

# ELK HOOF DISEASE

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Report to Legislature | 2023



WASHINGTON STATE UNIVERSITY  
**College of Veterinary Medicine**



## 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 FY2022-2023 and FY2024-2025 biennia.

The WSU CVM team dedicated to addressing elk hoof disease in 2023 included one full-time 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. Research by our team resulted in publication of three scientific articles 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.*** Results from our initial study conducted at the Elk Research Facility were published in a scientific journal (PLoS ONE 18(8): e0289764). The study provided the first direct evidence that treponeme-associated hoof disease (TAHD) can be transmitted to elk through soil contaminated with affected hooves and contributed additional evidence that TAHD is an infectious disease. Additional studies investigating transmission are ongoing using captive elk.

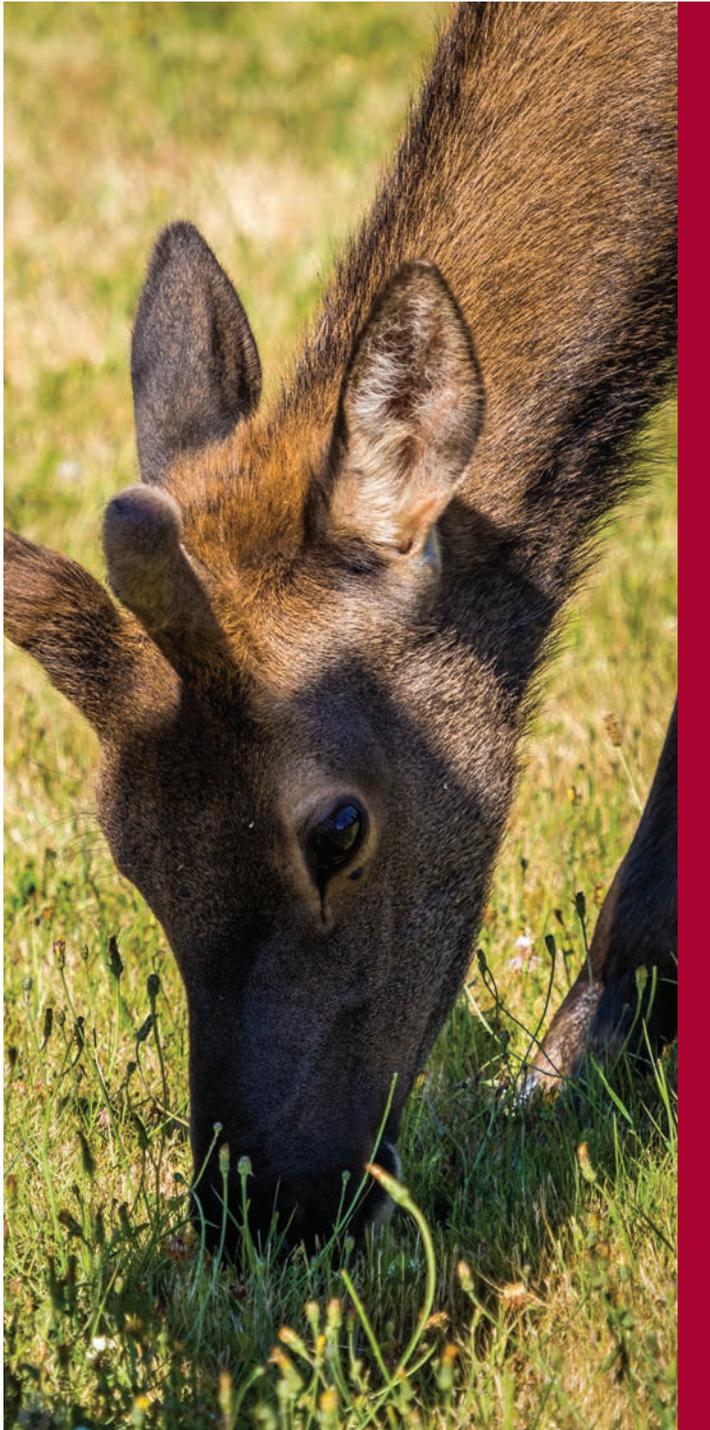
- **Study disease agents in the laboratory.** We used state of the art technology (16S amplicon sequencing) to sequence a specific portion of DNA from bacteria in hoof samples to investigate bacteria associated with a spectrum of microscopic hoof lesions to help elucidate the cause and manner of disease development of TAHD. In-depth data analyses are in progress, but preliminary data provide additional evidence of the presence of bacteria we previously associated with TAHD (e.g., *Treponema* and *Treponema*-like bacteria, *Mycoplasma*, and *Fusobacterium*) in samples from lesions.

While 16S amplicon sequencing can identify many species of bacteria, some of the bacteria that may be important in TAHD lesions are poorly characterized (i.e., have never been cultured in the lab or lack sequencing of full genomes). We developed an approach that used DNA sequence data obtained from shotgun sequencing of samples from elk with TAHD to reconstruct genomes and discover novel *Treponema* and *Treponema*-like bacteria. Findings contribute new information on the variety of *Treponema* bacteria associated with TAHD. The analysis approach will be used for additional work to further explore previously unknown bacteria in a larger sample of TAHD lesions.

- **Conduct regional surveillance and investigate risk factors.** Treponeme-associated hoof disease has been confirmed in Washington, Oregon, Idaho, and California. Working with wildlife managers, we collected hooves for surveillance and research uses from Washington and other western states. We did not detect TAHD in any new states or in species other than elk.

We integrated information from multiple data streams, from both community science and professional sources, to identify patterns in distribution and apparent prevalence of TAHD over space and time in western Washington. Results were published in a scientific journal (Transboundary and Emerging Diseases, 2023: 6685108).

- **Understand social aspects of the disease and communicate findings.** We conducted outreach by distributing information via a quarterly newsletter ([ElkTracks](#)), website, lay and scientific media, and in-person meetings. We also provided individual responses to inquiries received. We contributed to a virtual scientific meeting on TAHD to share information among wildlife managers and health professionals from state, federal, and tribal agencies. We presented scientific findings from WSU's research program at scientific meetings, including the Wildlife Disease Association, Washington Chapter of the Wildlife Society, and American College of Veterinary Pathologists conferences.
- **Ancillary projects.** We collaborated with WSU researcher Dr. Michael Skinner to investigate epigenetic changes (changes in molecular factors and processes around the DNA that alter which genes are turned on or turned off, which in turn modifies what proteins are made by the body) in elk with TAHD. Despite the lack of systemic infection, the disease may be exerting molecular-level effects in cells throughout the body. Results of our research were published in a high impact scientific journal (Scientific Reports, 13: 15378). Additionally, a study was initiated to investigate the utility of telemetry monitors on captive elk to detect behavioral changes associated with TAHD.



## BACKGROUND AND OVERVIEW

Hoof disease, known scientifically as treponeme-associated hoof disease (TAHD), is an emerging disease of elk in the U.S. 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 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 in FY2022-2023 and FY2024-2025.

The following report consists of two sections. The first section, Research and Outreach Accomplishments in 2023, summarizes accomplishments made implementing our research plan in 2023. 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 2023

## 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 sixth-year combined pathology residency/PhD candidate. Dr. Goldsmith's research focuses on pathogen discovery using metagenomics techniques. She received the Pathology of Wildlife and Zoo Animals student award from the American College of Veterinary Pathology and also second place in the graduate student category at the 25th Annual College of Veterinary Medicine Research Symposium for her poster titled "An etiopathologic investigation of treponeme-associated hoof disease, an emerging disease in free-ranging elk". Anticipated graduation 2025.
  - Holly Drankhan, DVM, is currently a fifth-year combined pathology residency/PhD candidate. Dr. Drankhan is developing a reliable disease transmission model using elk at the captive research facility. She was a 2023 recipient of the Fowler Emerging Infectious Diseases Graduate Fellowship and successfully completed her PhD preliminary examination in October. Anticipated graduation 2025.
  - Steven Winter, MS, is a PhD candidate. Steven is studying spatial and temporal distribution and risk factors of TAHD using computer modeling. He successfully completed his PhD preliminary examination in June. Steven received a departmental travel award to help support attendance and poster presentation at the Wildlife Disease Association annual conference. Anticipated graduation 2024.
  - Trent Hill began a master's degree program in fall 2023. Trent is evaluating behavioral changes associated with TAHD infection in captive elk using telemetry data collected from ankle monitors. Anticipated graduation 2025.
  - Thomas LeClair, MS, is a third-year veterinary student. Thomas continued work he initiated during a summer research program following his first year of veterinary school. Thomas investigated pathogens present in elk hooves with TAHD using shotgun sequencing of bacterial DNA.



- **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.
- **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 2023.
- **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.
- **Elk procurement.** We added seven juvenile elk (about 9 months old) to our captive herd in March for use in future studies on 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 studies.** We published a scientific journal article reporting findings from our first TAHD transmission study (Robinson MS project). This study was the first conducted in the WSU Elk Research Facility and provided direct evidence that TAHD can be transmitted through soil contaminated with affected hooves. The findings also provide additional evidence that TAHD is an infectious disease. Results were published in a peer-reviewed scientific journal, PLoS ONE 18(8): e0289764 [doi.org/10.1371/journal.pone.0289764](https://doi.org/10.1371/journal.pone.0289764).

### TRANSMISSION AND LESION PROGRESSION OF TREPONEME-ASSOCIATED HOOF DISEASE IN CAPTIVE ELK (*CERVUS CANADENSIS*)

Zachary B. Robinson, Devendra H. Shah, Kyle R. Taylor, and Margaret A. Wild

**ABSTRACT:** Treponeme-associated hoof disease (TAHD) is a debilitating disease of free-ranging elk (*Cervus canadensis*) in the northwestern U.S. While treponemes are associated with lesions, the etiology and transmissibility between elk are unknown. Our objective was to determine whether the disease can be environmentally transmitted to captive elk. Four individually housed treatment elk and 2 control elk were challenged with soil mixed with inoculum prepared from free-ranging elk hooves from TAHD-positive elk or autoclaved hooves from normal elk, respectively. The inoculum for each group was applied to the interdigital space and added to pre-existing soil in each pen. Eight challenges were conducted at 1–4-week intervals and lesion development was assessed during a 138-day challenge period that was followed by a 170-day monitoring period to document lesion progression. All treatment elk, but no control elk, developed gross and histologic lesions consistent with TAHD. Treponema phylotypes similar to those in bovine digital dermatitis in cattle were detected using 16S rRNA gene amplicon sequencing from lesions in all treatment elk, but no control elk, during the challenge period. Lesions progressed from ulcerations in the interdigital space to extensive ulceration and underrunning of the hoof

capsule by 35 and 173 days following the initial inoculation, respectively. Lameness in treatment elk was correlated with lesion development ( $R=0.702$ ,  $p<0.001$ ), and activity of infected elk was reduced during the challenge ( $p<0.001$ ) and monitoring periods ( $p=0.004$ ). Body condition was significantly lower in treatment than control elk 168 days following the initial inoculation ( $p=0.05$ ) and at each individual elk's study endpoint ( $p=0.006$ ). Three of 4 treatment elk were euthanized when they reached humane endpoints, and one elk recovered. These results provide direct evidence that TAHD is a transmissible infectious disease in elk. As such, actions that reduce transmission risk can support disease management and prevention.

Grades	Normal	Grade I	Grade II	Grade III	Grade IV
<b>Description</b>	Grossly normal hoof	Cutaneous erosion or ulceration	Underrunning of hoof capsule	Sole ulcer – cavity in hoof capsule	Broken or sloughed hoof capsule
<b>A) Free-ranging</b>					
<b>B) Experimental</b>					

**Free-ranging vs. study elk TAHD lesions.** Hoof lesions indistinguishable from those in free-ranging elk with treponeme-associated hoof disease (TAHD) developed in study elk following experimental challenge with inoculum prepared from TAHD-affected hoof material mixed with soil. (A) Characteristic TAHD lesions from free-ranging elk graded as I to IV based on a previously described grading system (Han et al. 2019) provide a reference for comparison to lesions observed in (B) representative lesions on experimentally infected elk. Lesions progressed through the study period from grade 0 to III. Figure from Robinson et al. 2023.

Additionally, we began analyzing data from a follow-up study completed in 2022 to investigate whether TAHD could be transmitted to captive elk under replicated natural conditions in which elk were simply exposed to soil in pens where TAHD-affected elk were previously housed or where TAHD-affected hoof material was added on two occasions. Visual changes to the feet were limited and TAHD was not confirmed in any of the exposed elk. We are currently analyzing data to determine whether early changes to the bacterial community of the feet occurred and explore possible reasons why disease transmission was not observed.

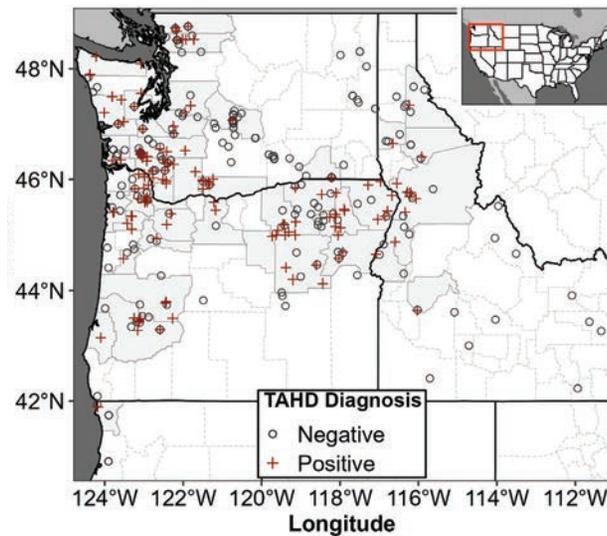
- ***Develop a reliable transmission model in captive elk.*** We conducted a pilot study and the initial phase of a study to develop a reliable TAHD transmission model in captive elk (Drankhan PhD project). We developed a boot system patterned after one used in cattle to expose elk to affected hoof material. Results were mixed, and not all exposed elk developed TAHD. Additionally, boots caused non-TAHD related lesions on feet that interfered with study implementation. We began examining modifications to the transmission model to be implemented in subsequent experiments.

### 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. In-depth data analysis is in progress, but preliminary data provide additional evidence of the presence of bacteria we previously associated with TAHD (e.g., *Treponema* and *Treponema*-like bacteria, *Mycoplasma*, and *Fusobacterium*) in samples from lesions.
- ***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). 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. A phylogenetic tree was constructed to compare newly discovered *Treponema*-like bacteria with those found in other host species, such as cattle. The construction of genomes of previously identified *Treponema* bacteria provides confidence in reliability of the technique and our preliminary results. A manuscript has been drafted and will be submitted to a scientific journal for publication.
- ***Compare hoof sampling techniques.*** We collected 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.



*Dr. Margaret Wild feeding an elk in the Elk Research Facility at Washington State University.*



## 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 2023 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 (9 elk), and for disease surveillance from Washington (2 elk), Oregon (16 elk), Idaho (4 elk), and Nevada (1 elk). This year we did not detect TAHD in any new states or in species other than elk.

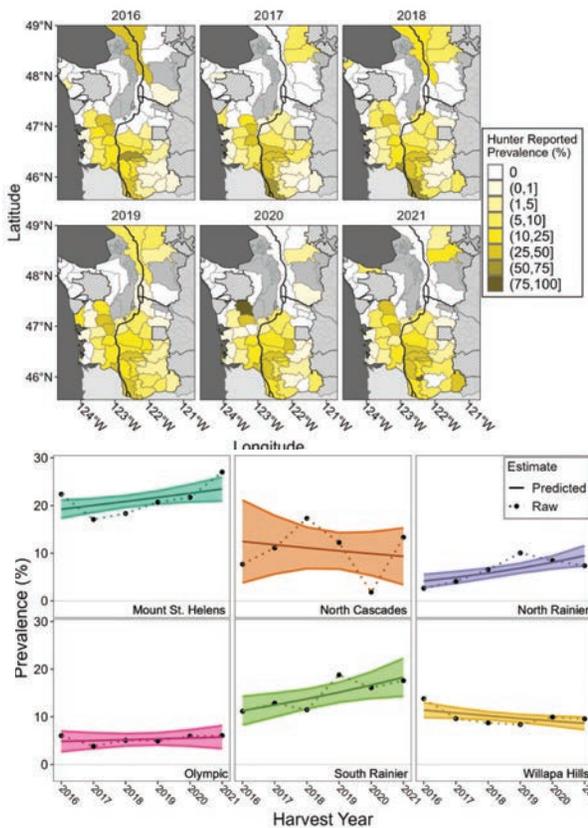
- TAHD distribution and apparent prevalence.** We integrated information from multiple data streams, from both community science and professional sources, to identify patterns in distribution and apparent prevalence of TAHD in space and time in western Washington (Winter PhD project). Results were published in a peer-reviewed scientific journal, *Transboundary and Emerging Diseases*, 2023: 6685108 <https://doi.org/10.1155/2023/6685108>.

## COMMUNITY SCIENCE STRATEGIES REVEAL DISTRIBUTIONAL PATTERNS OF TREPONEME-ASSOCIATED HOOF DISEASE IN WASHINGTON ELK (*CERVUS CANADENSIS*)

Steven N. Winter, María del Pilar Fernández, Erin Clancey, Kyle Garrison, Kristin Mansfield, and Margaret A. Wild

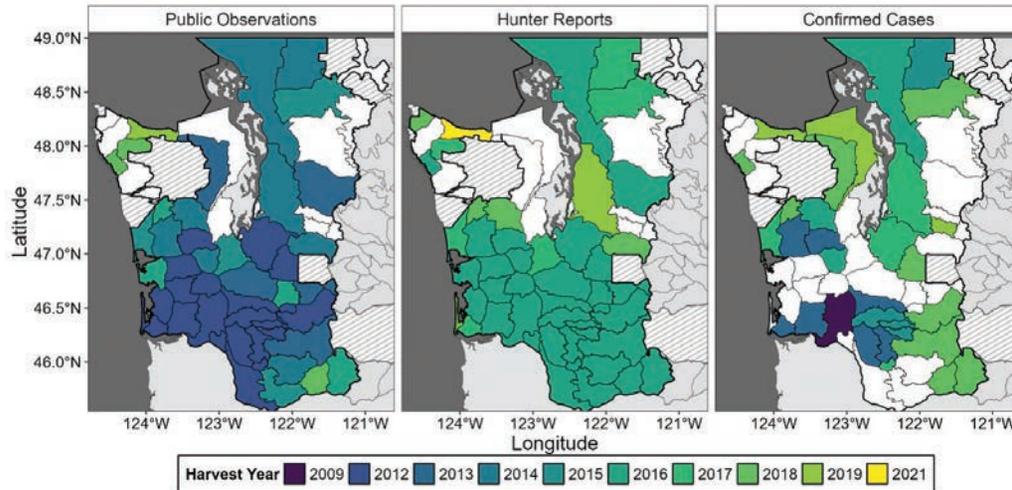
**ABSTRACT:** Treponeme-associated hoof disease (TAHD) is an emerging disease of conservation concern in elk (*Cervus canadensis*) in the Northwest USA. Elk with TAHD exhibit characteristic hoof lesions that are often accompanied by lameness and limping. The gold standard approach traditionally used for infectious disease surveillance is laboratory confirmation, which for TAHD is a histologic examination of abnormal elk hooves submitted by wildlife agencies. Diagnostic evaluation affords certainty in confirming TAHD; however, these examinations are also labor and resource intensive, and therefore, not conducive to the collection of sufficient data for epidemiologic investigations. In response, two community science (CS) surveillance strategies have been implemented in Washington State: public observations of limping elk from a web-based reporting tool and hunter reports of hoof abnormalities on harvested elk. Surveillance using CS strategies can be implemented widely and may be useful for describing broad distributional patterns of TAHD, despite their unknown relationship to laboratory-confirmed cases. We described and compared the spatial-temporal distribution of TAHD in western Washington game management units (GMU) using the two CS strategies to assess congruences and discrepancies between observed patterns. We used spatial scan statistics to identify possible core-affected and newly emerging areas at the GMU level. Lastly, we contrasted CS observations against confirmed case data to examine possible delays in TAHD detection and co-occurrence among surveillance strategies. We found public observations of limping elk often predated TAHD confirmations in GMUs by several years, while hunter-reported abnormalities predated confirmations in

GMUs by several months. High co-occurrence between the presence of apparent and confirmed cases under different surveillance strategies further supports the use of CS sources. This study capitalizes on wide-reaching CS data to provide new and complementary epidemiological information that can help guide future surveillance, management, and research efforts for this novel elk hoof disease.



**Spatial-temporal distribution of prevalence from hunter reports.** Western Washington game management unit (GMU) maps show raw estimates of the apparent prevalence of reports of hoof abnormalities collected annually by hunters during the 2016–2021 harvest seasons. Proportions were converted to percentages and binned for visualizations. Prevalence estimates are not adjusted by sample sizes and thus do not reflect uncertainty in estimates. Areas outside the Washington Department of Fish and Wildlife jurisdiction are shown in striped polygons; neighboring states (Oregon to south) and other GMUs outside of the study area in gray. Interstate highways shown in black lines are included as landmarks. Figure from Winter et al. 2023.

**Varying trends in Hunter-Reported prevalence over time in western Washington elk herd areas.** We evaluated trends in Hunter- Reported prevalence aggregated to game management units comprising designated elk herd areas (panels). Raw annual prevalence estimates are connected by dotted lines. Colored solid lines with ribbons show slopes and confidence intervals from a binomial generalized linear model. We identified significant positive slopes in prevalence over time for Mount St. Helens and North Rainier elk herd areas. We found a significant decreasing trend in prevalence in the Willapa Hills elk herd area. Effects of time on prevalence were less clear for Olympic, North Cascades, and South Rainier herd areas. Figure from Winter et al. 2023.



**Maps of harvest years with earliest game management unit-level detection from TAHD surveillance strategies.** Panels show the presence of apparent cases or Confirmed Cases collected under different TAHD surveillance strategies (panel labels) in Western Washington game management units (GMUs). GMUs were colored to represent older (dark blue) to more recent (yellow) harvest years in which respective cases were first identified. For community science strategies, many detections were first recorded in the year they were developed (i.e., Public Observations—2012, Hunter Reports—2016). Figure from Winter et al. 2023.

## 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 submitted 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 journal review process.
- **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 quarterly updates in “[ElkTracks](#)”. The listserv reaches nearly 200 recipients and continues to grow.
- **Stakeholder communication and meetings.** Updates on elk hoof disease research conducted at WSU were provided in presentations at three locations in western Washington in July. The presentations were organized with county commissioners in Wahkiakum, Skagit, and Cowlitz Counties. 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.
- **Outreach via media.** Our work on hoof disease received coverage in local and national media. Of particular note, our research on the apparent systemic epigenetic alterations in elk with hoof disease reported in the [Spokesman-Review](#) was picked up by multiple news wires. The Wildlife Management Institute featured our research in the January 2023 edition of the [Outdoor News Bulletin](#). We also shared our research by filming interviews for a planned upcoming episode of *Cal in the Field*.



*Elk at the Elk Research Facility at Washington State University.*

- **Website.** The [elk hoof disease website](#) was maintained to provide information on elk hoof disease and our research.
- **Scientific outreach.** In March 2023, we collaborated with the Rocky Mountain Elk Foundation (RMEF) and California Department of Fish and Wildlife (CDFW) to conduct the second annual virtual scientific meeting on elk hoof disease for wildlife managers and health professionals from state, federal, and tribal agencies. We presented scientific findings from WSU's research program at scientific meetings, including the Wildlife Disease Association, American College of Veterinary Pathologists, and Washington Chapter of the Wildlife Society conferences. Our work was also included in an article featuring WSU CVM research published in the American Journal of Veterinary Research (Mealey et al. 2023; [doi.org/10.2460/ajvr.23.03.0043](https://doi.org/10.2460/ajvr.23.03.0043)).

## 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, 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 for submission of hooves for disease surveillance and research, we provided updates on elk hoof disease and our research to the NWIFC and at the Invasive Species Webinar for Tribal Audiences.

## 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.

- **Epigenetic impacts.** We collaborated with researcher Dr. Michael Skinner, a professor in the WSU School of Biological Sciences, to investigate epigenetic changes (changes in molecular factors and processes around the DNA that alter which genes are turned on or turned off, which in turn modifies what proteins are made by the body). Despite the lack of systemic infection, TAHD may be exerting effects in cells throughout the body. We suspect that the epigenetic changes are a result of the body responding to infection with TAHD. A summary of findings was provided in the Fall 2023 edition of [ElkTracks](#). Results of our research were published in a high impact, peer-reviewed scientific journal, Scientific Reports 2023 13:15378 [doi.org/10.1038/s41598-023-42546-8](https://doi.org/10.1038/s41598-023-42546-8).



## **SYSTEMIC EPIGENOME-WIDE ASSOCIATION STUDY OF ELK TREPONEME-ASSOCIATED HOOF DISEASE**

Margaret A. Wild, Kyle R. Taylor, Eric E. Nilsson, Daniel Beck, and Michael K. Skinner

**ABSTRACT:** Treponeme-associated hoof disease (TAHD) is an emerging disease of elk (*Cervus canadensis*) in the U.S. Pacific West. Because environmental epigenetics is the primary molecular process that mediates environmental factor impacts on a host organism and disease, the role of epigenetics in TAHD etiology was examined. The current study was designed to examine potential effects of TAHD on systemic epigenetic modifications in infected elk over a range of TAHD lesion severity. Leg tendons that contain predominantly fibroblast connective tissue cells were used to isolate fibroblast cells for epigenetic analysis in unaffected and TAHD-positive male and female Roosevelt and Rocky Mountain elk. Differential DNA methylation regions (DMRs) between the unaffected and TAHD-positive elk were identified for both female and male elk. The presence of TAHD was associated with alteration of the connective tissue cell epigenetics, and DMR associated genes identified. Therefore, the infected elk were found to have a systemic epigenetic alteration that was associated with the disease, despite pathology being generally limited to feet. If the elk germline epigenetics is altered then generational transmission of susceptibility to TAHD may impact subsequent generations through epigenetic inheritance. This first study of epigenetic changes associated with disease in elk suggests that TAHD promotes a systemic effect on the elk epigenetics which could exert health impacts on the elk.

- **Monitoring lameness.** We collaborated with Advanced Telemetry Systems (ATS) to develop an “anklet” monitor that collects and transmits activity data to a receiver. We recruited a master’s degree student who began conducting pilot studies to investigate utility of the monitors to differentiate bedded, standing, and walking behavior in captive research elk. The objective of the study will be to validate reliability of data collected by the monitors and investigate changes in activity of captive elk with hoof disease.

# 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). In mid-2024, WSU will begin a search to replace the faculty lead for elk hoof disease research. A transition, ideally with a period of overlap with the current and new faculty lead, is anticipated in 2025.
- **Legislative reporting.** The next report covering the period January-December 2024 will be submitted by February 7, 2025. 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 used in continuing studies in 2024. We will continue to work with Starkey Experimental Forest in Oregon to obtain additional elk as deemed necessary for planned research.
- **Environmental transmission.** We will complete analyses of data from the study initiated in 2021 to investigate transmission of TAHD to captive elk naturally exposed to contaminated soil and determine whether results are rigorous enough for development of a draft manuscript for submission to a scientific journal.
- **Develop a reliable transmission model in captive elk.** We will conduct additional experiments to develop a reliable TAHD transmission model in captive elk (Drankhan PhD project). We will modify the transmission model based on results from experiments conducted in 2023 to increase the occurrence and severity of TAHD lesions and reduce unintended lesions from the challenge system. Concurrently, we will evaluate changes in behavior associated with development of TAHD lesions (see Ancillary projects below).



*Dr. Margaret Wild and team taking a hoof sample from one of the captive elk at the WSU Elk Research Facility.*

## Study disease agents in the laboratory.

- **Bacteria associated with microscopic lesion categories.** We will continue this investigation by conducting data analyses to determine bacteria that are overrepresented in different stages of microscopic lesions associated with TAHD to provide insight into cause and disease progression (Goldsmith PhD project). A manuscript will be prepared for submission to a scientific journal.
- **Pathogen discovery using genome reconstruction.** We will conduct shotgun sequencing and apply the technique we developed for genome reconstruction to a larger set of samples associated with different microscopic lesions (Goldsmith PhD project and Deb post-doctoral study). We anticipate that we will confirm the presence of our previously discovered *Treponema*-like bacteria and describe additional new pathogens associated with TAHD lesions. This work will also lead to the identification of genetic sequences specific to pathogens of interest so that diagnostic tests can be developed.
- **Compare hoof sampling techniques.** We will collect additional samples and extract DNA for comparison of three methods of sampling elk feet (Drankhan PhD project). Samples collected using a punch biopsy, skin scraping, and skin swab will be compared to determine if less invasive techniques (swabbing or scraping) are as reliable as a biopsy in characterizing the bacterial community present in a TAHD lesions.
- **Enrich *Treponema* for experimental challenges.** Our findings to date lend further support for the importance of *Treponema* or *Treponema*-like bacteria in TAHD lesions; however, other bacteria are also likely important in disease development. *Treponema* species and other potentially important species (e.g., *Mycoplasma*) are notoriously difficult to grow, or culture, in the laboratory. As an alternative to culture, we will develop laboratory methods that are selectively conducive to the growth of potential pathogens. These techniques will be applied in inoculum preparation for use and evaluation in transmission studies (Drankhan PhD project).
- **Metabolic analysis.** New technologies can profile metabolic changes in animals that are associated with disease. Led by WSU veterinary pathologist, Dr. Kyle Taylor, we will analyze 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.

## 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 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 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. Additionally, we will continue work with CDFW on a data-sharing agreement so that we can obtain geographic locations for their confirmed TAHD cases. These locations will be added to the national distribution map maintained by WSU.



*Captive elk in pen at the WSU Elk Research Facility.*

- **Modeling risk factors.** We will examine how conditions in landscapes (e.g., land cover types, vegetation phenology, elevation, soil types), climate, and human land-use changes (e.g., commercial forestry) correlate with TAHD occurrence across local and coarse scales (Winter PhD project). We will evaluate support for competing causal hypotheses of TAHD transmission risk accounting for available host characteristics (e.g., sex) using methods from Bayesian structural equation modeling. Identification of risk factors will inform management strategies and guide TAHD surveillance programs.
- **Mineral status of elk.** We will continue collaboration with CDFW to investigate the correlation of mineral levels in liver with the occurrence of TAHD (Winter PhD project). We will draft a manuscript reporting results from recent analyses for submission to a scientific journal.

## Understand social aspects of the disease.

- **Social science inquiry.** We are currently awaiting peer-review comments from a scientific journal on our manuscript that reports findings of the 2020 survey of Washington residents. We will finalize the manuscript for publication based on these reviews.
- **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 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 RMEF 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. 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

- **Investigate new monitoring techniques.** We will perform validation studies with captive elk to determine the reliability of data collected by the ankle monitor in identifying bedded, standing, and walking behavior. We will then use the monitors to evaluate changes in activity in captive research elk that develop TAHD (Hill MS project).

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# 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.



WASHINGTON STATE UNIVERSITY  
**College of Veterinary Medicine**

**Department of Veterinary Microbiology and Pathology**

[vetmed.wsu.edu/elk-hoof-disease](http://vetmed.wsu.edu/elk-hoof-disease)

