2019 Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Next step in helping Minnesota's moose: Understand brainworm transmission to find solutions
PROJECT MANAGER: Tiffany Wolf
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03f

APPROPRIATION AMOUNT: $400,000
AMOUNT SPENT: $393,021.19
AMOUNT REMAINING: $6,978.81

Sound bite of Project Outcomes and Results

We created new knowledge regarding the ecological context of *Parelaphostrongylus tenuis* transmission that will aid wildlife and forest managers considering management actions as they try to conserve Minnesota's at-risk moose population.

Overall Project Outcome and Results

Parelaphostrongylus tenuis, or brainworm, is an important cause of adult moose mortality in Minnesota. Our team employed innovative approaches to better understand *P. tenuis* transmission between white-tailed deer and moose for targeted management. In Activity 1, we conducted spatial analyses of movement data from collared deer, moose, and wolves on the Grand Portage Indian Reservation (GPIR) to explore seasonal habitat use by deer and moose under different levels of predation risk. We found that habitat overlap between moose and deer increased seasonally from winter to summer, primarily due to use by deer in association with wolf predation pressure. Importantly, predation risk resulted in greater spatial separation of deer and moose, resulting in reduced *P. tenuis* risk to adult moose. In Activity 2, we applied advanced molecular techniques to moose and deer feces to detect gastropods (i.e., snails and slugs) consumed (an understood mechanism for *P. tenuis* transmission). We detected gastropods in five moose samples out of more than 160 samples screened. We identified two species of upland, ground-dwelling snails in samples collected in autumn (*Punctum minutissimum* and *Zonitoides arboreus*) and one species of aquatic snail detected twice in samples collected during summer (*Helisoma trivolvis*). This is the first empirically documented consumption of gastropods by moose. Finally, in Activity 3, we produced novel *P. tenuis* genomic data, to which we applied habitat analyses to identify transmission patterns across the GPIR. We found that landscape likely influences *P. tenuis* transmission, with features such as wetlands and natural mineral licks playing an important role relative to the other landscape features. Our research has been published in *Science Advances* and *Journal of Wildlife Diseases*, presented to numerous scientific and lay audiences, covered by local and national media (e.g., Duluth News Tribune, MPR, Smithsonian Magazine), and considered in the drafting of the DNR's current moose management plan.

Project Results Use and Dissemination

We disseminated project findings through presentations, papers, and popular media. The primary resources for managers/scientists are peer-reviewed papers on the non-lethal effects of predators on brainworm transmission and on moose gastropod consumption. We disseminated our work to scientists and managers at conferences and seminars. We also created an infographic about moose gastropod consumption that is available on

YouTube. Our dissemination outside the academic setting includes organizations such as the Minnesota Native Plant Society, Cook County High School, Fox and Hounds Club and media outlets such as Outdoor News, Minnesota Public Radio, Smithsonian Magazine, WTIP radio, and Duluth News Tribune.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan Final Report

Today's Date: 1/26/24

Final Report

Date of Work Plan Approval: June 5, 2019

Project Completion Date: June 30, 2023

PROJECT TITLE: Next step in helping Minnesota's moose: Understand brainworm transmission to find solutions

Project Manager: Tiffany Wolf

Organization: University of Minnesota

College/Department/Division: Veterinary Population Medicine Department

Mailing Address: 495 Animal Science/Veterinary Medicine, 1988 Fitch Ave.

City/State/Zip Code: St. Paul, MN 55108

Telephone Number: 612-625-0492

Email Address: wolfx305@umn.edu

Location: Northeastern Minnesota, specifically Cook, Lake and St. Louis counties.

Total Project Budget: $400,000

Amount Spent: $393,021.19

Balance: $6,978.81

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03f as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

Appropriation Language: $400,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to identify key habitats and vectors of brainworm transmission between deer and moose that may be targeted by resource management to mitigate moose exposure to this deadly condition.

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

I. PROJECT STATEMENT: Effective restoration of Minnesota (MN) moose will require continued investment and research to build on the important discoveries that have determined causal factors of moose mortality. Brainworm, Parelaphostrongylus tenuis, infection was diagnosed in 25-33% of adult moose mortalities in northeast MN in previous LCCMR funded research. We propose innovative approaches to understand brainworm transmission between white tailed deer and moose to identify vulnerabilities in transmission to enable habitat management practices that benefit moose. We will use landscape analysis to:

- Characterize habitat overlap by deer and moose,
- Prioritize snail/slug species as possible transmission vectors based on ingestion by moose and deer
- Identify patterns of brainworm transmission by mapping the parasite's population genetics.

This project directly addresses the LCCMR priority to develop foundational natural resource data through data acquisition, research, and analysis.

A 2017 workshop of MNDNR, tribal, and university experts determined that we do not know enough about where and how moose are exposed to brainworm within their habitat and what mitigation strategies may be most effective. In response, we have assembled a multidisciplinary team of researchers to tackle the highest research priorities identified in the 2017 workshop. We are building robust datasets of collared moose and deer movements in northeastern MN and are adapting novel metagenomics techniques to noninvasively evaluate snail/slug ingestion by moose and brainworm transmission patterns in deer. Each data set will be linked to the landscape to identify vulnerabilities in brainworm transmission that may be exploited to protect moose.

Brainworm larvae are shed by white tailed deer, the definitive host, and mature in an intermediate host—one of several species of terrestrial snails and slugs—before becoming infectious to ungulates. When a moose ingests an infected snail while browsing, the larvae migrate to meningeal tissue. Rather than remaining there, as in deer, the larvae tunnel through brain and spinal tissue, resulting in neurological disease and often, death. Few management options have been proposed for controlling this parasite other than controlling deer. We would like to change that.

II. OVERALL PROJECT STATUS UPDATES:

Amendment Request as of 10/22/2019

We are requesting funds be shifted from the personnel budget line to professional/technical/service contracts.

- Personnel budget (Under allocation for Post-doctoral Researchers) would decrease by $12,311
- Professional /Technical /Services Contracts would increase by $12,333

The need for this reallocation is due to a recent change of employment by Nick Fountain-Jones (NFJ) from his post-doctoral position here at University of Minnesota to University of Australia, Tasmania. NFJ has been a critical part of the research team, facilitating study design, with the skill set to lead some of our intended analyses and mentor our PhD student on several of the research activities. Moving these funds to service contracts will allow us to maintain his effort on this project by supporting him through a professional contract. The reallocation of these funds also requires a shift in the total amount of FTE directly funded and funded through contracts to reflect the shift of FTE by NFJ on this project as personnel to contract.

Amendment Approved by LCCMR 11/1/2019.

First Update March 1, 2020

Since commencement of the project in July 2019, our first priorities have been building the necessary team of personnel and dedicating staff time in the field to collect moose and deer fecal samples. As such, we have a newly hired post-doctoral researcher with extensive skills in spatial ecology who started in November and has been making efficient progress with existing data related to Activity 1. The Grand Portage Band procured collars for 25 moose (which were captured by our team and fitted with collars in February 2020) and 14 deer (captures in progress) for the collection of additional movement data related to this Activity. In December 2019 through January 2020, seven aerial surveys were flown with fixed wing and rotor craft to survey moose and deer. We had students and technicians in the field July-October 2020 to collect moose and deer fecal samples related to Activities 2 and 3; collections of deer pellets resumed in December 2019 and continue in areas used by deer in the winter. The Grand Portage Band has provided substantial logistical support, including staff assistance, ATV use, office space, computing facilities, and GPS collar data.

Second Update September 1, 2020
Despite some of the challenges related to COVID-19 (hibernation of all University of Minnesota research from March-early June), our team continues to make progress on all Activities related to this work plan. In particular, we have recently completed the primary objective of Activity 1 and are in the process of developing a manuscript for scientific peer-review. In addition to this, our team hired a field conservation dog team to assist in the collection of deer pellet samples in May and June in some of the areas of deer summer range on the Grand Portage reservation that are particularly challenging to reach and survey. The overwhelming success of the dog team in "sniffing out" these hard-to-find samples allowed us to achieve the spatial coverage of the reservation we were hoping for and complete all deer pellet sampling related to Activity 3. The Grand Portage Department of Biology and Environment contributed additional support (financial and logistical) to employ the field conservation dog team. Finally, our team was one of the first non-essential research teams to return to campus and field research earlier this summer following the initial COVID-19 shutdown, which allowed us to continue moving forward on laboratory processing of samples shortly after field collection.

Third Update March 1, 2021
In the last project reporting period we accomplished a couple of notable milestones. In particular, we completed and submitted a manuscript to be reviewed for publication describing the work around our primary objectives related to Activity 1. The manuscript is currently under review for publication in the Proceedings for the National Academy of Sciences. We are currently exploring how moose-deer habitat overlap has varied over time as an approach to future forecasting overlap and potential impacts of *P. tenuis* on moose. In October, we completed the field sampling component of Activity 2, which means field sampling has now been completed for all aspects of the project. Outside of field sampling, we have focused on optimizing laboratory protocols and preparing samples for molecular screening. One of the biggest challenges related to progress in the laboratory has been related to substantial delays in the acquisition of laboratory materials due to the COVID-19 pandemic, where manufacturers of these materials are experiencing a large demand for reagents used in molecular testing. Thus, we are continuing to navigate COVID-19 challenges as we work toward meeting project objectives.

Amendment Request March 1, 2021
Due to COVID-19 related delays in shipment of laboratory materials needed for Activities 2 and 3, we are requesting to postpone the projected completion dates for the first outcomes of both activities to November 2021.
Amendment Approved by LCCMR 3/19/2021.

Fourth Update September 1, 2021
Over the past reporting period our team has continued to make progress in all Activities. At this stage of the project, all computational analyses and objectives of Activity 1 have been completed and we are in the process of writing up and/or publishing our results, with two manuscripts at different stages of this process. Activities 2 and 3 have involved intensive lab work to process field samples for molecular analysis. The molecular analyses proposed for Activity 2 have recently been completed by the University of Minnesota Genomics Center (UMGC), and our team will soon be analyzing those results through a bioinformatics pipeline we developed to efficiently search for and identify gastropod genetic sequences in these data. Activity 3 has involved the most intensive

laboratory processing in order to extract *P. tenuis* larvae from deer pellets and then extract larval DNA. After several rounds of protocol optimization, our team has recently assembled a data set of over 100 *P. tenuis* DNA extracts that we have been more recently prepped by UMGC for genetic sequencing. While this is a major milestone, laboratories around the university are still experiencing delays related to supply chain backorders in association with the ongoing COVID-19 pandemic. Thus, the next stage of molecular analysis could take anywhere between 3-6 months for completion.

AMENDMENT REQUEST September 1, 2021
We are requesting a budget amendment to shift funds ($34,312) budgeted for a Grand Portage contract to support biology technician time in data collection and management to personnel at UMN to manage, analyze and publicly archive new data assembled or produced through this project. Over the course of this project, Grand Portage has successfully recruited substantial external funding to support their staff on this project and have not needed the originally requested funds in support of the work plan. In shifting the unused funds to UMN personnel, we can more efficiently assemble, manage, analyze and archive data sets associated with this project with an additional personnel (Research scientist) over the remaining life of the project. This staff member will also be involved in outreach and reporting efforts to enhance the reach of our project.

Amendment Approved by LCCMR 9/24/21.

Fifth Update March 1, 2022
Over the past reporting period our team has made exciting progress. In the previous period, we reported the completion of Activity 1. Since then, we have successfully published our findings in *Science Advances* and disseminated our findings further through scientific conferences and the popular media. We have also now completed all activities associated with Activity 2 and are currently in the process of writing up those findings in a manuscript soon to be submitted for peer-reviewed publication. While Activity 3 has seen the most challenges and pandemic-related slow-downs, we are pleased to report that all laboratory processing and genomic sequencing is now complete. Importantly, we've recently received from the University of Minnesota Genomics Center a complete set of 145 sequenced *P. tenuis* genotypes on which we've recently begun some exploratory analyses to understand this new and unique data set. In the coming months we will develop landscape genomic models in which we will use these new data to identify areas on the landscape where barriers to or enhanced transmission may exist.

AMENDMENT REQUEST as of March 1, 2022
We are formally requesting a no-cost extension through December 2022 to fully complete the milestones proposed for Activity 3. This Activity has been impacted the most by supply chain disruptions in laboratory supplies and personnel associated with the ongoing COVID-19 pandemic, while also requiring additional procedures to optimize sample processing protocols to enhance the success of downstream DNA sequencing applications. A no-cost extension would provide our personnel with the support needed to achieve the proposed outcomes as well as broadly disseminate our research findings to the scientific community and general public.

Pending legislative action 3/24/2022

We are also requesting a budget amendment to shift $2,440 from Budget Line 21 related to UMGC molecular services and $1,600 from Budget line 26 related to travel by Co-I Escobar for project meetings to Budget Line 23 for disposable supplies related to sampling and laboratory processing. We were able to accomplish in our own laboratory more of the molecular techniques we had originally planned for UMGC, thus have overspent the projected costs from Line 21 and underutilized those in Line 23. Meetings with Co-I Escobar also occurred online because of COVID-related travel restrictions, thus these funds were not spent.

Approved by LCCMR 3/24/2022

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
As of this report, our team has now completed Activities 1 and 2 of this workplan. We continue to disseminate our research findings related to Activities 1 and 2 through presentations to both local and national audiences, and we have recently submitted a scientific manuscript for peer-reviewed publication in the Journal of Wildlife Disease. At this stage, we have been working to analyze the genomic data produced in Activity 3 as well as assemble the landscape-level variables we plan to examine in association with these genomic data through a set of landscape genomic models we are concurrently building. We are on track to complete this final Activity by the conclusion of the project period.

AMENDMENT REQUEST as of September 1, 2022
We are requesting a budget amendment to shift unspent travel funds from Budget line 27, in the amount of $4508, to new Budget line 24, publication costs. Line 27 of the budget was originally intended to cover travel to collect samples from the field. Given that we were able to reduce our expected travel expenses associated with field sampling and this aspect of the project is now complete, these unspent funds are no longer needed. However, we are now in a phase where we are preparing manuscripts for publication, and we did not budget for publication costs in our original budget. Publication costs range from $1,200 to $3,500, thus we anticipate this shift of funds in the budget would allow us to cover the costs of 2-3 of four manuscripts we anticipate emerging from this work.

Amendment Approved by LCCMR 9/15/2022

Seventh Update as of March 1, 2023:
We continue to disseminate our research findings related to Activities 1 and 2 through presentations to both local and national audiences. We soon anticipate acceptance for publication of the work conducted in Activity 2 in the Journal of Wildlife Disease, with a third manuscript describing our sequencing of the *P. tenuis* genome currently in review for publication (an effort associated with Activity 3 research). Since our last update our team has made important strides in the exploratory analysis of the genetic and spatial structuring of our *P. tenuis* genomic data and are now finalizing the landscape genetics models to understand what landscape features might influence the observed genomic patterns. As such, we are on track to complete this final Activity by the conclusion of the project period.

Amendment Request as of 1/24/24
Final out-of-state costs ended up costing slightly more than budgeted and travel expenses within the state costing a little less than budgeted. To correct this, we request to increase out of state travel by $150 from $1,100 to $1250 and decrease Minnesota travel by $150 from $4,324 to $4,174.

Amendment Approved by LCCMR 1/26/24

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):
Parelaphostrongylus tenuis, or brainworm, is an important cause of adult moose mortality in Minnesota. Our team employed innovative approaches to better understand *P. tenuis* transmission between white-tailed deer and moose for targeted management. In Activity 1, we conducted spatial analyses of movement data from collared deer, moose and wolves on the Grand Portage Indian Reservation (GPIR) to explore seasonal habitat use by deer and moose under different levels of predation risk. We found that habitat overlap between moose and deer increased seasonally from winter to summer, primarily due to use by deer in association with wolf predation pressure. Importantly, predation risk resulted in greater spatial separation of deer and moose,

resulting in reduced *P. tenuis* risk to adult moose. In Activity 2, we applied advanced molecular techniques to moose and deer feces to detect gastropods (i.e., snails and slugs) consumed (an understood mechanism for *P. tenuis* transmission). We detected gastropods in 5 moose samples (out of >160 samples screened). We identified two species of upland, ground-dwelling snails in samples collected in autumn (*Punctum minutissimum* and *Zonitoides arboreus*) and 1 species of aquatic snail detected twice in samples collected during summer (*Helisoma trivolvis*). This is the first empirically documented consumption of gastropods by moose. Finally, in Activity 3, we produced novel *P. tenuis* genomic data, to which we applied habitat analyses to identify transmission patterns across the GPIR. We found that landscape likely influences *P. tenuis* transmission, with features such as wetlands and natural mineral licks playing an important role relative to the other landscape features. Our research has been published in *Science Advances* and *Journal of Wildlife Diseases*, presented to numerous scientific and lay audiences, covered by local and national media (e.g., Duluth News Tribune, MPR, Smithsonian Magazine), and considered in the drafting of the DNR's current moose management plan.

III. PROJECT ACTIVITIES AND OUTCOMES:

Activity 1: *Mapping risks of brainworm transmission across moose range to identify options for mitigation.* There is risk of brainworm transmission to moose where deer and moose overlap, but our preliminary data show that deer and moose only overlap in portions of their range. We hypothesize that spatial analysis will reveal landscape and climatic features that are distinct where moose and deer overlap, providing the opportunity to manage for specific habitat types that prevent overlap. Accordingly, our team will 1) quantify spatial overlap by deer and moose across their distributions, 2) characterize landscape and climatic features associated with deer and moose abundance, and 3) create a map of brainworm transmission risk across moose range. We will use deer and moose movement data collected by the Grand Portage Department of Biology and Environment (where high densities of collared moose and deer are studied) to develop the model to predict overlap and transmission risk across the larger northeastern MN region.

ENRTF BUDGET: $ 155,267

Outcome	Completion Date
1. Quantify spatial overlap of deer and moose across different habitat types	*Dec 2020*
2. Identify landscape and climatic variables most important for deer-moose overlap	*July 2021*
3. Create a spatial risk map of brainworm transmission based on deer-moose overlap (1) and landscape features (2)	*July 2021*

First Update March 1, 2020

In the past 4 months we have made advances toward proposed outcomes. We hired a post-doctoral researcher with strong expertise in spatial ecology, who started in November 2019 and has been making great strides with existing data from collared moose and deer. Achievements include the creation of an automated, computational pipeline to download, merge, clean and store spatial data collected by GPS-collars over the past 10 years in Grand Portage, as well as the creation of a landscape database that will be combined with the collaring database and used in planned analyses. This GPS collaring database includes 64 deer, 90 moose and 29 wolf individuals; the latter of which, though not an explicit component of this Activity, were included in this effort for inclusion as needed. The landscape database includes habitat, roads and elevation data for Minnesota and Southeastern Canada.

We have also started running analyses on habitat selection by deer and moose. Because most individuals migrate, trajectories were empirically split into winter, dispersal, and summer phases before habitat selection analyses were performed. Since predators (wolves) could exert behavioral changes on prey (deer and moose), we also incorporated the spatial distribution of pack territories as an additional variable affecting habitat selection. Preliminary results allowed us to assess how deer and moose are using the landscape and their spatial overlap, which is the first step towards a risk map of brainworm transmission.

Overall, deer appeared to be more generalist in their habitat preference, and responded strongly to wolf territories, while moose showed stronger preferences for specific habitat. Interestingly, moose and deer responded differently to wolves, yielding an overall decrease in spatial overlap when wolf pressure was intense. These findings suggest that wolves may play a "hidden", important role in brainworm transmission by spatially separating deer and moose.

We are on track for reaching our outcomes in the projected timeline. In 2020 we will refine maps and data, as well work to extrapolate a risk map for the entire moose distribution.

Second Update September 1, 2020

We have recently completed analyses related to both outcomes originally proposed for July 2021. We identified landscape variables linked to deer-moose spatial overlap, and then, built a risk map that predicts brain worm risk for moose across the study area. In doing so, climatic variables were not used as we determined that we do not have enough climate variability within the spatial scale at which we have worked. Although climate variables were not used, we did incorporate seasonal effects in our analysis to determine how space use and overlap by deer and moose differed between summer and winter months. We incorporated wolf GPS data into our analysis to create a predation risk map based on wolf movement that was also used for building the brain worm risk map (an addition to our original research plan). This inclusion allowed us to understand the role of this top predator on brain worm transmission risk to moose. We are currently developing a manuscript describing the findings related to the three outcomes described above. COVID-19 research hibernation did not impact progress on this activity, although computational power has been considered the main challenge for these achievements, which we overcame by using computing clusters available through the University of Minnesota.

While ahead of schedule, we continue to work with Luis Escobar (project collaborator) in using ecological niche modeling as another tool to evaluate the spatial risks associated with brainworm.

Third Update March 1, 2021

As noted in the previous Activity 1 update, we have been ahead of schedule in meeting the proposed objectives for this activity. As a result, we were able to complete the writing and submission of a manuscript detailing our objectives, methods, and findings, which was submitted for review to the journal Proceedings of the National Academy of Sciences. We continue to work with moose and deer distribution data using alternative methods and modeling approaches to explore moose-deer habitat overlap as a risk of *P. tenuis* transmission. In particular, we are currently using historical moose, deer, gastropods and *P. tenuis* range data, combined with landscape and climate data, to characterize variation in cervid ranges in association with these other variables. The goal would then be to forecast future host, vector and parasite overlap based on predicted changes in landscape and climate, and verify if the future outcome (i.e. level of overlap) had occurred in the past with subsequent moose population persistence.

Fourth Update September 1, 2021

We are excited to report that the objectives of this Activity have now been completed and we are in the process of publishing results. In review of our accomplishments, we completed computational analyses to characterize the spatial overlap of deer and moose across different habitat types using a collection of GPS data accrued over a decade of moose and deer collaring research in Grand Portage. A key finding was that overlap varied seasonally, and was primarily based on seasonal habitat use and the utilization of elevation by deer in response to wolf predation pressure. Importantly, we found that wolf predation risk resulted in greater spatial separation between deer and moose, which results in reduced *P. tenuis* transmission risk to moose. We are currently revising a manuscript describing this study for publication in the journal Science Advances. Subsequent to these analyses, we utilized a different approach to understand how climate might influence species overlap and *P. tenuis* transmission. In this analysis, we examined species distributions at a continental scale over much of the history of white-tailed deer, moose, *P. tenuis*, and three species of gastropod intermediate hosts to forecast how anticipated changes in climate will impact overlap. Key findings from this analysis are that 1) deer and moose range overlap is currently at its highest magnitude if we consider the previous 20,000 year history of moose in North America, and 2) the ranges of these two cervids are expected to shift further north in response to climate change over time, but 3) the magnitude of overlap will be approximately the same as it is today. Thus, *P. tenuis* is expected to remain a health concern for moose along the southern extent of their range. An open evolutionary question in this relatively new moose-parasite system is: will moose adapt to *P. tenuis* or will southern range expansion by moose be limited by the parasite. A manuscript describing this work is currently in development with a goal to submit for review for publication by the end of 2021.

Fifth Update March 1, 2022

We are pleased to report that since the completion of this Activity, we have successfully published our findings related to the spatial overlap between deer and moose and *P. tenuis* risk, under the influences of wolf pressure (see summary of findings in September 1, 2021 update) in a December 2021 issue of the journal *Science Advances* (https://www.science.org/doi/10.1126/sciadv.abj5944). Following a College of Veterinary Medicine media release (https://vetmed.umn.edu/news/wolves-might-help-moose-avoid-acquiring-deadly-parasite-deer-study-shows), study findings were covered at a local, regional, and national level through Grand Marais's WTIP, Duluth News Tribune, Minnesota Public Radio, and Smithsonian Magazine. Study findings were also disseminated through the North American Moose Conference held in December 2021, and we are looking forward to additional opportunities for further dissemination and incorporation into UMN educational materials in 2022.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

This Activity is complete, although our team continues to disseminate findings through presentations, as well as by contributing to the Minnesota Department of Natural Resources Expert Elicitation Study on the northeast Minnesota moose decline. Details on dissemination updates are provided below.

Seventh Update as of March 1, 2023:

This Activity is complete, although our team continues to disseminate findings through presentations, as well as by contributing to the Minnesota Department of Natural Resources Expert Elicitation Study on the northeast Minnesota moose decline. Details on dissemination updates are provided below.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

Through this activity, we conducted spatial analyses of movement data from collared deer, moose and wolves on the Grand Portage Indian Reservation (GPIR) to explore seasonal habitat use by deer and moose under different levels of predation risk. The goal was to better understand how deer and moose use habitats across a shared landscape, such that managers might have more information related to species selection when it comes to forest management. Importantly, habitat overlap with deer is a recognized risk factor for *P. tenuis* transmission to moose. We found that habitat overlap between moose and deer increased seasonally from winter to summer, primarily due to use by deer in association with wolf predation pressure. Importantly, predation risk resulted in greater spatial separation of deer and moose, resulting in reduced *P. tenuis* risk to adult moose. This discovery is important for managers as strategies, such as wolf management, are considered to benefit moose (to reduce direct mortality through predation). The results of this work have been presented to numerous scientific and lay audiences, covered by the local, regional, and national media, described in a 2021 publication in the journal *Sciences Advances,* and considered in the drafting of the DNR's latest moose management plan.

Activity 2: *Identify slugs and snails consumed by deer and moose through fecal DNA analysis.* Terrestrial snails and slugs (gastropods) are known intermediate hosts in brainworm transmission, but which species are most important in transmission remains unclear. To address this gap, we will collect 180 deer and moose pellet samples throughout the transmission season (spring-fall) and use genetic metabarcoding to assess gastropod consumption over habitat types in Grand Portage, where a high density of deer and moose are studied. We hypothesize that if moose and deer are exposed through gastropod consumption, then we can identify key species and habitats for transmission through fecal analysis for gastropod DNA. With this knowledge, management actions such as prescribed burning can be used to reduce gastropod populations in specific habitats.

ENRTF BUDGET: $123,566

Outcome	Completion Date
1. Identify and characterize the frequency of snail/slug species consumption by deer and moose overtime through fecal DNA analysis of pellet samples	*November 2021*
2. Identify which snail/slug species are primary diet components across seasons	*July 2022*

First Update March 1, 2020

A large amount of effort in these past 6 months were dedicated to field activities to obtain the necessary samples. We collected 113 moose pellet samples from July-October 2019 and are on track to double this sample size by next Fall. Thus far, we have submitted 20 moose pellets to the University of Minnesota Genomics Center for DNA extraction, PCR, and DNA sequencing, and have results for these samples. We also submitted 39 deer pellet samples that have undergone the same laboratory procedures. To build capacity within our lab, we are currently performing DNA extraction and PCR on the remaining samples in-house. So far, we have extracted DNA from 50 pellets and will perform PCR within the next month.

We have run sequenced samples through our bioinformatics pipeline, and recognized several errors in the process. We are thus using this time early in the project to work through these issues with experts to improve both the quality and speed of the pipeline, which will produce reliable results more efficiently. We aim to generate results from the modified pipeline within the next several months. With this new pipeline in place, we will be on track to meet outcomes according to the projected timeline.

Second Update September 1, 2020

Field sampling for this activity was subject to a brief delay due to the hibernation of research in response to the COVID-19 outbreak. However, we have made good progress, having collected 52 moose samples from 38 individuals, since June 2020 (29 in June, 23 in July). All of these samples have been processed in preparation for DNA extraction, PCR, and sequencing, which we will conduct in Fall 2020. We will continue to collect ~20 moose fecal samples/month through October, with the goal of accumulating a sample size of ~120 moose fecal samples in 2020. This exceeds our original goal of 80 moose fecal samples this summer. We deemed the extra sampling necessary given that DNA extractions from some 2018 and 2019 samples yielded insufficient DNA for analysis. In addition to increasing sample size, we are also amending our sample handling and preparation protocols to minimize freeze-thaw cycles that may degrade DNA. Accordingly, we've extracted DNA from 28 additional 2019 moose samples under these new protocols.

In terms of molecular work, we received sequencing data from the University of Minnesota Genomics Center for the 20 moose pellets and 39 deer pellets mentioned in the March 2020 update. In processing this new data, we have amended our bioinformatic pipeline to reduce false positive results, which allowed us to detect with high confidence the snail, *Punctum minutissum,* in one of our 2018 moose fecal samples. While we did not find any other definitive snail hits in our 2018 samples, this novel finding provides strong proof of concept for our bioinformatic pipeline and the efficacy of this project. Based on the literature, *P. minutissum* has never been examined for *Parelaphostrongylus tenuis*, but this finding suggests that further understanding of its role in transmission may be important.

Third Update March 1, 2021
Since the last report, we completed field collections of moose pellets through the end of October 2020. We collected 112 moose pellet samples over the period of June-October 2020, providing us with an overall total of 246 moose pellet samples collected from June through October 2018-2020. At this stage, we have met and exceeded our pellet sampling goals for this Activity. Following collection, we prepared each sample by randomly selecting 3 pellets from each pellet group, removing the outer layer of the pellet to minimize DNA contamination from gastropods moving across the feces, and homogenizing them together to enhance our chances of detecting snail/slug DNA. All samples have now been prepped in this way and are ready to undergo DNA extraction methods ahead of molecular screening. At this stage, we are awaiting the necessary kits and reagents to be shipped from the manufacturer; shipments have been substantially delayed due to high demands related to COVID-19 diagnostic testing globally. In the meantime, we have put additional efforts into optimizing the downstream molecular methods, which should improve our ability to identify any gastropod DNA present in the samples. However, given the delays in obtaining the necessary materials for DNA extraction, as well as the potential for similar delays in downstream applications, we now recognize that this will delay our timeline beyond July 2021 for the identification of slugs and snails in the moose pellet samples.

Fourth Update September 1, 2021
We've recently completed the molecular analysis of all moose pellet samples through the University of Minnesota Genomics Center. This achievement follows intensive laboratory work over the past 6 months to process moose pellet samples, extract DNA, perform PCR to amplify a mitochondrial gene segment, and then sequence those PCR products. As a result of these activities, we have 4.4 billion bases of sequencing data from 162 fecal samples (155 moose samples, 7 deer samples). Over the course of this period, we also optimized the bioinformatics pipeline developed by our team to screen the resulting genetic sequences specifically for gastropod gene sequences, the next step toward completing the objectives of this Activity. We are currently on track to complete this activity on the projected timeline. We will also be describing our team's work on this project through a presentation to be delivered at the end of August 2021 to an international virtual conference for the Wildlife Disease Association.

Fifth Update March 1, 2022
We are excited to report that over this reporting period we completed all analyses associated with this Activity and are currently in the process of writing up our findings for peer-reviewed publication. Over the course of this period, DNA samples that we sequenced were subjected to a highly sensitive and efficient bioinformatic pipeline that allowed us to compare our sequences to those of the same mitochondrial gene from all species of Gastropoda that have been archived in the Barcode of Life Database. Though this process, we were able to detect gastropods in 5 moose pellet samples. More specifically, we detected two species of upland, ground-dwelling snails in fecal samples that were collected in autumn (*Punctum minutissimum* on September 14, 2018; *Zonitoides arboreus* on September 17, 2019) and 1 species of aquatic snail was detected twice from fecal samples collected during the summer (*Helisoma trivolvis* on June 20, 2019 and June 24, 2020). We additionally detected *Punctum minutissimum* in a pilot sample that did not have associated metadata. This is the first time any researcher has empirically documented consumption of gastropods by moose, an understood mechanism for the infection of moose with *P. tenuis*. Although we do not have enough gastropod detections to do a formal analysis of gastropod consumption patterns by moose, these results point to exciting new research directions. Also during this period, we described our team's work on this project through local, national and international scientific conferences, including the College of Veterinary Medicine's Graduate Student Seminar series, the North American Moose Conference, and the international conference of the Wildlife Disease Association.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
Since last update, we completed additional steps to confirm the accuracy of our putative gastropod detections with a phylogenetic analysis. In doing so, we compared the genetic sequences found in our samples with publicly available sequences from the genuses of the same gastropods. This phylogenetic analysis confirmed our previous species identifications with high confidence. We have now compiled our findings into a manuscript, which was submitted as a full-length research article to the Journal of Wildlife Diseases for review for publication. This manuscript is currently awaiting review assignments.

Seventh Update as of March 1, 2023:
Since the last update, we received reviews on the manuscript we sent to Journal of Wildlife Diseases. Reviews were very positive but also noted concerns that our molecular test for gastropods might not detect all consumption events. While we recognize this to be a limitation of the technique, we generated further sequence data to test the repeatability of the method, an important feature that increases confidence in the methodology. In other words, we explored whether we could re-detect gastropods in new DNA extractions from our original positive fecal samples. Due to limited resources and time, we could not extensively explore this question, but we were able to demonstrate repeatability in a mini experiment with minimal sequencing data. This preliminary analysis suggests our test is reasonably sensitive. With changes to the manuscript, we adequately addressed the reviewer's original major comments, and this manuscript is accepted for publication by the Journal of Wildlife Diseases pending minor edits.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):
It is understood that *P. tenuis* transmission to moose occurs from the inadvertent consumption of an infected intermediate gastropod host, though which species of slugs and snails are most important for *P. tenuis* transmission to moose has been unknown. In Activity 2, we applied advanced molecular techniques to moose and deer feces to detect the DNA of gastropods (i.e., snails and slugs) consumed. With the collection of almost 250 fecal samples and DNA analysis of >150 of those, we successfully detected gastropods in 5 moose samples. We identified two species of upland, ground-dwelling snails in samples collected in autumn (*Punctum minutissimum* and *Zonitoides arboreus*) and 1 species of aquatic snail detected twice in samples collected during summer (*Helisoma trivolvis*). This is the first empirically documented consumption of gastropods by moose. Our study reveals that further work on this aspect of transmission is needed, particularly since two of the detected snails have not previously been screened for their potential as *P. tenuis* hosts. This work also demonstrates the utility of DNA screening for the detection of cryptic interactions between species that are relevant for parasite transmission. Our results have been disseminated through numerous scientific and lay presentations and has recently been published by the *Journal of Wildlife Diseases*.

Activity 3: *Identify patterns of brainworm transmission across the landscape by mapping the parasite's population genetics.* We will genotype the larvae transmitted by deer from 150 fecal samples collected across habitats in Grand Portage, where a high density of deer are studied. We hypothesize that the genetic population of brainworm is sufficiently heterogeneous to characterize transmission across the landscape. Ultimately, by mapping the parasite's population genetics, we can describe gene flow of the parasite and identify natural landscape barriers to transmission that might be exploited in future mitigation efforts.
ENRTF BUDGET: $121,167

Outcome	**Completion Date**
1. Genetic analysis of brainworm larvae from deer pellet samples in northeastern MN for a goal of 150 deer samples	*November 2021*
2. Identify where natural landscape barriers to transmission might exist through spatial autocorrelation analysis of parasite genetics	*July 2022*

First Update March 1, 2020
We collected deer pellets from January-December 2019 (field efforts were initiated ahead of this grant through other funding mechanisms). The Grand Portage Indian Reservation (GPIR) was spatially broken up into 1 km2 survey cells from which we targeted the collection of 2 fecal samples from each of 50 randomly selected survey cells that fell within deer summer and winter range and 25 additional samples collected opportunistically from cells that fall outside of primary deer range but within primary moose range in GPIR. Over the course of the 2019, we collected 121 deer fecal samples, screened 93 samples for *P. tenuis* larvae, and found larvae in 66 samples (71%). Larval counts ranged from 0.3-762 larvae/gram feces. Field sampling is ongoing and will continue through 2020.

We continue the validation of a restriction site associated DNA sequencing (RADseq) protocol developed by University of Minnesota Genomics Center (UMGC), which we are using to achieve Activity 3 goals. We submitted 97 previously banked samples for RADseq, 70 of which were suitable for this analysis. Through these analyses, we recognized the need for a reference genome to deal with contaminant DNA from other lungworm infections that might be present in some samples. Reliable reference genomes do not exist for *P. tenuis*; hence, we are working with UMGC to develop a high-quality whole genome sequence. We acquired the requisite samples for this, extracted sufficient DNA from those and are currently in the process of sequencing the genome. We anticipate the completion of sequencing and bioinformatics in the coming months and anticipate moving forward with the genotyping of larval samples by RADseq shortly thereafter.

Second Update September 1, 2020
We continued to collect deer pellets January-June 2020. Because of low deer densities and thick vegetation on deer summer range, we used a field conservation dog team (employing a scat-detecting dog) in May and June to increase efficiency and success in deer pellet collection. We found this to be a highly effective technique over our traditional survey methods; the dog detected 74 deer pellets in very low-density deer areas. Getting samples from this area provides important coverage for our landscape genetic analyses, and likely would not have been possible without use of a dog. This was also timely, as it was implemented at a time when our university personnel were restricted from field and laboratory activities in response to the COVID-19 outbreak. Prior to this, we had collected 53 samples from winter deer range in 2020, leading to a total of 127 deer samples collected in 2020, meeting project objectives. Ninety-three samples collected this year have already undergone initial processing using Baermann's floats to extract *P. tenuis* larvae, with 63 being positive for *P. tenuis* (68%). We have collected 455 samples total for this project and consider sampling complete.

We also completed a whole genome sequence of *P. tenuis* using PacBio sequencing technology, which gives us high confidence in genome completeness. Although not originally anticipated as necessary, we determined the need for this novel resource early and it will contribute to the success of both this project and future *P. tenuis* research. In particular, we were able to align DNA sequence reads we generated from 97 larval samples to the whole genome sequence to effectively and efficiently eliminate contaminating sequences from the database. However, this also led to the realization that many of our sequences had problematic contamination and will therefore be resequenced. Although this is a setback in the lab pipeline, the creation of the whole genome sequence allowed us to catch this early in data generation and analysis and explore alternative approaches to reduce contamination prior to sequencing, which have been successful elsewhere in reducing contamination to acceptable levels.

Third Update March 1, 2021
At this stage of Activity 3, all field sampling has been completed. Since the last update, all samples collected in the field have gone through initial stages of laboratory processing, which involves the identification of positive samples (i.e. those containing *P. tenuis* larvae) and the extraction of those larvae from the fecal material. In summary, we collected 455 fecal samples, and 271 (60%) of those samples were positive for *P. tenuis*, with larvae ranging from <1 to 842 larvae per gram of feces. In the last update, we described the recognition of

problematic contamination early in this pipeline, which we identified as having been associated with this stage of processing. Thus, new steps have now been developed and incorporated to clean the larvae and reduce contamination from inhibiting downstream procedures. We currently have a small sample set undergoing molecular sequencing and genotyping through the University of Minnesota Genomics Center using the newly generated *P. tenuis* genome we created early in 2020. Thus far, all quality control indicators suggest that the newly optimized protocols may have resolved the previous contamination issues. Once this has been fully confirmed with the current small test sample set, we will move forward in submitting the full sample set for molecular sequencing and genotyping. We are experiencing the same delays in laboratory reagent acquisition as described for the previous Activity, where orders from manufacturers are delayed by the demand for laboratory materials for diagnostic testing related to the COVID-19 pandemic. Thus, we anticipate completion of our first milestone, originally targeted for July 2021, will also be delayed.

Fourth Update September 1, 2021

This Activity has been the most challenging in regard to the development and optimization of laboratory protocols, though we continue to make progress. Much of the time over the past reporting period was focused on enhancing the amount of DNA we could extract from *P. tenuis* larvae (collected from 277 deer pellet samples) while reducing contamination associated with their origin from fecal samples. The quality and quantity of DNA directly influences downstream sequencing analyses and associated results, hence this was an important step to get right. At this stage we have completed the extractions and have sufficient DNA samples from 104 larval samples, which are now in the queue for sequencing and genotyping by the University of Minnesota Genomics Center. While this is a major milestone, laboratories around the university are still experiencing delays related to supply chain backorders in association with the ongoing COVID-19 pandemic. Thus, the next stage of molecular analysis could take anywhere between 3-6 months for completion, which has the potential to further delay downstream landscape genetic analysis. Another accomplishment in the current reporting period was the upload of the *P. tenuis* genome sequenced by our team to National Center for Biotechnology Information (NCBI). NCBI is a national database that makes genomic data publicly available for further scientific research. We are currently working with other *P. tenuis* researchers to identify genes on the *P. tenuis* genome and publish those results.

Fifth Update March 1, 2022

This Activity has been the most challenging to progress, although the critical efforts put forth by our team to optimize the laboratory protocols for decontaminating and extracting larval DNA were extremely successful in the downstream production of quality sequence data. To summarize our approach, we cleaned larvae to remove as much contaminant matter as possible. We then used forensic DNA extraction kits to maximize our DNA yield from the microscopic larvae. We amplified the resulting DNA to concentrations necessary for downstream analyses. We then performed RAD-seq, a sequencing technique in which the entire genome is subsampled using enzymes, on 151 larvae DNA samples. We compared the sequences generated to a whole genome sequence of brainworm, which allowed us to identify and remove contaminant DNA sequences. From these processes, we were able to obtain an adequate number of brainworm sequences from 145 of the 151 samples, and we identified reliable markers for genotyping brainworm based on those samples. Preliminary analyses found substantial genetic structuring of the *P. tenuis* population on the Grand Portage Indian Reservation, which suggests that the landscape-level modeling to be performed in the next several months will yield informative and useful results.

Due to delays associated with the COVID-19 pandemic (supply chain disruptions, etc) and additional time spent optimizing laboratory protocols, we are approximately 3 months behind our projected schedule for this Activity’s milestones. Therefore, we are formally requesting a no-cost extension to our project (see amendment request above) for uninterrupted support of our personnel as our team completes the computational and spatial modeling needed to achieve Outcome 2 of this Activity.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Since our last update, we've performed additional exploratory analyses on this new and unique *P. tenuis* data set to better understand how the genetics of the sample set vary and how the population is structured. We found that different methodological approaches to these exploratory analyses produced conflicting results, where some analyses suggested that this sample set was both spatially and genetically structured (in other words, we could identify clusters of genetically similar *P. tenuis* on the landscape indicative of transmission), but another analysis indicated a lack of genetic variation between individuals. Thus, we continue to apply these various methods in different ways to gain more insight on these seemingly disparate results. This will ensure that we optimally process our data for the proposed landscape genomics modeling.

Accordingly, we have also created a pipeline for the landscape genomics modeling effort. With this modeling, we will examine which landscape variables best explain the observed patterns of *P. tenuis* genetic structuring we have observed. The landscape variables we plan to examine include terrain ruggedness, rivers, roads, deer migration routes, habitat type, winter and summer deer range, distance to Lake Superior, distance to the nearest mineral lick, and Euclidean (i.e., straight-line) distance. As with the data processing, we are concurrently working to optimize these draft models. Such optimization is typical for landscape genomics studies, especially when dealing with high numbers of genetic markers, such as in our study. We are on track to finalize this analysis and have a draft of the resulting manuscript by the project end.

Finally, we presented this work at the Wildlife Disease Association Conference in Madison and for the University of Minnesota College of Veterinary Medicine Points of Pride Research Day.

Seventh Update as of March 1, 2023:

Since our last report, we reached concordance between results from our exploratory analyses of *P. tenuis* genetic and spatial structuring. We achieved this by creating stricter criteria for data inclusion and further optimization of the analytical pipeline. These more rigorous results show that genetically related *P. tenuis* larvae can be found in closer proximity to each other on the landscape than unrelated larvae, and qualitatively suggests that rivers, elevation, and deer dispersal routes may drive these spatial patterns of genetic clustering. We are now quantitatively testing this relationship through landscape genomics modeling. A fundamental goal of this modeling approach is understanding how different landscape features might be expected to affect the flow of genes (i.e., through the movement of individuals between populations and their reproduction). We model gene flow through different landscape features by creating “resistance surfaces”, which quantify how a given landscape feature (for instance, high vs low elevation) impedes or enhances the biological flow of genes. These methods have often been applied to the study of gene flow between vertebrate populations, but we are using the flow of *P. tenuis* larval genes as a proxy for transmission.

Over the last reporting period, we used our *P. tenuis* genomic data to optimize resistance surfaces for elevation, landcover type, deer dispersal patterns, roads, and rivers. These resistance surfaces are computationally intensive to create, but they will provide value to the scientific community once openly shared at the end of this project. Using our resistance surfaces and other quantitative variables (e.g., distance to Lake Superior, distance to nearest mineral lick), we are exploring the relative importance of various features in shaping *P. tenuis* gene flow with machine learning modeling. This is the final component of our analysis and will provide insight on how the landscape might be managed to reduce *P. tenuis* gene flow, and thus transmission.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

Since our last report, we finalized the landscape genomics analyses. In summary, the landscape likely influences *P. tenuis* transmission, and features such as wetlands and natural mineral licks may play an important role relative to the other landscape features (e.g., habitat type, proximity to Lake Superior, roads, rivers, deer dispersal/migration habitat preferences). Our finding of mineral licks as important to *P. tenuis* transmission is particularly interesting, as recent camera trap data from our study site demonstrated that deer and moose exhibit behaviors conducive to transmission in these areas. This is a novel finding in the study of *P. tenuis*. We stop short of recommending management actions, as more work is needed to understand the strength and direction of associations between mineral licks or wetlands and *P. tenuis* transmission, particularly at a larger spatial scale. We have summarized these findings into a draft manuscript, which is currently being reviewed and edited by coauthors. We plan on submitting to the peer-reviewed journal *Molecular Ecology* as soon as possible. By the time of publication, all genetic data generated by this project will also be made publicly available in the National Center for Biotechnology Information Sequence Read Archive.

IV. DISSEMINATION:

Description: The core intent of this project is to provide science-based information for assessing and managing risks associated with brainworm transmission between deer and moose. Our goal is also to communicate our findings in both policy and scientific arenas. In order to reach a broad range of stakeholders, we anticipate the following public and scientific communications.

1) Tribal and regional audiences:
    - local public radio program will provide updates on study results
    - results and recommendations will be made to the Grand Portage Tribal Council, where a large portion of the study will be conducted
    - we intend to engage Minnesota's media community to deliver updates and results of this study through the popular press

2) Minnesota Department of Natural Resources:
    - this project stems partly from the findings of the LCCMR-funded moose mortality study, thus we will regularly share findings from this study to colleagues in the Wildlife Health Program.

3) Scientific Audience
    - we intend the submission of at least three scientific articles submitted to peer reviewed journals. These publications will communicate scientific results, as well as sampling methods, diagnostic methodologies and system-based analyses.
    - we will submit scientific abstracts to the International and North American Moose meetings, among other scientific meetings, to share findings through oral presentations.

4) Education/Extension programs
    - this project will be used as a platform to deliver STEM education through experiential learning to tribal students in Grand Portage.
    - Drs. Wolf and Forester have appointments in the UMN Colleges of Veterinary Medicine (ecosystem health) and Food, Agricultural, and Natural Resource Sciences. They teach courses to undergraduate, graduate and professional students, as well as in the Grand Challenge Curriculum. These findings will be incorporated into already developed case studies in all of the above.
    - a website is currently under construction to showcase and disseminate information about ongoing ecosystem health research projects conducted by Dr. Wolf and other faculty in the College of Veterinary Medicine. This project is planned for inclusion in the earliest version of the new website (yet to be officially named and launched).

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.

First Update March 1, 2020

We are still in the early stages of this project, but within the last 6 months presented project goals and preliminary data at the following events:

- Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2019. Presentation: Understanding Brainworm Landscape Genomics on Grand Portage Indian Reservation to Prevent Minnesota Moose Declines. Ecosystem Health Seminar, University of Minnesota

- Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2019. Poster: Understanding Brainworm Landscape Genomics on Grand Portage Indian Reservation to Prevent Minnesota Moose Declines. Points of Pride Research Day Poster Competition at UMN College of Veterinary Medicine

We have also had the opportunity to discuss our project more broadly through the media:

- Outdoor News, "Researchers digging deeper into effects of brainworm on moose." By Javier Sanchez.
- https://www.ifallsjournal.com/news/outdoors/helping-minnesota-s-moose/article_c1022a01-70ba-5b71-9576-4f6c05434b48.html

Second Update September 1, 2021

In the past 6 months, we presented updates on to Activities 1 and 3 at the University of Minnesota One Health Division Research Seminar and have a first manuscript in preparation:

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2020. Presentation: Understanding How Landscape Features Influence Brainworm Transmission in Minnesota. *UMN College of Veterinary Medicine One Health Division Research Seminar, University of Minnesota*

Oliveira-Santos, L.G.R., Moore, S., Severud, W., Forester J.D., Wolf, T. 2020. Presentation: Spatial ecology of brainworm disease: Looking at movement of vertebrate hosts and their predators. *UMN College of Veterinary Medicine One Health Division Research Seminar, University of Minnesota*

In preparation: Oliveira-Santos, L.G.R., Severud, W.J., Moore, S., Forester, J.D., Garwood, T., Escobar, L., Wolf, TM. Unraveling nonlethal predator cascade effect on brain worm transmission to moose. To be submitted to Proceedings of the National Academic Sciences (PNAS) in 2020.

Third Update March 1, 2021

In the past 6+ months, we have disseminated our work through four presentations (two national, two local), development of an infographic for the 2020 UMN College of Veterinary Medicine annual research event, which can be disseminated to a broad public audience, and the submission of a manuscript communicating Activity 1 findings.

Presentations:

Wolf TM, Moore SA, Swader T, Travis D. Moose Population Decline in Minnesota: Evaluating Ecosystem Health Through Tribal-Academic Partnerships. American Veterinary Medical Association 2020 Virtual Convention, August 20-22, 2020.

Moore SA, Wolf TM, Isaac EJ, Severud W, Oliveira-Santos LGR, Garwood T, Chenaux-Ibrahim Y. Mooz (Moose) Ecology and Health update. MNDNR and Tribal Moose Management Meeting. October 27, 2020.

Moore SA, Wolf TM, Isaac EJ, Severud W, Oliveira-Santos LGR, Garwood T, Chenaux-Ibrahim Y. Expanding a Tribal Moose Collaborative to Develop a Research Framework for Regional Collaboration on Mooz (Moose) Ecology and Health. Bureau of Indian Affairs Midwest Region Great Lakes Restoration Initiative Annual Meeting. December 2, 2020.

Wolf TM**.** Moose Decline in Minnesota: Evaluating Ecosystem Health Through Tribal-Academic Partnerships. Minnesota Native Plant Society, December 4, 2020.

Infographic:
Garwood T, Moore S, Fountain-Jones N. Larsen P., Wolf T. 2020. Infographic: Which Slugs or Snails Transmit Brainworm to Moose? *UMN College of Veterinary Medicine Office of the Associate Dean of Research YouTube Channel*. Video Link: https://www.youtube.com/watch?v=WRxK-oZhCxI

Manuscript submission:
Oliveira-Santos, L.G.R., Severud, W.J., Moore, S., Forester, J.D., Garwood, T., Escobar, L., Wolf, TM. Unraveling nonlethal predator cascade effect on brain worm transmission to moose. Proceedings of the National Academic Sciences (PNAS). *In review*.

Fourth Update September 1, 2021
Presentations:
Wolf TM. The indirect impacts of a parasite on a community: *Parelaphostrongylus tenuis* and moose. The Fox and Hounds Club Breakfast Seminars, June 2, 2021, Minnetonka, MN.

Wolf TM. Moose health in Grand Portage. Minnesota Department of Natural Resources Moose Research Meeting, July 28-29, 2021, Virtual conference.

Garwood T, Moore SA, Fountain-Jones NM, Larsen PA, Wolf TM. Using metabarcoding to study effective contact for brainworm transmission between moose and gastropods. 69th Wildlife Disease Association-14th European Wildlife Disease Association Joint Virtual Conference, August 31 - September 2, 2021.

Garwood T. Investigating brainworm ecology to reduce aberrant transmission to Minnesota moose. Comparative and Molecular Biosciences PhD Preliminary Examination Seminar, April 19, 2021, virtual seminar.

Manuscript submission:
Oliveira-Santos L.G.R., Severud W.J., Moore S., Forester J.D., Garwood T., Escobar L., Wolf TM. Unraveling nonlethal predator cascade effect on brain worm transmission to moose. Science Advances *In revision*.

Fifth Update March 1, 2022

Presentations:
Garwood, T. “Disease Studies in Moose and Bighorn Sheep,” Student Fisheries, Wildlife, and Conservation Sciences Club, University of Minnesota. (February 9, 2022)

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. “Using metabarcoding to study effective contact for brainworm transmission between moose and gastropods,” College of Veterinary Medicine Graduate Student Seminar, University of Minnesota, Virtual. (February 3, 2022).

Wolf, T. M., Oliveira-Santos, L. G. R., Severud, W., Forester, J., Isaac, E. J., Chenaux-Ibrahim, Y., Garwood, T., Escobar, L. E., Moore, S. A. "Grey wolves reduce *Parelaphostrongylus tenuis* transmission risk from white-tailed deer to moose," 54th North American Moose Conference and Workshop, Virtual. (December 3, 2021).

Garwood, T., 2021. "Investigating brainworm ecology to reduce aberrant transmission to Minnesota Moose," Three Minute Thesis Competition, College of Veterinary Medicine, University of Minnesota, Virtual. (September 9, 2021).

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. M. "Using metabarcoding to study effective contact for brainworm transmission between moose and gastropods," 54th North American Moose Conference and Workshop, Virtual. (December 3, 2021).

Garwood T, Moore SA, Fountain-Jones NM, Larsen PA, Wolf TM. "Using metabarcoding to study effective contact for brainworm transmission between moose and gastropods," 69th Wildlife Disease Association-14th European Wildlife Disease Association Joint Virtual Conference. (August 31 - September 2, 2021).

Publications:
Oliveira-Santos, L. R., Moore, S. A., Severud, W. J., Forester, J. D., Isaac, E. J., Chenaux-Ibrahim, Y., Garwood, T., Escobar, L. E., & Wolf, T. M. (2021). Spatial compartmentalization: A nonlethal predator mechanism to reduce parasite transmission between prey species. Science Advances, 7(52). doi: 10.1126/sciadv.abj5944

Media Communications:

Date	Medium	Outlet
12/27/21	T. Wolf interview with Euan Kerr on MN moose, wolves and brainworm	Minnesota Public Radio
12/30/21	https://www.duluthnewstribune.com/sports/northland-outdoors/wolves-may-help-keep-deer-and-brainworm-away-from-moose	Duluth News Tribune
12/30/21	https://www.wtip.org/wolves-could-play-key-role-preventing-parasite-killing-minnesota-moose	WTIP, Grand Marais
2/1/21	https://www.smithsonianmag.com/smart-news/plummeting-minnesota-moose-populations-may-recover-with-help-from-wolves-180979484/	Smithsonian Magazine

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:
Presentations:
Wolf, TM. Moose Mysteries: Understanding the factors driving Minnesota's moose decline. National Veterinary Summer Scholars Symposium, St. Paul, Minnesota (August 6, 2022). *Invited presentation*.

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. Poster: Exploration of landscape feature determinants of Parelaphostrongylus tenuis transmission in northeastern Minnesota. Annual Wildlife Disease Association Conference, Madison, Wisconsin (July 23-29, 2022)

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2022. Video Abstract: Landscape feature determinants of Parelaphostrongylus tenuis transmission in northeastern Minnesota. Points of Pride Research Day Poster Competition at College of Veterinary Medicine, University of Minnesota (April 5, 2022)

Wolf TM. Unlocking the mystery of moose mortality in Minnesota: the interaction of species behavior, predators, and disease. Macalaster College, St. Paul, Minnesota (March 3, 2022). *Invited presentation*.

Manuscript submissions:
Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2022. Using metabarcoding to study effective contact for brainworm transmission between moose and gastropods. Journal of Wildlife Diseases*, In review*

Seventh Update as of March 1, 2023:
Scientific Presentations:
Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. Poster: Exploration of landscape feature determinants of Parelaphostrongylus tenuis transmission in northeastern Minnesota. The Wildlife Society's 29th Annual Conference, Spokane, Washington (November 6-10, 2022).

Garwood, T. 2022. Presentation: Seeking Solutions for Minnesota's Moose Decline: Illuminating Parelaphostrongylus tenuis ecology with next-generation sequencing. SDSU Natural Resource Management Fall Seminar Series, Brookings, South Dakota. (September 23, 2022). Invited.

Wolf TM, Oliveira-Santos LGR, Severud WJ, Forester JD, Isaac EJ, Chenaux-Ibrahim Y, Garwood T, Escobar LE, Moore SA. Grey wolves reduce Parelaphostrongylus tenuis transmission risk from white-tailed deer to moose. International Wolf Symposium, Brooklyn Park, Minnesota. (October 15, 2022).

Wolf, T. M. Moose Mysteries: Understanding the factors driving Minnesota's moose decline. National Veterinary Summer Scholars Symposium, St. Paul, Minnesota. (August 6, 2022). Invited.

Wolf, T. M. Unlocking the mystery of moose mortality in Minnesota: the interaction of species behavior, predators, and disease. Macalaster College Biology Department Seminar Series Macalaster College, St. Paul, Minnesota. (March 3, 2022). Invited.

Teaching and Outreach:
Garwood, T.J. Guest Lecture for WL 411: "Genetics, Genomics, and their Applications in Wildlife Conservation: Principles of Wildlife Management", South Dakota State University, Brookings, South Dakota. (September 21, 2022). In person.

Wolf T.M. A balancing act: the conflicting impacts of wolves on moose survival and health. International Wolf Center Webinar Series, virtual event with 84 registrants. (February 15, 2023).

Manuscripts submitted for publication or in prep:
Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2023. Species in the feces: DNA metabarcoding to detect potential gastropod hosts of *Parelaphostrongylus tenuis* consumed by moose (Alces alces). Journal of Wildlife Disease, *Accepted pending minor edits*

Garwood, T., Richards, J., Macchietto, M., Gerhold, R., Garbe, J., Kania, S., Fountain-Jones, N., Larsen, P., Wolf, T., 2023. A de novo whole genome assembly and annotation of *Parelaphostrongylus tenuis*. Journal of Nematology*, In review*

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2023. Exploration of landscape feature determinants of *Parelaphostrongylus tenuis* transmission in northeastern Minnesota. Molecular Ecology, *In preparation*

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

Selected Teaching and Outreach:

Garwood, T.J. Guest Lecture for WL 411: "Genetics, Genomics, and their Applications in Wildlife Conservation: Principles of Wildlife Management", South Dakota State University, Brookings, South Dakota. (September 21, 2022). In person.

Wolf T.M. A balancing act: the conflicting impacts of wolves on moose survival and health. International Wolf Center Webinar Series, virtual event with 84 registrants. (February 15, 2023).

Wolf, TM Moose mysteries: Unraveling the epidemiology of *Parelaphostrongylus tenuis* transmission to moose. School of Environmental Studies, Fall lecture, 2021, 2022.

Wolf, T. M. Unlocking the mystery of moose mortality in Minnesota: the interaction of species behavior, predators, and disease. Macalaster College Biology Department Seminar Series Macalaster College, St. Paul, Minnesota. (March 3, 2022). Invited.

Garwood, T. 2022. Presentation: Seeking Solutions for Minnesota's Moose Decline: Illuminating Parelaphostrongylus tenuis ecology with next-generation sequencing. SDSU Natural Resource Management Fall Seminar Series, Brookings, South Dakota. (September 23, 2022). Invited.

Garwood T, Moore S, Fountain-Jones N. Larsen P., Wolf T. 2020. Infographic: Which Slugs or Snails Transmit Brainworm to Moose? *UMN College of Veterinary Medicine Office of the Associate Dean of Research YouTube Channel*. Video Link: https://www.youtube.com/watch?v=WRxK-oZhCxI

Most Recent Presentations:

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. Landscape feature determinants of Parelaphostrongylus tenuis transmission in northeastern Minnesota (Poster). Annual Wildlife Disease Association Conference, Athens, Georgia. (July 30 - August 4, 2023).

Garwood, T. Parelaphostrongylus tenuis's role in Minnesota's moose decline: Investigating it's relative importance, ecology, and potential management by integrating modern field, molecular, and statistical tools (Presentation). Comparative and Molecular Biosciences Defense Seminar, College of Veterinary Medicine, University of Minnesota. (July 27th, 2023).

Wolf, T. M. "Parasite Transmission as a Key Aspect for Understanding a Moose Population Decline," II Symposium of Ecoparasitology CAPES - Coordination for the Improvement of Higher Education Personnel, Campo Grande, Brazil. (June 6, 2023). Invited.

Garwood, T., Severud, W., Windels, S., Wuenschmann, A., Isaac, E., Moore, S., Wolf, T. Cause-specific mortality of moose in Grand Portage Indian Reservation and Voyageurs National Park, Minnesota over 12 years: Brainworm's contribution to the decline and indications of wolf prey switching (Presentation). 55th North American Moose Conference and Workshop, Grand Portage, Minnesota. (May 22-26, 2023).

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. Landscape feature determinants of Parelaphostrongylus tenuis transmission in northeastern Minnesota (Presentation). 55th North American Moose Conference and Workshop, Grand Portage, Minnesota. (May 22-26, 2023).

Garwood, T., Moore, S., Fountain-Jones, N. Larsen, P., Wolf, T. Which Slugs or Snails Transmit Brainworm to Moose? (Presentation). Fond du Lac Tribal & Community College Earth Week Seminars, Cloquet, Minnesota. (April 19, 2023). Invited.

Publications

Oliveira-Santos, L. R., Moore, S. A., Severud, W. J., Forester, J. D., Isaac, E. J., Chenaux-Ibrahim, Y., Garwood, T., Escobar, L. E., & Wolf, T. M. (2021). Spatial compartmentalization: A nonlethal predator mechanism to reduce parasite transmission between prey species. Science Advances, 7(52). doi: 10.1126/sciadv.abj5944

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2023. Species in the feces: DNA metabarcoding to detect potential gastropod hosts of *Parelaphostrongylus* tenuis consumed by moose (*Alces alces*). Journal of Wildlife Disease, 59(4): 00-00 (pre-released).

Garwood, T., Richards, J., Macchietto, M., Gerhold, R., Garbe, J., Kania, S., Fountain-Jones, N., Larsen, P., Wolf, T., 2023. A de novo whole genome assembly and annotation of Parelaphostrongylus tenuis. Journal of Nematology, *in 2nd review.*

Garwood, T., Moore, S., Fountain-Jones, N., Larsen, P., Wolf, T. 2023. Exploration of landscape feature determinants of *Parelaphostrongylus tenuis* transmission in northeastern Minnesota. Molecular Ecology, *In preparation*

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

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

Explanation of Use of Classified Staff: N/A

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

Enter Total Estimated Personnel Hours for entire duration of project: 7997.6	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 3.85

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

Enter Total Estimated Contract Personnel Hours for entire duration of project: 2,007.2	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: 0.97

VI. PROJECT PARTNERS:

Name	Title	Affiliation	Role
Veterinary Population Medicine; Fisheries, Wildlife and Conservation Biology; UMN Genomics Center		University of Minnesota	Lead project partner

Name	Title	Affiliation	Role
Grand Portage Dept. of Biology and Environment – Seth Moore As of 3/11/22, the project manager confirms that Dr. Seth Moore is not deriving any direct personal financial benefit from this project.	Director	Grand Portage Band of Lake Superior Chippewa	Co-leading project partner
Luis Escobar	Assistant Professor	Virginia Tech	Collaborating partner

A. Partners outside of project manager's organization receiving ENRTF funding
Biologist (TBD), Grand Portage Department of Biology and Environment, Grand Portage Band of Lake Superior Chippewa, data acquisition and management in Grand Portage
Biology technician (TBD), Grand Portage Department of Biology and Environment, Grand Portage Band of Lake Superior Chippewa, field logistics and data acquisition
Luis Escobar, PhD, Assistant Professor, Department of Fish and Wildlife Conservation, Virginia Tech, Co-Lead on Activity 1.
B. Partners outside of project manager's organization NOT receiving ENRTF funding
Seth Moore, PhD, Director of Biology and Environment, Grand Portage Band of Chippewa, Project Lead for Grand Portage

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The proposed activities will help fill high priority research gaps in understanding the ecology of brainworm transmission to moose with a primary intent to inform management. The goal of this research is the identification of key areas in transmission that may be effectively targeted by resource management to mitigate moose exposure. We expect additional research will arise from this endeavor, which will further hone management decisions. In particular, we will use the data derived from this project in future proposals to NSF programs (NSF-EEID, https://nsf.gov/funding/pgm_summ.jsp?pims_id=5269 and NSF-NHCS, https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=13681).

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet
D. Acquisition, Easements, and Restoration Requirements
E. Research Addendum

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet
Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03f
Project Manager: Tiffany Wolf
Project Title: Next step in helping Minnesota's moose: Understand brainworm transmission to find solutions
Organization: University of Minnesota
Project Budget: $400,000
Project Length and Completion Date: 4 years, July 2023
Today's Date: 1/26/24

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET		**Revised Budget 1/24/24**	**Amount Spent**	**Balance**
BUDGET ITEM				
Personnel (Wages and Benefits)		$ 347,055		$ 2,280
UMN Assistant Professor: Tiffany Wolf* - $42,987 (9.5% salary, 34.2% fringe) 9.5% FTE x 3 yrs; James Forester (9-month appointment) - $9,217 (2% salary, 34.2% fringe) 2% FTE x 3yrs *Wolf holds a 12-month contract appointment which stipulates recruitment of 25% of salary and fringe for research effort from externally funded contracts or grants. Wolf is the overall project manager, organizing all personnel across activities, as well as directly supervising and mentoring project post-doctoral researchers.	$ 52,204		$ 52,369	
Graduate Research Associate: Tyler Garwood - $140,220 (50% salary + 77.4% benefits) 50% FTE x 3yrs Garwood will participate in all research activities associated with Activities 2 and 3 as part of his thesis research	$ 140,220		$ 158,194	
Post-doctoral Researcher: TBD - $120,319 (100% salary + 23.0% fringe) 100% FTE x 2 yr A full-time post-doctoral researcher in spatial epidemiology will be hired in Year 1 to perform all analyses of Activity 1 under the mentorship of Forrester and	$ 120,319		$ 106,712	
Researcher 5: William Severud - $34,312 ($28,380 salary +$5,932 fringe) 52% FTE x 6 months Severud will facilitate data processing, analysis, management, and archival, as well as outreach and reporting activities associated with this project.	$ 34,312		$ 27,500	
Professional/Technical/Service Contracts				
Nick Fountain-Jones Contract: $12,311 ($32.50/hour compensation) 9% FTE x 2 yrs Fountain-Jones is a community ecologist and post-doctoral researcher who mentor the graduate research assistant and contribtue to analyses of Activity 2 and 3.		$ 12,311	$ 8,222	$ 4,089
Virgnia Tech Contract: Luis Escobar, Assistant Professor (9-month appointment): $7,241 (2% salary + 7.75% fringe) 2% FTE x 3 yrs. Escobar is a biogreographer with specialized expertise in spatial and ecological niche modeling. He joined this research team 2 years ago when he held a post-doctoral position at University of Minnesota and will continue as Co-Investigator on this project in his new faculty position at Virginia Tech, given his expertise and input in the development of the project goals and design. He will have a leading role in Activity 1.		$ 7,802	$ 7,802	$ 0
UMGC metagenomics and molecular services for Activities 2 and 3 (Metabarcoding (180 samples x $40/sample) = $7,222; RadSeq (150 = $6,138)		$ 13,360	$ 13,351	$ 9
Equipment/Tools/Supplies				$ -
Disposable supplies: sample collection and preservation, laboratory processing		$ 8,540	$ 8,540	$ 0
Publishing costs		$ 4,508	$ 4,308	$ 200
Computer software for data analysis		$ 1,000	$ 600	$ 400
Travel expenses in Minnesota				$ -
2 twelve-week trips to Grand Portage (GP) from St. Paul by grad RA to collect field data for Activities 2 and 3 ($7,932), 4 1-week trips to GP by various project personnel to accomplish project activities, 1 three-day trip/year by Co-Investigator (Escobar) from Blacksburg, VA to St. Paul for annual in-person project meetings and activities ($2,500). Round trip travel to GP includes mileage ($350/round trip), lodging for student ($64/week) or faculty ($60/night), and a weekly meal budget ($80/week).		$ 4,174	$ 4,174	$ (0)
Other				$ -
Out of state travel: This funding supports travel to VA and/or lodging in VA for the 4-week duration of mentorship, while the RA will be responsible for maintaining their permanent residence in MN during this temporary time away. 1 4-week trip to Blacksburg, VA by post-doctoral RA to work under direction of Escobar in year 1 for Activity 1 ($1,100 to cover either round trip travel and/or lodging during project work - additional funds will be obtained from other funding sources). Having the RA work directly under the mentorship and unique expertise of Co-Investigator Escobar over several weeks is the most efficient approach to accomplishing objectives under Activity 1; Or travel to 1-2 scientific conferences to present project findings (e.g. International or North American Moose Conference, Wildlife Disease Association, Native American Fish and Wildlife conference): airfare ($500) and conference registration or lodging ($322). Note: most of these conferences meet outside of MN, but presentation of findings at these large conferences will facilitate broad dissemination of findings, and position MN as a leader in moose and brainworm research.		$ 1,250	$ 1,250	$ 0
COLUMN TOTAL		$ 400,000	$ 393,021	$ 6,979

OTHER FUNDS CONTRIBUTED TO THE PROJECT	**Status (secured or pending)**	**Revised Budget 1/24/24**	**Spent**	**Balance**
Non-State: College of Veterinary Medicine's Population Systems Signature Program Grant, Project Title: Untangling Parelaphostrongylus tenuis transmission in a declining moose population, Project Manager: Tiffany Wolf	secured	$ 64,098	$ 64,098	$ -
Non-State: College of Veterinary Medicine's contract with Grand Portage for moose research, covers an additional 12% personnel for Tiffany Wolf on project, supplies, and travel between St. Paul and Grand Portage.	secured	$ 47,535	$ 47,535	$ -
In kind: In Kind from Virginia Tech (covers approximately 5% additional personnel time for Luis Escobar's contribution to the project)	secured	$ 20,000	$ 20,000	$ -
In kind: In Kind Services from Grand Portage Band of Chippewa (Grand Portage NRM will provide in-kind support for the three-year project in a total amount of $259,210.00 which includes salary from personnel time (including Seth Moore at 5% FTE) with corresponding 13.06% fringe, in-kind support of collaring moose and deer, equipment (GPS, computer, printer, digital camera). Not included in calculations are vehicle use, fuel costs, travel costs, and miscellaneous supplies and equipment required to conduct operations over the two-year period.): $259,210;	secured	$ 259,210	$ 383,684	$ (124,474)

PAST AND CURRENT ENRTF APPROPRIATIONS	**Amount legally obligated but not yet spent**		**Spent**	
Past appropriation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04g, Project Title: Chemicals of emerging concern in subsistence species used by Minnesota Chippewa, Project manager: Seth Moore	$ 106,056	$ 400,000	$ 400,000	$ -
Past appropriation: ML 2016, Ch 186, Sec 2, Subd 6a, Project Title: Understanding the benefits and limitations of using goats for invasive plant control, Project manager: Tiffany Wolf	$ 242,882	$ 445,533	$ 317,700	$ 127,833
Past appropriations:			$ -	$ -

Which Slugs or Snails Transmit Brainworm to Moose?

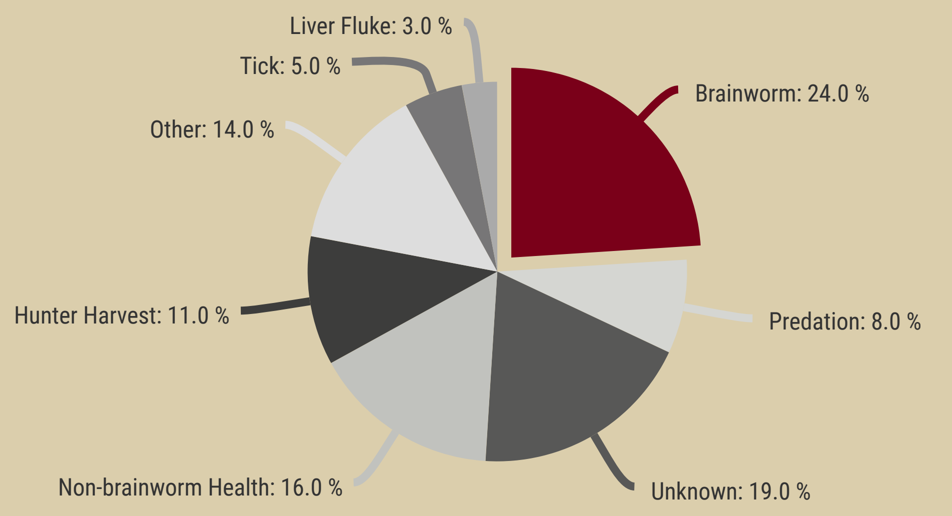
University of Minnesota
College of Veterinary Medicine

What is killing Minnesota moose?

58%
MOOSE POPULATION DECLINES SINCE 2006

24%
OF MOOSE MORTALITY DUE TO BRAINWORM

#1
CAUSE OF MOOSE MORTALITY IS BRAINWORM



Cause-specific Mortality Study by Grand Portage Department of Biology and Environment

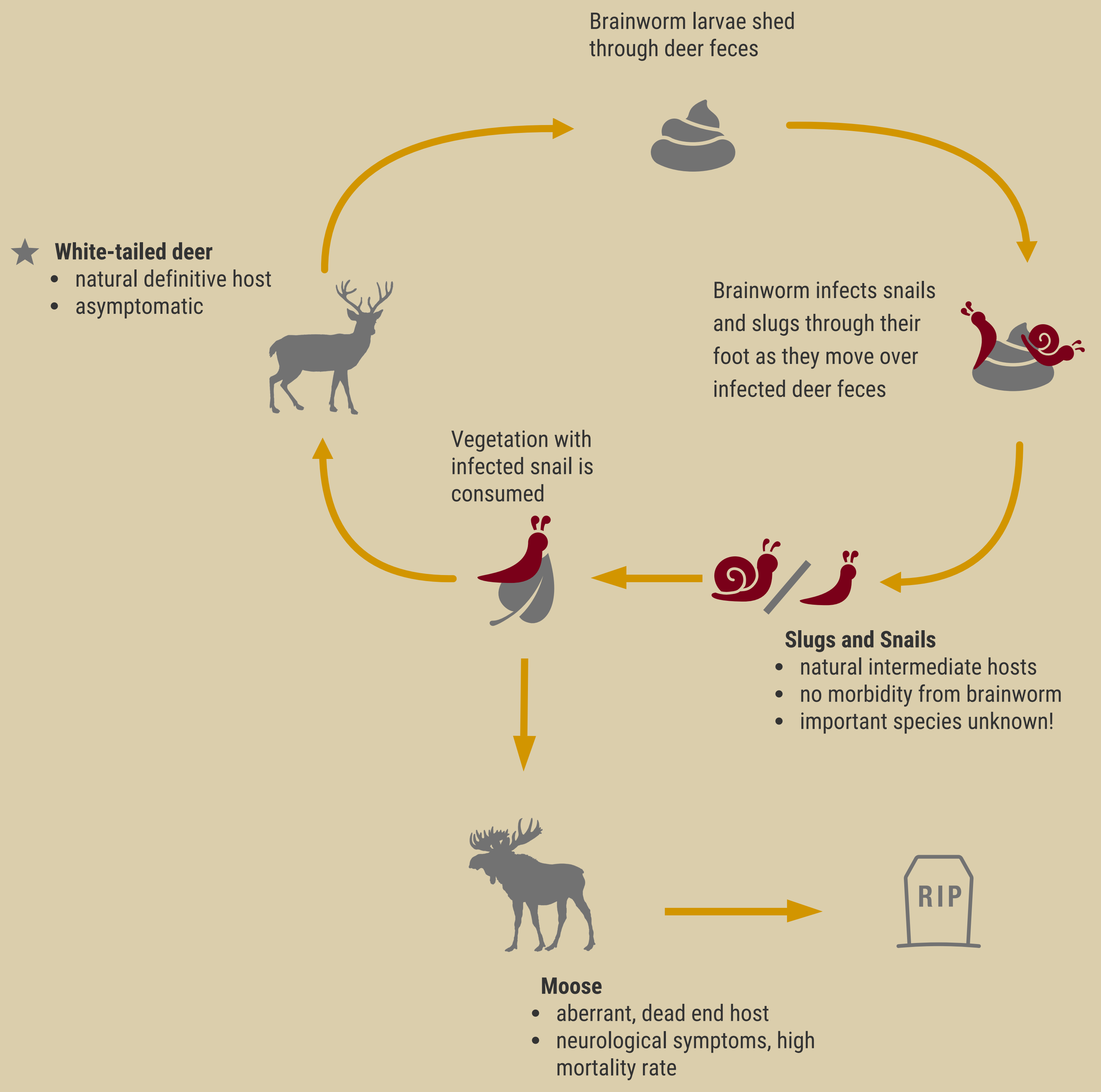
Reasons to save Minnesota moose

Moose hunting is both a treaty right and source of cultural preservation for Minnesota Anishinaabe people.

Wildlife viewing is a $75.9 billion industry in the United States.[1] Moose hunting garners state conservation revenue.

Moose structure aquatic vegetation by grazing and cycle nutrients between terrestrial and aquatic environments.[2]

How do snails and slugs transmit brainworm?[3]



Brainworm larvae shed through deer feces

★ **White-tailed deer**
- natural definitive host
- asymptomatic

Brainworm infects snails and slugs through their foot as they move over infected deer feces

Vegetation with infected snail is consumed

Slugs and Snails
- natural intermediate hosts
- no morbidity from brainworm
- important species unknown!

Moose
- aberrant, dead end host
- neurological symptoms, high mortality rate

How do we find out which snails and slugs moose eat?

DNA barcoding identifies slug and snail species in moose feces. Barcoding a species in a sample is similar to checking out at the grocery store.

Each item in the store has a tag with a barcode

The grocery store has a database of barcodes and corresponding cost

Matching the barcode on the item with the barcode in the database gives you the cost

Similarly, fragments of DNA present in all animals serve as "barcodes" for species identity.[3] Scientists built databases of these DNA fragments sequences for as many species as possible.

Each species consumed by the moose has a unique DNA fragment

The lab has a database of DNA fragment sequences and corresponding species

Matching DNA in the sample with DNA in the database gives you the species

How can we use this information to conserve moose?

Wildlands Management

If we learn about the ecology of important slug and snail host species, we can manage the landscape to reduce their abundance in moose range.

Carrier Competency Studies

Little is known about which slug and snail species are capable of harboring a brainworm infection. Knowing which species moose consume helps prioritize species for carrier competency studies.

Infection Risk Maps

Accounting for the distribution of all hosts in multi-host transmission systems is an important component of assessing transmission risk.

What you can do to help save moose

This and other important research aimed at preventing the Minnesota moose decline is supported both by larger funding sources and citizens like you! Please support our research by scanning this QR code and contributing:

Funding and support from:

Grand Portage Chippewa

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND

Van Sloun Foundation

University of Minnesota
GENOMICS CENTER

University of Minnesota
College of Veterinary Medicine

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1) National FHWAR Survey, 2016, US Fish and Wildlife Service
2) Bergman & Bump, 2015, Freshwater Biology, 60, 1635-1646
3) Lankester, 2010, Alces, 46, 53-70
4) Hebert et al, Proc. Royal Soc. B, 2003, 270, 313-321

Contact Tyler Garwood (garwo010@umn.edu), Dr. Tiffany Wolf (wolfx305@umn.edu), or Dr. Seth Moore (samoore@boreal.org) for more information