EXECUTIVE SUMMARY

Cases of limping elk exhibiting characteristic hoof lesions reported to the Washington Department of Fish and Wildlife (WDFW) increased markedly in Southwest Washington beginning in 2008. In response to intense stakeholder concern regarding the severity and spread of the disease, in 2017 the Washington State Legislature unanimously passed Senate Bill 5474 to designate Washington State University College of Veterinary Medicine (WSU, CVM) as the state lead in developing a program to monitor and assess causes of and potential solutions for elk hoof disease. Funding of $1,169,900 was provided to WSU CVM for the FY2020-2021 biennium.

In 2019, WSU CVM implemented a Research Plan developed in 2018 and recruited students and support staff to make initial progress toward addressing the four principle areas of inquiry:

- **Study the disease cause(s) and contributing factors in captive elk.** The $1.3 million facility is 90+% complete and finishing touches are being conducted with an expectation of housing animals in the facility in January 2020. One tame elk was obtained; however, challenges securing elk that have not been exposed to hoof disease in Washington required planning for alternative sources of elk for research. Wild elk for the first study will be captured in coordination with WDFW in early 2020. The objective of that initial study is to determine whether elk hoof disease is infectious and transmissible to otherwise healthy elk through natural routes of transmission in captivity.

- **Study disease agents in the laboratory.** We conducted metagenomics analyses (looking at all the genetic material in a sample to determine which organisms are present) in a pilot study of samples collected from diagnostic case submissions. Findings were reported in a plenary presentation at the Wildlife Disease Association annual conference. We collected samples from elk for metagenomics study to compare disease-causing agents from four geographic areas.
• **Conduct regional surveillance.** In 2019, we collaborated with wildlife managers to examine hoof samples from 75 hunter-harvested elk or elk found deceased. The geographic distribution of hoof disease continues to expand. Surveillance to date suggests that some areas in Washington and adjacent states that were once thought to be free of elk hoof disease may harbor the disease at low levels, but further investigation is needed in 2020. We examined deer and other wildlife with hoof lesions and to date TAHD has not been diagnosed in species other than elk. We also initiated computer modeling to explore risk factors associated with occurrence of elk hoof disease in Washington. Additionally, an ancillary project was initiated to investigate the occurrence and potential causes of antler asymmetry reported by stakeholders in Southwest Washington.

• **Understand social aspects of the disease.** In collaboration with the WSU Social and Economic Sciences Research Center, we completed a qualitative study of stakeholders’ opinions on elk hoof disease in Washington. Between January and May 2019, 11 focus groups were conducted with various stakeholders. The objectives were to learn about the levels of awareness and range of opinions on the disease. Data collected were used to help form a general public survey for the second stage of research that will be performed in early 2020. We conducted outreach via the website, media, legislative briefings, and stakeholder meetings. Additionally, a listserv was launched to provide regular research updates to subscribers.
OVERVIEW

Hoof disease, known scientifically as Treponeme-associated hoof disease (TAHD), is an emerging disease of elk in Washington. Prior to 2008, only sporadic cases of limping elk with hoof deformities had been reported to the Washington Department of Fish and Wildlife (WDFW). In 2008, those reports increased substantially, particularly in Southwest Washington. The disease has now been identified in elk herds across most of Western Washington, as well as east of the Cascades in the Trout Lake and Walla Walla areas. Additionally, cases have been diagnosed in Northern Oregon and Western Idaho.

Elk with hoof disease have characteristic ulcers on their feet with associated overgrown, broken, or sloughed hooves. Affected elk are debilitated and, according to preliminary research by WDFW, experience higher mortality which may lead to population level impacts. This disease has the potential to devastate the elk population in Washington and because of the interaction of wild elk with domestic livestock, is also of concern by other Washington stakeholders including the livestock industry.

In response to intense stakeholder concern, in 2017 the Washington State Legislature unanimously passed Senate Bill 5474 to designate Washington State University College of Veterinary Medicine (WSU, CVM) as the state lead in developing a program to monitor and assess causes of and potential solutions for elk hoof disease. A $1,519,900 biennial budget was allocated to WSU CVM to address this effort beginning on July 1, 2017. At that time no elk hoof disease program existed at WSU, and a new program was created from the ground up. The legislature continued funding for the FY2020-2021 biennium at a level of $1,169,900.

This report consists of two sections. Section I, Research Plan, was developed in 2018 and defines the research approach that guides our work in the period 2019-2021. This background information establishes the context for Section II, Research and Outreach Accomplishments in 2019, which summarizes accomplishments made implementing the plan in 2019.
I. RESEARCH PLAN

Phase One: 2019-2021

The first step in establishing the research program was to define research goals and identify key research questions. In alignment with Senate Bill 5474, the goal of WSU elk hoof disease research is to identify the cause(s) of the disease and how to successfully manage it in the wild. Achieving this goal will require an incremental multi-pronged biological and social science research approach implemented over multiple years. The first phase of work addresses foundational questions and will be conducted with a three-year horizon (2019-2021). Successive phases of work will build on findings from these initial studies. The four principle areas of inquiry for these studies are:

- **Study the disease cause(s) and contributing factors in captive elk.** We will use captive elk in a controlled environment to investigate the cause(s) of hoof disease and contributing factors that make elk more or less susceptible.
  
  **Need:** The definitive cause(s) of hoof disease are not known and are required for effective management as well as to identify risk to other species. Recent studies led by WDFW have identified Treponeme species associated with hoof lesions; however, it is unknown whether these bacteria are the primary cause of disease, or secondary invaders. Extensive stakeholder concern exists regarding elk exposure to herbicides, fertilizers, and habitat changes as a cause or contributing factor for disease. Controlled studies are needed to investigate the individual and collective impacts of pathogens and other contributing factors to disease.

  **Approach:** Initial work will focus on development of a disease challenge model to determine if the disease 1) is infectious and contagious and 2) can be reliably reproduced in elk following exposure to infectious material. Based on results, modifications to the challenge model will be investigated. For example, addition of contributing factors, such as reduced nutritional status or exposure to herbicides, may be required to reproduce disease.

- **Study disease agents in the laboratory.** We will use state-of-the-art technology to identify pathogens associated with hoof disease.
  
  **Need:** Many pathogens, including Treponeme species, are not easily cultured using standard techniques. Advanced approaches are needed to identify pathogens in samples collected from free-ranging and captive research elk to determine which organisms are, and are not, contributing to disease. This work is needed to guide improvement of methods to isolate the causative agent(s) and develop tests to detect, and potentially treat, them.

  **Approach:** Initial work will use metagenomics (looking at all the genetic material in a sample to determine which organisms are present) to identify bacteria associated with hoof disease in general, and at specific points during progression of the disease.
• **Conduct regional surveillance.** We will collaborate with WDFW and other wildlife management agencies to collect hoof samples for diagnostic investigation.

*Need:* Disease surveillance and monitoring is key to documenting where a disease occurs and to estimate prevalence. It provides baseline data to measure changes in the future and can also be used to identify risk factors for disease occurrence.

*Approach:* In collaboration with wildlife managers, we will collect and perform diagnostic evaluation of hoof samples from across Washington and other states in the northwest to document where TAHD occurs. Surveillance samples can also be used to address additional research questions. Initially we will focus additional collections from four geographically distinct areas to investigate whether or not the pathogens involved are the same in every area to determine if one disease outbreak is spreading, or if multiple independent outbreaks are occurring. Additionally, we will overlay disease distribution data collected from surveillance efforts with potential risk factors to investigate if disease occurrence is correlated with particular locations or environmental factors.

• **Understand social aspects of the disease.** Implement outreach and education efforts that are grounded in an understanding of stakeholder’s beliefs, values, and concerns about hoof disease and elk management.

*Need:* Effective outreach and education is an important companion to the implementation of biological research, particularly when addressing wildlife issues with multiple opposing stakeholder perspectives. Information gained from social science inquiry can guide outreach and education efforts and contribute to setting goals for research and management.

*Approach:* Initial research will be conducted in collaboration with the WSU Social and Economic Sciences Research Center (SESRC). We will use focus groups of interested stakeholders to gather qualitative information regarding public opinion on hoof disease. This information will be used to develop a questionnaire for a statewide survey that will provide statistical representation of public opinion. Additionally, we will conduct program development work to guide outreach and education efforts, while concurrently seeking to increase public awareness through media outlets.

In addition to these WSU research priorities, we will support related WDFW and tribal research and management as requested. This includes providing staff support for field work or diagnostic investigations, providing diagnostic services for hoof samples submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL), and conducting collaborative research. This level of cooperation requires a commitment to communication that will be addressed in part through regularly scheduled quarterly meetings between WSU and WDFW staff.
II. RESEARCH AND OUTREACH ACCOMPLISHMENTS IN 2019

General

• Recruited students. This year we recruited two additional graduate students to work on elk hoof disease. Students contribute to research while gaining education. Current students that are contributing to research described in this report include:

  o Elizabeth Goldsmith, DVM, is a second-year combined pathology residency/PhD student. Dr. Goldsmith’s research focuses on pathogen discovery using metagenomics techniques.

  o Holly Drankhan, DVM, is a first-year combined pathology residency/PhD student. Dr. Drankhan will conduct research on disease cause and transmission in elk at the captive research facility.

  o Zachary Robinson is student in the first-year of a Master’s degree program. Zach grew-up in Southwest Washington observing elk with hoof disease. He will study disease development and transmission in elk at the captive research facility.

  o Dylan Conradson is a second-year veterinary student participating in a CVM Research Scholars Program. Dylan is using computer modeling to conduct a spatial epidemiology analysis of disease and also investigate antler asymmetry observed in Washington elk.
Hired staff. Scientific assistant, Elizabeth Wheeler, was hired to manage the lab, oversee diagnostic case submissions and processing, and coordinate research animal care.

Donations and grants. We pursued donations and grants to supplement state funding.

- The Rocky Mountain Elk Foundation donated $100,000 to support construction of the elk research facility.
- A grant proposal titled “Investigating the etiology and developing a diagnostic tool for an emerging hoof disease in elk using a genomic approach” was submitted to the Morris Animal Foundation, Wildlife/Exotics funding section. The proposal was one of 77, out of a total 169 received, that advanced for full review. Notifications regarding funding decisions will be made early 2020.

Study the disease cause(s) and contributing factors in captive elk.

- Construction of captive elk facility. A contractor was selected and ground broken for construction of the elk research facility in spring. The facility includes 10 isolation pens, a handling facility, and two 1.5 acre holding pastures. The facility is 90+% complete and finishing touches are being conducted with an expectation of housing animals in the facility in January 2020. Coordination with the WSU environmental safety and animal care programs contributed to ensuring compliance with applicable standards and regulations. The donation from the Rocky Mountain Elk Foundation and a significant private donation supplemented state funds, including minor capital improvement funding, to ensure completion of the approximately $1.3 million facility.
- Animal care. A protocol to establish a captive elk herd was developed and approved by the WSU Institutional Animal Care and Use Committee (IACUC). A scientific collection permit was obtained from WDFW to capture and hold elk in captivity.
- Elk procurement. Alternative approaches were required to secure elk when initial efforts to obtain our goal of four female neonatal elk calves were unsuccessful. No female orphan calves were available from the wild in Washington and our disease surveillance detected TAHD in elk at the semi free-ranging Washington zoo facility that was intended to serve as the source of healthy research elk. Because previous exposure to hoof disease could bias experimental results, other sources for elk that have not been exposed
Elk S19, otherwise known as Salix, was the first elk calf acquired by Washington State University’s College of Veterinary Medicine for its Elk Hoof Disease Research Program.
to hoof disease are needed. Unfortunately, availability of elk from herds where no signs of hoof disease have been documented in Washington are limited. We are continuing discussions with WDFW regarding sources of elk to bring into captivity for use as breeding stock and research animals. In the short-term, we have identified wild elk that can be captured and brought into captivity for our first study in early 2020.

One weaned male calf about 5-months-old was obtained from a Washington-licensed rehabilitator when he was not suitable for release back to the wild. The calf was picked up as an orphan near Mount Rainer and bottle-raised. Elk S19, or Salix, was castrated and housed temporarily in a secure wildlife pen until construction on the elk research facility is complete.

**Study disease agents in the laboratory.**

- **Performing metagenomics.** We conducted metagenomics analyses in a pilot study of samples collected from diagnostic case submissions in collaboration with CVM veterinary microbiologist, Dr. Devendra Shah. Findings were reported in a plenary presentation at the Wildlife Disease Association annual conference titled “Further inquiry into the etiology of hoof disease in elk using bacterial metagenomics analysis.”

  *Presentation abstract: Cases of limping elk (*Cervus elaphus*) exhibiting characteristic hoof lesions reported to the Washington Department of Fish and Wildlife increased markedly in southwest Washington in 2008. Currently, the disease has been documented in localized areas across western and southern Washington, northern Oregon, and western Idaho. Lesions are characterized by deformed, overgrown, broken or sloughed hooves, often with severe sole ulcers, extensive laminar necrosis, and pedal osteomyelitis. Spirochetes are observed on histology. In previous studies, Treponema species similar to those reported in digital dermatitis of cattle and sheep were isolated sporadically from lesions, and the presence of treponemes in tissues was supported by immunohistochemistry and PCR. Thus, the disease is currently diagnosed as treponeme-associated hoof disease (TAHD) based on gross lesions and histological presence of spirochetes. While treponeme-associated, additional investigation is warranted to determine the suite of organisms that might contribute to etiology. For example, digital dermatitis in livestock is generally considered to be a polymicrobial disease and predictable changes in the bacterial microbiota have been described through the progression of hoof lesions in cattle. We hypothesize a similar process may occur in elk. In a pilot study, we compared the bacterial (16S rRNA) metagenomes in biopsies collected postmortem from healthy (n=4) and diseased (n=4) elk feet. The bacterial diversity was reduced in the diseased state when compared to control samples. Results supported treponeme association; Spirochetes (Treponema spp.), as well as Tenericutes (Mycoplasma spp.) and Fusobacteria (Fusobacterium spp.), were the most predominant bacteria Phyla associated with lesions. Proteobacteria (Halomonas spp.) were overrepresented in control samples. Ongoing studies are evaluating larger numbers of samples collected from different lesion grades and geographically distant from the area of initial disease detection. Findings are critical for epidemiological investigation, developing a disease challenge model, and identifying potentially effective mitigation actions.*

- **Training.** WSU faculty and a PhD student attended an intensive week long training on metagenomics analysis titled Genomics of Disease in Wildlife in June 2019.
Conduct regional surveillance.

- **Diagnostic testing at WADDL.** Led by WADDL pathologist Dr. Kyle Taylor with support from scientific assistant Elizabeth Wheeler, we evaluated hooves from Washington and surrounding states. In 2019, we coordinated submission for WADDL examination of hooves from 52 elk and three deer from Washington, six elk from Oregon, and 17 elk and one deer from Idaho. In collaboration with WDFW hunter surveillance efforts, we also collected hoof samples for metagenomics from an additional 34 elk. TAHD has not been diagnosed in other wildlife species; however, we examine deer with hoof lesions to monitor for potential spillover of disease from elk to deer.

- **Spatial epidemiology.** The geographic distribution of hoof disease continues to expand. Surveillance to date suggests that some areas in Washington and adjacent states that were once thought to be free of elk hoof disease may actually harbor the disease at low levels. For example, we have diagnosed cases in the Olympic Peninsula and Western Idaho at a low prevalence and in some cases in elk with minor lesions that were not observed to be lame prior to death. These findings suggest that additional statewide surveillance is warranted to determine the true distribution of disease.

We initiated an investigation of risk factors associated with occurrence of elk hoof disease in Washington. A veterinary student, Dylan Conradson, participating in a research scholars program performed the analysis and computer modeling work. Preliminary results suggest some risk factors that may contribute to disease occurrence (e.g., precipitation), but additional work is required to refine the model. That work is scheduled to be performed in summer of 2020.

Understand social aspects of the disease.

- **Social science inquiry.** The WSU SESRC, led by Dr. Lena Le, completed a qualitative study of stakeholders’ opinions on elk hoof disease in Washington (Data Report 19-010). Between January and May 2019, 11 focus groups were conducted with various stakeholders, including three with conservationists, two with hunters, two with the general public, two with tribal affiliates, one with livestock producers, and one with individuals from the timber industry. Two supplemental focus groups were held with individuals from WDFW who were familiar with, or had worked directly with, the issue of elk hoof disease. The objectives were to learn about the levels of awareness and range of opinions on the disease. Four prominent themes arose from the data: perceived causes of, and contributing factors to, elk hoof disease; concerns regarding elk management; suggestions for how the disease
should be managed; and participants’ trusted sources of information. Data collected were used to help form a general public survey for the second stage of research that will be conducted in early 2020. The survey was submitted to the WSU Institutional Review Board and certified as exempt research. Results from the survey will be used to inform WSU research and outreach strategies and inform wildlife management officials in their decision-making processes.

• Listserv launched. A listserv was created using Mailman so that interested individuals can sign-up to receive regular email updates on our research activities. Currently, the list reaches 120 recipients and grows weekly.

• Stakeholder meetings. We continued to conduct informal as well as formal meetings with stakeholders to hear their concerns and share progress on our research. Coordination through WSU Extension resulted in increased connections with the timber and agricultural industries. Formal presentations included:
  o Family Forest Field Day, McCleary
  o Skagit Ag Leaders Breakfast
  o Wahkiakum County Commission special meeting
  o Latah Wildlife Association
  o Latah County Fair

• Legislative briefings and presentations. In addition to responding to routine inquiries, briefings were provided to interested state and federal legislators or their staff, and county commissioners as requested.

• Outreach via media. WSU’s work on hoof disease was covered in in regional newspaper stories focused primarily on the Rocky Mountain Elk Foundation donation, facility construction and planned research, and acquisition of research elk. Local radio, such as Pullman Radio KQQQ, has reported on research facility construction and KRFP, Moscow, Idaho aired an hour interview with Dr. Wild. An interview was also provided to Jon Gosch for his article in the Columbia Insight.

• Website. The elk hoof disease website was maintained to provide up-to-date information on elk hoof disease and our research. It also includes a link to a donation account.

Collaborate with WDFW and Tribes

• Collaborative disease investigation. WSU collaborated with state wildlife management agencies in Washington, Idaho, and Oregon and the Northwest Indian Fisheries Commission (NWIFC) to obtain hooves from elk harvested or found recently deceased in locations of interest for disease surveillance and/or for collection of research samples. Expertise at WSU assists collaborators in detecting the disease and findings from all locations help inform our understanding of the disease in Washington. These collaborations are critical for our research.

• Quarterly meetings with WDFW. In addition to regular communications, we conducted in person meetings quarterly in Olympia to plan and coordinate efforts.
ANCILLARY PROJECTS

During the course of planned research, unexpected new and important questions often arise. Addressing these questions must be prioritized to avoid over extending resources, but in some cases opportunistic projects that can be supported will be added to the research program. This is particularly true for projects that can be conducted by WSU students.

- **Antler asymmetry.** During discussions with stakeholders in Southwest Washington, concerns were voiced about antler asymmetry and its potential association with the high prevalence of hoof disease. A veterinary student, Dylan Conradson, is using hunter harvest data routinely collected by WDFW to investigate whether antler asymmetry occurs at a higher rate in Southwest Washington than other areas of the state where hoof disease is uncommon. If a difference is confirmed, we hypothesize that the asymmetry results from either hoof lesions or mineral deficiencies. Previous research on deer has documented that damage to a limb (similar to elk with hoof disease) can result in an antler anomaly on the opposite side. Alternatively, mineral deficiencies may contribute to both hoof and antler abnormalities.
NEXT STEPS

Since the passage of Senate Bill 5474, active disease surveillance conducted collaboratively between WSU, WDFW, and other agencies has resulted in diagnosis of the disease over a broader geographic area than was previously described. Unfortunately, the disease has expanded from a primarily local concern in Southwest Washington to a statewide issue. Moreover, with continuing cases in Oregon and recent detections in Idaho, it has emerged as a multi-state regional issue. The broader geographic range amplifies the need for research on this emerging disease. Moving forward our research will reflect this broader scope.

General

- **Staffing.** We will collaborate with another CVM researcher to hire a post-doctoral fellow who will work one quarter time on elk hoof disease laboratory investigations.
- **Legislative reporting.** The next report covering the period January-December 2020 will be submitted by February 12, 2021.

Study the disease cause(s) and contributing factors in captive elk.

- **Captive elk facility.** The captive elk facility is scheduled for completion in January 2020. Following approval by the WSU IACUC, we will move the first elk, S19, into the facility. Minor facility modifications will continue as needed to refine operations.
- **Animal care.** In early 2020 a protocol for conducting the first research project on elk in the facility will be submitted to the WSU IACUC. Additionally, we will work with the WSU Institutional Biosafety Committee to obtain authorization to work with infectious disease agents. Studies will commence after these approvals are secured.
- **Elk procurement.** We will continue to work with WDFW to secure a source of elk to establish a captive breeding herd. These discussions will need to include the possibility of bringing elk in from outside Washington. In the short-term, we will collaborate with WDFW to capture about 12 healthy elk and up to eight elk affected with hoof disease to conduct the first trial in the captive facility.
- **Natural exposure trial.** In the initial study, we will determine whether elk hoof disease is infectious and transmissible to otherwise healthy elk through natural routes of transmission. We will expose healthy elk to elk with hoof disease in captivity and monitor disease progression.

Study disease agents in the laboratory.

- **Performing metagenomics.** We will continue collecting samples from the four identified geographic locations selected for comparison. In the interim, the first batch of samples collected in 2019 will be submitted for genetic sequencing to identify bacteria present. PhD student Elizabeth Goldsmith and a post-doctoral fellow will analyze data and begin drafting reports.
- **Collaborative metagenomics investigation.** We are awaiting data from our collaborators at USDA Agricultural Research Service. When the data is provided, we will conduct analyses and draft a joint manuscript.
Conduct regional surveillance.

- **Diagnostic testing at WADDL.** Disease surveillance will continue using samples collected from hunter harvests, WDFW removals, and elk found recently deceased. The priority will continue to be on new geographic areas as well as in Game Management Units (GMUs) where suspect cases of the disease have been observed but diagnostic testing has not confirmed TAHD. We will propose to WDFW to conduct surveillance in select areas in Central and Eastern Washington to determine if the disease is present and undetected in those areas. We will also examine hoof samples from neighboring states to determine the geographic extent of the disease.

- **Spatial epidemiology.** The veterinary research student scholar will continue investigating risk factors for elk hoof disease during participation in the summer 2020 program. We will collaborate with a statistician to enhance the rigor of the preliminary modeling and draft a report that may be submitted for publication as appropriate.

Understand social aspects of the disease.

- **Social science inquiry.** The statewide survey will be launched in January 2020. Sampling will focus on counties where hoof disease has been documented and two non-affected counties for comparison. Results will be compiled in summer and a manuscript drafted for submission to a scientific journal.

- **Outreach.** Outreach via the listserv, website, media, legislative briefings, and stakeholder meetings will continue. Scientific presentations will also be made to wildlife managers and veterinarians.

Collaborate with WDFW and Tribes

- **Quarterly meetings with WDFW.** In addition to regular communications, we will continue quarterly meetings.

- **Tribes.** We plan to schedule meetings with the NWIFC and Cowlitz Tribe to promote outreach.

Ancillary projects

- **Antler asymmetry.** Data compilation and analysis will continue.