EXECUTIVE SUMMARY

In 2008, cases of limping elk exhibiting characteristic hoof lesions reported to the Washington Department of Fish and Wildlife (WDFW) increased markedly in Southwestern Washington. By 2017, and in response to stakeholder concern regarding the intensity and spread of the disease, the Washington State Legislature unanimously passed Senate Bill 5474 to designate Washington State University’s 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 2021, WSU CVM continued implementation of a Research Plan developed and submitted in 2018. Although the COVID-19 pandemic and associated restrictions to limit spread and protect human health slowed some research activities, we made progress toward addressing the four principal areas of the plan, as well as ancillary areas of inquiry:

- **Study the disease cause(s) and contributing factors in captive elk.**
  We completed the first study conducted in the new elk research facility on the Pullman campus. Our preliminary results indicated that TAHD can be transmitted to otherwise healthy elk through exposure to soil contaminated with hooves from affected elk. Four treatment elk developed moderate grade lesions indistinguishable from TAHD in wild elk about 4 months following experimental exposure. Unexposed control elk did not develop TAHD. Additional studies investigating transmission are ongoing. Ten juvenile elk were obtained from the Starkey Experimental Forest, Oregon, for use in future studies.

- **Study disease agents in the laboratory.** We used state of the art technology to sequence DNA from bacteria in hoof samples to determine what types of bacteria were present. This technique, called metagenomic analysis, was conducted on a subset of diagnostic case submissions and was also used to investigate bacteria present in lesions of captive elk that developed TAHD following the experimental challenge described above. The results in both cases suggest that hoof lesions arise from an infectious disease process commonly associated with *Treponema* or *Treponema*-like bacteria; however, a few other types of bacteria, e.g., *Mycoplasma*, are also
routinely associated with TAHD lesions and are worthy of further investigation. We continued hoof sample collections for a larger study that will use metagenomic analysis to investigate the importance of *Treponema* and other types of bacteria in lesions.

**Conduct regional surveillance.** Our research program at WSU reported distribution of TAHD in the Pacific West in a manuscript accepted for publication by the *Journal of Wildlife Diseases*. Findings were from surveillance conducted in Washington, Oregon, Idaho, and California in 2018-2020. With this baseline distribution documented, in 2021 we focused sample collection primarily on areas (generally at the county level) where TAHD had not been previously detected and to obtain samples for other research. Working with state wildlife managers, we collected hooves from Washington (74 elk), Oregon (7 elk, 1 deer), Idaho (8 elk, 1 deer), California (15 elk), Montana (2 elk), and Nevada (3 elk). We have not detected TAHD in any samples from Montana or Nevada or from any sampled deer.

**Understand social aspects of the disease.** We conducted outreach by providing information via a listserv (169 recipients), website, and the lay and scientific media. We also provided individual responses to all inquiries received. We presented research findings and answered questions from the public in a virtual meeting conducted via Zoom in September. We also shared information through a podcast with Wildlife Health Connections, by producing a series of 1-minute videos which introduce our research team and their work, and through presentation of scientific seminars.

**Ancillary projects.** Three additional studies were conducted in response to stakeholder interest. In the first, we used hunters’ observations reported in harvest data routinely collected by WDFW that were shared with WSU to determine that, as suspected by stakeholders, antler asymmetry occurs at a higher rate in elk with hoof lesions. A manuscript reporting the findings was submitted to the *Journal of Wildlife Management*. We also collaborated to investigate epigenetic effects (changes in gene activity and expression separate from the DNA sequence) that may influence susceptibility of elk to TAHD. Preliminary results indicate some differences in elk with and without TAHD and further analysis is underway. Finally, we initiated an investigation using hair to determine differences in mineral levels of elk with and without TAHD.
OVERVIEW

Hoof disease, known scientifically as Treponeme-associated hoof disease (TAHD), is an emerging disease of elk in the Pacific West. 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 Southwestern Washington. The disease has now been identified in elk herds across much of Western Washington, as well as sporadic locations east of the Cascades. Additionally, cases have been diagnosed in Oregon, Idaho, and Northern California.

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 Washington’s elk populations and because of the interaction of wild elk with domestic livestock, it 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’s 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. At that time no elk hoof disease program existed at WSU, and a new program was literally created from the ground up, including the construction of an elk research facility. The legislature continued funding for the FY2020-2021 biennium at a level of $1,169,900.

The following report consists of two sections. The first section, Research and Outreach Accomplishments in 2021, summarizes accomplishments made implementing our research plan in 2021. The second section is our Research Plan, which was developed in 2018 and defines the research approach to guide our work for the period 2019-2021, and is attached as an Addendum at the end of this report for reference.
RESEARCH AND OUTREACH ACCOMPLISHMENTS IN 2021

General

- **Student training.** Students contribute to research while gaining education. Current students that are contributing to research described in this report include:
  - Elizabeth Goldsmith, DVM, is a fourth-year combined pathology residency/PhD student. Dr. Goldsmith’s research focuses on pathogen discovery using metagenomic techniques. Anticipated graduation 2025.
  - Holly Drankhan, DVM, is a third-year combined pathology residency/PhD student. Dr. Drankhan conducts research on disease cause and transmission in elk at the captive research facility. Anticipated graduation 2025.
  - Zachary Robinson is in the second year of a master’s degree program. Zach grew-up in southwest Washington observing elk with hoof disease. He is studying disease development and transmission in elk at the captive research facility. Anticipated graduation 2022.
  - Steven Winter, MS, began a PhD program in 2021. Steven will study spatial distribution and risk factors of TAHD using computer modeling. Anticipated graduation 2025.

- **Staffing.** A scientific assistant, Charlie Park, manages the laboratory, oversees diagnostic case submissions and processing, and coordinates research animal care.

- **Donations and grants.** We actively pursue donations and grants to supplement state funding.
  - The CVM Fowler Emerging Disease Research Endowment Fund contributed $14,286 toward elk hoof disease research.

- **Impact of COVID-19 pandemic.** Although the COVID-19 pandemic and associated restrictions to limit spread and protect human health slowed some research activities, we made progress toward addressing the four principal areas of our research plan, as well as three ancillary projects.
Study the disease cause(s) and contributing factors in captive elk

- **Captive elk facility.** We maintained the elk research facility, which was constructed in 2020, and completed minor upgrades to its laboratory space. The first research experiment was completed at the facility in 2021.

- **Animal care and biosafety.** Coordination with the WSU Environmental Health and Safety office and animal care oversight programs contributed to ensuring compliance with applicable standards as well as state and federal regulations. Protocols for holding and conducting research on captive elk were developed and approved by the WSU Institutional Animal Care and Use Committee (IACUC), a federally mandated oversight group. In response to the COVID-19 pandemic, safe work practices and protocols were followed to protect the health of employees, students, and research animals. No exposures of elk to COVID-19 were known to occur.

- **Elk procurement.** We added 10 juvenile female elk (about 10 months old) to our captive herd in April. These individuals will be used in future studies of TAHD transmission. Elk were obtained from the Starkey Experimental Forest, Oregon. The Starkey Forest is a vital resource since TAHD has not been diagnosed within the unit and elk are accessible during captures conducted for ongoing ecological studies.

- **Environmental transmission study.** Following a delay due to the COVID-19 pandemic restrictions, we initiated the first study of TAHD transmission in October 2020 and completed the first phase in March 2021 (Robinson master’s degree project). In this study we asked the question, can TAHD be transmitted to healthy captive elk through soil contaminated by hooves of affected elk? Six healthy captive elk lived in individual biosecure stalls, a portion of which were covered in soil. At about 2-4 week intervals, study elk were exposed to an inoculum made of soil mixed with minced hooves from wild elk that had been collected and frozen quickly after death to preserve any bacteria and other germs living on and within the hooves. Four of the six study elk were exposed to TAHD-affected hooves (treatment elk), while two other elk were treated identically except they were exposed to hooves without TAHD (control elk). Inoculum was placed between the toes on each foot and held it in place with a wrap on two feet of each elk. This ensured contact between the foot and inoculum. Additional inoculum was mixed with the soil in each pen. We observed early lesions suggestive of TAHD in the four treatment elk 2-3 months after the study began. By 4 months, all four treatment elk had
moderate grade lesions indistinguishable from TAHD in wild elk based on visible lesions, microscopic lesions, and bacterial DNA present. None of the control elk exposed to normal hooves developed visible or microscopic foot lesions or had Treponema bacteria present, indicating that the affected hoof material was the source of lesions in treatment elk. These preliminary results indicate that TAHD can be transmitted through soil contaminated with affected hooves. The findings also provide further evidence that TAHD lesions are a result of an infectious disease. We are further analyzing results and preparing a manuscript for submission to a scientific journal. Additionally, we initiated a follow-up study to investigate whether TAHD can be transmitted in the captive elk without wrapping feet to hold the inoculum in place.

Study disease agents in the laboratory

- **Metagenomic analysis.** We continued metagenomic analyses of samples in collaboration with WSU CVM veterinary microbiologist, Dr. Devendra Shah. We conducted metagenomic analyses (looking at genetic material in a sample to determine all the types of bacteria that are present) in samples collected from a subset of diagnostic case submissions. We used the same technique to investigate bacteria present in lesions of captive elk that developed TAHD following experimental challenges as described above. In both cases, results suggested that hoof lesions arise from an infectious disease process commonly associated with *Treponema* or *Treponema*-like bacteria; however, a few other types of bacteria, e.g., *Mycoplasma*, are also routinely associated with TAHD lesions and are under further investigation. Results from the initial inquiry to identify bacteria present in samples from across the known range of TAHD are reported in a manuscript titled *Surveillance for an emergent hoof disease in elk (Cervus elaphus) in the U.S. Pacific West supplemented by 16S rRNA gene amplicon sequencing* that is in press with the *Journal of Wildlife Diseases*.

- **Sample collection for metagenomic analysis.** We continued hoof sample collection and analysis for a larger study in order to conduct a thorough investigation into the bacterial community of the elk hoof and identify potential pathogens associated with hoof disease (Goldsmith PhD project). In conjunction with disease surveillance efforts, to date we have collected and archived biopsies from over 100 cases representing both TAHD-affected and normal hooves for use in this metagenomic analysis.
Conduct regional surveillance

- **Diagnostic testing.** Led by Washington Animal Disease Diagnostic Laboratory (WADDL) pathologist and faculty member Dr. Kyle Taylor and with support from graduate students and scientific assistant Charlie Park, we evaluated hooves from Washington and surrounding states. We reported distribution of TAHD in the Pacific West in the manuscript accepted for publication by the *Journal of Wildlife Diseases* (see above). Findings were from surveillance conducted in Washington, Oregon, Idaho, and California in 2018-2020. With this baseline distribution documented, in 2021 we focused sample collection primarily on areas (generally at the county level) where TAHD had not been previously detected, on cases of management concern (e.g., abnormal deer hooves), and to obtain samples for other research. Working with state wildlife managers, we collected hooves from Washington (74 elk), Oregon (7 elk, 1 deer), Idaho (8 elk, 1 deer), California (15 elk), Montana (2 elk), and Nevada (3 elk). We have not detected TAHD in any samples from Montana or Nevada or from any sampled deer.

- **Spatial distribution and risk.** Preliminary analysis examining risk factors for elk hoof disease and its distribution indicated that a more complete and rigorous evaluation was needed to understand this complex disease. Fundamentally, diseases may often appear to occur randomly until additional analysis is conducted across numerous factors such as geography, topography, environmental disturbances (both natural and human-caused), etc. After such analysis, it is often found that patterns can be described and, in some cases, predicted as a result of this work. As a result, a new student began a PhD program in 2021 to focus on disease ecology and spatial epidemiology of TAHD. The objective of this work is to understand why the disease occurs where it does and to help us understand if predisposing factors, like environmental conditions or human alterations, are important in disease occurrence (Winter PhD project).
An elk receives treats during training at the WSU Elk Research Facility
Understand social aspects of the disease

• **Social science inquiry.** We continued collaboration with the WSU Social and Economic Sciences Research Center (SESRC), led by Dr. Lena Le, to draft a scientific manuscript that reports findings from the 2020 survey of public perceptions of elk hoof disease in Washington. Based in part on these findings, we assisted WDFW in developing questions to ask in a supplemental hunter survey to understand acceptance and participation in WDFW’s pilot elk hoof disease incentive permit program.

• **Communicate through a Listserv.** Respondents to the SESRC survey expressed strong support for learning of progress on hoof disease via a listserv. Individuals who were signed-up for our listserv received Research Updates and other information on our research activities in 2021. Currently, the list reaches 169 recipients and continues to grow.

• **Stakeholder meetings.** Informal correspondence continued, but meetings with stakeholders were markedly reduced this year due to COVID-19 restrictions. We presented research findings and answered questions from the public in a virtual meeting (meeting starts at timestamp 1:33 of the recording) conducted via Zoom in September. We also shared information via a podcast with Wildlife Health Connections and through scientific seminars presented virtually to the 14th biennial Deer and Elk Workshop and the Trilateral webinar series.

• **Outreach via media.** With the support of CVM communications specialist Charlie Powell, WSU’s work on hoof disease received coverage in local and regional media. Of particular note, a story aired on Northwest Public Radio and one article that originally ran in the Lewiston Tribune was picked up by Yahoo news for broad distribution.

• **Website.** The elk hoof disease website was maintained to provide up-to-date information on elk hoof disease and our research. To supplement written materials on the website, six members of the research team recorded 1-minute videos introducing themselves and their research to further communicate our work to the public.

Collaborate with WDFW and Tribes

• **Collaborative disease investigation.** WSU collaborated with state wildlife management agencies in Washington, Idaho, Oregon, California, Montana, and Nevada 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. Findings from all locations help inform our understanding of the disease in Washington and are critical for our research.

• **Scientific meeting.** WSU researchers collaborated with the Rocky Mountain Elk Foundation (RMEF) and the California Department of Fish and Wildlife (CDFW) to plan a virtual scientific meeting on elk hoof disease for wildlife managers in early 2022.

• **Collaboration with WDFW.** In addition to regular communications, we conducted virtual meetings about quarterly to share information and plan and coordinate work. WDFW efforts have been critical for obtaining fresh elk hooves for research. We also coordinated to opportunistically collect hooves from about 50 elk submitted to WDFW as part of their pilot elk hoof disease incentive permit program. Hooves will be used for metagenomics research.
Ancillary projects

During the course of any planned research, unexpected new and important questions often arise. Addressing these questions must be prioritized to avoid overextending resources, but in some cases opportunistic projects that can be supported are added to the research program. This is particularly true for projects that are of particular interest to stakeholders.

- **Antler asymmetry.** During discussions with stakeholders in Southwestern Washington, concerns were voiced about antler asymmetry and the potential association with the high prevalence of hoof disease. We collaborated with a U.S. Geological Survey wildlife researcher, Dr. Glen Sargeant, and used hunter harvest data routinely collected by WDFW between 2016-2018 that was shared with us to investigate whether antler asymmetry occurs at a higher rate in elk with hoof lesions. Results indicate that elk with hoof abnormalities are more likely to exhibit asymmetric antlers. Previous research on deer has documented that trauma to a limb (similar to elk with hoof disease) can result in an antler anomaly in an individual; however, impacts from a transmissible disease affects a much larger proportion of a population. Our manuscript titled *Association of antler asymmetry with hoof disease in Washington elk* is under review for publication in the *Journal of Wildlife Management*.

- **Epigenetic impacts.** While we now know that TAHD lesions are caused by bacterial infection, other factors could alter an elk’s susceptibility to developing disease. We are collaborating with researcher Dr. Michael Skinner, a professor in the WSU School of Biological Sciences, to investigate epigenetic transgenerational inheritance of disease (increased susceptibility of future generations due to “ghosts” in their genome). We determined that cells in the tendon from an elk’s foot submitted for TAHD diagnosis could be used for epigenetic analysis. We collected approximately 70 samples from Roosevelt and Rocky Mountain subspecies of elk with and without TAHD for analysis by Dr. Skinner’s lab. Preliminary results revealed differences called epimutations (changes in the chemical structure of DNA that regulate genome activity independent of DNA sequence) in elk with TAHD and differences between the subspecies of elk. If confirmed through additional analyses, this suggests a systemic effect, meaning that many different cells throughout the body are affected. These changes can be passed to subsequent generations and potentially change their susceptibility to TAHD. Cause of the epimutations would require additional research.
• **Mineral status of elk.** Another factor that may change an elk’s susceptibility to TAHD is the status of important minerals in the body. For example, a deficiency in selenium, copper, or zinc or an excess of heavy metals like arsenic or chromium, may increase the likelihood that an elk would develop TAHD when exposed to the causative bacteria. We analyzed minerals in hair collected opportunistically from feet submitted for TAHD surveillance. We are comparing mineral status of approximately 70 elk with and without TAHD to determine if mineral concentration correlates with TAHD status.

**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 Southwestern Washington to a statewide issue. Moreover, with continuing cases in Oregon, and detections in Idaho and California, it has emerged as a multi-state regional issue. The broader geographic range amplifies the need for continued research on this important emerging disease. Moving forward our research will reflect this broader scope.

**General**

• **Research Team.** We will maintain the current level of staffing and hire a post-doctoral assistant to increase laboratory capacity for bacterial genome sequencing.

• **Legislative reporting.** The next report covering the period January-December 2022 will be submitted by February 10, 2023. Legislators and their staff are welcome to contact Dr. Margaret Wild at any time to ask questions or receive additional information as it is developed.

• **Update research plan.** The initial research plan we developed (Phase One) covered 2019-2021 (See Addendum). In 2022, we will review and update the research plan for the coming years.

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

• **Maintain and procure captive elk for study.** Elk that were procured in 2021 will be used in continuing studies in 2022. Approximately 10-12 additional elk calves will need to be procured for studies in 2022 and 2023. We will continue to work with Starkey Experimental Forest in Oregon to obtain elk; however, if chronic wasting disease is detected in Oregon (CWD is nearing the eastern border of both Oregon and Washington), our source of elk may be terminated if Oregon elk are not allowed to enter Washington.
• **Environmental transmission trial.** The results of our initial transmission study conducted in 2020-2021 will be reported in a scientific publication. We will complete the study initiated in 2021 investigating transmission of TAHD to elk naturally exposed to contaminated soil. In that study, we exposed healthy elk to soil where TAHD-positive elk had walked and to TAHD positive hooves in soil to determine if TAHD is highly transmissible in the environment.

• **Plan next study with captive elk.** We plan to develop a model for reliable TAHD transmission to captive elk (Drankhan PhD project). Pilot studies for technique development will be conducted in 2022.

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**Study disease agents in the laboratory**

• **Performing metagenomics.** Three studies focused on metagenomic analysis are in progress. The manuscript reporting findings from the first study using surveillance cases will be published in the *Journal of Wildlife Diseases* (see description above). For the second study (Goldsmith PhD), we will continue to collect samples and develop a grading system to sort lesions based on microscopic changes. The bacterial community of samples from each grade will be determined using metagenomic analysis in the coming year. The third study was initiated in 2018 in collaboration with the USDA Agricultural Research Service using samples from Southwestern Washington. We are currently awaiting data from our collaborator and will conduct analyses when data is provided and then jointly draft a manuscript to report findings.

• **Sequence novel bacterium.** Our initial metagenomic analyses routinely identified a unique spirochete (a group of spiral shaped bacteria that includes *Treponema* species) in TAHD lesions. The organism is referred to as phylotype 19 (PT19) based on the DNA sequence of a small section of its genome. We will conduct additional genome sequencing of this bacterium and determine how it is related to *Treponema* species that affect livestock. Findings will provide insights into the current range of distribution of this bacterium geographically and in different animal species.

• **Culture bacteria.** Our findings to date lend further support for the importance of *Treponema* or *Treponema*-like bacteria in hoof lesions. *Treponema* species are notoriously difficult to grow, or culture, in the laboratory. As a result, *Treponema* from elk hoof lesions have not yet been successfully cultured in the U.S. We will develop methods to culture bacteria and begin examining hoof lesion samples in the WSU College of Veterinary Medicine laboratory of Dr. Devendra Shah.
Conduct regional surveillance

- **Diagnostic testing at WADDL.** Disease surveillance will continue using samples submitted by wildlife agencies. Priority will continue to be on game management units (GMUs) in Washington where suspect cases of the disease have been observed but diagnostic testing has not confirmed TAHD and in new geographic areas (at the game management unit or county level) where TAHD has not been previously diagnosed. We will also collaborate to examine hoof samples from other states to determine the geographic extent and study progression of the disease.

- **Spatial epidemiology.** We will integrate data on elk distribution and TAHD occurrence from multiple data streams for preliminary analyses. We will also identify risk factors for disease occurrence and begin compiling data on each that will ultimately be used to model risk of disease occurrence.

Understand social aspects of the disease

- **Social science inquiry.** Data analysis from the research conducted in collaboration with SESRC in 2020 will be completed and the draft manuscript finalized for submission to a scientific journal.

- **Outreach.** Outreach via the listserv, website, media, legislative briefings, and stakeholder meetings will continue. Additionally, presentations to Washington’s legislators and staff can be arranged. Communication with the scientific community will occur through publications and presentations.

Collaborate with WDFW and Tribes

- **Collaborative disease investigation.** We will continue collaboration with WDFW, NWIFC, and other wildlife agencies to obtain hooves from elk harvested or found recently deceased in locations of interest for disease surveillance, for collection of research samples, and in joint research projects. Expertise at WSU assists managers in Washington and across the northwest in detecting the disease. Findings from all locations help inform our understanding of the disease in Washington and are critical for our research.

- **Scientific meeting.** We will co-host with RMEF and CDFW a virtual scientific meeting with state, federal, and tribal wildlife managers and veterinarians to share information, identify knowledge gaps, and solicit input on research priorities.

- **Collaboration with WDFW.** In addition to regular communications, we will continue quarterly meetings. We will also continue to collaborate on procuring elk hooves for disease surveillance and research. Additionally, WDFW will contribute important data for spatial epidemiology research.

- **Native American Tribes.** We will continue collaboration with Native American tribes to share information and obtain hoof samples. We will schedule virtual meetings as requested to promote outreach.
Ancillary projects

- **Antler asymmetry.** We will finalize the manuscript for publication in the *Journal of Wildlife Management*. We will also share these research findings via outreach to stakeholders.

- **Epigenetic impacts.** We will conduct final analyses and draft a manuscript for submission to a scientific journal. Following peer review, findings will be shared via outreach to stakeholders.

- **Mineral status of elk.** We will finalize statistical analyses and complete drafting a manuscript for submission to a scientific journal. Following peer review, findings will be shared via outreach to stakeholders. We will also collaborate with CDFW to conduct further investigations into the correlation of mineral levels in liver with the occurrence of TAHD.
Researchers examining infected elk hooves
ADDENDUM

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 be conducted with a three-year horizon (2019-2021). Successive phases of work will build on findings from these initial studies. The four principal 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 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 genetic material in a sample to determine which bacteria 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.