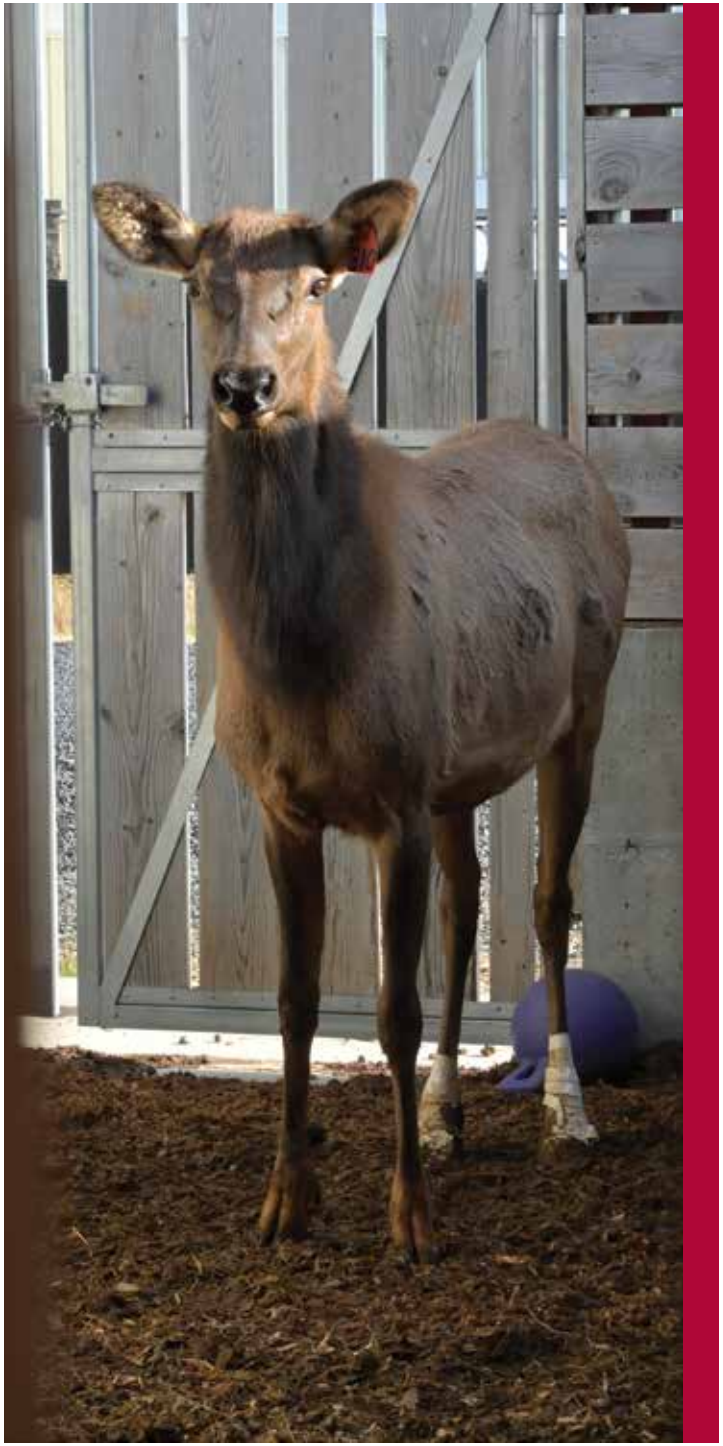
WASHINGTON STATE UNIVERSITY
College of Veterinary Medicine

ELK HOOF DISEASE

Report to Legislature | 2024



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 $1,170,000 was provided to WSU CVM for the FY2024-2025 biennium.

The WSU CVM team dedicated to addressing elk hoof disease in 2024 included one faculty member, one post-doctoral fellow, three PhD students, one master's student, and one scientific assistant. Additional WSU faculty, staff, and students and non-WSU collaborators also made significant contributions. In mid-2024, WSU began a search to replace the faculty lead for elk hoof disease research. Research by our team resulted in publication of one scientific article that significantly contributed to the current limited published scientific literature on elk hoof disease.

During the year, we made progress toward addressing the four principal areas of the Research Plan, as well as ancillary areas of inquiry.

- ***Study the disease cause(s) and contributing factors in captive elk.*** We conducted a study to develop a reliable transmission model for elk hoof disease, also called treponeme-associated hoof disease (TAHD), in captive elk (Drankhan PhD project). Our modified experimental approach, which included use of enriched bacterial cultures in addition to minced TAHD lesion material, successfully reproduced TAHD quickly and used far fewer inoculations than our previously reported model suggesting it could be used in future studies to investigate host risk factors and immune response and potential inter-species transmission of TAHD. We are currently conducting data analysis and drafting a manuscript for submission for publication in a scientific journal.

- ***Study disease agents in the laboratory***. We used state of the art technology (16S amplicon sequencing) to sequence a specific portion of DNA from bacteria in hoof samples to investigate bacteria associated with a spectrum of microscopic hoof lesions to help elucidate the cause and manner of disease development of TAHD (Goldsmith PhD project). We detected bacteria previously associated with TAHD (e.g., *Treponema* and *Treponema*-like bacteria, *Mycoplasma*, and *Fusobacterium*), as well as some additional bacteria of interest providing further evidence that TAHD is a polybacterial disease. Preparation of a manuscript for publication in a scientific journal is underway.

  We conducted an even more in-depth investigation of bacteria using shotgun sequencing that provides DNA sequences from throughout the bacterial genome (Deb post-doc project). We developed and applied an approach that used these data to reconstruct genomes of novel bacteria present in TAHD lesions. We published a manuscript in the journal *Applied and Environmental Microbiology* (doi.org/10.1128/aem.00105-24) reporting the discovery of six new types of bacteria: four in the family *Treponemataceae* and two in the genus *Treponema*. Additionally, we identified new "strains" of two *Treponema* bacteria associated with digital dermatitis. Additionally, we used shotgun sequence data to identify unique DNA sequences that allowed us to develop probes to microscopically label four potentially important TAHD pathogens we previously identified. These probes will be used to study bacterial association with lesions and will inform development of new diagnostic tests.

- ***Conduct regional surveillance and investigate risk factors.*** TAHD has been confirmed in elk in Washington, Oregon, Idaho, and California. We continued working with state wildlife managers to collect hooves for surveillance and research uses from Washington and other western states. TAHD was confirmed in several new game management units (GMUs), but not in any new states or in species other than elk.

  We investigated risk factors associated with TAHD in two projects (Winter PhD project). In the first, we did not find evidence that lower mineral levels were associated with higher TAHD occurrence; however, copper and selenium in both TAHD-affected and unaffected elk were below previously reported reference ranges in the elk populations sampled, which obscures interpretation of results (manuscript accepted for publication in the *Journal of Wildlife Diseases*). Currently, we do not recommend mineral supplementation due to the risk of congregation of elk promoting disease transmission. In the second study, we evaluated how hoof abnormalities were associated with local land cover, topography, and soil characteristics, as well as how TAHD cases at the GMU-level were associated with precipitation prior to the harvest season. A manuscript reporting findings is in preparation. These findings can be used to focus surveillance and design future field-based studies to evaluate management approaches.

- ***Understand social aspects of the disease and communicate findings***. We conducted outreach using multiple approaches including distributing information via a newsletter (ElkTracks), website, lay and scientific media, in-person meetings, and scientific conferences. We reached a national audience by being featured in an episode of *Meateater*'s *Cal in the Field* (available on YouTube). We also revised a scientific manuscript that reports findings from the 2020 survey of public perceptions of elk hoof disease by the general public and hunters in Washington for publication in the journal *Human Dimensions of Wildlife*. Collaborations with WDFW, tribes, and other wildlife management agencies were key for outreach and research success.

- ***Ancillary projects.*** We conducted a study to investigate the utility of activity monitors on captive elk to remotely detect behavioral changes associated with TAHD (Hill MS project). Data analysis is underway. We also collected hooves from bison to conduct initial surveillance for hoof lesions and investigate the bacteria present on their hooves.

BACKGROUND AND OVERVIEW

Elk hoof disease, known scientifically as treponeme-associated hoof disease (TAHD), is an emerging disease of elk in the northwestern U.S. 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 (Han et al. 2019). Affected elk become debilitated and, according to preliminary research by WDFW, experience higher mortality that 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 to 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 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 literally created from the ground up, including the construction of an elk research facility. The legislature continued biennial funding at a level of $1,170,000 through FY2024-2025.

The following report consists of two sections. The first section, Research and Outreach Accomplishments in 2024, summarizes accomplishments made implementing our research plan in 2024. The second section is our Research Plan, which was developed in 2018 and defines the research approach to guide our work for the period 2019-2024 and is attached as an Addendum at the end of this report for reference.

RESEARCH AND OUTREACH ACCOMPLISHMENTS IN 2024

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 currently a seventh-year combined pathology residency/PhD candidate. Dr. Goldsmith's research focuses on pathogen discovery using metagenomic techniques. Her work is also providing DNA sequences of pathogens that will be used in the future to develop new diagnostic tests for TAHD. She presented a poster on her research at the American College of Veterinary Pathologists conference, attendance at which was supported by a departmental travel grant. Anticipated graduation 2025.
    - Holly Drankhan, DVM, is currently a sixth-year combined pathology residency/PhD candidate. Dr. Drankhan is developing a reliable disease transmission model using elk at the captive research facility. She is the recipient of an NIH T32 training grant (grant number T32AI007025). Anticipated graduation December 2025.
    - Steven Winter, MS, is a PhD candidate. Steven is studying spatial and temporal distribution and risk factors of TAHD using computer modeling. The third scientific manuscript from Steven's PhD work, which examined associations between TAHD and liver mineral levels, was accepted for publication in the *Journal of Wildlife Diseases*. He is completing the final phase of his work investigating risk factors for TAHD occurrence in southwestern Washington. Steven presented his research at The Wildlife Society (TWS) annual conference. He received a departmental travel award and TWS grant to help support attendance at the conference. Anticipated graduation May 2025.
    - Trent Hill is a master's student in the WSU School of the Environment. Trent is evaluating behavioral changes associated with TAHD infection in captive elk using telemetry data collected from ankle monitors. Trent presented a poster on his research at the TWS conference. Anticipated graduation 2025.


Dr. Elizabeth Goldsmith

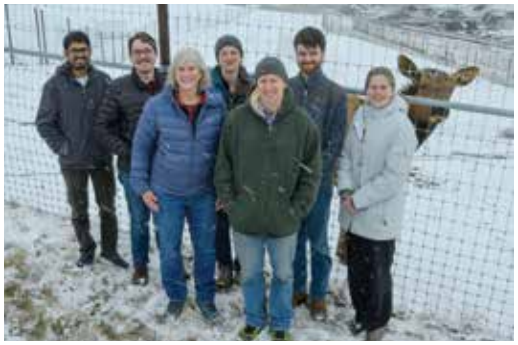

Dr. Holly Drankhan


Steven Winter


Trent Hill


Dr. Sushanta Deb


Charlie Park



- ***Post-doctoral fellow.*** Sushanta Deb, PhD, joined our research lab in fall 2022. Dr. Deb brings extensive experience with computer analysis of complex genetic codes (a field called bioinformatics). He is applying his knowledge to discover and describe bacteria associated with TAHD. His work led to discovery of new *Treponema* and *Treponema*-like bacteria. Dr. Deb's findings were published in the journal *Applied and Environmental Microbiology* and were delivered in an oral presentation at the Lameness in Ruminants international conference and in a poster presentation at the American Society of Microbiology Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Pipelines.
- ***Staffing.*** A scientific assistant, Charlie Park, manages the laboratory, oversees diagnostic case submissions and processing, and coordinates research animal care. Veterinary and undergraduate students assist with laboratory tasks and animal care.

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. Research to develop a reliable TAHD transmission model was conducted at the facility in 2024.
- ***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 maintained and approved by the WSU Institutional Animal Care and Use Committee (IACUC), a federally mandated oversight group.
- ***Develop a reliable transmission model in captive elk.*** We conducted a study to develop a reliable TAHD transmission model in captive elk (Drankhan PhD project). Following mixed results in initial transmission studies in 2023, we modified the experimental technique and added enriched bacterial cultures to our challenge inoculum. *Treponema*, and some other TAHD-associated bacteria, do not grow well under standard laboratory conditions; however, we adapted techniques to enhance their growth. These enriched bacterial cultures were used in addition to minced lesion material from wild elk that had died from TAHD to create inocula that were applied to the feet of captive elk under experimental conditions. We observed foot lesions consistent with TAHD in all five experimentally challenged elk within 28 days. Two control elk that were treated identically, but without the bacterial culture and with hooves from healthy wild elk, did not develop TAHD lesions. We are currently conducting further evaluation of tissues collected during the experiment and drafting a manuscript for submission to a scientific journal. This experimental approach successfully reproduced TAHD quickly and used far fewer inoculations than our previously reported model (Robinson et al. 2023) suggesting it could be used in future studies to investigate host risk factors and immune response and potential inter-species transmission of TAHD.

Study disease agents in the laboratory.

- ***Bacteria associated with microscopic lesion categories.*** The objective of this work is to investigate bacteria associated with a spectrum of microscopic hoof lesions to elucidate the cause and manner of disease development of TAHD (Goldsmith PhD project). Samples from 123 elk were categorized based on the microscopic changes observed and paired samples were analyzed to determine bacteria that were present in each. Bacteria were identified by sequencing a portion of DNA extracted from foot tissue using a technique called 16S amplicon sequencing. Preparation of a manuscript for publication in a scientific journal is underway. Findings support the presence of bacteria previously associated with TAHD (e.g., *Treponema* and *Treponema*-like bacteria, *Mycoplasma*, and *Fusobacterium*), as well as some additional bacteria of interest. These bacteria were found in samples collected across the geographic range of TAHD, with minor variations, whether elk were from areas with high or low prevalence of TAHD. Together, these findings provide further evidence that while *Treponema* are important, TAHD is a polybacterial disease.

- ***Pathogen discovery using genome reconstruction.*** We conducted an even more in-depth investigation of bacteria than can be achieved with the 16S amplicon sequencing described above. We used shotgun sequencing that provides DNA sequences from throughout the bacterial genome that can be used to more specifically identify bacteria at the species or strain-level. Further, sequences can be assembled to reconstruct genomes of previously uncharacterized bacteria to discover new species or strains of bacteria (Deb post-doctoral study, and in collaboration with Dr. Devendra Shah, Texas Tech University). This approach is particularly useful in studying diseases like TAHD that are associated with bacteria that are challenging, if not currently impossible, to grow in the laboratory. We used a series of steps to reconstruct genomes from known, as well as previously uncharacterized, *Treponema*-like bacteria. We discovered six new types of bacteria: four in the family *Treponemataceae* and two in the genus *Treponema*. Additionally, we identified new "strains" of two bacteria associated with digital dermatitis, *Treponema pedis* and *Treponema phagedenis*. Currently, it is unclear whether these bacteria are unique to elk or to the geographic area of the northwestern U.S. (i.e., no genomes from *Treponema* in livestock from the western U.S. are available in the databases that could be used for comparison). These findings were published in a peer-reviewed scientific journal, *Applied and Environmental Microbiology*, June 2024, doi.org/10.1128/aem.00105-24.

DISCOVERY OF NOVEL TREPONEMES ASSOCIATED WITH PODODERMATITIS IN ELK (CERVUS CANADENSIS)
Sushanta Deb, Margaret A. Wild, Thomas LeClair, Devendra H. Shah.

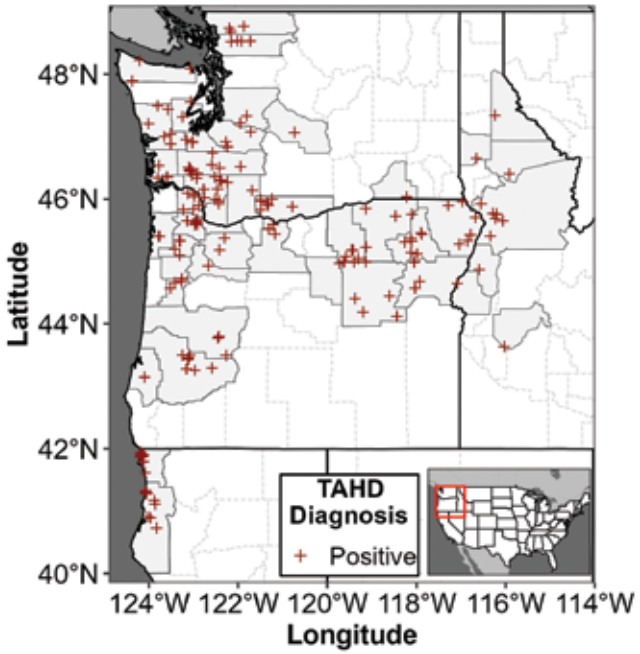
ABSTRACT: Pododermatitis, also known as treponeme-associated hoof disease (TAHD), presents a significant challenge to elk (*Cervus canadensis*) populations in the northwestern USA, with *Treponema* spp. consistently implicated in the lesion development. However, identifying species-specific *Treponema* strains from these lesions is hindered by its culture recalcitrance and limited genomic information. This study utilized shotgun sequencing, *in silico* genome reconstruction, and comparative genomics as a culture-independent approach to identify metagenome-assembled *Treponema* genomes (MATGs) from skin scraping samples collected from captive elk experimentally challenged with TAHD. The genomic analysis revealed 10 new MATGs, with 6 representing novel genomospecies associated with pododermatitis in elk and 4 corresponding to previously identified species—*Treponema pedis* and *Treponema phagedenis*. Importantly, genomic signatures of novel genomospecies identified in this study were consistently detected in biopsy samples of free-ranging elk diagnosed with TAHD, indicating a potential etiologic association. Comparative metabolic profiling of the MATGs against other *Treponema* genomes showed a distinct metabolic profile, suggesting potential host adaptation or geographic uniqueness of these newly identified genomospecies. The discovery of novel Treponema genomospecies enhances our understanding of the pathogenesis of pododermatitis and lays the foundation for the development of improved molecular surveillance tools to monitor and manage the disease in free-ranging elk.

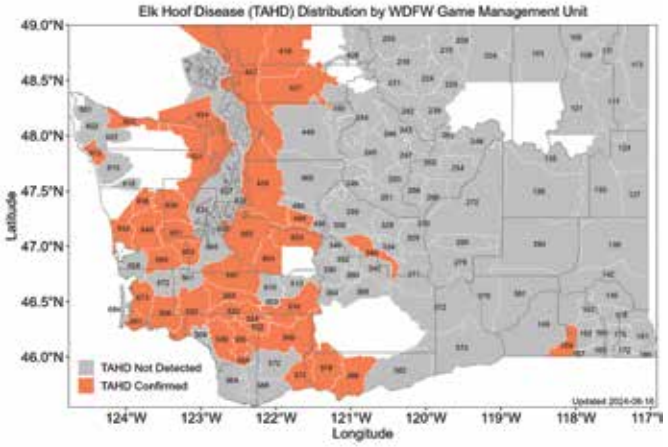
IMPORTANCE: Treponema spp. play an important role in the development of pododermatitis in free-ranging elk; however, the species-specific detection of Treponema from pododermatitis lesions is challenging due to culture recalcitrance and limited genomic information. The study utilized shotgun sequencing and in silico genome reconstruction to identify novel Treponema genomospecies from elk with pododermatitis. The discovery of the novel Treponema species opens new avenues to develop molecular diagnostic and epidemiologic tools for the surveillance of pododermatitis in elk. These findings significantly enhance our understanding of the genomic landscape of the Treponemataceae consortium while offering valuable insights into the etiology and pathogenesis of emerging pododermatitis in elk populations.

We conducted further investigations into the occurrence and prevalence of these novel pathogens by evaluating a larger number of known TAHD-positive and TAHD-negative samples. These data are being analyzed.

- ***Association of putative pathogens with histologic lesions.*** We also used shotgun sequence data collected for the genome reconstruction studies to identify unique DNA sequences of four potentially important pathogens we previously identified (see section above on "*Bacteria associated with microscopic lesion categories*"; Goldsmith PhD project). These sequences were used to develop probes that label these potentially important pathogens. When samples of TAHD lesions are examined under the microscope, a color change is observed where the probe finds the DNA sequence of the specific pathogen of interest using a test called an *in situ* hybridization (ISH) assay. This test allows a veterinary pathologist to determine if the specific pathogen is present, and if so, how it is (or is not) associated with a microscopic TAHD lesion. The findings are used to determine if the pathogen is important in the occurrence of TAHD lesions. The results will lead to development of an improved diagnostic test for TAHD. The technique for using ISH was developed and a pilot study was conducted to determine if the probes would work on samples of the elk hoof tissue. Results were encouraging that ISH will be a useful technique.
- ***Compare hoof sampling techniques.*** We continued to collect sets of samples (punch biopsy, skin scraping, and skin swab) from hooves submitted for research and surveillance for use in a study that will compare three methods of sampling elk feet. DNA was extracted from a subset of the samples. The objective of the study (Drankhan PhD project) is to determine if less invasive techniques (swabbing or scraping) are as reliable as a biopsy in characterizing the bacterial community present in TAHD lesions.
- ***Metabolic analysis.*** New technologies can profile metabolic changes in animals that are associated with disease. Led by WSU veterinary pathologist, Dr. Kyle Taylor, we analyzed serum (the liquid portion of blood) and preserved hoof skin samples from elk with and without TAHD using metabolomics and proteomics, respectively. Metabolomics measures metabolites and low molecular weight molecules that are formed in response to a stressor, such as disease. Proteomics characterizes and quantifies proteins in place in a tissue sample. The approaches are connected and may be used to identify biomarkers or contributing factors for disease. Analysis of results is in progress.

Drs. Margaret Wild and Holly Drankhan continue elk hoof research.





Conduct regional surveillance and investigate risk factors.

- ***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 previously reported findings from surveillance conducted in Washington, Oregon, Idaho, and California in 2018-2020 (Wild et al. 2022). With this baseline distribution documented, in 2024 we focused sample collection primarily on areas (generally at the county or game management unit level) where TAHD had not been previously detected, on cases of management concern, and to obtain samples for research purposes. Working with state, federal, and tribal wildlife managers, we collected hooves for research uses from Washington (20 elk), and for disease surveillance from Washington (9 elk, 1 deer), Oregon (12 elk), and Idaho (5 elk, 1 moose). We completed a data sharing agreement with the California Department of Fish and Wildlife (CDFW) to obtain location of TAHD cases diagnosed in California. We maintained a national map of locations of TAHD cases in elk. TAHD has not been detected in wildlife species other than elk.

  This year we did not detect TAHD in any new states or areas distant to previously known distribution, although TAHD was confirmed in several new game management units (GMUs). We continue to solicit submission of abnormal hooves from several Washington GMUs (501, 503, 504, 510, 513, 568, 572, 618, 658, 672, 684) in an attempt to confirm TAHD where it is suspected to occur but has not been diagnosed using laboratory techniques.

- ***Mineral status of elk.*** We continued collaboration with CDFW to investigate the correlation of mineral levels in liver with the occurrence of TAHD (Winter PhD project). We did not find support for our hypothesis that lower mineral levels contributed to higher TAHD occurrence in the populations sampled; however, copper and selenium in both TAHD-affected and unaffected elk were below previously reported reference ranges, so we cannot discount suboptimal levels of these minerals as potential risk factors for TAHD in elk in California. Nevertheless, given our current understanding, we do not recommend mineral supplementation due to the risk of congregating elk and promoting disease transmission.

We submitted a manuscript reporting our research results that was accepted for publication in the *Journal of Wildlife Diseases*.

- ***Risk factors for TAHD occurrence.*** We aimed to better understand the role of environmental features in the occurrence of TAHD (Winter PhD project). Sufficient data were available only from elk in an endemic area of disease in southwestern Washington. We evaluated how hoof abnormalities were associated with local land cover, topography, and soil characteristics, as well as how TAHD cases at the GMU-level were associated with precipitation prior to the harvest season. A manuscript reporting findings is in the final stages of preparation and will be submitted to a scientific journal in early 2025. These findings can be used to focus surveillance and design future field-based studies to evaluate potential management approaches.

Understand social aspects of the disease and communicate findings.

- ***Social science inquiry.*** We continued collaboration with the WSU Social and Economic Sciences Research Center (SESRC), led by Dr. Lena Le, and revised a scientific manuscript that reports findings from the 2020 survey of public perceptions of elk hoof disease by the general public and hunters in Washington. The manuscript is currently in the final review process by the journal.
- ***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. We provided updates in "ElkTracks". The listserv reaches nearly 200 recipients and continues to grow.

- ***Media coverage and outreach***. Our research on hoof disease received coverage in local and national media. Of particular note, elk hoof disease and our research were the focus of an episode of *Meateater's Cal in the Field*. The hour-long episode is available on YouTube and has received 190,000 views since its release in September. On the local level, our research was featured in a story in the Columbian.

  Informal correspondence also continued, with timely responses to inquiries from stakeholders, legislators, and the media. Presentations were also given to WSU veterinary and wildlife students and Wahkiakum High School.

- ***Website.*** The elk hoof disease website was maintained to provide information on elk hoof disease and our research.

- ***Scientific outreach.*** We presented scientific findings from WSU's research program at scientific conferences, including The Wildlife Society, American College of Veterinary Pathologists, Lameness in Ruminants, and the American Society of Microbiology Conference on Rapid Applied Microbial Next-Generation Sequencing and Bioinformatic Pipelines.

Collaborate with WDFW and tribes

- ***Collaborative disease surveillance.*** WSU collaborated with wildlife managers in Washington, Idaho, Oregon, California, the US Fish and Wildlife Service, Muckleshoot tribe, Makah tribe, 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.

- ***Collaboration with WDFW.*** In addition to regular communications, we conducted quarterly virtual meetings to share information and plan and coordinate work.

- ***Tribal collaboration.*** In addition to coordination with several tribes for submission of hooves for disease surveillance and research, we met with the Yakama Nation Tribal Council as part of a WSU CVM team and provided an update on elk hoof disease and our 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.

- ***Activity monitors.*** We deployed activity monitors developed in collaboration with Advanced Telemetry Systems (ATS) to investigate changes in behavior associated with TAHD in captive research elk (Hill MS project). We conducted a pilot study and confirmed the reliability of telemetry data collected by the ankle monitors in identifying bedded, standing, and walking behavior. We then used the monitors to collect data on elk activity during the TAHD challenge study (see above). If accurate and reliable, similar technology may be used to provide insights into impacts of TAHD on free-ranging elk.

- ***Evaluation of bison hooves.*** The susceptibility of bison to TAHD or a similar disease of cattle called bovine digital dermatitis is not known. However, because wild and captive-raised bison overlap range with elk in some areas and given the phylogenetic similarity of bison and cattle, bison may be a species at risk of developing digital dermatitis similar to that in elk or cattle. To obtain baseline information on the presence or absence of foot lesions and bacteria associated with digital dermatitis in cattle and elk, we collaborated with Turner Ranches to obtain feet from 23 bison at slaughter. A subset of the hooves were sampled according to our TAHD protocol and examined with histopathology and 16S sequencing to identify bacteria present. Analyses are in progress.

A captive elk is sampled and examined during a challenge study.

NEXT STEPS

Since the passage of Senate Bill 5474, active disease surveillance conducted collaboratively between WSU and state, tribal, and federal wildlife agencies 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 Washington, Oregon, 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. Our research will continue to reflect this broader scope, while focusing on the application of findings to Washington.

General

- ***Research Team.*** We will maintain staff and students to implement the Research Plan (see Addendum). WSU CVM will continue the job search with the objective of hiring a new faculty member to lead elk hoof disease research in mid-2025. A transition, ideally with a period of overlap with the current and new faculty lead, is anticipated in late 2025. An updated Research Plan will be developed by the new faculty lead. Most, or all, of the current graduate students will graduate in 2025.
- ***Legislative reporting.*** The next report covering the period January-December 2025 will be submitted by February 6, 2026. 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 captive elk for study.*** Elk that were previously procured will be maintained. We will continue to work with Starkey Experimental Forest in Oregon to obtain additional elk as deemed necessary for planned research.
- ***Develop a reliable transmission model in captive elk.*** Studies with captive research elk to develop a transmission model have been completed. We will analyze data and draft a manuscript reporting findings for publication in a scientific journal (Drankhan PhD project). Concurrently, we will analyze data and draft a manuscript reporting changes in activity associated with development of lesions during the challenge study (Hill MS project; see Ancillary projects page 18).

Study disease agents in the laboratory.

- ***Bacteria associated with microscopic lesion categories.*** Data for this study have been collected and analyzed (Goldsmith PhD project). A manuscript will be prepared for submission to a scientific journal early in 2025.
- ***Pathogen discovery using genome reconstruction.*** We will conduct further investigations into the occurrence and prevalence of novel *Treponema* and *Treponema*-like bacteria pathogens by evaluating known TAHD-positive and TAHD-negative samples. Data will be analyzed and a manuscript submitted for publication in a scientific journal. The results will contribute to development of improved diagnostic tests for TAHD.
- ***Association of putative pathogens with histologic lesions.*** Results from a pilot study indicated that the four probes we developed are able to label specific pathogens of interest in TAHD lesions. We will use the probes to investigate the association of these pathogens with microscopic TAHD lesions (Goldsmith PhD project). We will determine whether these probes, and potentially additional probes, should be pursued and used to develop diagnostic tests for TAHD. These diagnostic tests could include an ISH that would be used to definitively confirm TAHD by examining samples under a microscope and/or a PCR to screen for TAHD-associated pathogens in samples.
- ***Compare hoof sampling techniques.*** We will analyze samples collected and conduct analyses to compare results from the three different sample types (Drankhan PhD project). A manuscript will be prepared for submission to a scientific journal. Study results will inform our protocol for sample collection.
- ***Metabolic analysis.*** Metabolomic and proteomic results from serum and preserved hoof skin samples from elk with and without TAHD will be `analyzed to identify biomarkers or potential contributing factors for disease.

Conduct regional surveillance and investigate risk factors.

- ***Disease surveillance.*** Disease surveillance will continue using samples submitted by wildlife agencies. Priority will continue to be on 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 GMU 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 distributional progression of the disease.
- ***Mineral status of elk.*** A manuscript reporting research findings has been accepted and will be published in the *Journal of Wildlife Diseases*.
- ***Modeling risk factors.*** We will complete data analysis and prepare a manuscript for submission to a scientific journal in early 2025. Identification of risk factors will inform management strategies and guide TAHD surveillance programs.

Understand social aspects of the disease.

- ***Social science inquiry.*** Final manuscript revisions have been submitted following review of our manuscript that reports findings of the 2020 survey of Washington residents. We anticipate publication in the journal *Human Dimensions of Wildlife* in 2025.
- ***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. We will pursue another national venue (e.g., print media, podcast) in 2025 to increase awareness of TAHD. 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 and collection of research samples. Expertise at WSU assists managers in Washington and across the northwest in detecting the disease. Findings from all locations help inform our understanding of TAHD in Washington and are critical for our research.
- ***Scientific meeting.*** We will co-host with Rocky Mountain Elk Foundation and CDFW the third annual virtual scientific meeting with state, federal, and tribal wildlife managers and veterinarians to share information, identify knowledge gaps, and solicit input on research priorities. We will also provide research updates to collaborators as requested.
- ***Collaboration with WDFW.*** In addition to regular communications, we will conduct quarterly meetings. We will also continue to collaborate on procuring elk hooves for disease surveillance and research. We will pursue a data transfer and use agreement between WSU and WDFW so that WSU TAHD surveillance data from Washington can be maintained and managed on a WDFW data management site.
- ***Native American Tribes.*** We will continue collaboration with Native American tribes to share information and obtain hoof samples. We will schedule in-person or virtual meetings as requested to promote outreach.

Captive elk in pen at the WSU Elk Research Facility.

Activity monitor on a captive elk at the WSU Elk Research Facility.

Ancillary projects

- ***Activity monitors.*** All data have been collected and will be analyzed to evaluate changes in activity in captive research elk that developed TAHD (Hill MS project). A manuscript will be prepared and submitted for publication in a scientific journal.
- ***Evaluate bison hooves.*** We will compile results on microscopic changes observed in bison feet and conduct 16S analysis to determine what bacteria are present. Based on results, we will determine whether preparation of a manuscript to submit for publication is warranted.
- ***Post-mortem changes associated with TAHD.*** We will summarize data from post-mortem examinations of elk with TAHD that have been conducted at WADDL and collaborate with CDFW to obtain reports from examinations that have been conducted on elk with TAHD in California. We will recruit a student to compile data and prepare a manuscript for submission to a scientific journal.

REFERENCES

Han S., K. G. Mansfield, D. S. Bradway, T. E. Besser, D. H. Read, G. J. Haldorson, D. P. Alt, and J. H. Wilson-Welder. 2019. Treponeme-associated-hoof disease of free-ranging elk (*Cervus elaphus*) in southwestern Washington State, USA. Veterinary Pathology 56: 118-132.

Robinson, Z. B., D. H. Shah, K. R. Taylor, and M. A. Wild. Transmission and lesion progression of treponeme-associated hoof disease in captive elk (*Cervus canadensis*). 2023. PLoS ONE 18(8): e0289764.

Wild, M. A., D. Shah, K. R. Taylor, K. Garrison, K. Mansfield, J. Burco, S. N. Winter, M. L. Drew, S. Han, R. Bildfell, and B. A. Munk. 2022. Surveillance for an emergent hoof disease in elk (*Cervus elaphus*) in the U.S. Pacific West supplemented by 16S rRNA gene amplicon sequencing. Journal of Wildlife Diseases. 58: 487-499.

ADDENDUM

RESEARCH PLAN
Phase One: 2019-2024

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 was initially conducted with a three-year horizon (2019-2021). Program evaluation led to extension and expansion of the plan to incorporate a six-year period (2019-2024). 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 required for effective management as well as to identify risk to other species. Treponema species are 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 Treponema 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 and investigate risk factors.*** 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.

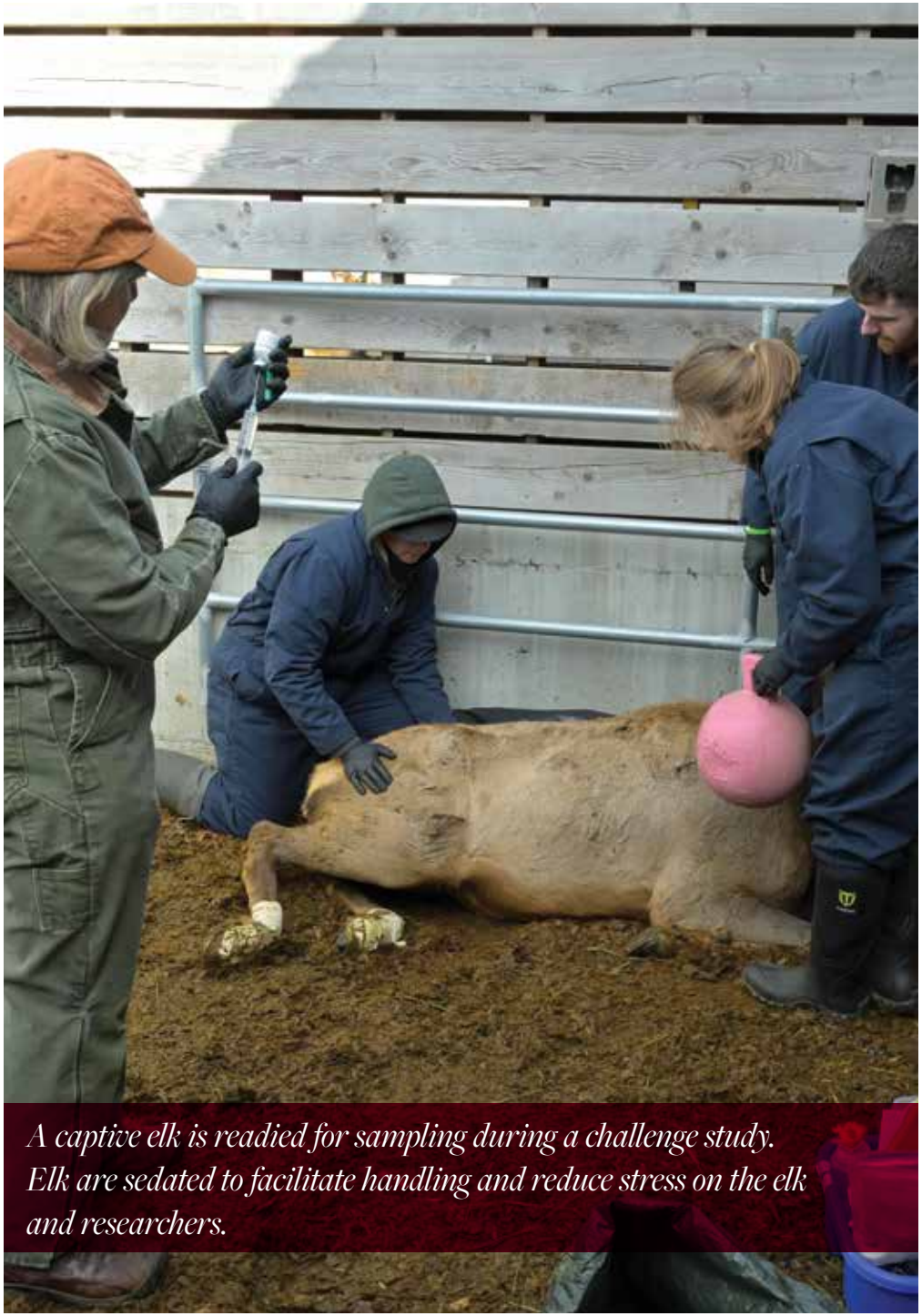
- ***Understand social aspects of the disease and communicate findings.*** Implement outreach and education efforts that are grounded in an understanding of stakeholder's beliefs, values, and concerns about hoof disease and elk management.

  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.

Washington State University is recognized as a leader in elk hoof disease research. As a result, wildlife agencies outside of Washington also seek collaborations. Such collaborations provide access to additional datasets and research opportunities and will be pursued when they also contribute to the understanding and management of hoof disease in Washington.



A captive elk is readied for sampling during a challenge study. Elk are sedated to facilitate handling and reduce stress on the elk and researchers.

WASHINGTON STATE UNIVERSITY

College of Veterinary Medicine

Department of Veterinary Microbiology and Pathology

vetmed.wsu.edu/elk-hoof-disease