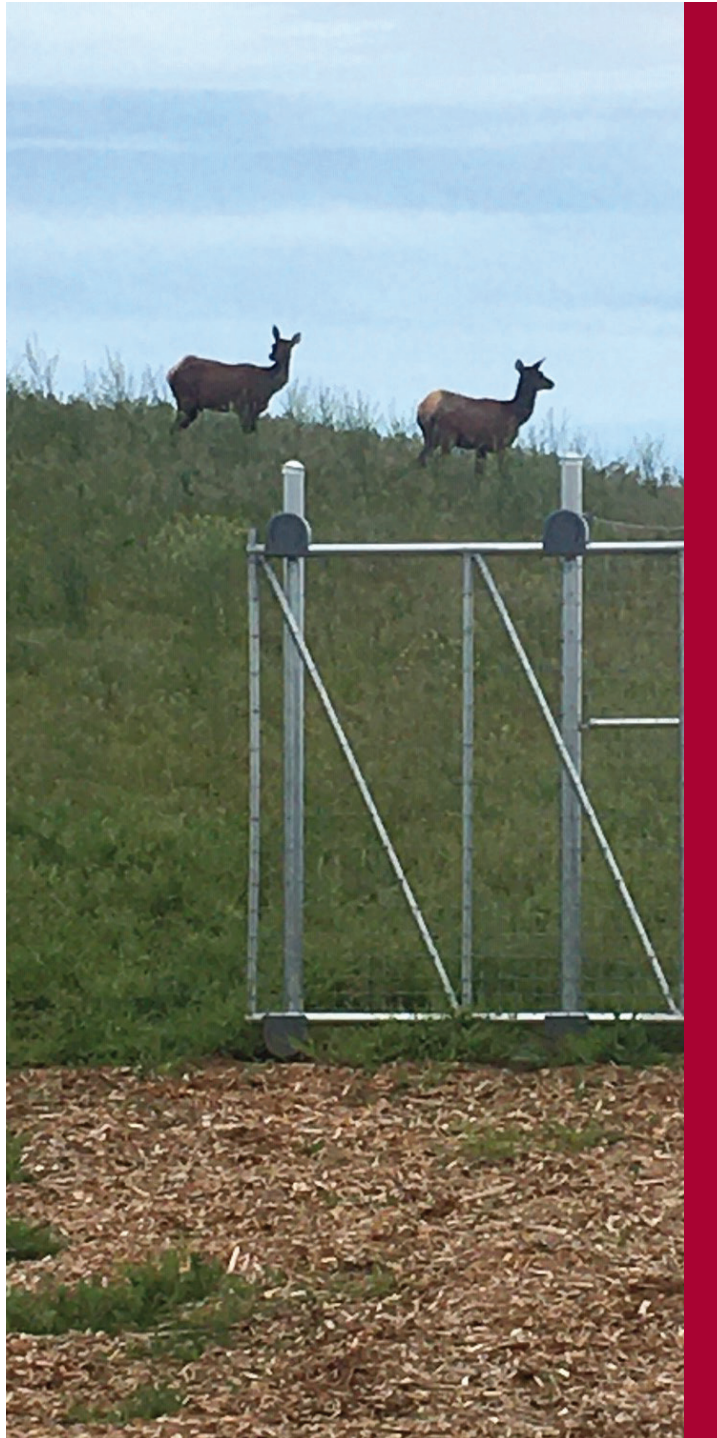


ELK HOOF DISEASE

Report to Legislature | 2020



College of
Veterinary Medicine



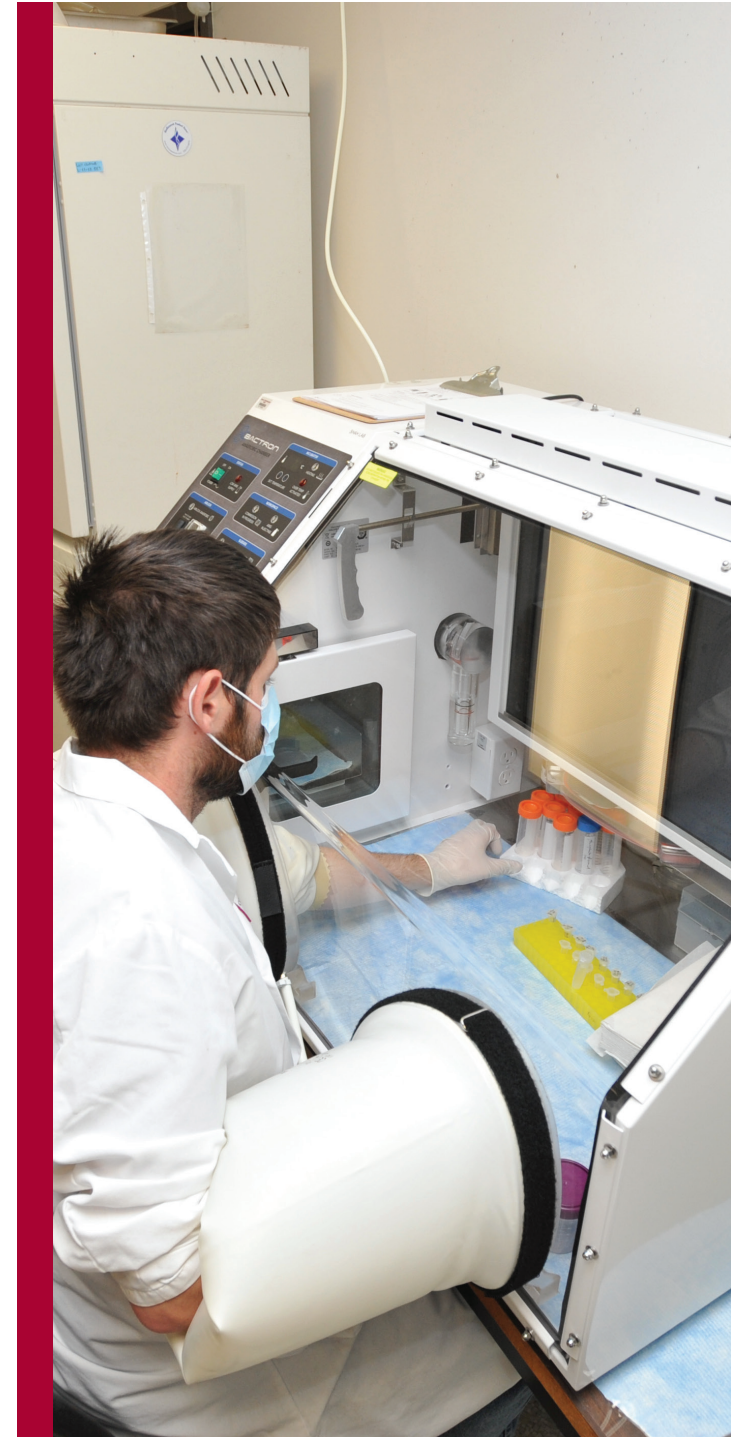
EXECUTIVE SUMMARY

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 intensity and spread of the disease, 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. Funding of \$1,169,900 was provided to WSU CVM for the FY2020-2021 biennium.

In 2020, WSU CVM continued implementation of a Research Plan developed 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 principle areas, as well as ancillary areas, of inquiry:

- *Study the disease cause(s) and contributing factors in captive elk.* The \$1.3 million facility was completed in January 2020. Twelve healthy wild elk calves were captured in cooperation with WDFW and transported to join one tame calf already at the facility to establish the inaugural research herd in February. Due to COVID-19 restrictions, we were not able to capture elk affected with Treponeme-associated hoof disease (TAHD) to study disease transmission between affected and healthy elk in captivity as planned. Instead, we initiated a revised study to investigate environmental transmission of TAHD in October. This marked a major milestone as the first study conducted on hoof disease in captive elk and the first in our new research facility.

- *Study disease agents in the laboratory.* We conducted metagenomic analyses (looking at all the genetic material in a sample to determine which organisms are present) in samples collected from diagnostic case submissions. Findings were reported in presentations at two virtual scientific conferences. Results lend support to the hypothesis that hoof lesions arise from an infectious disease process commonly associated with *Treponema* or *Treponema*-like bacteria. Interestingly, we identified DNA sequences from an as yet unidentified *Treponema*-like bacteria in the vast majority of lesions. We also continued hoof sample collection for metagenomic analysis in a future larger study.
- *Conduct regional surveillance.* In 2020, we coordinated with wildlife agencies for submission of hooves from 55 elk from Washington, eight elk and two deer from Oregon, 15 elk and two deer from Idaho, 17 elk from South Dakota, and six elk from California. Surveillance resulted in TAHD detection for the first time at a feedground in central Washington and in the state of California. We have not detected TAHD in sampled deer.
- *Understand social aspects of the disease.* We conducted outreach via a listserv, website, the media, legislative briefings, and stakeholder meetings. In collaboration with the WSU Social and Economic Sciences Research Center (SESRC) we completed a survey of Washington residents' (general public and hunters) opinions on elk hoof disease. Findings are summarized in a [technical report](#) titled *Public Perceptions of Elk Hoof Disease in Washington State (2020)*. Results from the survey are being used to inform our research and outreach strategies and were shared with WDFW to inform their decision-making processes.
- *Ancillary projects.* Two additional studies were initiated in response to stakeholder interest. In the first, we used hunters' observations reported in harvest data routinely collected by WDFW to investigate whether antler asymmetry occurs at a higher rate in elk with hoof lesions. Preliminary results support stakeholder observations that elk with hoof lesions are more likely to exhibit asymmetric antlers. Additionally, we initiated collaboration on a study to investigate epigenetic effects (changes in gene activity and expression) that may result from herbicide exposure in elk.



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 Southwest 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 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'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. 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 2020, which summarizes accomplishments made implementing the approach in 2020.



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 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 2020

General

In 2020, WSU CVM continued implementation of the Research Plan developed 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 principle areas, as well as two ancillary areas, of inquiry.

- *Student training.* 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 third-year combined pathology residency/PhD student. Dr. Goldsmith's research focuses on pathogen discovery using metagenomics techniques.
 - o Holly Drankhan, DVM, is a second-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 completed his first-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.
 - o Dylan Conradson participated in a College of Veterinary Medicine Research Summer Scholars Program following his second year of veterinary school. Dylan investigated antler asymmetry and its association with hoof disease in Washington elk.
 - o Vinay Bandarupalli, PhD, is a postdoctoral research associate. Dr. Bandarupalli was hired through Dr. Devendra's Shah's lab and contributes one quarter time to metagenomics and bacteriology research on elk hoof disease.
- *Staffing.* A scientific assistant, Elizabeth Wheeler, 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 completed elk research facility.

- o The CVM Fowler Emerging Disease Research Endowment Fund contributed \$15,000 toward elk hoof disease research.
- o Private individuals and businesses contributed over \$2000 in cash and services to support research efforts.
- o 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, but not selected for funding.

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

- *Captive elk facility.* Construction of the \$1.3 million captive elk facility was completed in late January 2020. The facility includes 10 individual animal pens, a handling facility, and two 1.5 acre holding pastures.
- *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 and 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 scientific collection permit from WDFW was maintained to capture and hold elk in captivity. Biosafety approval for conducting Biosafety Level 1 (BSL-1) research at the facility was obtained from WSU Environmental Health and Safety. In response to the COVID-19 pandemic, safe work practices and protocols were established to meet requirements to protect the health of employees, students, and research animals. No COVID-19 cases were diagnosed in any individuals involved in the WSU elk hoof disease research program.
- *Elk procurement.* Our initial objective was to obtain wild or captive elk to establish a captive breed herd at the elk facility. Unfortunately, availability of elk from herds where no signs of hoof disease have been documented in Washington are limited. Through collaboration with WDFW, 12 healthy wild elk calves (about 8-9 months old) were captured and transported to join one tame calf already at the facility to establish the inaugural research herd in February. These elk were captured near Yakima, Washington.



Dr. Wild examines an anesthetized captive elk for signs of hoof disease.



- *Environmental transmission study.* Unfortunately, response to the COVID-19 pandemic precluded plans to capture elk affected with TAHD that were needed to study disease transmission between affected and healthy elk in captivity. As a result of COVID-19 restrictions, the initial penned animal study was postponed by 7 months and the design was modified. Rather than study elk-to-elk transmission, we are studying disease transmission to healthy elk through exposure to infected hoof material “inoculated” into soil substrate in the individual biosecure pens. This study was initiated in late October and will continue into 2021, when we anticipate having preliminary results.

Study disease agents in the laboratory.

- *Metagenomic analysis.* We conducted metagenomic analyses of a subset of samples collected from diagnostic case submissions. These analyses were conducted in collaboration with WSU CVM veterinary microbiologist, Dr. Devendra Shah. Results lend support to the hypothesis that hoof lesions arise from an infectious disease process and that *Treponema* or *Treponema*-like bacteria are commonly associated with these lesions. We do not know yet if additional predisposing factors are necessary to promote lesion development and disease progression. Interestingly, we identified DNA sequences from an as yet unidentified *Treponema*-like bacteria in the vast majority of lesions. The identity and potential role of this currently unknown *Treponema*-like bacteria is intriguing and will be a focus in future studies. A manuscript reporting these findings is in preparation and will be submitted to a scientific journal for publication. Findings were also reported at the virtual annual conference of The Wildlife Society and by invitation to the virtual meeting of the United States Animal Health Association, Committee on Wildlife Health.

Presentation abstract: Distribution and Etiologic Investigation of An Emergent Hoof Disease of Elk in the Pacific West

Margaret A. Wild, Devendra Shah, Kyle Taylor, Venkata Vinay Bandrupalli

Recent surveillance has identified expansion of the known distribution of an emergent hoof disease in free-ranging elk (*Cervus elaphus*). The disease was initially investigated in 2008-2009 following a marked increase in limping elk observed in Southwest Washington. The disease is now locally endemic and has also been detected at lower prevalence to sporadically in other areas of Washington, Oregon, Idaho, and California. Characteristic lesions include

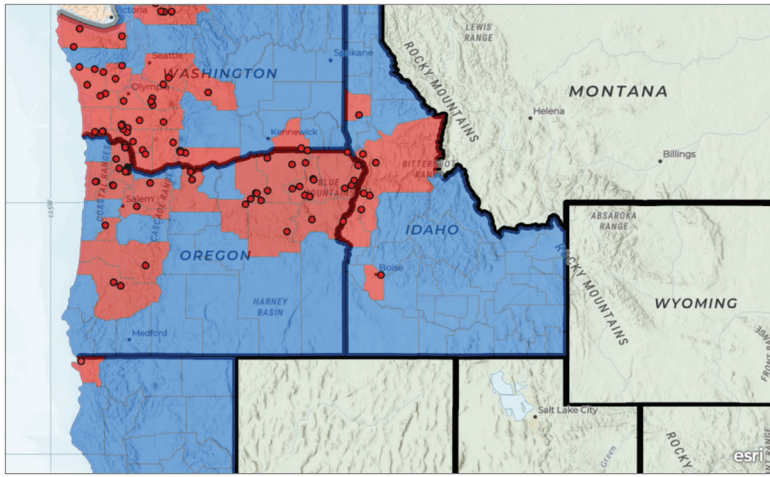


ulceration of the interdigital space, undermining of the heel bulb, and breakage or sloughing of the hoof capsule with associated lameness and debilitation. Spirochetes are routinely observed within areas of eroded epithelium with marked suppurative inflammation on histologic examination. In previous studies of elk from Southwest Washington, immunohistochemistry, PCR, and culture detected *Treponema* spp. in a majority of samples examined. Cultured isolates were similar to those reported in digital dermatitis of cattle and sheep. Thus, the disease is currently diagnosed as treponeme-associated hoof disease (TAHD). While treponeme-associated, additional investigation conducted over a broader geographic range is necessary to further investigate the etiology and refine the case diagnosis. Digital dermatitis in livestock is generally considered to be a polybacterial disease. We hypothesize a similar process may occur in elk. In a preliminary investigation, we compared the bacterial (16S rRNA) metagenomes in biopsies collected postmortem from affected and unaffected elk (n=32) from across the known distribution of disease. Results supported treponeme association, although as with previously reported methods, 16S analysis failed to detect *Treponema* in all samples classified positive by gross and histologic examination. Uncultured members of the phylum Spirochaetae were more commonly detected than typical bovine digital dermatitis *Treponema* phylotypes in analyzed samples. In addition to Spirochetes, other potential pathogens including Tenericutes (primarily *Mycoplama* spp.) and Fusobacteria, were overrepresented in lesions as compared to normal feet. Further investigation of the bacterial consortium of hoof lesions, as well as expanded disease surveillance in free-ranging and captive elk, are warranted and necessary to more fully understand this emergent disease.

- *Sample collection for metagenomic analysis.* A study was designed 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. Samples were collected opportunistically in most cases, but submission of hooves was also solicited from state and federal wildlife managers in California and South Dakota to obtain normal hooves for comparison. Interestingly, this led to the first detection of TAHD in California (see below).

Conduct regional surveillance.

- *Diagnostic testing.* Led by Washington Animal Disease Diagnostic Laboratory (WADDL) pathologist and faculty member Dr. Kyle Taylor and with support from scientific assistant Elizabeth Wheeler, we evaluated hooves from Washington and surrounding states. We focused surveillance on areas where the disease had not been previously detected (e.g., central and eastern Washington) and other cases of management interest (e.g., deer with abnormal hooves). In 2020, we coordinated with state and tribal wildlife agencies for submission of hooves from 55 elk from Washington, eight elk and two deer from Oregon, and 15 elk and two deer from Idaho. As in the past, we did not detect TAHD in deer samples. We also examined hooves from 17 elk from Wind Cave National Park, South Dakota, and six elk from northern California to serve as presumed normal hooves for comparison.



- *Spatial distribution.* Surveillance has resulted in the continued expansion of the known geographic distribution of hoof disease. In 2020, we detected TAHD for the first time in California and identified southward expansion in Oregon and Idaho. We also confirmed cases for the first time on an elk feedground in central Washington (Kittitas County). A suspicious case was also identified in eastern Washington, but more investigation is needed to confirm the status of this case. These findings highlight the need for continued statewide, as well as regional, surveillance to identify the true distribution of TAHD.

Understand social aspects of the disease.

- *Social science inquiry.* The WSU SESRC, led by Dr. Lena Le, completed a survey of Washington residents' opinions on elk hoof disease. Findings are summarized in a [technical report](#) titled *Public Perceptions of Elk Hoof Disease in Washington State (2020)*. Two groups were targeted for the survey: a general population sample of Washington residents and Washington resident elk hunters. Questions focused on five topics related to how respondents: seek information, trust entities involved in wildlife management, describe their awareness of elk hoof disease and their level of support for management actions and strategies, and wildlife value and belief dimensions. Among entities compared, WSU received the highest support to find a workable solution to elk hoof disease. To help maintain and grow this trust, results from the survey are being used to inform our research and outreach strategies and were shared with WDFW to inform their decision-making processes. A manuscript is in preparation for submission to a scientific journal to report findings.
- *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 five Research Updates as well as other information on our research activities in 2020. Currently, the list reaches over 150 recipients and continues to grow.
- *Stakeholder meetings.* Informal correspondence continued, but meetings with stakeholders were markedly reduced this year to due COVID-19 restrictions. Invited formal virtual presentations included:
 - o Northwest Indian Fisheries Commission
 - o Cowlitz Tribe
 - o Pacific Northwest Forest Vegetation Management workshop
 - o Society of Veterinary Hospital Pharmacists conference

- *Outreach via media.* WSU's work on hoof disease was covered in local and regional newspapers (including stories on disease spread in the [Yakima Herald](#) and [Lewiston Tribune](#)), an [online journal](#), and an interview on [Washington Ag Network radio](#).
- *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, Oregon, and California as well as with federal managers in Washington and South Dakota, 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.
- *Wildlife managers' meeting.* Following the WADDL diagnosis of TAHD in northern California, WSU participated in a virtual meeting of wildlife managers from the four TAHD-affected states. This forum provided an opportunity to share research and management information and plan collaborative efforts.
- *Collaboration with WDFW.* In addition to regular communications, we conducted virtual meetings quarterly to share information and plan and coordinate work. WDFW efforts have been critical to procuring elk for captive research and for obtaining fresh elk hooves for research. We also coordinated with WDFW on the SESRC survey and to obtain data collected on elk hoof disease in their mandatory hunter harvest reporting.

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 are of particular interest to stakeholders.

- *Antlers asymmetry.* During discussions with stakeholders in Southwest Washington, concerns were voiced about antler asymmetry and the potential association with the high prevalence of hoof disease. We used observations of hunters reported in harvest data routinely collected by WDFW to investigate whether antler asymmetry occurs at a higher rate in elk with hoof lesions. We are currently analyzing that data for publication in a scientific journal and dissemination to stakeholders. Preliminary results indicate that

elk with hoof lesions are more likely to exhibit asymmetric antlers. 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, other factors, such as mineral deficiencies may contribute to both hoof and antler abnormalities.

- *Epigenetic impacts from herbicides.* Herbicide use in forestry practices is frequently cited by stakeholders as a significant concern related to the occurrence of hoof disease. Findings from the SESRC survey (cited above) confirmed this concern in a portion of respondents. We are collaborating with WSU researcher Dr. Michael Skinner, a professor in the WSU School of Biological Sciences. His research has demonstrated the ability of environmental toxicants to promote epigenetic transgenerational inheritance of disease (increased susceptibility of future generations due to “ghosts” in their genome) in laboratory animals. The first step in potential application of this research to elk hoof disease was to determine if an appropriate tissue sample for analysis could be collected from hoof samples received for disease investigation. A promising tissue was identified and we are currently collecting samples for future analysis.

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 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 enroll on additional graduate student to study the spatial epidemiology of hoof disease.
- *Legislative reporting.* The next report covering the period January-December 2021 will be submitted by February 11, 2022. 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.

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

- *Maintain and procure captive elk for study.* Elk that were procured in 2020 will be used in continuing studies in 2021. Approximately 10-12 additional elk calves will need to be procured for studies in late 2021 and 2022. We will continue to work with WDFW to secure a source of elk, ideally from a herd where TAHD has not been diagnosed. These discussions will need to include the possibility of bringing elk in from outside Washington.

- *Environmental transmission trial.* The study initiated in late 2020 is in progress and will continue. Stage 2 of this study will be implemented when treatment elk (those exposed to affected hooves in pen soil in stage 1) develop hoof lesions. In stage 2, four additional healthy elk will be exposed to pens which housed elk with hoof lesions. The objective of these studies is to investigate natural transmission of TAHD through disease-contaminated soil.
- *Plan next study with captive elk.* Based on findings from the environmental transmission study, we will prioritize the next disease transmission study to be conducted in captive elk in 2021.

Study disease agents in the laboratory.

- *Performing metagenomics.* Three studies focused on metagenomic analysis are in progress. In the first study, a manuscript will be submitted for publication to report findings from a subset of diagnostic cases examined in 2020 (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 Southwest 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.
- *Culture bacteria.* Our findings to date lend further support for the importance of *Treponema* or *Treopnema*-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, including the new *Treponema*-like bacteria identified in our metagenomic analysis, and examine hoof lesion samples in the WSU CVM laboratory of Dr. Devendra Shah.

Conduct regional surveillance.

- *Diagnostic testing at WADDL.* Disease surveillance will continue using samples collected from hunter harvests, removals by management agencies, 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 in Washington. We will also collaborate to examine hoof samples from neighboring states, expanding efforts to Montana and Nevada, to determine the geographic extent of the disease.
- *Spatial epidemiology.* Preliminary analysis conducted by a summer veterinary research scholar examining risk factors for elk hoof disease indicated that a more complete and rigorous evaluation was needed to understand this complex disease. As a result, a PhD student has been recruited to start work in 2021 to focus on disease ecology and spatial epidemiology. 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.



Understand social aspects of the disease.

- *Social science inquiry.* Data from the research conducted in collaboration with SESRC in 2020 will be analyzed and a manuscript will be submitted for publication 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, presentations, and an annual virtual meeting with affected states.

Collaborate with WDFW and Tribes

- *Quarterly meetings with WDFW.* In addition to regular communications, we will continue quarterly meetings. We will also continue to collaborate on procuring elk hooves, elk for captive studies, and on other research.
- *Tribes.* We will continue collaboration with tribes to obtain hoof samples. We will schedule virtual meetings as requested to promote outreach.

Ancillary projects

- *Antler asymmetry.* Data analysis will be completed. We will draft a manuscript for submission to a scientific journal and provide outreach through lay publications.
- *Epigenetic impacts from herbicides.* We will compile samples and select a subset for analysis through our collaborator as funding allows.