</b>	<b>\$273,112</b>	<b>-</b>
<b>Contracts and Services</b>										
Stuart Lichtenberg, University of WI	Sub award	Collaborative sample testing, and assistance in R&D related to application of RT-QuIC to soil samples				0.22		\$2,878	\$2,878	-
							<b>Sub Total</b>	<b>\$2,878</b>	<b>\$2,878</b>	<b>-</b>
<b>Equipment, Tools, and Supplies</b>										
	Tools and Supplies	Soil sampling and analysis supplies	Expenses include consumables and other supplies needed for transect surveys, soil sampling, and laboratory testing.					\$33,562	\$33,562	-
	Tools and Supplies	Recombinant protein production	Expenses related to the production of protein substrate needed for RT-QuIC reactions.					\$16,500	\$15,554	\$946
	Equipment	Computing equipment	A PC laptop computer is needed to perform several analyses requiring Windows-based programs.	X				\$1,345	\$1,345	-
							<b>Sub Total</b>	<b>\$51,407</b>	<b>\$50,461</b>	<b>\$946</b>



<b>Capital Expenditures</b>										
							<b>Sub Total</b>	-	-	-
<b>Acquisitions and Stewardship</b>										
							<b>Sub Total</b>	-	-	-
<b>Travel In Minnesota</b>										
	Miles/ Meals/ Lodging	*Lodging \$96/night, M&E at \$46/day for teams of 5, \$42/day 2 vehicles rental, for ~4, 3-day trips of 500mi roundtrip each.	Travel between UMN and Beltrami Co. farm for sampling purposes.					\$5,373	\$5,373	-
							<b>Sub Total</b>	<b>\$5,373</b>	<b>\$5,373</b>	-
<b>Travel Outside Minnesota</b>										
	Conference Registration Miles/ Meals/ Lodging	Conference travel would involve approximately \$200 registration fee, \$300 airfare and \$500 hotel expenses for 3 nights lodging each for two team members.	Conference presentation to disseminate project results to relevant audiences.	X				\$835	\$835	-
							<b>Sub Total</b>	<b>\$835</b>	<b>\$835</b>	-
<b>Printing and Publication</b>										
	Publication	Publication costs range \$2-3,000 per manuscript; we anticipate the publication of 2-3 manuscripts from this original research.	Publication will allow broad dissemination of research results to the scientific community to build on our findings.					\$2,195	\$2,195	-
	Printing	Scientific posters (2-3) and handout materials	For dissemination of findings and outreach.					\$200	\$200	-
							<b>Sub Total</b>	<b>\$2,395</b>	<b>\$2,395</b>	-
<b>Other Expenses</b>										
							<b>Sub Total</b>	-	-	-

							<b>Grand Total</b>	<b>\$336,000</b>	<b>\$335,054</b>	<b>\$946</b>
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## Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
<b>Equipment, Tools, and Supplies</b>		Computing equipment	A PC computer is necessary for the project-related work (e.g., to use Windows-based analytical software) and will but used solely for project-related work. At the conclusion of this project, the computer will be transferred to Center for Prion Research for future use on wildlife projects after this project is completed.
<b>Travel Outside Minnesota</b>	Conference Registration Miles/Meals/Lodging	Conference travel would involve approximately \$200 registration fee, \$300 airfare and \$500 hotel expenses for 3 nights lodging each for two team members.	We intend to present our work at national and international conferences to disseminate new knowledge from this project. These are the best venues to share the latest knowledge with the broadest scientific audiences, demonstrating Minnesota as a leader in research on CWD.

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
State						
			State Sub Total	-	-	-
Non-State						
			Non State Sub Total	-	-	-
			Funds Total	-	-	-

## Attachments

### Required Attachments

#### *Visual Component*

File: [f18d8246-208.docx](#)

#### *Alternate Text for Visual Component*

The visual graphic contains a map of the Beltrami County carcass dumpsite and summarizes project goals and outcomes....

### Supplemental Attachments

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

Title	File
Regents of University of Minnesota Support Letter	<a href="#">782d1a40-691.pdf</a>
Background Check Certification Form	<a href="#">947c3154-a14.pdf</a>
Dissemination Activities	<a href="#">c0b8a261-101.docx</a>
Final Visual Component	<a href="#">a3a29f6b-b79.pdf</a>

## Difference between Proposal and Work Plan

#### *Describe changes from Proposal to Work Plan Stage*

01/07/22 We have updated our collaborator information to include a change in affiliation of collaborator Joel Pedersen, the addition of collaborator, Stuart Lichtenberg at University of Wisconsin, and a change in the name on the service contract in the budget from Joel Pedersen to Stuart Lichtenberg, for collaborative sample testing. This change is necessitated based on a change of affiliation by Joel Pedersen from University of Wisconsin to John Hopkins. Dr. Lichtenberg is Dr. Pedersen's lead scientist on prion research in soil and will be completing the soil research at University of Wisconsin through the completion of this project. Dr. Pedersen and Dr. Lichtenberg will both be collaborating on this project, but the work will be completed by Dr. Lichtenberg in the University of Wisconsin lab.

## 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 agree travel expenses must 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 Commissioner's Plan applies.

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

Yes

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

Yes

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

No

**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	<ul style="list-style-type: none"> <li>Budget - Capital, Equipment, Tools, and Supplies</li> </ul>	We are requesting an amendment to re-budget equipment and supply costs to include the purchase of a computer. This purchase is necessary to complete planned analyses requiring Windows-based software by our graduate students to meet project goals. We request to move \$2,000 from supplies to the purchase of a PC laptop computer and accessories.	October 7, 2022	Yes	October 28, 2022
2	Amendment Request	<ul style="list-style-type: none"> <li>Budget - Personnel</li> <li>Budget - Professional / Technical Contracts</li> <li>Budget - Capital, Equipment, Tools, and Supplies</li> <li>Budget - Travel and Conferences</li> </ul>	Stuart Lichtenberg was subcontracted through UW-Madison but has recently joined MNPRO. We would like to move remaining UW subcontract funds to support Dr. Lichtenberg's effort on the project as UMN personnel and to conference travel (\$2,000) to disseminate research at the 4th International Prion Conference occurring in June 2023 in Denver. These funds will support the participation of 3 MNPRO researchers at this important conference.	May 9, 2023	Yes	May 9, 2023
3	Completion Date	Previous Completion Date: 06/30/2023 New Completion Date: 06/30/2024	This project was, when launched, was a new line of research for MNPRO. Given the complexity of working with soils, it has taken time to build a team with the right expertise to optimize RT-QuIC for detection of prions in these complex matrices. Thus we have made progress, but it has taken more time than anticipated. Activity 2 (screening Beltrami soil samples) cannot be completed by the original completion date, hence the extension request.	May 9, 2023	Yes	May 9, 2023

4	Amendment Request	<ul style="list-style-type: none"> <li>• Budget - Capital, Equipment, Tools, and Supplies</li> <li>• Budget - Travel and Conferences</li> <li>• Budget - Printing and Publication</li> </ul>	We are requesting to shift money in the budget to cover expenses associated with ongoing work, particularly lab supplies (e.g., recombinant protein production) and printing materials. To do this, we want to shift potentially excess funds from travel (Minnesota and out of state) and publication to accommodate these lab expenses.	September 28, 2023	Yes	October 9, 2023
5	Amendment Request	<ul style="list-style-type: none"> <li>• Budget - Capital, Equipment, Tools, and Supplies</li> <li>• Budget - Travel and Conferences</li> <li>• Budget - Printing and Publication</li> </ul>	We request to move unspent funds from travel, equipment, publications and printing to supplies and MNProtein production, based on anticipated expenses in the final months of this grant. For example, we don't expect any further travel or publication expenses prior to June, but we will continue with experimentation in the lab.	April 24, 2024	Yes	May 17, 2024



# Status Update Reporting

## Final Status Update August 14, 2024

**Date Submitted:** August 14, 2024

**Date Approved:** October 15, 2024

### Overall Update

Research involved optimizing RT-QuIC for prion detection in soils (Activity 1) with the goal of characterizing environmental contamination at the Beltrami County deer carcass dumpsite (Activity 2). Importantly, soil types found at the dumpsite are unrepresented in the soil-prion literature, yet our early soil composition profile analyses (Activity 2) demonstrated that they contain compounds known to contribute both to prion binding (e.g., clay minerals) and RT-QuIC interference (e.g., high levels of organic matter, such as humic acids). Thus, a major undertaking was optimization of the soil extraction protocol. False positivity was a problematic feature of these soils that we recognized early on, and we continue to investigate what compounds are driving this characteristic and how to resolve it. However, successful optimization efforts allowed us to conduct testing of all soil samples collected in 2021 in association with carcass materials at the dumpsite. Using RT-QuIC, we detected prion activity in 20% of the soils samples from the dumpsite. No soil samples from outside the periphery of the dumpsite tested positive. While we are confident that these research efforts document soil prion contamination at the dumpsite, we will be confirming results through alternate testing beyond the life of this grant.

### Activity 1

Over the life of this project, MNPRO has pursued several lines of inquiry related to the development and optimization of RT-QuIC for soils. These efforts have included characterizing the seeding activity of various soil types in RT-QuIC, experimental manipulation of the prion extraction methodology to reduce false positivity observed with certain soil types, and development of methods to identify other proteins in soils that may be mimicking prion seeding activity in RT-QuIC. Discoveries and advancements include: 1) standardization of the test soil sample quantity through drying, such that better comparisons might be made between samples, 2) sample extraction dilution and other extraction modifications to reduce false positivity, 3) identification of the lowest concentration of prions in soil that can be detected with current methods, 4) development of a bioinformatic pipeline that will enable further study into the identification of proteins in soil that may be interfering in soil using mass spectrometry, and 5) identification of peptides that may be used in mass spectrometry to enable the confirmation of prion protein sequences in samples that test positive by RT-QuIC. Much of this research is ongoing, but these early insights create a strong foundation for the continued study of environmental prion contamination.

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

### Activity 2

The primary aim was to screen for environmental contamination at the Beltrami County deer carcass dumpsite. Early on, soil composition analyses demonstrated that we were working with soils of the Alfisol and Histosol orders, soil types associated with bogs and hardwood forests, respectively. We also learned that they contained levels of clay (5.5-13%) and organic matter (3-35%) that are known to play roles in prion binding and RT-QuIC interference. Thus, preliminary efforts involved characterizing the baseline activity of these soils in RT-QuIC when in the absence of prion contamination in order to confidently discriminate RT-QuIC activity in the presence of prions. Following these efforts, we were able to screen 201 soil samples collected from on (n=124) and off (perimeter, n=15) the dumpsite, the CWD-positive farm where the carcasses originated (n = 33) and a stream bed that ran through the farm (n = 29). We observed prion activity in 20% of the dumpsite samples, and a single sample from a central feeding area of the farm. While we are confident these results provide evidence of dumpsite soil contamination, we are working with external collaborators to confirm RT-QuIC

results with alternate testing methods to fully understand the extent of contamination.

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

### **Dissemination**

Conference Abstracts: this work was presented at 3 conferences or scientific research events in 2024.

Teaching: research activities associated with this grant were presented in 3 guest lectures on CWD for teaching purposes in 2023 and 2024.

Outreach: research activities associated with this grant were shared in presentations delivered at a Leech Lake community event in March 2024 and with the Minnesota Indian Affairs Commission in February 2024.

Media Mentions:

June 6, 2024, CVM News: Cracking the CWD case: Researchers use forensic techniques to investigate an illegal deer dump.

<https://vetmed.umn.edu/news/cracking-cwd-case-researchers-use-forensic-techniques-investigate-illegal-deer-dump>

May 30, 2024, CVM News: CVM Researchers Receive Grant from US Department of the Interior to Study Environmental Transmission of Chronic Wasting Disease. <https://vetmed.umn.edu/news/cvm-researchers-receive-grant-us-department-interior-study-environmental-transmission-chronic>

March 7, 2024, Dept. of Forestry Resources: Prions in MN Waterways: Discovery Helps Water Managers Plan.

<https://forestry.umn.edu/news/prions-mn-waterways>

August 14, 2023, CVM News: An Innovative Lens on Prions in the Environment.

<https://vetmed.umn.edu/news/innovative-lens-prions-environment>

A full list of dissemination activities over the life of this grant are attached.

# Status Update Reporting

## Status Update April 1, 2024

**Date Submitted:** March 25, 2024

**Date Approved:** May 17, 2024

### Overall Update

MNPRO continues efforts to optimize RT-QuIC for prion detection in soils, through experimentation with the extraction process as well as efforts to identify non-prion protein compounds in the soil that may be interfering with RT-QuIC. While ongoing experimentation is primarily focused on the soil types found at the Beltrami County dump site, which are particularly problematic in generating patterns of false seeding (i.e., false positivity), much of these efforts lay the groundwork for validation across soil types. As these optimization efforts to continue, we refined the current RT-QuIC extraction process in such a way as to eliminate false seeding activity by diluting the extraction product that is tested by RT-QuIC. Prion spiking experiments demonstrate that 1) prion is still detectable though 2) it is at the expense of detection sensitivity. In other words, in order to eliminate false positive results, we recognize that low concentrations of prions will be much more difficult to detect. Thus, we applied the revised protocol to screen soil samples associated with carcass materials at the Beltrami County dumpsite, which was completed by the end February. RT-QuIC results are currently undergoing analysis.

### Activity 1

To date, the MNPRO team continues experimental work to identify interfering proteins in soil that might be removed through further modification of the soil extraction process that precedes RT-QuIC. Over the last reporting period we performed metaproteomics analysis of two soil samples (one containing prion as determined by RT-QuIC and one presumed to be negative of prion but containing interfering substances) to determine what proteins were present in these samples. To do this, we worked with experts from the UMN Center for Metabolomics and Proteomics to build a soil protein reference database by which we could compare amino acids from our samples. The initial results of our two soil samples were inconclusive; a peptide was identified but was not able to be validated. During discussions of these results, it was suggested that sugar groups associated with the prion protein's natural biochemistry may interfere with the mass spec. As a result, we have re-processed these samples using a secondary treatment to remove the sugar groups, and resubmitted for mass spectrometry analysis. We are currently awaiting the results of our resubmission; if validated, a panel of 12 negative soil samples of varying compositions will be prepared in the same manner and evaluated.

### Activity 2

Over the previous reporting period, we have seen delays in obtaining PMCA results from the Bartz Lab (Creighton University) for comparative testing. Within the MNPRO lab, we have determined through preliminary experimentation with alternate fluorescent dyes that optimization and validation of that approach for prion detection by RT-QuIC is a much more complex process, beyond the scope of the current grant. Thus, we've moved forward in revising the soil extraction procedure in such a way that it minimizes false seeding at the expense of sensitivity (i.e., prion concentrations at lower levels will not be detectable). Given current limitations in soil testing, we considered this more conservative approach is best, as it maximizes our confidence that any RT-QuIC seeding is associated with prion in a sample and not interfering substances. As of February, we have now completed all laboratory analysis of soils samples collected from the Beltrami County dump site with data analysis underway.

### Dissemination

Nothing new to report.

# Status Update Reporting

## Status Update October 1, 2023

**Date Submitted:** September 28, 2023

**Date Approved:** October 9, 2023

### Overall Update

The MNPRO environmental research team has been diligently working over the past reporting period to better understand and resolve the complexities of soil matrices that challenge accurate detection of CWD prions by RT-QuIC. While this seems a monumental task (and explains why few teams across the country are working on this challenge), our team is tackling this problem from different angles. One approach was to optimize the protein extraction process (a step separating proteins from the soils prior to RT-QuIC testing) to ensure that negative soils (those obtained outside the Beltrami carcass dumpsite) showed no evidence of false positive RT-QuIC activity. We then began testing the Beltrami dump site samples, which revealed more variability in RT-QuIC seeding activity than expected from a reliable assay. Thus, we are leveraging our partnership with the Bartz Lab (Creighton University) and expertise in MNPRO to apply different screening methodologies that will help us better interpret what we are observing with RT-QuIC. Simultaneously, we continue experiments to identify the amino acid sequences contained within these soil samples to identify interfering proteins and experimentation with different strategies to remove non-proteinaceous interfering substances. We have also disseminated insights from these efforts at scientific conferences and the media.

### Activity 1

The MNPRO team continues to explore several lines of inquiry related to 1) the optimization and standardization of soil processing for RT-QuIC and 2) the identification and remediation of soil substances that interfere with the RT-QuIC reaction (e.g., nonspecific seeding). For example, several steps of the soil extraction process were identified as areas where variations in processing by researchers may occur; these are being addressed through improved protocol standardization. Recent experiments were conducted examining amino acid sequences of proteins extracted from a subset of soil samples, including one from outside of the Beltrami dump site. MNPRO researchers are currently working through existing proteomic databases and conducting taxonomic analyses of the recovered sequences as another approach to confirm true prion detection or identify the substances causing false positive seeding within these soils. Finally, because humic acids, known to interfere with RT-QuIC, are ubiquitous in soils, we are exploring new methods for removing these humic acids from soil samples prior to the extraction of proteins. While we are making progress on this Activity, we are still working toward solutions that enhance the reliability and reproducibility of the RT-QuIC technique for soils.

### Activity 2

Since our last update, we completed all soil spiking experiments and began screening samples collected from locations in the Beltrami dump site where positive carcass material had been recovered. We began by retesting a subset of samples that were collected during the first site visit in 2021 and characterized as positive by Dr. Lichtenberg in the Pederson lab (UW-Madison). Unfortunately, we were unable to reliably reproduce those original results, and we also observed what we have considered to be “false positive seeding” by presumed negative soils from outside of the dumpsite, despite optimization of the protocol. Thus, to help our team interpret these conflicting results, we are undertaking two new endeavors: 1) the subset of dumpsite samples have been sent to the Bartz Lab (Creighton University) for retesting by protein misfolding cyclic amplification (PMCA), and 2) we are also retesting the same subset with new RT-QuIC equipment and dyes that may allow differentiation between true prion amplification and nonspecific seeding. Using these different methodologies will provide additional data that we expect will allow us to better differentiate between true and spontaneous seeding in RT-QuIC.

## Dissemination

### Conference Abstracts

Grunklee, M., Wille, E., Ferguson-Kramer, V., Schwabenlander, M., Lichtenberg, S., Karwan, D., Larsen, P., Wolf, T. (2023, May 30 – June 2). Detection of Chronic Wasting Disease in Soil at a White-Tailed Deer Carcass Dumpsite [Poster Presentation]. 4th International Chronic Wasting Disease Symposium, Denver, CO, United States.

Schwabenlander, M., Bartz, J., Carstensen, M., Fameli, A., Glaser, L., Larsen, R., Li, M., Lindsey, L., Oliver, J., Shoemaker, R., Rowden, G., Stone, S., Walter, W., Wolf, T., Larsen, P. (2023, May 30 – June 2). Implementing a veterinary forensics approach to investigate chronic wasting disease at a deer carcass disposal site [Conference Presentation]. 4th International Chronic Wasting Disease Symposium, Denver, CO, United States.

Wille, E. A., Karwan, D. L., Lichtenberg, S. S., Grunklee, M., Rowden, G., Ferguson-Kramer, V., Schwabenlander, M., Wolf, T. M., Larsen, P. A. Hydrological Transport and Persistence of Chronic Wasting Disease, Gordon Research Conference on Catchment Science: Interactions of Hydrology, Biology and Geochemistry, Andover, New Hampshire. (June 18, 2023).

### Media Mentions

<https://vetmed.umn.edu/news/innovative-lens-prions-environment>

<https://vetmed.umn.edu/news/cvm-researchers-receive-grant-us-department-interior-study-environmental-transmission-chronic>

# Status Update Reporting

## Status Update April 1, 2023

**Date Submitted:** May 9, 2023

**Date Approved:** May 9, 2023

### Overall Update

This project was a new area of research for MNPRO, yet at this time we have grown a team of researchers and students that are effectively working together to conduct a variety of experiments to advance this research. In particular, our team has conducted a variety of experiments to optimize the RT-QuIC protocol for soils to standardize the procedure and reduce variability in assay performance across different soil types. The latter has been the most challenging hurdle to overcome because of the complex and variable nature of soils found in nature. However, much of this work is being conducted using soil types identified and collected from near the Beltrami dumpsite in order to meet our goal of screening soil samples from the dumpsite to better characterize environmental risk. Once our experiments to characterize RT-QuIC performance with these soil types is completed (which we expect will be late spring), we will begin a series of prion spiking experiments using these same soil types in an effort to create an RT-QuIC signal threshold that will allow us to confidently distinguish prion contaminated and uncontaminated soils from the Beltrami dumpsite.

### Activity 1

We have recruited two additional researchers to MNPRO to focus specifically on RT-QuIC with soils, Victoria Ferguson-Kramer and Stuart Lichtenberg. Dr. Ferguson-Kramer has been evaluating both the assay and sample processing components of soil detection. For example, the addition of silica nanoparticles to RT-QuIC has been examined as a signal enhancement technology in other projects at MNPRO. We evaluated this technology with soil samples, and though preliminary results do not demonstrate enhancement, experiments are ongoing. We have also observed that a significant degree of 'noise' may arise from certain soil types, making interpretation of low prion concentration samples difficult. We are exploring ways to degrade these interfering substances without compromising the assay, and are working to identify the compounds that cause interferences. Finally, some general improvements to the soil assay have been addressed. By drying soil samples prior to extraction, no loss in positive prion signal is seen, yet we can more precisely control inputs to the extraction process. Dr. Lichtenberg has provided a variety of soil types that are well-characterized in both composition and prion contamination, which have been integral to ongoing experiments. We continue optimization experiments, but expect this will need to continue beyond the intended completion date.

### Activity 2

Representative off-dump site soil samples were assayed for their content, including total organic carbon (TOC), total nitrogen, cation exchange capacity and texture. These characteristics vary between soils and, as we know from previous studies, can play a role in prion binding and/or interfere with RT-QuIC performance (e.g., inhibiting prion detection or creating false-signals). We have been using these soil samples that are uncontaminated with prions from the dumpsite to optimize RT-QuIC and establish baseline signaling thresholds, above which we can be confident that signaling is associated with prions in a sample instead of interfering substances. Preliminary experiments showed variability in RT-QuIC performance between the different soil types from the dumpsite. For example, soils with higher TOC and coarser mineral fractions produced a more consistent RT-QuIC signal, whereas those with low TOC and fine mineral texture have shown wide signal variability. Better characterization of RT-QuIC performance with these soil types through prion-spiking laboratory experiments will facilitate discrimination of uncontaminated and prion-contaminated soils from the dumpsite. That work is occurring now and will inform our next step: screening of the dump site soil sample set. Screening dumpsite soils will take several months to achieve (beyond the original project completion date).

### Dissemination

Grunklee, Madeline, E. Anu Wille, Victoria Ferguson-Kramer, Marc D. Schwabenlander, Stuart Siegfried Lichtenberg,

Diana L. Karwan, Peter A. Larsen, Tiffany M. Wolf. 2023. Detection of Chronic Wasting Disease in Soil at a White-Tailed Deer Carcass Dumpsite. College of Veterinary Medicine Graduate Programs - Admitted Students Day Poster Session.

Dr. Peter A. Larsen. Minnesota Center for Prion Research & Outreach: Chronic Wasting Disease Update. Minnesota Legislature, Joint: House Environment and Natural Resources Finance and Policy and Senate Environment, Climate and Legacy. 7 Feb 2023

# Status Update Reporting

## Status Update October 1, 2022

**Date Submitted:** October 7, 2022

**Date Approved:** October 28, 2022

### Overall Update

Over the past reporting period MNPRO has continued to collect environmental samples from the Beltrami County dump site, building a critically important sample archive that can be used to study the persistence and transport of prions over time in areas of environmental contamination. This included a spring sampling event, where water runoff was characterized and sampled, and a summer sampling event where additional soil samples were collected from established transects at positive carcass sites. During the latter sampling event, soil samples were also collected offsite to characterize the soil composition of the two soil types found at the dump site. Soil composition analysis will help us understand the characteristics and properties of the soil that might facilitate CWD prion binding and/or transport through natural environmental processes (e.g., water runoff). We also continue to develop and optimize methods for RT-QuIC detection of prions in soils, collaborating across four independent labs on this effort.

### Activity 1

We continue to work toward the development and optimization of methods for prions in soil. The primary challenge is in the development of methods to effectively extract prions from the soil matrix such that other compounds within the soil that cause false positive results in RT-QuIC can be eliminated from the reaction matrix. We have independent teams working within MNPRO and University of Wisconsin, Madison conducting separate experiments that examine different solutions to this challenge. We're also collaborating with Rodrigo Morales of University of Texas, Houston, where CWD-prion spiked soil samples were created by his lab for sharing across labs to advance this work using different soil types. These samples have been shipped to the Bartz Lab at Creighton University where some initial experiments will be performed. Dr. Stuart Lichtenberg, an international leader in prion/soil research from the Pedersen Lab, has accepted a position with MNPRO and will join in early 2023. Dr. Lichtenberg's addition to the MNPRO team will allow for more direct advancement of the soil and environmental CWD testing protocols.

### Activity 2

The MNPRO team continued sampling the Beltrami dump site during 2022 spring and summer seasons. The goal is to collect and archive soil samples associated with positive carcass sites within the dump site, as well as areas where water from snowmelt flows off the site. Thus, in spring 2022 the MNPRO team focused primarily on collecting water runoff and characterize how water flowed from the site to identify other areas on the site where soil may need to be examined. In Summer 2022, the team returned again to resample and archive soil from previous CWD-positive carcass sites for future study of prion persistence and environmental transport as laboratory methods are refined. The team also collected soil samples offsite representative of the two soil types found at the dump site. These samples will be used for soil composition analyses to understand the characteristics and properties of the soil that might facilitate binding or transport through natural environmental processes (e.g., water runoff). Offsite samples are being used in these analyses to avoid potential prion contamination with these more standard soil analyses provided by service laboratories. Soil composition samples are currently being processed and prepared for submission to the service laboratory.

### Dissemination

University of Minnesota College of Veterinary Medicine current issues course. Marc Schwabenlander presented "Advancing CWD Diagnostics" as part of the series of topical discussions in the CVM issues course led by Dr. Joni Scheftel. March 17, 2022. Number of participants: 25.

University of Minnesota College of Veterinary Medicine Profiles. The MNPRO team was highlighted for their CWD



research, including the work at the Beltrami County dump site. <https://profiles-vetmed.umn.edu/article/leading-hunt>  
April 29, 2022.

70th Annual International Conference of the Wildlife Disease Association. Marc Schwabenlander presented a poster “Implementing veterinary forensics to investigate Chronic Wasting Disease at a deer carcass disposal site” at the WDA annual meeting in Madison, WI. 23–29 July 2022. Number of participants: several hundred.

# Status Update Reporting

## Status Update April 1, 2022

**Date Submitted:** April 6, 2022

**Date Approved:** April 7, 2022

### Overall Update

In conjunction with an LCCMR EIF award, the MNPRO team began collecting samples for CWD soils research over three separate sampling trips to the Beltrami County carcass dump site during summer and fall 2021. Field activities were focused on collecting an initial set of soil and debris samples from carcass locations within the dump site as well as from locations where CWD was not expected to be outside of the dump site (i.e., negative control samples). Sampling grids were established at all CWD-positive carcass sites (n=12) and two CWD-negative control sites (n=2; one where a non-cervid carcass was identified and another where there was no carcass). The goals of this initial effort were to establish baseline data of CWD detections in soils associated with infected carcasses. Over the course of this project period we collected 170 soil samples from 45 sites, which are awaiting RT-QuIC testing. During this project period, we also established additional sampling objectives for the spring 2022 sampling season that will 1) involve long term monitoring of soil grids and predicted spread based on hydrological modeling of water runoff and 2) inform a risk assessment of the dump site.

### Activity 1

Environmental sampling for prions, like those causing CWD, is research that is in its infancy. Since the inception of this grant we have assembled a team of UMN researchers and the Pedersen Laboratory of UW-Madison to develop and refine soil sampling strategies for CWD detection. The Pedersen lab has been researching CWD detection in soils for several years, using multiple diagnostic assays, including RT-QuIC. Through this collaboration, new methods underlying RT-QuIC examination of soils for the detection of CWD prions have been transferred to the MNPRO laboratory. This is a key step in accomplishing the objectives of this activity, particularly related to Outcome 1. The initial work in the Pedersen Lab over the past reporting period demonstrates the complexity of the soil matrices at the dump site, which are characterized by high organic matter content, affecting consistent RT-QuIC performance and prion detection. Thus, experiments that involve spiking various soil matrices, including that found at locations near the dump site, with various dilutions of prion are being performed to optimize protocols for soil processing and prion detection. We have extended our collaboration to include the lab of Rodrigo Morales, where ongoing soil experiments are also being performed to optimize prion detection.

### Activity 2

Our team has completed a number of initial steps that contribute to the proposed Outcomes for this Activity. First, the MNPRO team began collecting samples for CWD soils research over three separate sampling trips to the Beltrami County carcass dump site during summer and fall 2021. During these trips, our team completed initial site assessments, established sampling grids associated with all CWD positive carcass locations, and collected an initial sample set from each of these grids. An initial subset of soil samples were screened during this period, which 1) confirmed the presence of CWD prions in soil associated with carcass sites and 2) informed further sampling efforts, including the collection of negative control samples from locations away from the dump site. Our team, in partnership with the MN DNR, has also assembled spatial data sets of the area that will be used to create a site-level sampling grid that will help us better understand how prions might be disseminated across the site as a result of scavenging, surface water flow, etc. This site-level sampling grid will be informed by preliminary hydrological modeling and implemented in the spring sampling season.

### Dissemination

Beltrami County 2021 CWD Public Meetings. Conducted by the MN Department of Natural Resources, MN Board of

Animal Health, MNPRO on Chronic Wasting Disease. Bemidji High School commons, September 28, 2021. Kelliher High School commons, September 29, 2021. Number of participants: ~130.

County Board of Commissioners Meetings. Dr. Larsen presented to both the St. Louis (Sept. 7th) and Winona Co. (Sept. 28th) Board of Commissioners regarding the biology and ecology of CWD prions. He highlighted MNPRO's activities and research insights from the Beltrami Co. carcass dumpsite. Number of participants 20-30.

University of Minnesota Sustainable Forests Education Cooperative. Marc Schwabenlander presented "Chronic Wasting Disease Research and Outreach: Potential Implications for Natural Resource Management" as part of the 2021 Forestry Webinar Series. October 19, 2021. Number of participants: 30 (plus recording available for those registrants unable to attend).

Minnesota Board of Animal Health (BAH) Fall work conference. Marc Schwabenlander presented an update on RT-QuIC technology, research, and future implementation to the BAH and USDA staff. November 16, 2021. Number of participants: 55.