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New Hampshire Fish and Game Department

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Glenn Normandeau
Executive Director

February 23, 2016

Her Excellency, Governor Margaret Wood Hassan
and the Honorable Council
State House
Concord, NH 03301

Requested Action

Authorize the New Hampshire Fish and Game Department to enter into a sole source contract with the Tufts Cummings School of Veterinary Medicine, Northeast Wildlife Disease Cooperative (Vendor Code 163424) to coordinate and administer development of a blood serum based ELISA test for *Paralaphostrongylus tenuis* (brainworm) infection in moose and deer at a cost of \$9,216.00 effective the date of Governor and Council approval through December 31, 2017. Funding is 75% federal funds, 25% Other (Game Management Funds).

Funding is available for this contract as follows with the authority to adjust encumbrances in each of the state fiscal years through the Budget Office if needed and justified:

03 75 75 751520-21580000 - Wildlife Program - Game Management

20-07500-21580000-304-500841 Research & Management	<u>FY 2016</u>	<u>FY 2017</u>
	\$4,608.00	\$4,608.00

Explanation

Paralaphostrongylus tenuis (brainworm) is a meningeal worm parasite of white-tailed deer which are their natural host. While white-tailed deer are unaffected by *P. tenuis* infection, other native cervids such as moose can become infected by transmission through intermediate hosts (terrestrial snails and slugs). Infection of moose by *P. tenuis* is usually fatal. It is believed that *P. tenuis* may be a significant mortality factor for moose in southern New Hampshire in higher deer density areas.

Currently, *P. tenuis* infection is diagnosed by using post-mortem examination of brain and spinal cord sections which is a time consuming and expensive technique with low sensitivity. This makes diagnosis of *P. tenuis* infection impractical on dead moose and impossible on living moose. Development of an enzyme-linked immunosorbent assay (ELISA) diagnostic test for *P. tenuis* infection would provide a practical means of testing living and recently killed moose using blood serum samples. Additionally, sera from white-tailed deer could be collected to assess the distribution and prevalence of *P. tenuis* infection of deer as an aid to understanding the effect of such factors as deer density and intermediate host abundance on deer to moose transmission of *P. tenuis*.

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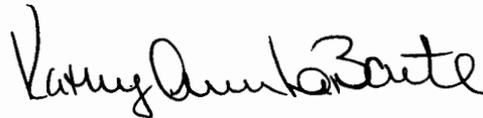
Development of this test will not only contribute to New Hampshire's understanding of moose mortality factors, but will provide a similar benefit to adjacent New England states which are also experiencing moose population declines. Due to the regional nature of the potential problem and the benefit which will be provided by development of this ELISA test, the states of New Hampshire, Maine and Vermont are cooperatively funding this project, each contributing one-third of the total cost.

Development of this test will be coordinated and administered through the Northeast Wildlife Disease Cooperative (NWDC) at the Cummings School of Veterinary Medicine at Tufts University. Additional expertise will be provided through the NWDC by the veterinary diagnostic laboratories at the College of Veterinary Medicine at Cornell University and the College of Veterinary Medicine at The University of Tennessee, Knoxville, both of whom are uniquely skilled given their ongoing work with *P. Tenuis* and ELISA testing, to conduct this highly specialized work. This is a sole source contract request because, as a participant in this three state partnership (NH, ME and VT), the net benefit for New Hampshire is a two-thirds savings on total project costs.

Respectfully submitted,



Glenn Normandeau
Executive Director



Kathy Ann LaBonte, Chief
Business Division

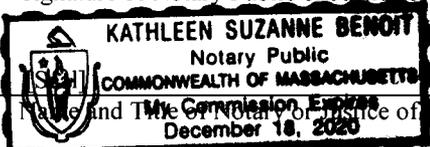
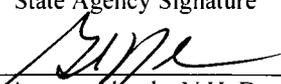
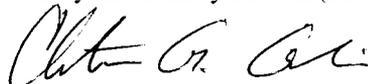
Notice: This agreement and all of its attachments shall become public upon submission to Governor and Executive Council for approval. Any information that is private, confidential or proprietary must be clearly identified to the agency and agreed to in writing prior to signing the contract.

AGREEMENT

The State of New Hampshire and the Contractor hereby mutually agree as follows:

GENERAL PROVISIONS

1. IDENTIFICATION.

1.1 State Agency Name NH Fish and Game Department		1.2 State Agency Address 11 Hazen Drive, Concord, NH 03301	
1.3 Contractor Name Tufts Cummings School of Veterinary Medicine		1.4 Contractor Address Tufts University, 200 Westboro Road, Grafton, MA 01536	
1.5 Contractor Phone Number 508-887-4933	1.6 Account Number 20-07500-21580000-304-500841	1.7 Completion Date 12/31/17	1.8 Price Limitation \$9,216.00
1.9 Contracting Officer for State Agency Glenn Normandeau, Executive Director		1.10 State Agency Telephone Number 603-271-2461	
1.11 Contractor Signature 		1.12 Name and Title of Contractor Signatory Barbara Gardner, Associate Director	
1.13 Acknowledgement: State of Massachusetts ^{Commonwealth} , County of Suffolk On <u>March 2, 2016</u> , before the undersigned officer, personally appeared the person identified in block 1.12, or satisfactorily proven to be the person whose name is signed in block 1.11, and acknowledged that s/he executed this document in the capacity indicated in block 1.12.			
1.13.1 Signature of Notary Public or Justice of the Peace <div style="display: flex; align-items: center;">  <div> Kathleen Suzanne Benoit </div> </div>			
1.13.2 Name and Title of Notary or Justice of the Peace Kathleen Suzanne Benoit Senior Research Administrator			
1.14 State Agency Signature 		1.15 Name and Title of State Agency Signatory Glenn Normandeau, Executive Director	
1.16 Approval by the N.H. Department of Administration, Division of Personnel (if applicable) By: _____ Director, On: _____			
1.17 Approval by the Attorney General (Form, Substance and Execution) (if applicable) By:  On: <u>3/9/16</u>			
1.18 Approval by the Governor and Executive Council (if applicable) By: _____ On: _____			

2. EMPLOYMENT OF CONTRACTOR/SERVICES TO BE PERFORMED. The State of New Hampshire, acting through the agency identified in block 1.1 ("State"), engages contractor identified in block 1.3 ("Contractor") to perform, and the Contractor shall perform, the work or sale of goods, or both, identified and more particularly described in the attached EXHIBIT A which is incorporated herein by reference ("Services").

3. EFFECTIVE DATE/COMPLETION OF SERVICES.

3.1 Notwithstanding any provision of this Agreement to the contrary, and subject to the approval of the Governor and Executive Council of the State of New Hampshire, if applicable, this Agreement, and all obligations of the parties hereunder, shall become effective on the date the Governor and Executive Council approve this Agreement as indicated in block 1.18, unless no such approval is required, in which case the Agreement shall become effective on the date the Agreement is signed by the State Agency as shown in block 1.14 ("Effective Date").

3.2 If the Contractor commences the Services prior to the Effective Date, all Services performed by the Contractor prior to the Effective Date shall be performed at the sole risk of the Contractor, and in the event that this Agreement does not become effective, the State shall have no liability to the Contractor, including without limitation, any obligation to pay the Contractor for any costs incurred or Services performed. Contractor must complete all Services by the Completion Date specified in block 1.7.

4. CONDITIONAL NATURE OF AGREEMENT.

Notwithstanding any provision of this Agreement to the contrary, all obligations of the State hereunder, including, without limitation, the continuance of payments hereunder, are contingent upon the availability and continued appropriation of funds, and in no event shall the State be liable for any payments hereunder in excess of such available appropriated funds. In the event of a reduction or termination of appropriated funds, the State shall have the right to withhold payment until such funds become available, if ever, and shall have the right to terminate this Agreement immediately upon giving the Contractor notice of such termination. The State shall not be required to transfer funds from any other account to the Account identified in block 1.6 in the event funds in that Account are reduced or unavailable.

5. CONTRACT PRICE/PRICE LIMITATION/PAYMENT.

5.1 The contract price, method of payment, and terms of payment are identified and more particularly described in EXHIBIT B which is incorporated herein by reference.

5.2 The payment by the State of the contract price shall be the only and the complete reimbursement to the Contractor for all expenses, of whatever nature incurred by the Contractor in the performance hereof, and shall be the only and the complete compensation to the Contractor for the Services. The State shall have no liability to the Contractor other than the contract price.

5.3 The State reserves the right to offset from any amounts otherwise payable to the Contractor under this Agreement those liquidated amounts required or permitted by N.H. RSA 80:7 through RSA 80:7-c or any other provision of law.

5.4 Notwithstanding any provision in this Agreement to the contrary, and notwithstanding unexpected circumstances, in no event shall the total of all payments authorized, or actually made hereunder, exceed the Price Limitation set forth in block 1.8.

6. COMPLIANCE BY CONTRACTOR WITH LAWS AND REGULATIONS/ EQUAL EMPLOYMENT OPPORTUNITY.

6.1 In connection with the performance of the Services, the Contractor shall comply with all statutes, laws, regulations, and orders of federal, state, county or municipal authorities which impose any obligation or duty upon the Contractor, including, but not limited to, civil rights and equal opportunity laws. This may include the requirement to utilize auxiliary aids and services to ensure that persons with communication disabilities, including vision, hearing and speech, can communicate with, receive information from, and convey information to the Contractor. In addition, the Contractor shall comply with all applicable copyright laws.

6.2 During the term of this Agreement, the Contractor shall not discriminate against employees or applicants for employment because of race, color, religion, creed, age, sex, handicap, sexual orientation, or national origin and will take affirmative action to prevent such discrimination.

6.3 If this Agreement is funded in any part by monies of the United States, the Contractor shall comply with all the provisions of Executive Order No. 11246 ("Equal Employment Opportunity"), as supplemented by the regulations of the United States Department of Labor (41 C.F.R. Part 60), and with any rules, regulations and guidelines as the State of New Hampshire or the United States issue to implement these regulations. The Contractor further agrees to permit the State or United States access to any of the Contractor's books, records and accounts for the purpose of ascertaining compliance with all rules, regulations and orders, and the covenants, terms and conditions of this Agreement.

7. PERSONNEL.

7.1 The Contractor shall at its own expense provide all personnel necessary to perform the Services. The Contractor warrants that all personnel engaged in the Services shall be qualified to perform the Services, and shall be properly licensed and otherwise authorized to do so under all applicable laws.

7.2 Unless otherwise authorized in writing, during the term of this Agreement, and for a period of six (6) months after the Completion Date in block 1.7, the Contractor shall not hire, and shall not permit any subcontractor or other person, firm or corporation with whom it is engaged in a combined effort to perform the Services to hire, any person who is a State employee or official, who is materially involved in the procurement, administration or performance of this

Contractor Initials BO
Date 3/2/16

Agreement. This provision shall survive termination of this Agreement.

7.3 The Contracting Officer specified in block 1.9, or his or her successor, shall be the State's representative. In the event of any dispute concerning the interpretation of this Agreement, the Contracting Officer's decision shall be final for the State.

8. EVENT OF DEFAULT/REMEDIES.

8.1 Any one or more of the following acts or omissions of the Contractor shall constitute an event of default hereunder ("Event of Default"):

8.1.1 failure to perform the Services satisfactorily or on schedule;

8.1.2 failure to submit any report required hereunder; and/or

8.1.3 failure to perform any other covenant, term or condition of this Agreement.

8.2 Upon the occurrence of any Event of Default, the State may take any one, or more, or all, of the following actions:

8.2.1 give the Contractor a written notice specifying the Event of Default and requiring it to be remedied within, in the absence of a greater or lesser specification of time, thirty (30) days from the date of the notice; and if the Event of Default is not timely remedied, terminate this Agreement, effective two (2) days after giving the Contractor notice of termination;

8.2.2 give the Contractor a written notice specifying the Event of Default and suspending all payments to be made under this Agreement and ordering that the portion of the contract price which would otherwise accrue to the Contractor during the period from the date of such notice until such time as the State determines that the Contractor has cured the Event of Default shall never be paid to the Contractor;

8.2.3 set off against any other obligations the State may owe to the Contractor any damages the State suffers by reason of any Event of Default; and/or

8.2.4 treat the Agreement as breached and pursue any of its remedies at law or in equity, or both.

9. DATA/ACCESS/CONFIDENTIALITY/PRESERVATION.

9.1 As used in this Agreement, the word "data" shall mean all information and things developed or obtained during the performance of, or acquired or developed by reason of, this Agreement, including, but not limited to, all studies, reports, files, formulae, surveys, maps, charts, sound recordings, video recordings, pictorial reproductions, drawings, analyses, graphic representations, computer programs, computer printouts, notes, letters, memoranda, papers, and documents, all whether finished or unfinished.

9.2 All data and any property which has been received from the State or purchased with funds provided for that purpose under this Agreement, shall be the property of the State, and shall be returned to the State upon demand or upon termination of this Agreement for any reason.

9.3 Confidentiality of data shall be governed by N.H. RSA chapter 91-A or other existing law. Disclosure of data requires prior written approval of the State.

10. TERMINATION. In the event of an early termination of this Agreement for any reason other than the completion of the Services, the Contractor shall deliver to the Contracting Officer, not later than fifteen (15) days after the date of termination, a report ("Termination Report") describing in detail all Services performed, and the contract price earned, to and including the date of termination. The form, subject matter, content, and number of copies of the Termination Report shall be identical to those of any Final Report described in the attached EXHIBIT A.

11. CONTRACTOR'S RELATION TO THE STATE. In the performance of this Agreement the Contractor is in all respects an independent contractor, and is neither an agent nor an employee of the State. Neither the Contractor nor any of its officers, employees, agents or members shall have authority to bind the State or receive any benefits, workers' compensation or other emoluments provided by the State to its employees.

12. ASSIGNMENT/DELEGATION/SUBCONTRACTS. The Contractor shall not assign, or otherwise transfer any interest in this Agreement without the prior written notice and consent of the State. None of the Services shall be subcontracted by the Contractor without the prior written notice and consent of the State.

13. INDEMNIFICATION. The Contractor shall defend, indemnify and hold harmless the State, its officers and employees, from and against any and all losses suffered by the State, its officers and employees, and any and all claims, liabilities or penalties asserted against the State, its officers and employees, by or on behalf of any person, on account of, based or resulting from, arising out of (or which may be claimed to arise out of) the acts or omissions of the Contractor. Notwithstanding the foregoing, nothing herein contained shall be deemed to constitute a waiver of the sovereign immunity of the State, which immunity is hereby reserved to the State. This covenant in paragraph 13 shall survive the termination of this Agreement.

14. INSURANCE.

14.1 The Contractor shall, at its sole expense, obtain and maintain in force, and shall require any subcontractor or assignee to obtain and maintain in force, the following insurance:

14.1.1 comprehensive general liability insurance against all claims of bodily injury, death or property damage, in amounts of not less than \$1,000,000 per occurrence and \$2,000,000 aggregate; and

14.1.2 special cause of loss coverage form covering all property subject to subparagraph 9.2 herein, in an amount not less than 80% of the whole replacement value of the property.

14.2 The policies described in subparagraph 14.1 herein shall be on policy forms and endorsements approved for use in the State of New Hampshire by the N.H. Department of Insurance, and issued by insurers licensed in the State of New Hampshire.

14.3 The Contractor shall furnish to the Contracting Officer identified in block 1.9, or his or her successor, a certificate(s) of insurance for all insurance required under this Agreement. Contractor shall also furnish to the Contracting Officer identified in block 1.9, or his or her successor, certificate(s) of insurance for all renewal(s) of insurance required under this Agreement no later than thirty (30) days prior to the expiration date of each of the insurance policies. The certificate(s) of insurance and any renewals thereof shall be attached and are incorporated herein by reference. Each certificate(s) of insurance shall contain a clause requiring the insurer to provide the Contracting Officer identified in block 1.9, or his or her successor, no less than thirty (30) days prior written notice of cancellation or modification of the policy.

15. WORKERS' COMPENSATION.

15.1 By signing this agreement, the Contractor agrees, certifies and warrants that the Contractor is in compliance with or exempt from, the requirements of N.H. RSA chapter 281-A ("*Workers' Compensation*").

15.2 To the extent the Contractor is subject to the requirements of N.H. RSA chapter 281-A, Contractor shall maintain, and require any subcontractor or assignee to secure and maintain, payment of Workers' Compensation in connection with activities which the person proposes to undertake pursuant to this Agreement. Contractor shall furnish the Contracting Officer identified in block 1.9, or his or her successor, proof of Workers' Compensation in the manner described in N.H. RSA chapter 281-A and any applicable renewal(s) thereof, which shall be attached and are incorporated herein by reference. The State shall not be responsible for payment of any Workers' Compensation premiums or for any other claim or benefit for Contractor, or any subcontractor or employee of Contractor, which might arise under applicable State of New Hampshire Workers' Compensation laws in connection with the performance of the Services under this Agreement.

16. WAIVER OF BREACH. No failure by the State to enforce any provisions hereof after any Event of Default shall be deemed a waiver of its rights with regard to that Event of Default, or any subsequent Event of Default. No express failure to enforce any Event of Default shall be deemed a waiver of the right of the State to enforce each and all of the provisions hereof upon any further or other Event of Default on the part of the Contractor.

17. NOTICE. Any notice by a party hereto to the other party shall be deemed to have been duly delivered or given at the time of mailing by certified mail, postage prepaid, in a United States Post Office addressed to the parties at the addresses given in blocks 1.2 and 1.4, herein.

18. AMENDMENT. This Agreement may be amended, waived or discharged only by an instrument in writing signed by the parties hereto and only after approval of such amendment, waiver or discharge by the Governor and Executive Council of the State of New Hampshire unless no

such approval is required under the circumstances pursuant to State law, rule or policy.

19. CONSTRUCTION OF AGREEMENT AND TERMS.

This Agreement shall be construed in accordance with the laws of the State of New Hampshire, and is binding upon and inures to the benefit of the parties and their respective successors and assigns. The wording used in this Agreement is the wording chosen by the parties to express their mutual intent, and no rule of construction shall be applied against or in favor of any party.

20. THIRD PARTIES. The parties hereto do not intend to benefit any third parties and this Agreement shall not be construed to confer any such benefit.

21. HEADINGS. The headings throughout the Agreement are for reference purposes only, and the words contained therein shall in no way be held to explain, modify, amplify or aid in the interpretation, construction or meaning of the provisions of this Agreement.

22. SPECIAL PROVISIONS. Additional provisions set forth in the attached EXHIBIT C are incorporated herein by reference.

23. SEVERABILITY. In the event any of the provisions of this Agreement are held by a court of competent jurisdiction to be contrary to any state or federal law, the remaining provisions of this Agreement will remain in full force and effect.

24. ENTIRE AGREEMENT. This Agreement, which may be executed in a number of counterparts, each of which shall be deemed an original, constitutes the entire Agreement and understanding between the parties, and supersedes all prior Agreements and understandings relating hereto.

EXHIBIT A

Scope of Services

A: Objective

To develop an Enzyme-linked immunosorbent assay (ELISA) to be utilized as a diagnostic test for the determination of *Parelaphostrongylus tenuis* infection of cervid sera.

B: Proposed Methods for Test Development and Optimization

A *P. tenuis* antigenic protein, the aspartyl protease inhibitor ortholog (API), was detected by Duffy et al (2002). Expression of this protein has been confirmed in L1, L3, adult male and adult female worms. It is conserved among *P. tenuis*, does not have strong similarity with proteins in other parasites and is immunogenic in atypical hosts. The gene for this protein was optimized for expression in *E. coli* and synthesized with a His tag in the pET11 expression vector. Western blot assays using this API protein have produced excellent results using sera from moose and deer that were known positive as well as sera from cervids from regions where *P. tenuis* does not exist as our known negatives. Our next step is to map API epitopes using overlapping synthetic peptides followed by fine mapping. Peptides with the highest reactivity with positive sera and low reactivity with negative sera will be used as antigen for ELISA assays. The ELISA is substantially less expensive and less time consuming than a western blot. To perform ELISA assays a 96-well microplate (Immulon 2, Thermo Fisher Scientific, Rockford, IL) will be coated with 100 µl/well of an optimal concentration of API peptide and incubated at 4° C overnight in a humid chamber. Following incubation, wells will be washed 5 times with PBS containing 0.05% Tween20 (PBS-T). Wells will be blocked with 200µl 1.0% bovine serum albumin diluted in PBS-T and incubated for 1hr at 37°C in a humid chamber. After repeating the washing procedure, 100µl of sera from *P. tenuis* –infected cervids diluted in PBS-T will be added to each well, incubated, and washed again. HRP rabbit anti-cervid will be diluted in PBS-T and 100µl added to the wells. Incubation and washing of plates will be performed as in the previous step, followed by adding 100 µl of peroxidase substrate to each well (Pierce: Thermo Fisher Scientific, Rockford, IL). The reaction will be stopped by the addition of sulfuric acid to the wells and the absorbance read at 450nm using an enzyme immunoassay plate reader (Bio-Tek). Wells consisting of all reagents except antigen will be used in each plate to determine background reading and obtain the optical density (OD) for the various sera dilutions. Positive and negative sera will be used on each plate as controls. Results are interpreted by determining the OD level.

C: Testing

Following development and optimization of the test, the cost of testing 100 New Hampshire moose and/or deer serum samples for *P. tenuis* is included as part this contract.

EXHIBIT B

Method of Payment

1. The New Hampshire Fish and Game Department agrees to reimburse the contractor up to \$9,216.00 of approved project expenses.
2. Payment will be made within 30 days of receipt of a proper invoice.
3. The contractor shall submit invoices and provide a written report documenting project progress by June 30 each year and upon project completion.
4. Any publications or publicity regarding this project must recognize funding sources, appropriate grant number and cooperative arrangement with the New Hampshire Fish and Game Department.

EXHIBIT C

Special Provisions

The following special provisions modify, change, delete or add to the General Provisions of the contract.

1. General Provisions are amended as follows:

- a) The requirements of section 9, "Data/Access/Confidentiality/Preservation" are waived.
- b) The provisions of section 12, "Assignment/Delegation/Subcontracts" are waived.
- c) The requirements of section 14 "Insurance" are waived.



The Northeast Wildlife Disease Cooperative

Offering wildlife health and disease services to the Northeast U.S.

Phone: 508-887-4933

E-mail: nwdc@tufts.edu

<http://sites.tufts.edu/nwdc>

Northeast Wildlife Disease Cooperative Research Proposal

Development of a live animal serological ELISA assay for detection and surveillance of *Parelaphostrongylus tenuis* in cervids

Principal Investigators:

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Summary

We propose to develop a serological test that will allow for rapid screening of cervids for *Parelaphostrongylus tenuis* infection, the etiologic agent of meningeal worm (*i.e.* brain worm) infection. We foresee the test being used by wildlife researchers seeking to understand the transmission dynamics, epidemiology, attack rate, and geographical and host range of *P. tenuis*. We are also interested in using the ELISA to determine if subclinical or nonfatal *P. tenuis* infections predispose moose (*Alces alces*), elk (*Cervus canadensis*), and other cervids to other infectious diseases, loss of nutritional condition, trauma, or predation leading to cumulative negative population impacts. NWDC participants are functioning as research (Dr. Krysten Schuler) and administration (Dr. Julie Ellis) coordinators for this project. We see this as the first of many collaborative relationships both within and outside of the NWDC.

Introduction

Parelaphostrongylus tenuis is a metastrongylid parasite of white-tailed deer (*Odocoileus virginianus*) (Anderson, 2000; Lankester, 2001). The intermediate hosts are terrestrial slugs and snails. In the natural host, the white-tailed deer, the larvae spend up to 1 month migrating within the dorsal horns of the spinal cord without producing clinical signs and then reside in the meninges. In other species of ungulates, the response is quite different. *P. tenuis* infection in aberrant hosts leads to longer durations of spinal cord migrations and potential migrations within the cerebrum, cerebellum, or brainstem. Severe infections often lead to mortality characterized by chronic thrashing of affected animals prior to death. Clinical signs and lesions of nonfatal infections include extensive central nervous system damage leading to crippling disease, circling, incoordination, or weakness. ***Collectively, these clinical signs may predispose infected animals to predation and other infectious or non-infectious diseases.***

Numerous cervids including moose (*Alces alces*) and elk (*Cervus canadensis*) as well as domestic ungulates including sheep, horses, cattle, llamas and alpacas can all be affected by *P. tenuis* (Lankester 2001; Gerhold et al., 2010; Tanabe et al., 2010; Mitchell et al., 2011). Infection of *P. tenuis* is considered to be one of the most important diseases negatively influencing moose populations areas where moose and white-tailed are sympatric, especially in areas where deer populations exceed >5 deer/km² (Lankester, 2010). Currently, *P. tenuis* infection is diagnosed by post-mortem examination of brain or spinal cord sections. This technique has variable to low sensitivity and often animals are diagnosed as *P. tenuis* suspects. The construction of a live animal test would be a significant and sensitive diagnostic, research, and surveillance tool. In addition, it would allow for detection of subclinically infected animals which would assist with understanding the epidemiology of the disease. Furthermore sera collected from hunter killed deer can be used to determine the prevalence of infection and geographical range of *P. tenuis* infection in deer. Although the historic distribution of *P. tenuis* extended to the 100th meridian (Lankester, 2001), recent anecdotal evidence suggests that *P. tenuis* has extended beyond its historical range.

Study Objectives

Our goal is to develop an Enzyme-linked immunosorbent assay (ELISA) to be utilized as a diagnostic test for the determination of Parelaphostrongylus tenuis infection of cervid sera. In previous studies, ELISAs have shown to be an effective diagnostic tool for the detection of

antibodies to other pathogens (Blitvich et al., 2003; Van Der Heijden et al., 2010). Additionally, the developed ELISA will be employed to perform preliminary *P. tenuis* surveillance from banked cervid sera. After development, this test will be retained by the U. of Tennessee Diagnostic Laboratory as a traditional fee-for-service assay available to the public.

Study Design

Sample Collection:

Sera samples containing at least 1 ml should be collected from whole blood removed from moose. Sera tubes should be labeled with species of cervid, animal ID, and state and county of origin. Sera should be frozen at -20C until shipped to the University of Tennessee College of Veterinary Medicine, 2407 River Dr Knoxville, TN 37996. Prior to shipping, wrap tube lids with parafilm to minimize any leaking and place tubes in sealed ziplock bag. Ship frozen sera samples overnight with refreezable ice packs inside a Styrofoam or hard cooler. Blood can be collected from live moose or clotted blood from dead moose and placed in Vacutainer (red-top or serum separator) tubes. Tubes should be centrifuged at 1000-2000g for 10 minutes. Serum can be stored frozen prior to testing without impacting result quality. Up to 300 unknown moose sera will be tested during assay development.

Laboratory Development & Optimization:

A *P. tenuis* antigenic protein, the aspartyl protease inhibitor ortholog (API), was detected by Duffy et al (2002). Expression of this protein has been confirmed in L1, L3, adult male and adult female worms. It is conserved among *P. tenuis*, does not have strong similarity with proteins in other parasites and is immunogenic in atypical hosts. The gene for this protein was optimized for expression in *E.coli* and synthesized with a His tag in the pET11 expression vector. Western blot assays using this API protein have produced excellent results using sera from moose and deer that were known positive as well as sera from cervids from regions where *P. tenuis* does not exist as our known negatives. Our next step is to map API epitopes using overlapping synthetic peptides followed by fine mapping. Peptides with the highest reactivity with positive sera and low reactivity with negative sera will be used as antigen for ELISA assays. The ELISA is substantially less expensive and less time consuming than a western blot. To perform ELISA assays A 96- well microplate (Immulon 2, Thermo Fisher Scientific, Rockford, IL) will be coated with 100 µl/well of an optimal concentration of API peptide and incubated at 4° C overnight in a humid chamber. Following incubation, wells will be washed 5 times with PBS containing 0.05% Tween20 (PBS-T). Wells will be blocked with 200µl 1.0% bovine serum albumin diluted in PBS-T and incubated for 1hr at 37°C in a humid chamber. After repeating the washing procedure, 100µl of sera from *P. tenuis* – infected cervids diluted in PBS-T will be added to each well, incubated, and washed again. HRP rabbit anti-cervid will be diluted in PBS-T and 100µl added to the wells. Incubation and washing of plates will be performed as in the previous step, followed by adding 100 µl of peroxidase substrate to each well (Pierce: Thermo Fisher Scientific, Rockford, IL). The reaction will be stopped by the addition of sulfuric acid to the wells and the absorbance read at 450nm using an enzyme immunoassay plate reader (Bio-Tek). Wells consisting of all reagents except antigen will be used in each plate to determine background reading and obtain the optical density (OD) for the various sera dilutions. Positive and negative sera will be used on each plate as controls. Results are interpreted by determining the OD level.

Once developed, the ELISA will be used to preliminarily test banked sera of moose, elk, white-tailed deer and other cervids, including sera from known positive and known negative *P. tenuis*-infected animals. The ELISA will be an excellent test to determine *P. tenuis* infection from sera (and potentially other bodily fluids including cerebral spinal fluid) from live animals or achieved samples. The test will allow us to determine if there is a spectrum of sensitivity to *P. tenuis* in different subspecies or genotypes of moose and elk. We are also interested in using the ELISA to determine if subclinical or nonfatal *P. tenuis* infections predispose moose, elk, and other cervids to other infectious diseases, loss of nutritional condition, automobile or other trauma or predation. Furthermore sera collected from hunter killed deer can be used to determine the prevalence of infection and geographical range of *P. tenuis* infection in deer.

Expertise:

The principal investigator, Dr. Gerhold, is a Certified Wildlife Biologist and a veterinary parasitologist and has substantial previous lab experience with the *P. tenuis* diagnostics (Gerhold et al., 2010; Tanabe et al., 2010; Mitchell et al., 2011; Dobey et al., 2014). The co-investigator, Dr. Kania has vast experience with ELISA development and testing and production and purification of recombinant proteins and epitope mapping. In addition, the researchers are currently working on various parasitic infections (i.e. *Elaeophora schneideri* and other filarids) including those of moose and elk and this assay would greatly complement the understanding of population implications of these parasites.

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Budget

<u>Personnel</u>	Cost
1.PI Gerhold (estimate 2.5% effort)	\$3,100
2.Technician @ U of TN (5 weeks)	\$4,365
Personnel total	\$7,465
<u>Supplies, equipment, & travel</u>	
1. Recombinant protein expression and purification, synthetic peptides, and ELISA development	\$4,000
2. Laboratory supplies, shipping costs and miscellaneous	\$3,000
3. Travel & publication costs	\$2,000
Supplies, equipment, & travel total	\$9,000
Diagnostic ELISA testing of up to 300 archived moose sera samples	\$5,000
Subtotal	\$21,465
Univ TN overhead (15%)	\$3,219.75
Total to be paid to Univ TN	\$24,685
Tufts institutional assessment fee (12%)	\$2,962.17
TOTAL project cost	\$27,647

BUDGET JUSTIFICATION

PERSONNEL

Principal investigators:

Dr. Richard Gerhold will be integrally involved in overseeing and performing parasite collection, sera collection, and mentoring as well as report and manuscript preparation. The PI is requesting 2.5% of his salary and benefits for this project given the significant amount of time that he will spend on this project.

Dr. Krysten Schuler will be coordinating the project for NWDC, as well as collecting parasites and sera for test validation and sensitivity analysis.

Dr. Julie Ellis will be administering the project as Director of the NWDC.

Technician: The research technician (U of TN) will assist with laboratory procedures including antibody preparation, sample collection, and testing. The requested amount will cover the technician's salary and benefits for 5 weeks.

The research technician (Cornell) will be collecting blood and parasite samples from white-tailed deer and confirming infection through analysis of brain and/or feces.

No funds are being requested for Dr. Schuler or a technician at Cornell for their participation on this project.

MATERIALS/EQUIPMENT

ELISA construction and testing: Various items including reagents and chemicals will be utilized for construction of the ELISA. We estimate \$4,000.

Laboratory supplies: Various laboratory supplies including gloves, transfer pipettes, sample preparation solutions, various test tubes, molecular pipette tips, and decontaminate solutions will be used. We estimate \$3,000 for these supplies.

Travel: We are requesting \$2,000 for the PI to travel to a scientific conference (e.g. Wildlife Disease Association) to present the research data and costs to publish data.

Diagnostic testing of moose sera: Once ELISA is developed and validated, we will test up to 300 moose sera samples for *P. tenuis* for \$5,000. Contributing agencies will receive a sample allotment proportional to their contribution to the project total. For instance, if each state agency contributes one-third of the project total cost, they will receive one-third of the 300 allotment or 100 samples to be tested. Further sample testing will be available at the U. of TN diagnostic lab for \$20 per samples.



February 17, 2016

OFFICE OF THE PROVOST AND SENIOR VICE PRESIDENT

Thomas McGurty
Vice President for Finance and Treasurer
Tufts University

Mary R. Jeka
Senior Vice President and General Counsel
Tufts University

Dear Tom and Mary,

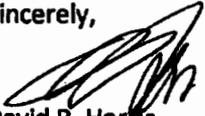
In order to enable them to perform more effectively, I hereby delegate the following signature authority to members of my staff:

1. **Vice Provost for Research-Diane L. Souvaine**
Associate Vice Provost for Research Administration-Deborah Grupp-Patruz
Sr. Associate Director, Research Administration (Boston)-Zoya Davis-Hamilton
Sr. Associate Director, Research Administration (Medford)-Maria Harlow
Associate Director, Research Administration (Boston)-Todd Conley
Associate Director, Research Administration (Boston)-Barbara Gardner
Associate Director, Research Administration (Medford)-Leigh Fallon Curley
are authorized to sign any and all agreements, grants, or contracts for any sponsored project in any amount.
2. **Vice Provost for Research-Diane L. Souvaine**
Sr. Director, Technology Transfer & Industry Collaboration-Larry Steranka
are authorized to sign any and all agreements related to University owned intellectual property regardless of monetary value including intellectual property management agreements, copyright, patent and trademark applications and related documents, material transfer agreements, confidentiality and confidential disclosure agreements, and documents relating to research collaborations and technology.
3. **Sr. Associate Director, Technology Transfer & Industry Collaboration-John Cosmopoulos**
Associate Director, Technology Transfer & Industry Collaboration-Martin Son
are authorized to sign material transfer agreements and confidential disclosure agreements regardless of actual or inferred monetary value.
4. **Vice Provost for Research-Diane L. Souvaine**
Associate Director, Sponsored Program Accounting (Medford)-Kathy Young
Sr. Associate Director, Sponsored Program Accounting (Boston)-Vacant
Assistant Director, Sponsored Program Accounting (Boston)-Deena Strabone
Assistant Director, Sponsored Program Accounting (Medford)-Susan Morrison
are authorized to sign any and all annual and final fiscal reports or vouchers requesting payment under sponsored projects.
5. **Vice Provost for Research-Diane L. Souvaine**
Associate Director, Business Operations & Finance-Kara E. Charmanski

are authorized to sign contracts for consultant and professional service engagements under \$250,000 and when a Tufts standard contract is used.

Nothing in this letter is intended to change the authority given to each of these individuals under the signature authorization policy in regard to the purchase of goods of services.

Sincerely,

A handwritten signature in black ink, appearing to read 'David R. Harris', written over a light blue horizontal line.

David R. Harris
Provost and Senior Vice President

cc: Diane L. Souvaine