



Environment and Natural Resources Trust Fund

2027 Request for Proposal

General Information

Proposal ID: 2027-284

Proposal Title: Moose Pathogen Surveillance and Genotyping Using Engorged Ticks

Project Manager Information

Name: Jonathan Oliver

Organization: U of MN - School of Public Health

Office Telephone: (515) 450-0984

Email: joliver@umn.edu

Project Basic Information

Project Summary: We will test moose blood found in engorged Winter ticks to determine what blood-borne pathogens are present in MN moose and develop a genotype database for identifying individual moose.

ENRTF Funds Requested: \$129,000

Proposed Project Completion: June 30, 2028

LCCMR Funding Category: Small Projects (G)

Secondary Category: Fish and Wildlife (D)

Project Location

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

Region(s): Metro

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

Statewide

When will the work impact occur?

In the Future

Narrative

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

As the distribution of white-tailed deer expands northward it overlaps more and more with that of Minnesota's moose population. This brings moose into contact with deer pathogens to which they are susceptible but poorly adapted to resist. For example, the brainworm, *Parelaphostrongylus tenuis*, causes minimal impacts in deer but is ultimately fatal in moose. Affected moose often develop large Winter tick (*Dermacentor albipictus*) burdens that result in patchy fur and "ghost moose" syndrome. Winter ticks bite both deer and moose and are associated with the transmission of several pathogens, some of which cause disease in deer and may have more severe impacts on non-resistant moose.

To understand, manage, and mitigate the new disease stressors that Minnesota's moose population is facing, we need to find out what pathogens are present in their blood. Capturing and sampling moose to detect bloodborne pathogens is expensive, complex, and causes stress to the animals. Previous collaring activities in Minnesota have resulted in the mortality of at least 12 moose (https://downloads.regulations.gov/FWS-R3-ES-2016-0061-0057/attachment_4.pdf). The potential for a non-invasive strategy for detecting disease-causing pathogens in moose is worth investigating to guide management to maintain the future health of this important cultural symbol.

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

Winter ticks are abundant on moose and feed by concentrating blood cells. Engorged ticks drop off of moose and are found in high numbers at bedding locations. We will use engorged ticks to perform surveillance for DNA from six bloodborne pathogens suspected to be present in moose. Of particular concern is *P. tenuis*, a nematode parasite of deer that causes lethal neurological damage in moose. To evaluate the efficacy of testing ticks for the presence of *P. tenuis*, tick samples associated with known positive or negative moose will be supplied by the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL).

Tick samples collected from bedding sites will be difficult to associate with individual moose if they are found to be positive for pathogens. We will develop a moose forensic database of genetic markers that will allow us to associate tick samples with specific individual moose. While initially this database will include only deceased moose, this work will lay the groundwork for a population-level database allowing DNA from moose ticks, fur, or tissues to be associated with individual, living moose. It will inform future management decisions using moose population genetics, disease transmission patterns, and the migration of new moose into the state.

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

1. Determine if engorged ticks taken from tested moose can be used to detect the presence of the *P. tenuis* nematode.
2. Detect the presence and prevalence of bloodborne bacteria and piroplasm pathogens associated with deer and moose in moose blood-engorged ticks.
3. Test methodology and begin building a moose blood-based forensic database to individuate specific moose.

Activities and Milestones

Activity 1: Test engorged Winter ticks for the presence of *P. tenuis* and other bloodborne pathogens

Activity Budget: \$36,656

Activity Description:

Engorged ticks will be tested for the presence of *P. tenuis*, and several tickborne pathogens associated with moose and deer: *Anaplasma phagocytophilum*, *A. odocoilei*, *Ehrlichia* spp., *Babesia odocoilei*, and *Theileria cervi*. Ticks will be supplied by the UMDVL and will be associated with deceased moose that the lab has previously tested for *P. tenuis* so we can compare known positive and negative moose samples with the engorged ticks. To avoid potential bias, researchers testing ticks will be blinded to moose infection status until after the tests are completed.

Testing of ticks for bloodborne pathogens will be performed using quantitative PCR targeting diagnostic genes for each of the pathogens listed. Five ticks will be tested from each moose with a goal of testing at least 20 moose for each of the six surveyed pathogens. This will determine what pathogens are in circulation in the blood of these moose, prevalence of infection, and efficacy of testing engorged ticks for *P. tenuis* detection. To help ensure acquisition of sufficient ticks for testing, collection by the Veterinary Diagnostic Lab would begin in Jan. 2027, prior to project initiation, and continue through April, 2028 so ticks can be collected over two seasons of activity.

Activity Milestones:

Description	Approximate Completion Date
Procure 1st set of engorged tick samples	July 31, 2027
Test 1st set of ticks for <i>P. tenuis</i> and bloodborne pathogens	November 30, 2027
Procure 2nd set of engorged tick samples	April 30, 2028
Test 2nd set of ticks for <i>P. tenuis</i> and bloodborne pathogens	May 31, 2028
Pathogen genetic data analysis	June 30, 2028

Activity 2: Develop a moose forensic database based on individual-specific genetic markers

Activity Budget: \$92,344

Activity Description:

Blood from engorged ticks will be used to produce a pilot database for individuating moose based on short-tandem repeat forensic DNA typing. Typing will be based on 12 autosomal microsatellites and one sex typing marker. These will provide sufficient genetic variation to tell moose apart from one another and will provide genetic validation of the sex of the sample. These 12 genetic markers have been previously validated as effective for forensic typing by a study performed in Alberta and Yukon produced in order to identify the origin of meat from poached animals (DOI: 10.1016/j.fsigen.2021.102536). We will evaluate the effectiveness of this set of genetic markers in Minnesota moose and begin the establishment of a moose genetic database for the state.

Genetic markers from each sample will be amplified in two multiplex PCR reactions then capillary electrophoresis will be performed to produce differentiable genotypes based on allele variation at each examined gene locus. Database construction and population genetic analysis will be based on the individual variation pattern of each moose blood sample. If blood from engorged ticks is insufficient to produce a genotype, preserved tissues from the moose of origin will be used instead.

Activity Milestones:

Description	Approximate Completion Date
Procure 1st set of engorged tick/tissue samples for genotyping	July 31, 2027
Perform genotyping lab work on 1st set of samples	January 31, 2028
Procure 2nd set of engorged tick/tissue samples for genotyping	April 30, 2028
Perform genotyping lab work on 1st set of samples	May 31, 2028
Build moose genetic database and descriptive statistical analysis	June 30, 2028

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Benjamin Cull	University of Minnesota - Department of Entomology	Dr. Cull is an expert in tick identification, surveillance, and laboratory. He will assist Dr. Oliver in processing samples, performing laboratory work, analyzing data, reporting results, and managing project staff.	Yes
Arno Wuenschmann	University of Minnesota - Veterinary Diagnostic Laboratory	Dr. Wuenschmann will provide tick samples from moose that have been tested for the presence of the <i>P. tenuis</i> nematode.	No

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.

Dissemination of our results will be important for producing an expansive bloodborne pathogen surveillance and moose genotype network. We will disseminate pilot project results to MN DNR, MN Board of Animal Health, and potential tribal and state agency collaborators interested in collecting engorged ticks for pathogen testing and/or genotyping. We will develop a peer-reviewed scientific publication for researchers and a factsheet to distribute to wildlife management personnel and lay people. The moose genetic database will be maintained at the Data Repository for U of M (DRUM) where it will be publicly available and all genetic data generated will be uploaded to the National Center for Biotechnology Information, a publicly available data hub managed by the federal National Institutes of Health.

Results will be presented at one or more regional professional conferences such as the Midwest Fish and Wildlife Conference or those hosted by local chapters of The Wildlife Society, Society of American Foresters.

In total, we will produce a final project report to document all findings, presentations at regional meetings and a factsheet will inform managerial and lay stakeholders of results, and peer-reviewed publications will provide the methods and results to the larger research community.

All dissemination products will acknowledge the ENRTF as the source of funding for this project. Presentations and the factsheet will show the ENRTF logo in addition to stating the funding source. Scientific publications will indicate the ENRTF as the funding source in the Acknowledgements section.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?

The proposed study is a pilot project designed to evaluate the feasibility of using engorged Winter ticks for the surveillance of moose infection by *P. tenuis* and other bloodborne pathogens, and to establish a methodological approach to the development of a moose forensic genotype database. Future efforts and funding support will be essential to turn these results into useful management tools for studying moose disease transmission and population genetics. Future funding for expansion and implementation will be sought from the ENRTF and federal funding agencies such as the USDA, NIH, and NSF.

Project Manager and Organization Qualifications

Project Manager Name: Jonathan Oliver

Job Title: Associate Professor

Provide description of the project manager's qualifications to manage the proposed project.

Jon Oliver has a PhD in Entomology and specializes in vector-borne diseases, especially those transmitted by ticks. He has extensive experience organizing and managing project performing tick surveillance in the Upper Midwest and evaluating ticks for the presence of pathogens using laboratory methods. Dr. Oliver has an established academic track record in genetic analyses and genomic surveillance of ticks and their associated host vertebrates. He has many collaborators in Minnesota including academics, city, county, and state agencies.

Organization: U of MN - School of Public Health

Organization Description:

The University of Minnesota is the flagship land grant university in Minnesota. The School of Public Health is ranked the #6 public university public health program in the nation.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
Jonathan Oliver		Project management, lab work, analysis, and report			36.6%	0.1		\$20,390
Benjamin Cull		Lab work, analysis, and staff management			36.6%	0.1		\$12,317
2 laboratory staff		Laboratory work and analysis			32.3%	0.5		\$88,081
							Sub Total	\$120,788
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Qiagen Dneasy Blood and Tissue kit (250 reactions)	DNA extraction from ticks and tissues					\$973
	Tools and Supplies	PCR Primers (30 primers)	Primers for PCR pathogen detection					\$450
	Tools and Supplies	Qiagen Multiplex PCR kits (200 reactions)	Reagents for quantitative PCR for moose genotyping					\$848
	Tools and Supplies	Genescan 400HD ROX dye size standard (800 reactions)	Reagent for quantitative PCR for moose genotyping					\$860
	Tools and Supplies	Hi-Di formamide 25 mL x 4	Reagent for capillary electrophoresis for moose genotyping					\$111
	Tools and Supplies	PCR Master Mix (500 reactions)	Reagent for PCR for pathogen detection					\$140
	Tools and Supplies	100 bp ladder (125 lanes)	Reagent for PCR for pathogen detection					\$90
	Tools and Supplies	Powerup SYBR green master mix for qPCR 5 mL	Reagent for quantitative PCR for genotyping					\$512
	Tools and Supplies	GeneMarker software (1 year license)	Software for analysis of moose genotypes					\$1,900
	Equipment	Sanger sequencing (120 samples)	Sanger sequencing to determine PCR product genetic sequence for pathogen detection					\$720

	Tools and Supplies	Capillary electrophoresis (40 samples)	Capillary electrophoresis to separate gene bands for genotyping					\$535
	Tools and Supplies	Misc. lab consumables (gloves, pipette tips, tubes, etc.)	Other small laboratory consumables					\$1,073
							Sub Total	\$8,212
Capital Equipment								
							Sub Total	-
Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								
							Sub Total	-
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
							Sub Total	-
Other Expenses								
							Sub Total	-
							Grand Total	\$129,000

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
---------------	---------------------	-------------	--

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
			Non State Sub Total	-
			Funds Total	-

Total Project Cost: \$129,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: [2d428011-929.pdf](#)

Alternate Text for Visual Component

A summary describing the problem of getting moose samples for disease detection and the approach of collecting engorged ticks for the detection of bloodborne pathogens. We will also begin development of a moose genetic database so that moose-associated samples can be matched to individual moose....

Supplemental Attachments

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

Title	File
U of Minnesota Sponsored Projects Admin letter	70b466c9-36a.pdf
Vet Diagnostic Lab letter of support	a128125d-dde.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

N/A

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

No

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

N/A

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

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing \$10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care,

treatment, education, training, instruction, or recreation to children")?

No

Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:

Kristina McReynolds, University of Minnesota Office of Sponsored Projects Administration

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

N/A