Elk Hoof Disease in Southwest Washington

Sandra Jonker, Ph.D.

WDFW Hoof Disease Public Working Group Meeting

14 January 2015

Agenda

- Welcome
- HDTAG Consensus Statement
- Understanding Hoof Disease Prevalence/ Distribution
- Hoof Disease Survival Study
- Hoof Disease Euthanasia Protocol
- Next steps
- Public Testimony

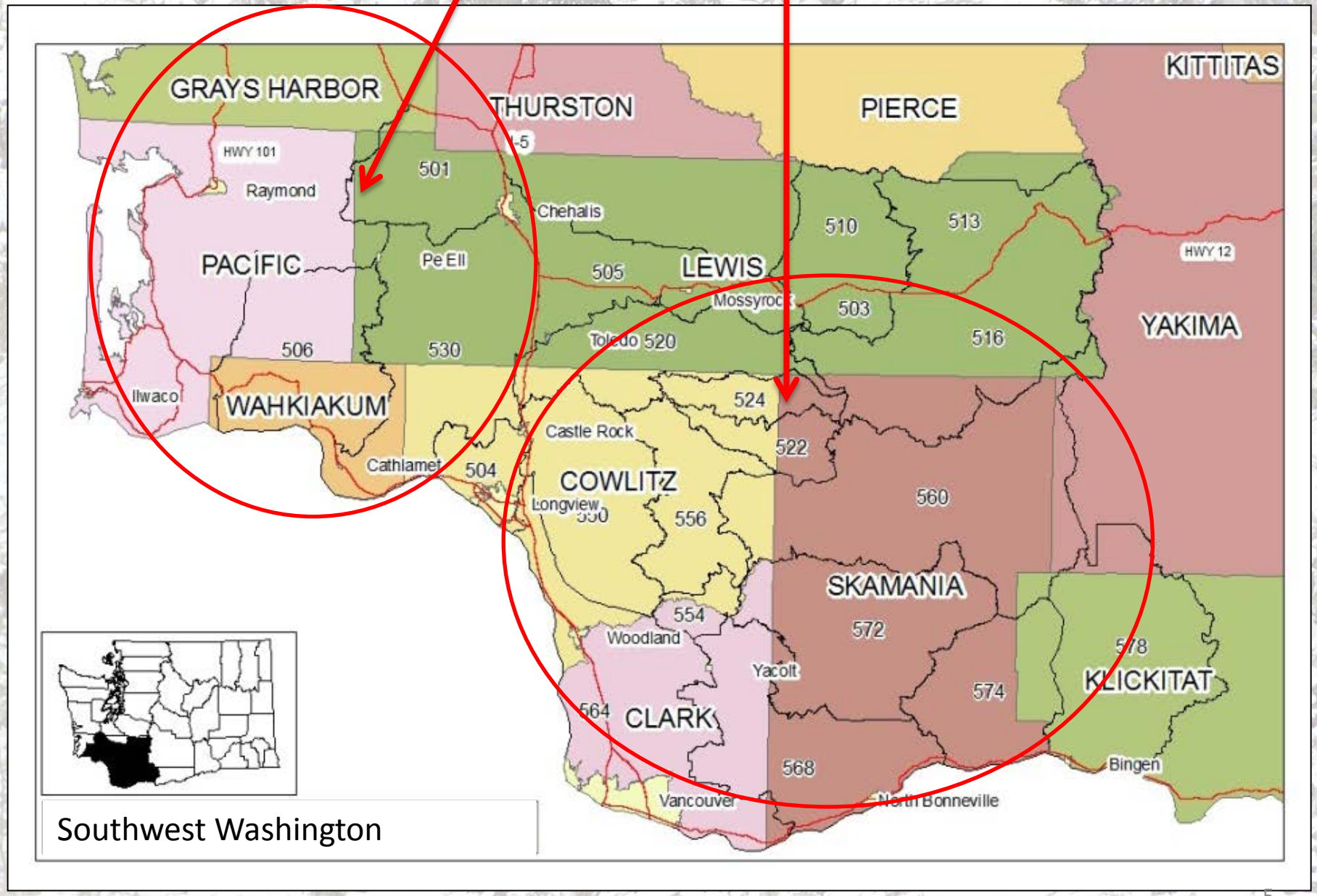
Public Testimony

- Members of the public are requested to fill out a Public Testimony Form
- Members of the public will be requested to provide their public testimony to the HDPWG in the order the Public Testimony Forms were received
- Each member of the public wishing to relay their comments will have 3 minutes each to do so
  - This time frame is provided to allow the opportunity for all members of the public to provide their testimony to the HDPWG

Hoof Disease Public Working Group

- **Understanding hoof disease in elk is a priority and WDFW is committed to the sound management of these important resources**
- **WDFW established the Public Working Group as we believe it is important to work together as we try to better understand and address this issue**
- **The purpose of this Working Group is to provide the opportunity to:**
  - **share information about the hoof disease phenomenon and WDFW activities,**
  - **discuss research and management questions with regard to hoof disease and solicit feedback, and**
  - **public outreach**

Willapa Hills and MSH Elk Herds



Collections

- **Four collections from affected and unaffected areas:**
  - **March 2009 : Adult elk**
  - **March 2013: 9-10 month elk**
  - **August 2013: 3-4 month calf elk**
  - **January 2014: 7-8 month calf elk**
- **Summary: 43 elk examined from March 2009 - Jan 2014**
- **Extensive analyses with multiple national and international partners including 5 independent labs…..**

Consensus
HDTAG June 3, 2014

- **Available evidence is most consistent with an infectious bacterial hoof disease**
- **The disease shares many features and most resembles treponeme-associated contagious ovine digital dermatitis (CODD)**
- **Environmental factors, including wet conditions, are likely important in disease initiation and propagation**

TAHD

- Diagnosis of Treponeme Associated Hoof Disease (TAHD)
- Continue to work with collaborators on additional TAHD-related studies
  - Based on identified information needs
    - Understanding of progression of disease
    - Inform management

Identified Information Needs
HDTAG June 3, 2014

- Being maintained in elk population?
- Elk movements/habitat use?
- Do elk develop immunity?
- Effects on survival & reproduction?
- Progression of disease over time (individual & herd)?
- How transmitted?
- Presence in environment (fecal & soil sampling)?

TAHD-related Studies

- How TAHD affects body condition and other health parameters
- How elks' immune system responds to TAHD
- What other bacteria are involved in the development of TAHD
- Are treponemes shed in the feces (we know this is the case in cattle)
- Whether some elk are genetically resistant (or susceptible) to developing TAHD

Prioritized Efforts

- **Prioritized 4 immediate questions/efforts to address and inform management:**
  - **Better understand prevalence of hoof disease in elk herds in Southwest Washington,**
  - **Better understand the distribution of hoof disease in elk herds in Southwest Washington,**
  - **Understand the impacts of hoof disease on elk survival and productivity, and**
  - **Remove elk severely affected with hoof disease**
    - **Euthanasia Protocol**

Understanding Hoof Disease Prevalence/Distribution

Distribution Effort

Wildlife Health
Elk Hoof Disease
Frequently Asked Questions
Report Limping Elk Observations
Report Dead Elk with Hoof Deformities
Elk Hoof Disease Public Working Group
Game Meat Safety

Found Injured Wildlife?

Contact a local Wildlife Rehabilitator

-- List by County --

For more information contact a WDFW Regional Office

Washington Department of Fish & Wildlife

Main Office
Natural Resources Building
1111 Washington St. SE
Olympia, WA 98501
360-902-2200
Get Directions

Mailing Address
600 Capitol Way N.
Olympia, WA 98501-1091

Phil Anderson
Director

Wildlife Health

How to report elk showing signs of hoof disease

State wildlife managers are seeking the public's help in determining the incidence and geographical distribution of hoof disease in southwest Washington elk herds.

The area of the map marked with **green hash lines** below is of primary interest in this effort, because more information is needed about the disease in that area. The department already has a substantial amount of documentation on diseased elk in the primary area of infection, which is marked with red crosshatches.

If you see elk that are limping or dead/harvested with hoof deformities in the area marked with **green hash lines**, please report your observations on this website.

As a reference point, the two colored/hash and crosshatched areas are divided into numbered Game Management Units (view GMU map).

Once you have submitted a report, a red "X" will appear on the master reporting map on the main Hoof Disease page.

Help Monitor Hoof Disease

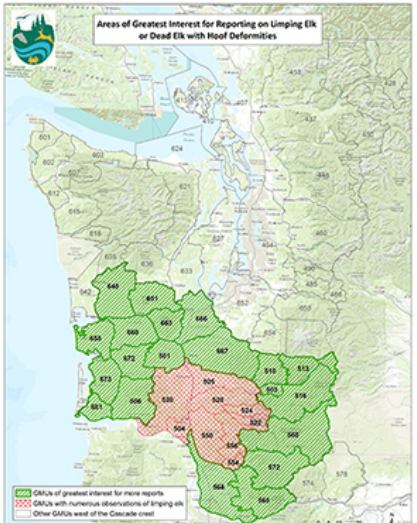
Wildlife managers are seeking reports on elk with hoof deformities observed in specific areas of southwest Washington.

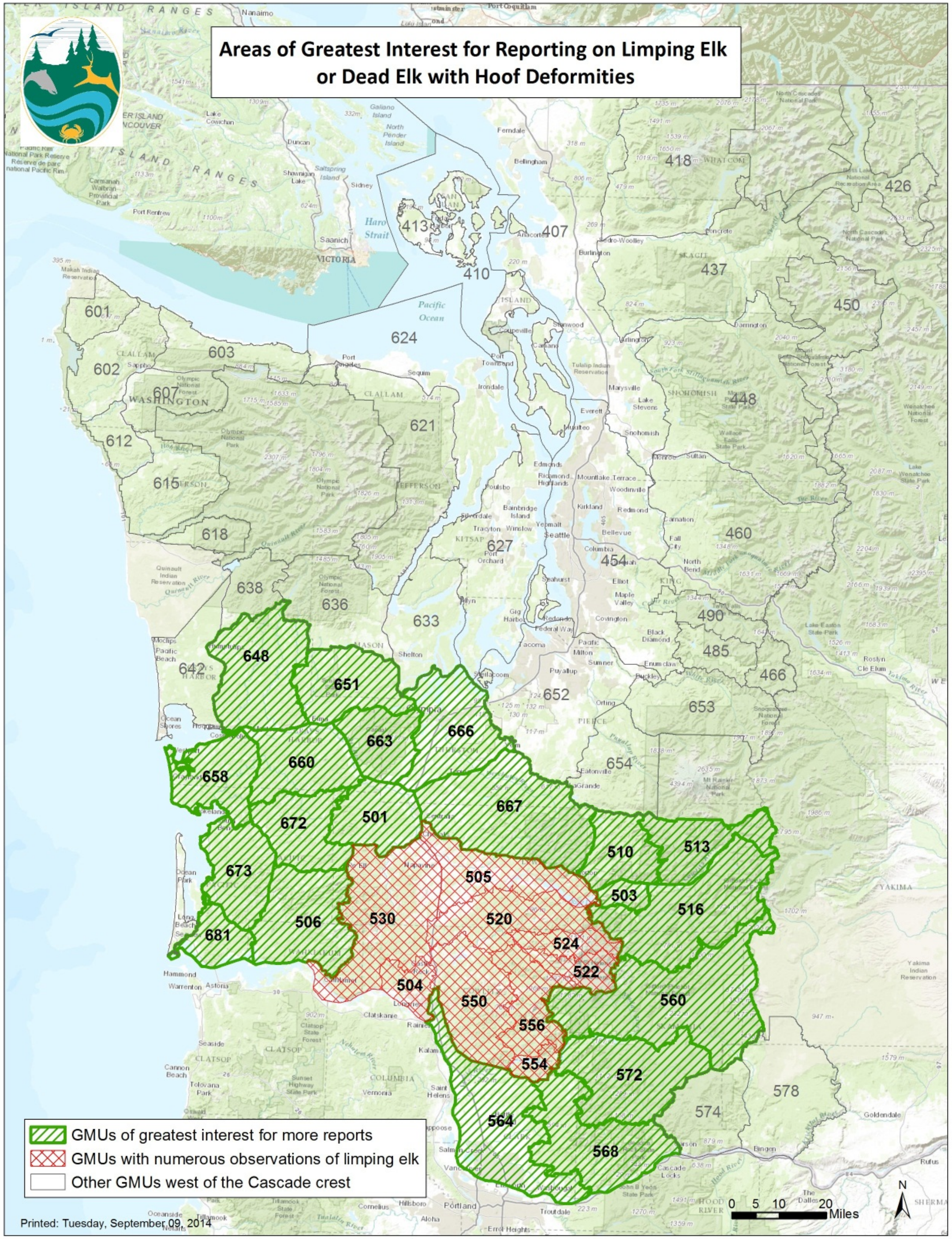
Report Limping Elk

Report Dead Elk with Hoof Deformities

The Washington Department of Fish and Wildlife thanks all who contribute to this important effort.

Areas of Greatest Interest for Reporting on Limping Elk or Dead Elk with Hoof Deformities





Areas of Greatest Interest for Reporting on Limping Elk or Dead Elk with Hoof Deformities
GMUs of greatest interest for more reports
GMUs with numerous observations of limping elk
Other GMUs west of the Cascade crest
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https://wdfw.maps.arcgis.com/apps/GeoForm/index.html?appi

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2. Select Location

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Search | Lat/Lon

Latitude: 46.86213, Longitude: -124.60492

3. Complete Form

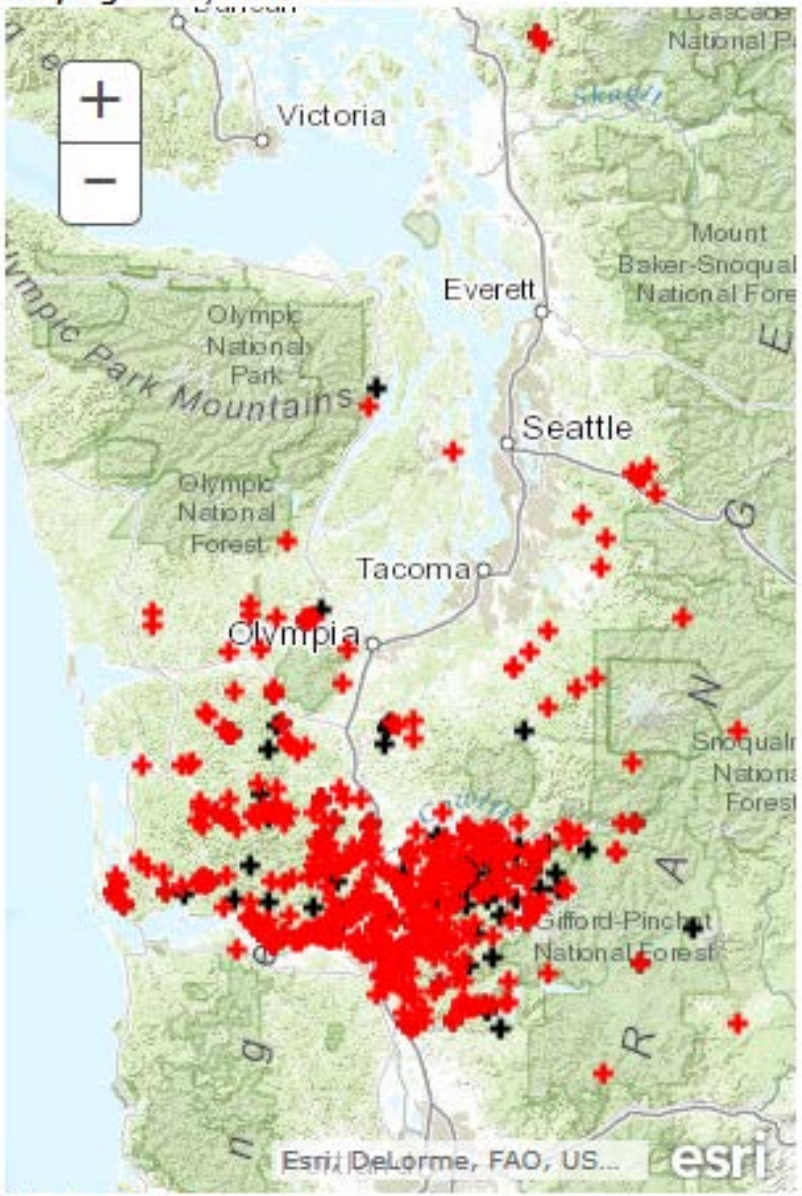
Add this information to the map.

Submit Entry

Publicly-Reported Limping Elk or Dead Elk with Hoof Deformities

(Observations from 2012 to present.)

Limping = Red, Dead = Black



View larger map

Distribution

Reports of Observations of Limping Elk to Date from Website Reporting Tool:

Report Type	Total # of Reports Since 2012	Total # of Reports After 09/15/2014	
Limping	494	101	
Dead	113	17	13/17 reported as harvested
Total	607	118	

Prevalence

- **Pilot Study August 2014**
  - **Region 5 with 18 volunteers**
  - **Region 6 with 13 volunteers**

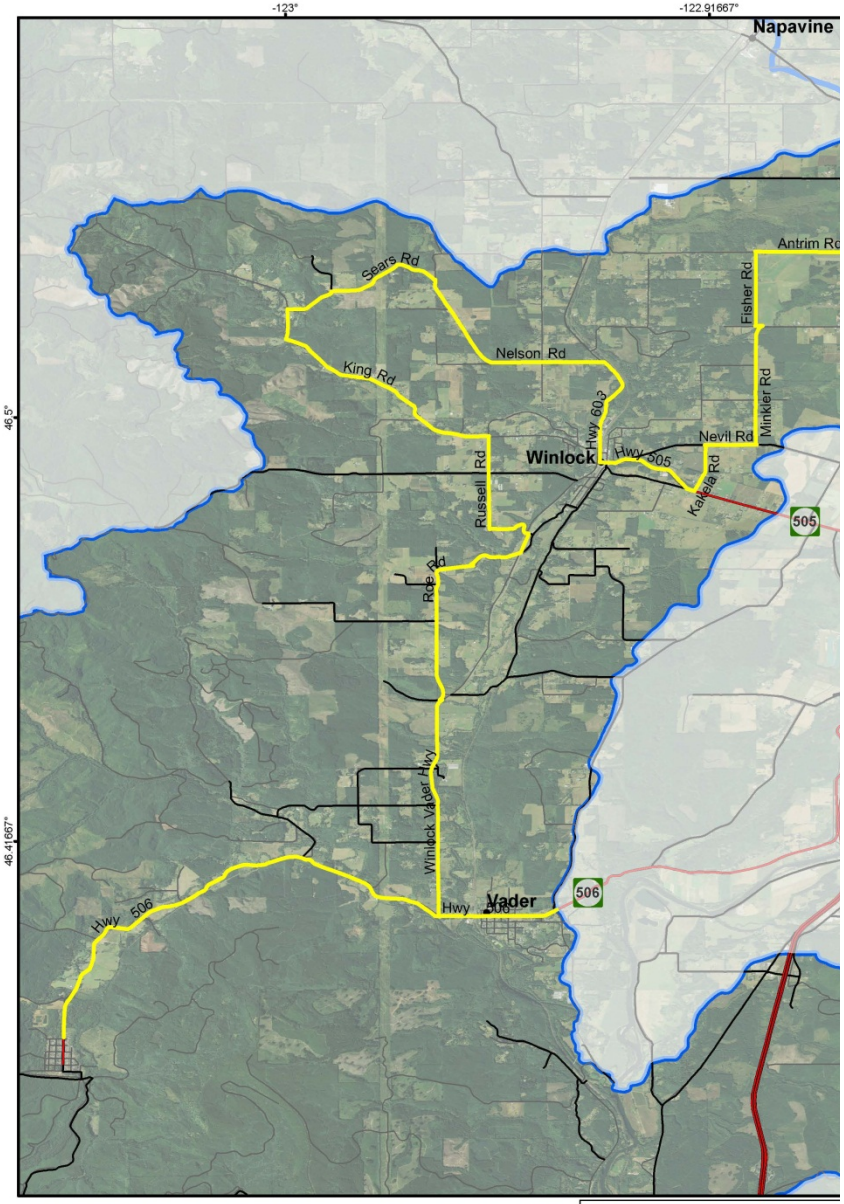
- **Tested two methods:**
  - **Route survey and Area survey**
    - **Based on HUC12 (Hydrologic Unit Codes = hierarchical watershed classification system)**

Elk Hoof Disease - Protocol Survey Map

Survey Route in HUC10: Ostrander - Cowlitz

Survey Date_____Surveyor Initials_____

(Mark the map with a dot and the ObsID# that corresponds to the observation on the f



Printed: 8/14/2014
Coordinates in WGS84

N

0 0.5 1 2 3 Miles

Map Scale: 1:112,651

Towns
Survey Route
HUC10
Highway/State Rou

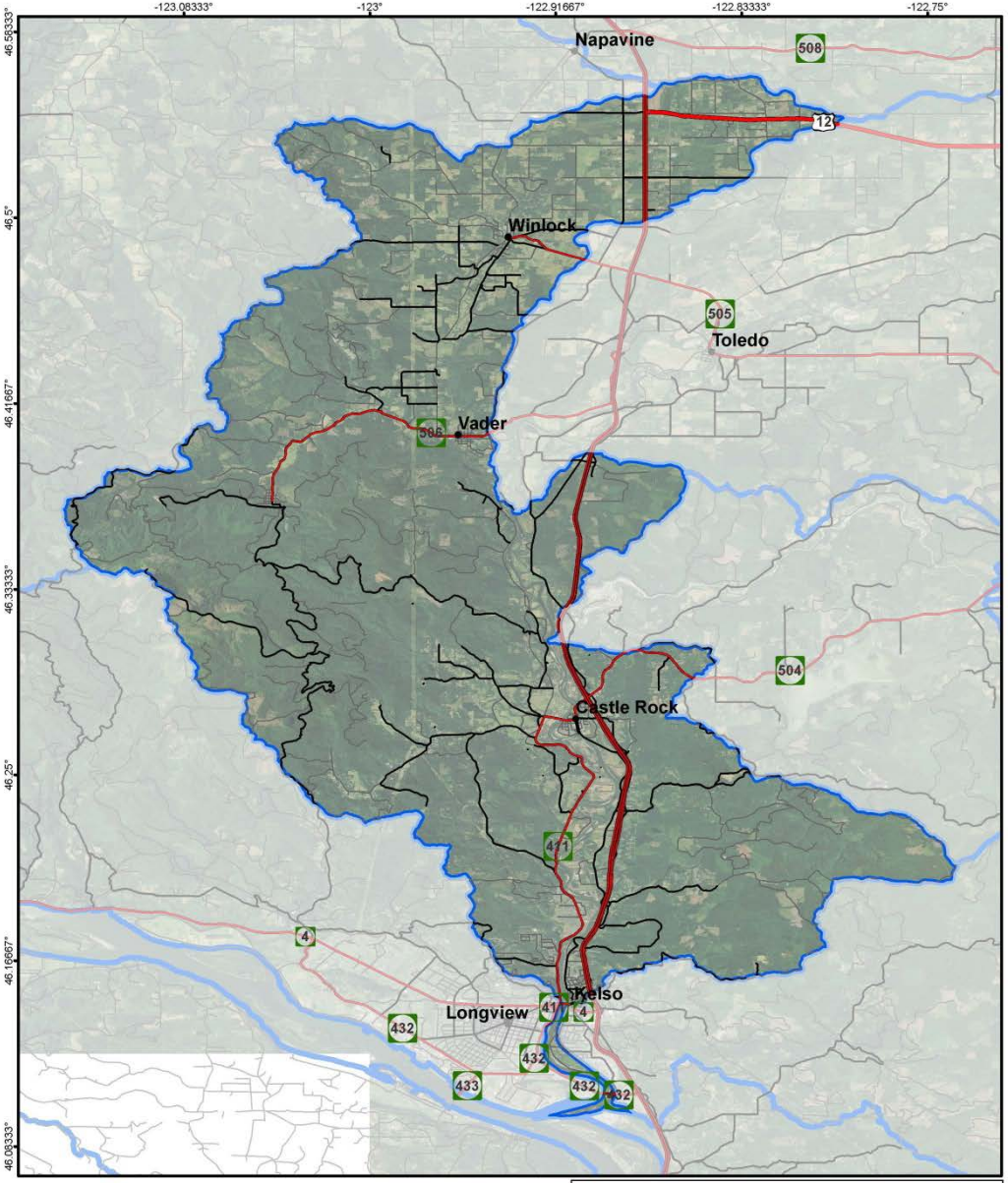
Elk Hoof Disease - Protocol Survey Map

Area Survey in HUC10: Ostrander - Cowlitz

Survey Date_____Surveyor Initials_____

(Mark the map with a dot and the ObsID# that corresponds to the observation on the form)

WDFW Region 5
Lewis County



Printed: 8/14/2014
Coordinates in WGS84

N

0 1 2 4 6 Miles

Map Scale: 1:252,960

Towns
Highway/State Route
Secondary Roads
HUC10
Other Roads

Prevalence

- **Pilot Study August 2014**
  - **Region 5 with 18 volunteers**
  - **Region 6 with 13 volunteers**

- **Tested two methods:**
  - **Route survey and Area survey**
    - **(2 observers/survey)**
  - **Two different times of day**
  - **Fixed length of time**

Survey Unit:_____ **Elk Hoof Disease - Protocol Survey Form** WDFW Region ____, _____County

Record effort and observation details below. For each record, add a location to the map. Addtionally, you may add coordinates to the notes below (use WGS84, decimal degrees, hddd.ddddd° format).

Survey Effort

Survey Date (MM/DD/YY)	Observer(s)	Survey Method (Circle One)	Survey Time (Military: HHMM)	Weather (Circle One)	Survey Mileage (Reset at start)
		AREA | ROUTE	Start_____ End_____	SUN | CLOUD | PRECIP	Total_____

Survey Observations (Alive - Individual or Herd)

Obs #	Time (HHMM)	Duration (Min) (Circle One)	Obs Method (Circle One)	Dist. (Yrd) (Circle One)	Total Count	Limp Count	Limp Cnt Acc. (Circle One)	Confidence in Obs (Circle One)	Observation Notes (Use addtional space below other values for this record)	(X Coord (Long) / Y Coord (Lat))
1		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
2		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
3		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
4		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
5		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
6		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
7		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
8		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
9		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		
10		<1 | 1 - 10 | >10	EYE | BINOC | SCOPE	<50 | 50+			Exact | Approx	Low | Avg | High		

Help/Codes

Weather:
If partly cloudy, put "Cloud"
If intermittent showers, put "Precip"

Mileage:
Only include miles during survey, not to and from survey unit

Accuracy of Limp Cnt:
If Limp Count=0, put line through all values (meaning "NA")

Dist.(Yrd):
Distance from elk in yards

Confidence in Observation:
Gauge the quality of your obs, relative to your previous observations. This is related to your experience (in general, the more times, the better you get) but also distance from elk; vegetation or fog partially obscuring your view; the size and movement of a herd; and many other factors that only you, the observer, will know about.

Obs Notes:
This is an optional field.
Include other info that you believe would be important to WDFW.
Examples include: overall condition of the herd, number of animals with severe limping or that are not mobile, and habitat type where observed.
Be as brief as possible!

Printed: Friday, August 15, 2014 10:22:56 AM Please return form to:

Prevalence

- **Pilot Study August 2014**
  - **19 observer pairs**
  - **Sighted 30 groups of elk in 31 effort units**
    - **i.e., on average (approximately) 1 group for every outing/100 miles/4 hours**
    - **Total of 250 individuals**
      - **Of these, 13% were limping in 12 groups**
  - **Pros and cons of each method**
  - **Learned from implementation effort to guide development of comprehensive effort this spring**

Prevalence

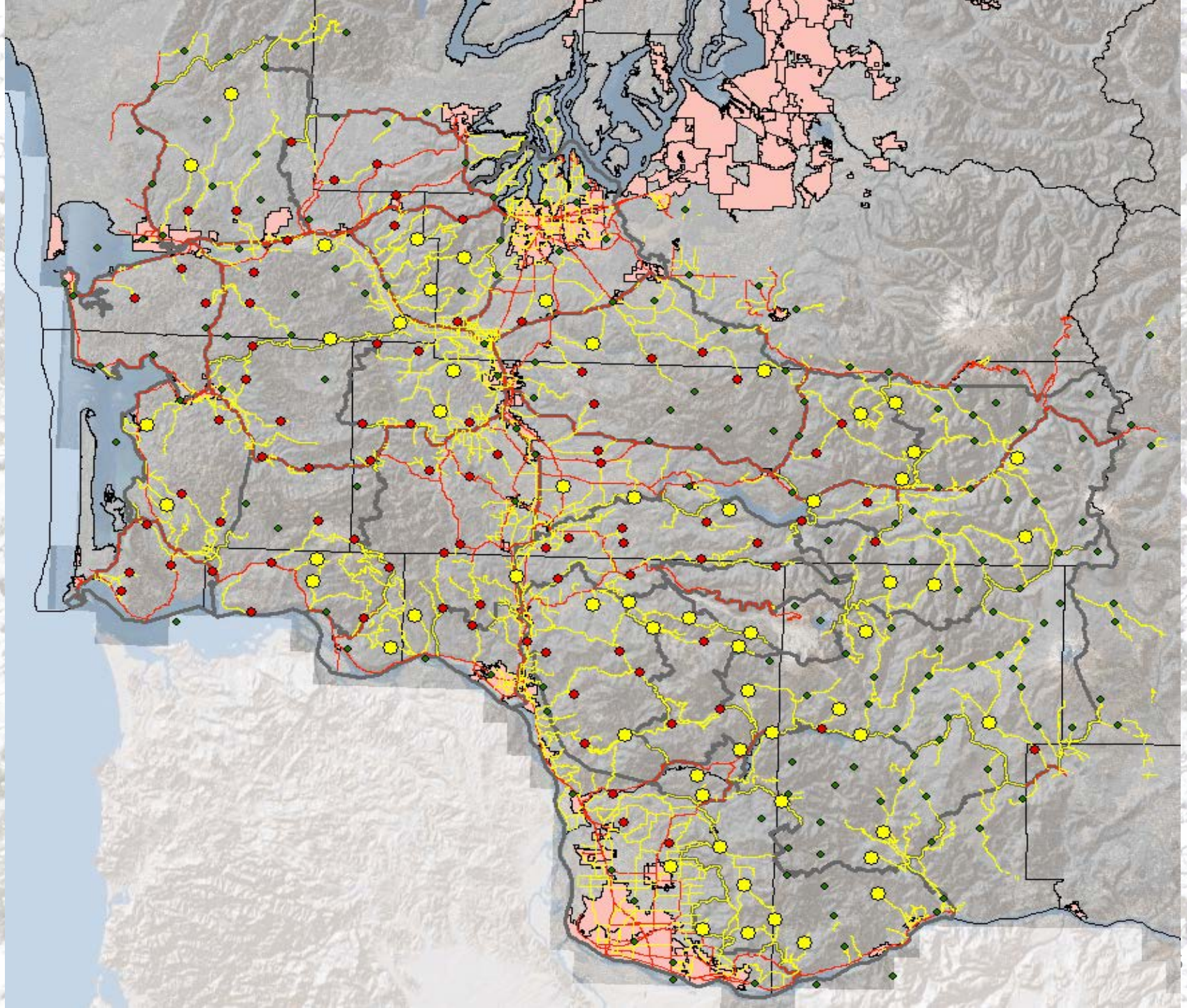
- **Determining a reliable estimate of the prevalence of disease at this scale presents many challenges**
  - **e.g., elk density, large geographic scale, access, staffing**
- **Consideration of:**
  - **Pilot study findings August 2014,**
  - **Distribution Survey Area based on the Game Management Units (GMU) developed for reporting incidental observations**
  - **Deliberations on multiple reasonable study designs**

Prevalence

- **Goal:**
  - **Understand the prevalence of hoof disease and monitor changes throughout a defined area within the range of the Mount St. Helens and Willapa elk herds**
- **Objectives:**
  - **Determine a geographic region to:**
    - **Monitor changes in the extent of the disease and**
    - **Proximate prevalence in areas where disease is known to occur**
  - **Develop a simplistic sampling strategy at landscape scale**
  - **Implement citizen science effort to accomplish study goals**
    - **Recruit and train volunteers to conduct surveys**

Prevalence Sampling Design

- "Grid" format to systematically cover the area
  - Sample points based on the HUC12s (watersheds) that intersected the study area
- Accessibility
  - Roads, public vs. private land ownership, etc.
- Prioritized 164 -184 points for volunteer and staff surveys based on land accessibility
  - Provide adequate sample to proximate prevalence and extent



Prevalence

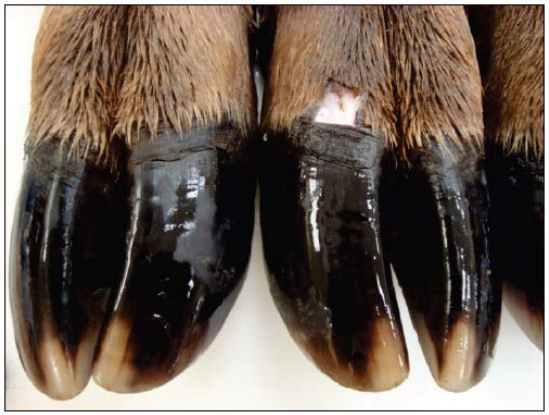
- Surveys in March and April
  - No time restriction set on the survey;
    - However volunteers and staff will be limited by:
      - survey distance, observation times for each group observed, and by speed (~ 4hours)
  - Complete data forms and enter data on-line
- Training sessions will be first week of March
- Outreach for volunteers
  - Project through Cervis

Hoof Disease Survival Study

Hoof Disease Study

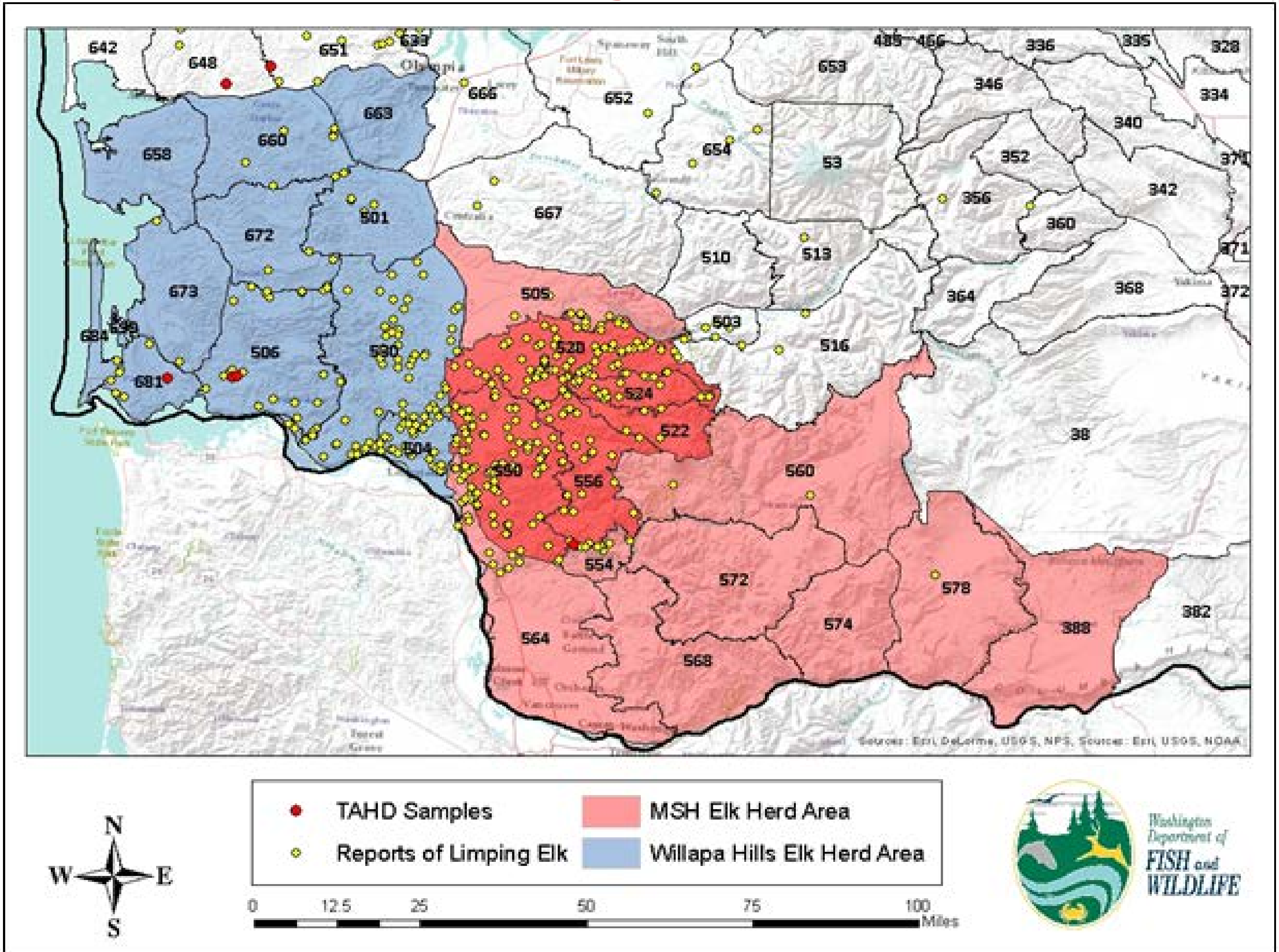
Potential Effects of TAHD

- May reduce survival of affected elk
- Secondary effect on nutritional condition
  - Reduced probability of conception
  - Limit the ability of a cow to support a calf
- Alter the way affected elk use the landscape



Han, S., and K. G. Mansfield (2014)

Study Area



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Sources: Esri, DeLorme, USGS, NPS, Sources: Esri, USGS, NOAA
TAHD Samples
Reports of Limping Elk
MSH Elk Herd Area
Willapa Hills Elk Herd Area
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Miles
Washington Department of FISH and WILDLIFE

Study Design

Objective 1: Estimate the effects of TAHD on survival of adult (>2 years old) female elk

Objective 2: Determine cause-specific mortality rates for adult female elk that have TAHD

- **Initiate captures in February 2015**
- **Radio-collar 60 affected elk and 20 non-affected elk**
- **Monitor radio-collared elk for 4 years (2015-2019)**
- **Identify covariates that affect survival**
  - **e.g., age, year, TAHD, weather events**
- **Compare survival estimates to the findings of McCorquodale et al. (2014)**

Study Design

Objective 3: Estimate the effects of TAHD on the pregnancy rates of adult female elk

- **Determine pregnancy status at time of capture**
  - **Ultrasonography**
  - **Pregnancy-Specific Protein B (PSPB)**

Study Design

Objective 4: Estimate the effects of TAHD on elk productivity (i.e., survivorship of calves)

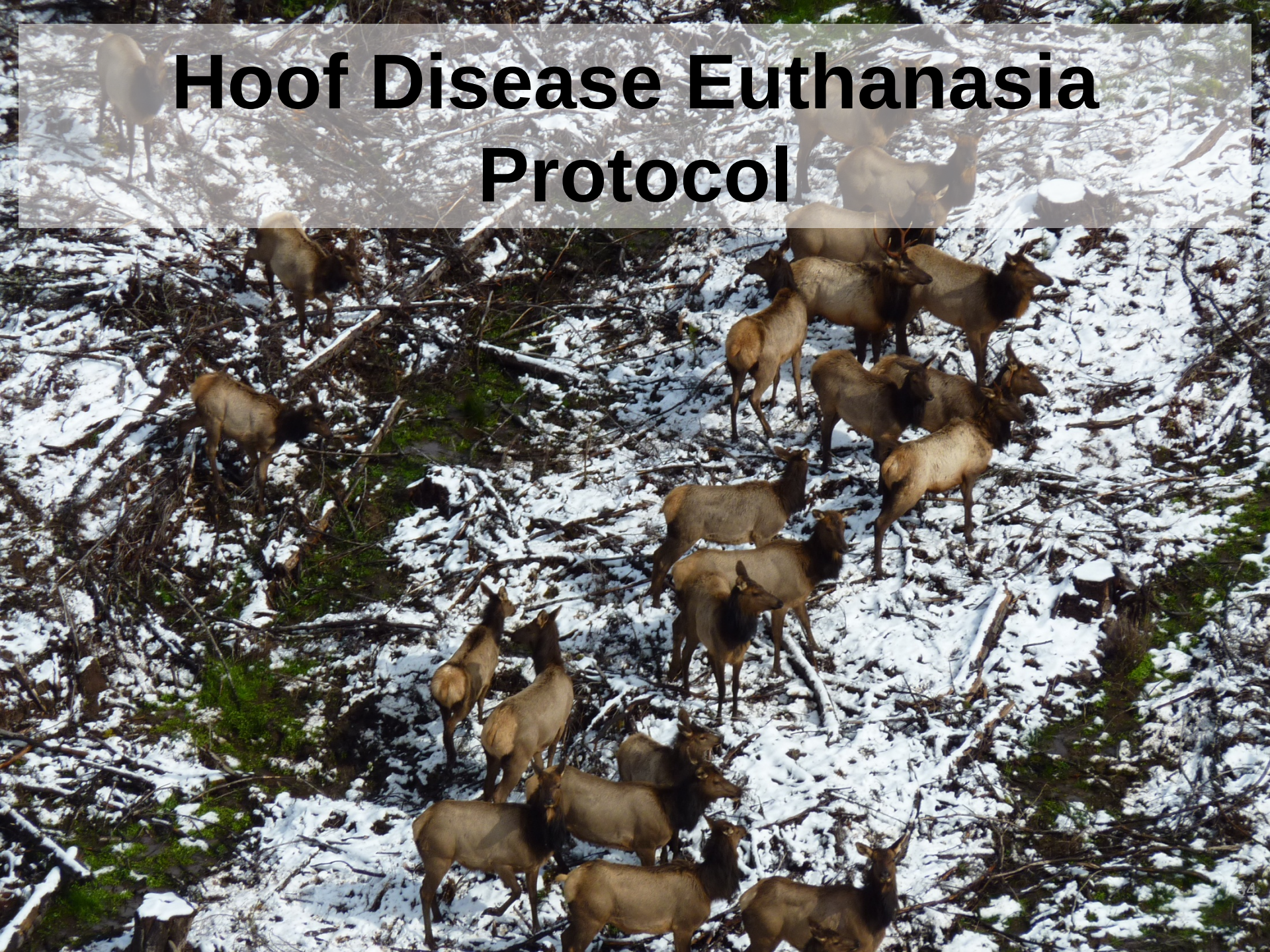
- **Calf-at-heel ratios from radio-collared elk**
- **Lactation rates from hunter harvested elk**
- **Will reassess the use of these approaches after Year 1**

Study Design

Objective 5: Estimate the effects of TAHD on the level of condition that hunter harvested adult female elk are able to achieve in autumn

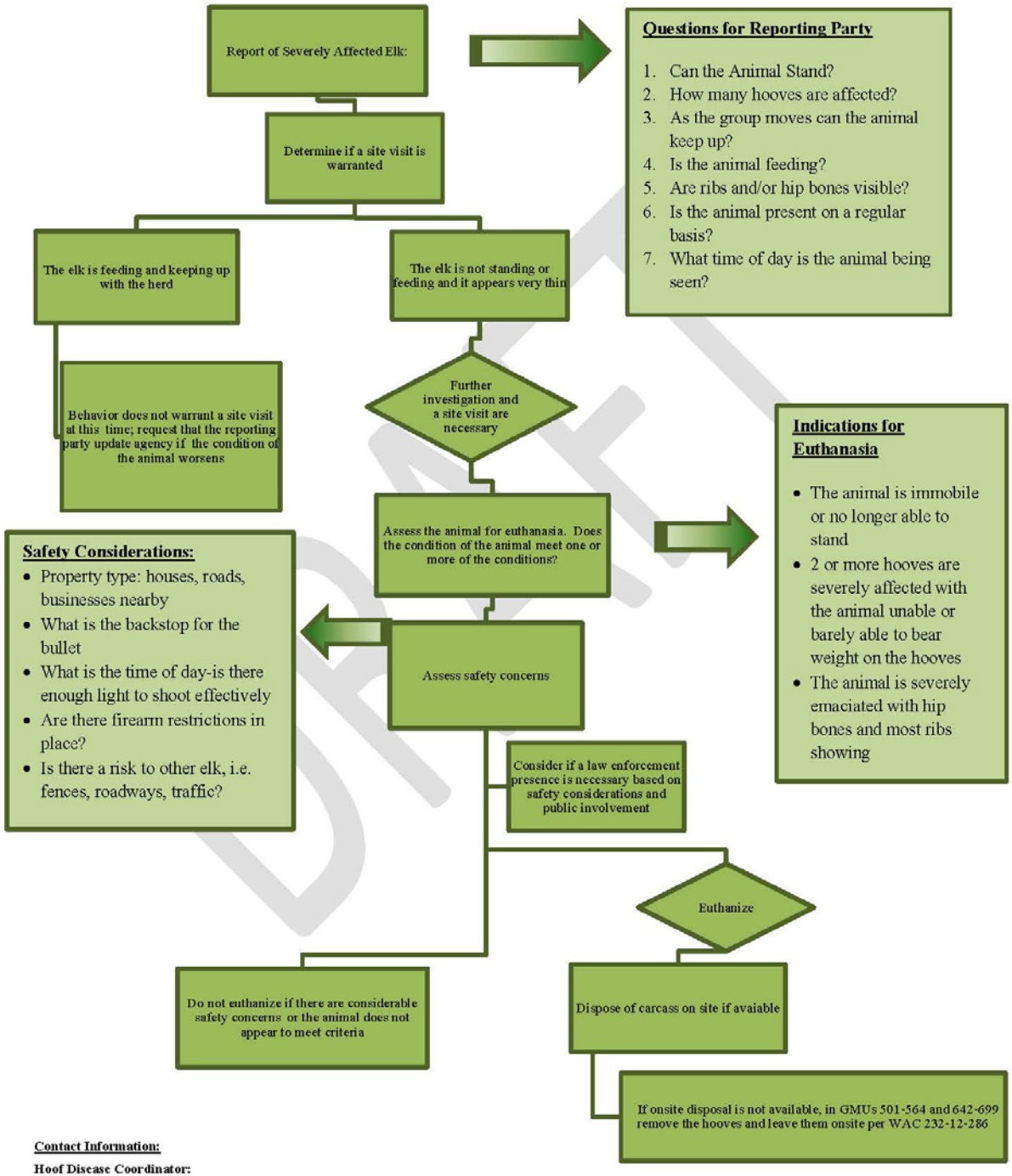
- **Solicit organs (heart, pericardium, kidneys) from antlerless permit holders**
- **Also ask hunters to:**
  - **Submit teeth**
  - **Determine lactation status**
  - **Determine presence of TAHD**
- **Estimate body condition (%IFBF) using modified Kistner scores**

Hoof Disease Euthanasia Protocol



SOP

- HDTAG and HDPWG agreed to removal for humane reasons
- Standard Operating Procedure for Lethally Removing Elk Severely Affected by Hoof Disease
- Guideline for euthanasia procedures
  - Each report will need to be evaluated on an individual basis


Report of Severely Affected Elk:
Determine if a site visit is warranted
The elk is feeding and keeping up with the herd
The elk is not standing or feeding and it appears very thin
Behavior does not warrant a site visit at this time; request that the reporting party update agency if the condition of the animal worsens
Further investigation and a site visit are necessary
Assess the animal for euthanasia. Does the condition of the animal meet one or more of the conditions?
Assess safety concerns
Consider if a law enforcement presence is necessary based on safety considerations and public involvement
Euthanize
Do not euthanize if there are considerable safety concerns or the animal does not appear to meet criteria
Dispose of carcass on site if avaiable
If onsite disposal is not available, in GMUs 501-564 and 642-699 remove the hooves and leave them onsite per WAC 232-12-286
Questions for Reporting Party
1. Can the Animal Stand?
2. How many hooves are affected?
3. As the group moves can the animal keep up?
4. Is the animal feeding?
5. Are ribs and/or hip bones visible?
6. Is the animal present on a regular basis?
7. What time of day is the animal being seen?
Indications for Euthanasia
• The animal is immobile or no longer able to stand
• 2 or more hooves are severely affected with the animal unable or barely able to bear weight on the hooves
• The animal is severely emaciated with hip bones and most ribs showing
Safety Considerations:
• Property type: houses, roads, businesses nearby
• What is the backstop for the bullet
• What is the time of day-is there enough light to shoot effectively
• Are there firearm restrictions in place?
• Is there a risk to other elk, i.e. fences, roadways, traffic?
Contact Information:
Hoof Disease Coordinator:
Brooke George 360-450-1202
WILDCOM: 360-902-2936

SOP

- WDFW staff with assistance from Master Hunters trained in criteria

Next Steps

- **Implementation!**
  - **Adaptive as we learn from these efforts with respect to management and research**
  - **2015-17 Budget Request is in Governor's budget**
- **Continue working with HDPWG and HDTAG as moving forward**
- **Assess/prioritize/address remaining information needs**


Thank you
….any questions….

Public Testimony

- Members of the public are requested to fill out a Public Testimony Form
- Members of the public will be requested to provide their public testimony to the HDPWG in the order the Public Testimony Forms were received
- Each member of the public wishing to relay their comments will have 3 minutes each to do so
  - This time frame is provided to allow the opportunity for all members of the public to provide their testimony to the HDPWG

Treponeme-associated Bacterial Hoof Disease in Elk in Southwest WA: Timeline of Events, Diagnostics, Research, & Management Efforts

1990's

- Sporadic reports of hoof deformities in the Cowlitz River Basin

2008

- Number of reports of abnormal hooves and lameness in elk as well as geographic scope increased significantly

2009

- WDFW conducted first clinical investigation on affected elk in March
- WDFW conducted a survey of hunters for an initial understanding of prevalence and distribution of limping elk

2010-2011

- Reviewed findings of 2009 investigation to guide future steps
- Comparison of copper and selenium levels from affected elk versus non-affected herds
- Consulted with national and international experts in wildlife disease

2012

- WSU College of Veterinary Medicine faculty convened to advise on diagnostic investigation
- WDFW Public meeting to share information
- Developed Online Hoof Disease Reporting Tool and informational materials on WDFW website

2013

- WDFW diagnostic collections of elk in March and August
- WDFW formalized Hoof Disease Technical Advisory Group to assess results of diagnostic investigation and to advise on further diagnostic approaches.
- WDFW formalized Hoof Disease Public Working Group to share information and discuss management options and research questions (meetings in October and December)
- Developed Hoof Disease Health/Safety Fact Sheet in partnership with Department of Health

2014

- WDFW diagnostic collections of elk in January
- Hoof Disease Public Working Group meetings in February and May
- Hoof Disease Technical Advisory Group reviewed results and reached consensus statement on treponeme-associated hoof disease in elk
- Fish & Wildlife Commission adopted new rule to leave hooves on site from harvested elk
- WDFW and citizen hosted public meetings (4)
- WDFW hired Hoof Disease Coordinator
- WDFW implemented a pilot prevalence study with volunteers to inform a larger effort for spring 2015
- Outreach for public assistance with an expanded effort to report limping elk or dead elk with hoof deformities on-line
- WDFW developing management approach based on input from HDTAG, HDPWG, & WDFW staff
  - Developing euthanasia criteria for severely affected elk
- Developing study design to implement long term elk survival study in 2015 to evaluate effect of hoof disease
- WDFW disseminating findings from diagnostic investigation with national and international forums and in peer-reviewed articles
- Legislative approved $200,000 supplemental budget for hoof disease investigation
- $180,000 Pittman-Robertson funds for hoof disease work
- $8,000 RMEF funds for sample analyses
- $250,000 legislative request for 2015-2017 biennium

JCM
Journals.ASM.org

Isolation of Digital Dermatitis Treponemes from Hoof Lesions in Wild North American Elk (*Cervus elaphus*) in Washington State, USA

S. R. Clegg,[a] K. G. Mansfield,[b] K. Newbrook,[a] L. E. Sullivan,[a] R. W. Blowey,[c] S. D. Carter,[a] N. J. Evans[a]

Department of Infection Biology, Institute of Infection and Global Health, School of Veterinary Science, University of Liverpool, Liverpool, United Kingdom[a]; Washington Department of Fish and Wildlife, Spokane Valley, Washington, USA[b]; University of Liverpool and Wood Veterinary Group, Gloucester, Gloucestershire, United Kingdom[c]

Since 2008, a large increase in the numbers of cases of lameness have been seen in wild North American elk (*Cervus elaphus*) from Washington State, USA. The most recent cases manifested as foot lesions similar both clinically and pathologically to those seen in digital dermatitis (DD) in cattle and sheep, a disease with a bacterial etiopathogenesis. To determine whether the same bacteria considered responsible for DD are associated with elk lameness, lesion samples were subjected to bacterial isolation studies and PCR assays for three phylogroups of relevant DD treponemes. The DD treponemes were isolated from lesional tissues but not from control feet or other areas of the diseased foot (including the coronary band or interdigital space), suggesting that the bacteria are strongly associated with DD lesions and may therefore be causal. In addition, PCR analysis revealed that all three unique DD treponeme phylotypes were found in elk hoof disease, and in 23% of samples, all 3 DD-associated treponemes were present in lesions. Sequence analysis of the 16S rRNA gene showed that the elk lesion treponemes were phylogenetically almost identical to those isolated from cattle and sheep DD lesions. The isolates were particularly similar to two of the three culturable DD treponeme phylotypes: specifically, the *Treponema medium*/*Treponema vincentii*-like and *Treponema phagedenis*-like DD spirochetes. The third treponeme culturable phylogroup (*Treponema pedis*), although detected by PCR, was not isolated. This is the first report describing isolation of DD treponemes from a wildlife host, suggesting that the disease may be evolving to include a wider spectrum of cloven-hoofed animals.

Diseases shared between wildlife and domesticated farm animals, such as brucellosis (1) and bovine tuberculosis in white-tailed deer (2), are notoriously difficult to manage. When wild animals are involved in the epidemiology of a disease which affects domestic animals, the effects on disease spread and control can be profound.

Treponemes can infect a wide range of hosts and tissues, causing a spectrum of diseases from syphilis in humans, periodontal disease in both companion animals and humans, and digital dermatitis (DD) in animals (3–5).

DD is an infectious foot disease causing severe lameness both in dairy and beef cattle worldwide (6, 7) and in sheep from the United Kingdom (8) and Ireland (9, 10). Although many bacteria can be isolated from a DD lesion, the most commonly observed bacteria belong to the genus *Treponema*. Cattle DD lesions generally contain spirochetes from several *Treponema* phylogroups, with previously isolated and characterized phylogroups identified as "*Treponema medium*/*Treponema vincentii*-like," "*Treponema phagedenis*-like," and "*Treponema denticola*/*Treponema putidum*-like" bovine (DD) spirochetes (11), with the latter now recognized as a new species, *Treponema pedis* (12). In addition, the same three unique, isolated phylogroups have been identified in bacterial cultures from DD foot lesions in sheep (9). The DD-associated treponemes are found in abundance in all DD lesions and are considered highly specific for DD lesions in both cattle and sheep, being undetectable in normal foot tissues. Current evidence suggests potential roles for the bovine gastrointestinal (GI) tract, manure and slurry, and hoof trimming equipment in the transmission of DD (13–15).

Currently, DD is very common in dairy cattle worldwide, particularly in those countries with intensive farming systems (16, 17). Furthermore, DD is present in beef cattle (18) and increasing in incidence in sheep (10) in the United Kingdom. Taken together, these data suggest that all cloven-hoofed animals are potential hosts for DD treponemes, a situation with similarities to that of the foot-and-mouth disease virus (7). Despite the identification of this widening host range, there have been no reports of treponemes being implicated in lameness in wild animals.

An outbreak of lameness in wild North American elk (*Cervus elaphus*) in Washington state, USA, has been reported since the mid-1990s, with an increased prevalence since 2008. Grossly, affected elk have deformed hooves that are asymmetrical, markedly elongated, and curved or broken or with sloughed horn. The disease pathology for elk showing such clinical signs has been described in detail (19).

Anecdotal information suggests that up to 80% of elk groups in the affected geographical area contain lame elk and that between 30 and 90% of individuals within a group are lame (20). This current study was designed to determine if this elk disease had the same infectious treponemal etiology as the DD lesions found in domesticated hooved species.

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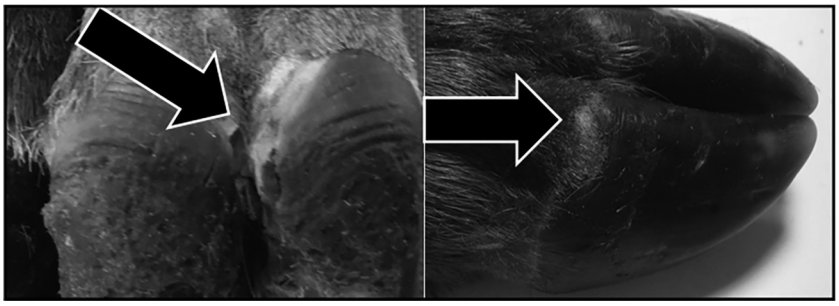

FIG 1 Photograph of an affected elk hoof with an early macroscopic lesion (indicated with an arrow) on the coronary band (right side) and a more typical foot lesion (left side) which shows more visual similarities to digital dermatitis.

MATERIALS AND METHODS

Animal distribution. Elk were sampled in 2013–2014 in southwest Washington. The study area included areas grazed by domestic cattle (*Bos taurus*) and sheep (*Ovis aries*); the DD status of the animals on this pasture was not determined. The terrain and study area have been discussed in more detail recently (19).

Sample collection. In the primary investigation, a variety of tissues were taken from seven young elk, representing four control animals (i.e., two unaffected animals from unaffected areas [elk 17 and 18] and two [elk 21 and 25] unaffected elk from an affected area) and three affected elk (elk 22 to 24). Biopsy samples were taken from the interdigital space, coronary band, and early gross macroscopic foot lesions (as judged by the attending veterinarian), where present (see Table 1). In addition, control samples were taken from the contralateral unaffected foot of affected animals (see Table 1). After the foot surface was cleaned by brushing and washing with sterile saline, a 3-mm punch biopsy specimen was taken from the center of the lesion and placed immediately in oral treponeme enrichment broth (OTEB) (Anaerobe Systems, Morgan Hill, CA, USA) containing rifampin (5 μg/ml) and enrofloxacin (5 μg/ml)). These samples were then transported with ice packs by courier from Washington to the University of Liverpool (~3 or 4 days) for microbiological analysis and were processed immediately for spirochete culture and DNA extraction for PCR. In addition, a second group of samples was collected from seven foot lesions and analogous foot tissues from 13 control tissues with no signs of lesions. These were processed blind, and results were collated after experimental work had been completed.

Isolation of spirochetes. Spirochete isolation attempts were made with all tissues taken from affected elk feet (coronary band, interdigital space, and lesions) and control elk samples. These bacterial isolations were done immediately upon arrival of samples, as described previously for cattle samples (11), using OTEB including rifampin (5 μg/ml) and enrofloxacin (5 μg/ml). Samples were inoculated into OTEB containing fetal calf serum (FCS) (Gibco, Paisley, United Kingdom) to maximize growth of *T. phagedenis*-like and *T. pedis* treponemes or containing rabbit serum (RS) (GE Healthcare Life Sciences, Buckinghamshire, United Kingdom) to maximize growth of *T. medium*/*T. vincentii*-like treponemes. All isolation attempts were carried out in an anaerobic cabinet (85% N_2, 10% H_2, and 5% CO_2; 36°C).

Passage of isolates was continued via fastidious anaerobe agar (FAA) plates, supplemented with 5% defibrinated sheep blood and antibiotics as described above, and single colonies from the plates were inoculated into further OTEB tubes to allow pure bacterial cultures to be obtained.

The second group of 20 elk samples, taken from 11 different animals, was inoculated into OTEB for culture as described above. The cultures were then examined by phase-contrast microscopy and analyzed by specific nested PCR assays to identify any specific treponeme phylogroups present, as described below.

DNA extraction. For isolation of bacterial genomic DNA from OTEB cultures, 2 ml of each culture was centrifuged (5,000 × *g*, 10 min, 4°C) in a bench-top centrifuge. DNA was then extracted from the cell pellet using Chelex-100, as previously described (21), and stored at −20°C.

PCR assays. Foot tissue and culture samples were subjected to nested PCR assays specific for the three DD-associated treponeme groups, "*T. medium*/*T. vincentii*-like," "*T. phagedenis*-like," and *T. pedis*, described previously (11, 12), with resulting PCR products encompassing 300 to 500 bp of the 16S rRNA gene. All hoof samples were also subjected to the *Treponema* genus PCR assay (22).

To validate the PCR assays, each experiment included positive controls (bovine DD treponeme genomic DNA from each of the three unique bovine DD treponeme phylogroups) and a negative control (water) as described previously (12), with all assays performed in triplicate. Characterization of isolates used PCR and gene sequencing of nearly the entire 16S rRNA gene, as described previously (11), with the sequencing outsourced to a commercial company (Beckman Coulter Genomics, Takeley, Essex, United Kingdom).

Sequencing and sequence analysis. Amplified PCR products were sequenced commercially, and the fragments of the 16S rRNA were assembled using the Chromas Pro sequence analysis package (Technelysium Pty. Ltd.) to produce a consensus gene sequence. Gene sequences were aligned using the software program CLUSTALW as implemented in the program MEGA 5.0 (23). The DNA alignment was subjected to analysis using the software program Modeltest, as implemented in the Topali interface (24), which revealed that the best-fit model was general time reversible (GTR). This was used to produce nucleotide maximum likelihood phylogenetic trees (bootstrap values based on 10,000 iterations).

Nucleotide sequence accession numbers. 16S rRNA gene sequences of isolates analyzed in this work are available in GenBank (accession numbers KM586666 to KM586673).

RESULTS

The pathology of lesions taken from elk feet has been described in detail recently (19), and an example is shown in Fig. 1. Briefly, a macroscopic description of the lesion pathology identified erosive lesions at the coronary band, underrun horn of the wall and sole, erosion of the pedal bone, and a red stippled appearance of exposed corium. It was this last appearance that initially suggested the similarity to DD lesions.

Spirochete isolations. Samples were taken from lesions, coronary bands, and interdigital spaces (IDS) from seven elk, three of which showed macroscopic coronary band lesions (Table 1). All six samples of lesional material taken from these three animals were positive for treponeme culture, subsequently confirmed by PCR. All control samples from unaffected elk feet (12 samples in total) were negative by DD treponeme-specific PCR assays and by culture. There was 100% correlation between PCR and isolation results, since every culture which was isolation positive was also

TABLE 1 Lesion and normal samples obtained from various foot sites from seven different elk[a]

Elk no.	Geographic location	Foot area	Isolation of treponemes using:		PCR result			
			FCS	RS	DD1	DD2	DD3	Treponeme whole genus
17	GH	Control	−	−	−	−	−	−
18	GH	Control	−	−	−	−	−	−
21	Lewis	IDS	−	−	−	−	−	−
22	Lewis	Control[b]	−	−	−	−	−	−
		Lesion 1	+ (elk 22af)	−	+	+	−	+
		Lesion 2	+ (elk 22f)	+ (elk 22R)	+	+	+	+
		IDS	−	−	−	−	−	−
23	Lewis	Lesion 1	+ (elk 23f)	+ (elk 23R)	+	+	−	+
		Lesion 2	−	+ (elk 23aR)	+	+	+	+
		Coronary band	−	−	−	−	−	−
		Control[b]	−	−	−	−	−	−
		Coronary band	−	−	−	−	−	−
24	Lewis	Control[b]	−	−	−	−	−	−
		Lesion 1	−	+ (elk 24R)	−	+	−	+
		Lesion 2	+ (elk 24f)	−	+	−	−	+
		Coronary band	−	−	−	−	−	−
25	Lewis	Coronary band	−	−	−	−	−	−
		IDS	−	−	−	−	−	−

[a] IDS, interdigital space. All samples were collected in summer 2013. Some elk had lesions on more than one foot, and each lesional sample was treated separately. Isolate names are shown in parentheses, and these are listed in the phylogenetic tree shown in Fig. 2 (where "f" indicates isolation using FCS, and "R" indicates isolation using RS). Control samples were taken from elk with no lesions found in an area considered to be unaffected, e.g., GH (Grays Harbor County), or from elk with no lesions found in areas known to be affected, e.g., Lewis County. All samples were cultured for treponeme isolation and analyzed by treponeme PCR, with only lesional material giving positive results. All other tissues, including control samples, were negative. DD1, DD2, and DD3 refer to the DD treponeme phylogroups, where DD1 is "*T. medium/T. vincentii-like*," DD2 is "*T. phagedenis*-like," and DD3 is "*T. pedis*."

[b] Each control sample was taken from the same anatomical area where the lesion was found but on an unaffected foot of the same elk. These were all found in Lewis County, WA.

PCR positive. Upon examination of the cultures by phase-contrast microscopy, the lesions were not highly contaminated with other bacteria, so it was possible to isolate a single discrete treponeme, which was analyzed further by 16S rRNA gene sequencing.

Spirochete isolations were also attempted from the second group of 20 biopsy samples taken from 11 elk. Thirteen of these samples were taken from elk not showing any signs of lameness or lesions (known as control elk), and seven samples were from foot tissues showing signs of potential DD-like disease (Table 2). Control samples were taken from the normal contralateral foot of animals with lesions, from normal feet of unaffected animals living within the area of endemicity (elk 4 and 5), and from normal feet of unaffected animals living in an unaffected area (elk 11 and 12).

As previously, all control elk samples were negative by isolation and by PCR (Table 2). However, three of the samples (33, 34, and 35) did have a bacterial organism which appeared to have a spirochetal morphology when viewed by phase-contrast microscopy but was subsequently shown by the diagnostic PCR assays not to be a treponeme. This organism requires further investigation. Of the seven elk showing signs of DD-like disease, spirochetes were isolated from five animals, with three of the samples containing two different phylogroups (*T. medium*/*T. vincentii*-like and *T. phagedenis*-like) of treponemes (Table 2). When cultured in OTEB, these samples proved to be highly contaminated with other unknown bacteria, so isolation of an individual treponeme for sequencing was not possible. The source of this bacterial contamination is unknown, but it may be due to delays in sample transport or to other bacteria present in lesion tissues. A negative-control OTEB tube remained free from bacterial growth, so contamination during culturing seems unlikely.

In total, for the second batch of 13 lesions investigated with the PCR assays, *T. medium*/*T. vincentii*-like, *T. phagedenis*-like, and *T. pedis* treponemes were detected in 54% ($n = 7$), 69% ($n = 9$), and 38% ($n = 5$), respectively. Three lesions contained three phylogroups, four contained two, and four contained just one phylotype.

16S rRNA gene analysis. Nine pure treponeme culture isolates were obtained from lesions taken from elk tested in the first group of samples and were subjected to 16S rRNA gene amplification with PCR prior to sequencing. One sequence produced an unreadable electropherogram and was excluded from future analysis. To determine the relationship of the eight elk treponeme isolates to those commonly found in domestic livestock (sheep, and cattle), the 16S rRNA gene sequences were compared to those from domestic livestock using phylogenetic analysis, with the results shown in Fig. 2. The sequences from these isolates are available in GenBank (see above).

Four treponemes with 16S rRNA gene sequences highly similar to those of *T. medium/T. vincentii*-like and four with high similarity to *T. phagedenis* were isolated from the elk foot tissues. The *T. phagedenis*-like elk spirochete 16S rRNA gene sequences were identical to each other and to isolate sequences from cases of clinical cattle DD, as well as sheep and similar human isolates.

Three of the four treponemes were closely related to *T. medium*, sharing 100% 16S rRNA gene nucleotide sequence identity. While the 16S rRNA gene sequence of one elk isolate was identical to dairy cattle *T. medium*/*T. vincentii*-like DD spirochete sequences from the United Kingdom (T19, T56m etc. [11]), the other three elk *T. medium/T. vincentii*-like DD spirochetes were more similar to human *T. medium* (25).

TABLE 2 Presence of spirochetes and PCR results from 20 elk samples taken from 11 different animals[a]

Elk no.	Sample no.	Sample type	Culture for spirochete growth				PCR result for treponeme group		
			FCS		RS				
			Spirochetes present	Treponeme whole genus	Spirochetes present	Treponeme whole genus	DD1	DD2	DD3
1	26	Lesion	+	+	+	+	+	+	+
1	37	Control	−	−	−	−	−	−	−
2	28	Lesion	−	−	−	−	−	−	−
2	50	Control	−	−	−	−	−	−	−
3	39	Lesion	−	−	−	−	−	−	−
3	40	Control	−	−	−	−	−	−	−
4	42	Control	−	−	−	−	−	−	−
5	47	Control	−	−	−	−	−	−	−
6	44	Control	−	−	−	−	−	−	−
6	38	Lesion	+	+	−	−	−	+	−
8	45	Lesion	+	+	−	−	−	+	+
8	41	Control	−	−	−	−	−	−	−
11	33[b]	Control	+	−	−	−	−	−	−
11	35[b]	Control	−	−	+	−	−	−	−
12	34[b]	Control	+	−	−	−	−	−	−
12	46	Control	−	−	−	−	−	−	−
13	29	Control	+	−	+	−	−	−	−
13	31	Lesion	+	+	−	−	−	+	−
16	36	Control	−	−	−	−	−	−	−
16	43	Lesion	+	+	+	+	+	−	+

[a] All samples were collected in January 2014. Where a lesion was present on one foot, a control sample was taken from the same animal, but from an unaffected foot ($n = 7$). In addition, four elk were tested which were unaffected by lameness and had no evidence of lesions. Culture using rabbit serum resulted in two treponemes from group 1, whereas culture using fetal calf serum resulted in four group 2 treponemes and three group three treponemes. Some of the lesions proved to be polytreponemal by PCR, whereas others were monotreponemal. DD1, DD2 and DD3 refer to the DD treponeme phylogroups, where DD1 is "*T. medium*/*T. vincentii*-like," DD2 is "*T. phagedenis*-like," and DD3 is "*T. denticola*/*T. putidum*-like." FCS is fetal calf serum, and RS is rabbit serum used for isolation of spirochetes.

[b] This control sample contained bacteria which appeared spirochetal when examined microscopically but later proved not to be treponemes when tested by PCR.

DISCUSSION

This is the first report of the isolation of DD-associated *Treponema* spp. from wild animals, with previous reports describing isolation from domesticated animals, including sheep, humans, and cattle (9, 26). The data presented here suggest that the range of hosts which treponemes are known to infect is expanding to now include elk.

The clearly detectable association of DD treponemes with elk foot lesions, based on detection and isolation of treponemes from only the lesion and no other part of the foot, or control feet, suggests that these bacteria are likely to be involved in the pathogenesis of the lesions. These lesions have many clinical and pathological (19) similarities with bovine DD and contagious ovine DD (CODD), as seen in cattle and sheep, respectively (9, 27). Recent studies have shown that isolated treponemes were capable of producing DD-like lesions in cattle feet, nearly fulfilling Koch's postulates for these spirochetal bacteria (26). In addition, there are a growing number of fluorescent *in situ* hybridization studies that substantially implicate the specific treponeme phylogroups as the considered etiological agents of DD (28–30).

Moreover, a range of metagenomic studies have identified the association of specific treponeme phylogroups with DD lesions in Europe, Japan, and the United States (31–34). In each of these studies, other bacterial genera were identified in DD lesions; however, only for the treponemes were there strong association data across all these studies.

In the elk, the high association of DD treponemes with the foot lesions and the lack of treponemes in unaffected tissues and control feet strongly suggest that DD treponemes may be implicated in this elk hoof disease, as they are in cattle and hoof diseases of other domestic livestock.

Nested PCR assays specific for three culturable DD treponeme phylogroups confirmed the isolation results for 9 of the 12 bacterial cultures grown in OTEB. The other three samples, although containing a spirochete-like microorganism when viewed microscopically, were in fact treponeme negative when tested by diagnostic PCR assays. This organism was not analyzed further. Due to the contaminated nature of these samples, 16S rRNA gene sequencing was not possible for these cultures.

In addition, and similarly to cattle and sheep lesions, the lesions from elk feet are generally polytreponemal, with bacteria belonging to two or three of the DD treponeme phylogroups according to the specific nested PCR assays. Previous studies have indicated that most DD lesions in cattle are polytreponemal (12, 22, 29, 30), and this is in agreement with lesions seen in elk described here. In this study, only 23% (3/13) of lesions were found to contain all three treponeme phylogroups when analyzed by PCR. This is significantly lower than the 74.5% of lesions reported for cattle. This may be due to wild animals having substantially less direct contact with animals (and their feet) infected with treponemes than is the case for housed dairy cattle, which usually show a much higher prevalence of DD than cattle on pasture (35).

Sequences of the 16S rRNA gene of the treponemes isolated from elk suggest that the bacteria found in the lesions are very similar and in some cases identical to those found in lesions in cattle and sheep (9, 35). This may suggest that elk are experiencing

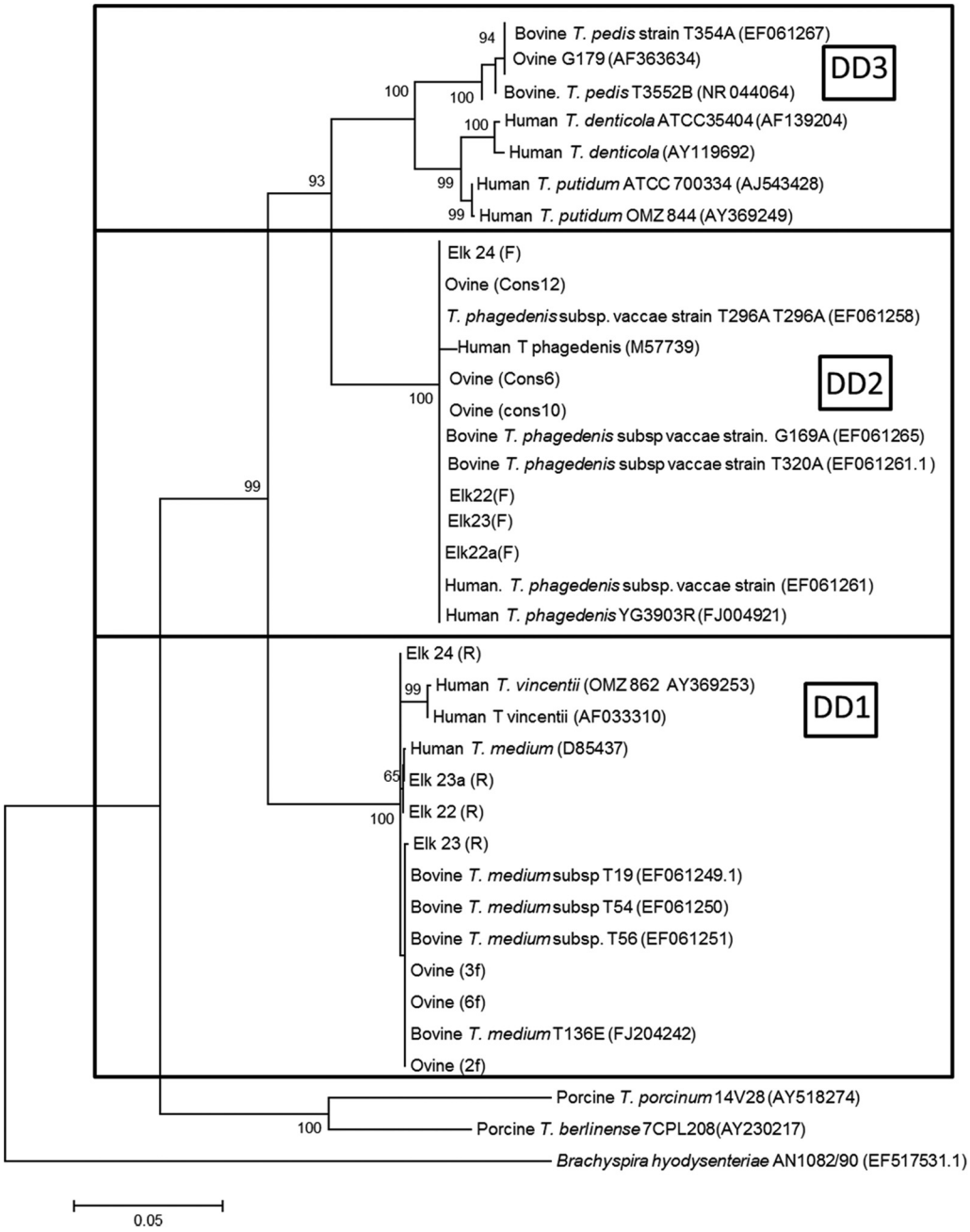

FIG 2 A maximum-likelihood tree (bootstrapped 10,000 times) for comparison of treponeme sequences isolated from elk to those isolated from cattle, humans, and sheep. (For clarity, bootstrap values below 65 were removed). Sequences from GenBank of human treponemes and other related treponemes are also shown, with the accession numbers in parentheses. The sequences from isolates in this study are labeled with the elk number and "F" or "R," indicating if they were isolated using fetal calf serum or rabbit serum. Key: DD1, DD2, and DD3 refer to the DD treponeme phylogroups, where DD1 is "*T. medium*/*T. vincentii*-like," DD2 is "*Treponema phagedenis*-like," and DD3 is "*T. denticola*/*T. putidum*-like."

a disease similar to that of farm ruminants, caused by the same bacteria, raising issues for potential transmission of disease between host species.

The clinical presentation of the lesions in elk is directly comparable with that of the lesions seen in DD in cattle and sheep. In sheep, the disease is frequently presented as severe lesions on the coronary band at the front of the hoof (36, 37). In dairy cattle, DD is mainly reported as a lesion at the rear of the foot between heel bulbs. However, there are many reports (in both Europe and the United States) showing that DD in cattle frequently manifests as a coronary band lesion at the front of the hoof in a manner similar to that of the initial lesion seen in sheep (36, 37). Whatever the presentation, the clear association of DD treponemes strongly suggests that we have identified another manifestation of the disease. Interestingly, DD treponemes have recently been associated with newly identified severe, nonhealing lesions in cattle feet, such as nonhealing white line disease and sole ulcers (38). This suggests that the DD treponemes are potent opportunistic secondary in-

vaders of other primary lesions, and this may be occurring in the elk feet. However, the extremely strong association of the DD treponemes with the elk lesions does suggest that they are primary invaders, as in cattle and sheep with DD, and lead to the ensuing severe pathogenesis.

Elk are wild animals, and their movement is currently uncontrolled. Thus, it is likely that they will travel much larger distances than domesticated cattle and sheep, which generally have much more controlled movements. While it might be considered that the elk may have originally contracted the bacteria while grazing on farmland previously used by sheep and cattle, they may now be considered to act as a potential reservoir of infection, spreading disease to other animals. The large territorial range of elk may mean that they have the potential to spread the bacteria over a larger range than domesticated animals, with implications for control, biosecurity, and disease management in both wild and domesticated animals (39).

This first-reported treponemal infection in wild animals may have far-reaching consequences for other animals, both wild and domesticated, and for disease management. Additionally, it suggests an expanding host range for the DD treponemes and that all cloven-hoofed animals could be susceptible to DD. Further studies will determine what preventative approaches and treatment measures can be considered to attempt to control the spread of this disease in elk and reduce the infection risk in other wildlife species.

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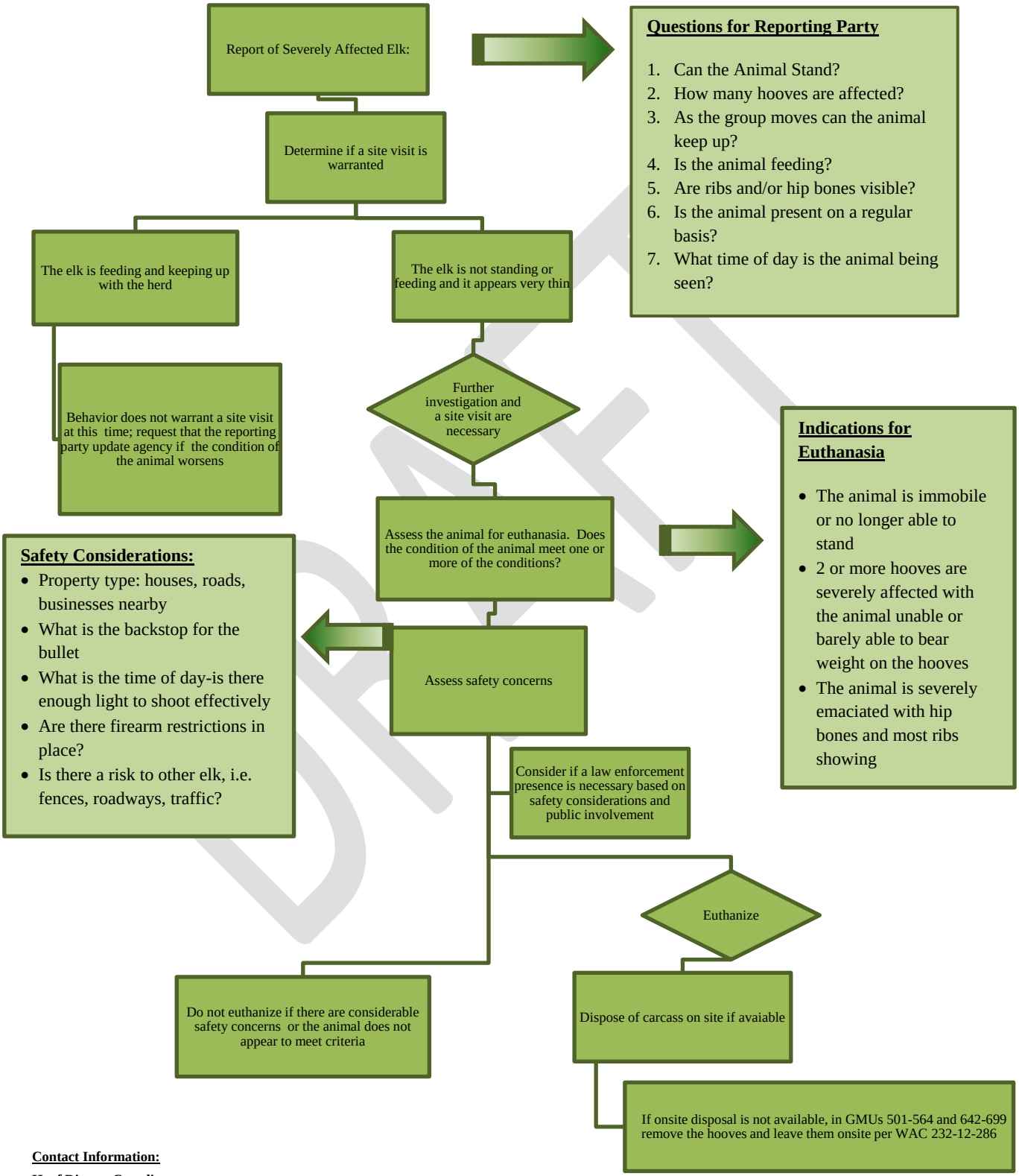
Standard Operating Procedure for Lethally Removing Elk Severely Affected by Hoof Disease

* This document is a guideline for euthanasia procedures. Each report will need to be evaluated on an individual basis and procedures will need to be adjusted based on unanticipated conditions in the field.



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STUDY PROPOSAL

for

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ASSESSING THE POTENTIAL EFFECTS OF TREPONEME ASSOCIATED HOOF DISEASE (TAHD) ON ELK POPULATION DYNAMICS IN SOUTHWEST WASHINGTON

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BACKGROUND AND RESEARCH NEED

Various hoof diseases have been reported worldwide in numerous free-ranging ungulates, including elk (*Cervus elaphus*; Murie 1930, Gray et al. 2001, Thorne et al. 2002), mule deer (*Odocoileus hemionus*; Wobeser et al. 1975), white-tailed deer (*O. virginianus;* Sleeman et al. 2009), moose (*Alces alces;* Flynn et al. 1977, Clauss et al. 2009), fallow deer (*Dama dama*; Lavin et al. 2004), reindeer (*Rangifer tarandus tarandus*; Handeland et al. 2010), roe deer (*Capreolus capreolus*; Handeland and Vikøren 2005), and mouflon (*Ovis gmelini musimon*; Volmer et al. 2008). The emergence of a new hoof disease in southwest Washington elk herds is unique in that bacteria in the genus *Treponema*, (aka "treponemes"), never previously associated with hoof diseases in any free-ranging ungulate, have been identified as causal (Clegg et al., 2014). Treponemes are strongly associated with two diseases of domestic livestock: bovine digital dermatitis of cattle (Evans et al. 2009), and contagious ovine digital dermatitis of domestic sheep (Sayers 2009). The Washington Department of Fish and Wildlife (WDFW) began receiving reports of lame elk with deformed hooves during the 1990s, but the number and geographical distribution of reports increased sharply by 2008 and have continued to increase in frequency and extent (Mansfield et al. 2011, WDFW unpublished data). To date, the public has reported multiple observations of limping elk within the range of five elk herds that occur west of the Cascade Mountain crest, and WDFW has collected biological samples within the range of the Mount St. Helens (MSH), Willapa Hills, and Olympic elk herds that researchers clinically diagnosed as treponeme-associated hoof disease (TAHD) (Figure 1; Clegg et al. 2014).

The severity of clinical signs of TAHD coupled with the seemingly rapid expansion of impacted areas has generated a great deal of concern for WDFW, other resource management agencies, hunters, tribes, and local citizens. Elk that are affected by TAHD often have severely overgrown and deformed hooves with sole ulcers and sloughed hoof walls (Han and Mansfield 2014). TAHD can occur in multiple limbs and can affect all age and sex classes (Clegg et al. 2014). In response to these concerns, WDFW is working with several specialists from around the world to understand the etiology of TAHD. In addition, WDFW has established a Hoof Disease Technical Advisory Group (HDTAG) and a Hoof Disease Public Working Group (HDPWG). The HDTAG has guided the diagnostic effort, provided input to research options, and provided review and input to management options. The HDPWG has provided input to management and research options and serves as a venue for WDFW to share information with

the public. However, it is difficult to assess what implications TAHD will have for the management of affected elk herds because the effects of TAHD on elk vital rates (e.g., survival, reproduction, etc.) are unknown.

Although severely affected elk are often times very emaciated, investigators do not believe declines in body condition are a direct effect of Treponemes. Unlike other bacterial diseases such as necrobacillosis that cause hoof deformities in elk, (Thorne et al. 2002), TAHD only involves the hooves; *Treponema spp*. have not been found to affect other organs or tissues (Han and Mansfield 2014; Clegg et al., 2014). Thus, observed declines in the body condition of severely affected elk, if related to TAHD, are likely a secondary effect that is associated with reduced mobility and subsequent inability of an elk to consume the quantity and quality of forage necessary to meet the basic energetic demands of metabolism and activity (9,500–10,500 kcal/day; Cook 2002).

It seems reasonable to assume that elk with advanced stages of TAHD may have a decreased probability of survival because their infirmities may predispose them to predation, harvest, severe weather events, or other types of disease (Bender et al. 2008). For example, mule deer with chronic wasting disease (CWD), prior to developing obvious clinical signs, have been shown to be more vulnerable to predation (Miller et al. 2008, Krumm et al. 2009), vehicle collisions (Krumm et al. 2005), and possibly harvest (Conner et al. 2000). This is an important consideration because the growth rate of large ungulate populations, such as elk, is highly sensitive to changes in adult female survival (Nelson and Peek 1982, Eberhardt 2002) and strongly correlated with the production and survival of juveniles (Gaillard et al. 2000; *see also* Smith and Anderson 1998, Raithel et al. 2007). When adult female and juvenile survival are concurrently reduced, populations would be expected to decline (Gaillard et al. 2000; *see also* Bender et al. 2007, McCorquodale et al. 2014). Consequently, if TAHD reduces the survival of affected cows and calves, it has the potential to have a negative effect on the population dynamics of impacted elk herds.

Although McCorquodale et al. (2014) monitored 16 adult female elk that had varying degrees of presumed TAHD (i.e., they had varying degrees of hoof deformities, but no lab samples were collected and tested) inferences from their work are limited. Twelve of 16 affected elk they monitored survived ≥ 1 year and of those that did not survive ≥ 1 year, all were harvest-related mortalities. In addition, 3 of 4 elk that were fitted with VHF collars that had a battery life of

several years survived until radio contact was lost 3-4 years after they were captured. Anecdotally, this indicates that, if TAHD negatively affects the natural survival of elk, it may take several years before it does so. We need to improve our understanding of how quickly TAHD progresses and if, and when, it may begin to predispose affected elk to mortality.

TAHD may also have the potential to affect the population dynamics of impacted elk herds because of its indirect effect on the nutritional condition of female elk. The nutritional condition of female ungulates can influence age at first breeding (Cook et al. 2004), timing of estrous and subsequent birth date (Andersen and Linnell 1998, Cook et al. 2004, Bishop et al. 2009), probability of conception (Cook et al. 2004, Cook et al. 2013), fetal development and survival (Verme 1969, Ozoga and Verme 1982), birth weight (Verme and Ullrey 1984, Keech et al. 2000, Lomas and Bender 2007), milk yield or composition (Landete-Castillejos et al. 2003, Tollefson 2007), and subsequent growth and survival of juveniles (Clutton-Brock et al. 1982, Bishop et al. 2009). For example, elk from the MSH herd area and other coastal regions of Washington are characterized by pregnancy rates for prime-aged females that are consistently depressed [Kuttel 1975 (74%), Smith 1980 (61%), Cook et al. 2013 (68-100%), McCorquodale et al. 2014 (71%)] because marginal summer-autumn nutrition limits the level of condition female elk are able to achieve during the breeding season (Cook et al. 2013). TAHD may further limit the ability of affected elk to improve their condition during the breeding season and therefore has the potential to reduce overall pregnancy rates even further, which could reduce demographic vigor.

Some hunters and local citizens attribute recent declines in the MSH elk herd to TAHD because the monitored portions of the MSH herd declined by 30-35% over a 4-year period (2009–2013; McCorquodale et al. 2014) that coincided with an increase in the prevalence and distribution of TAHD (WDFW, unpublished data). However, this period of population decline also occurred concurrently with a directed effort by WDFW to reduce the elk population through substantial increases in antlerless harvest because there was evidence that indicated the MSH elk herd was above ecological carrying capacity (McCorquodale et al. 2014). Moreover, density independent severe winter weather that occurred in 2012 likely contributed to the documented decline (McCorquodale et al. 2014). Because these three events overlapped temporally and elk with presumed TAHD represented <15% of the adult females that were monitored, McCorquodale et al. (2014) were not able to conclude whether or not TAHD was a contributing factor.

The number of elk that have TAHD and the effects of TAHD on elk vital rates, collectively, will determine what the long-term implications of TAHD are for the viability, and subsequent management, of impacted elk herds (Wobeser 2007). WDFW initiated a pilot effort in summer and fall of 2014 using citizen-scientists to estimate prevalence and distribution of TAHD within the range of the Willapa Hills and MSH elk herds, with a more comprehensive effort scheduled for the spring of 2015. Therefore, it is imperative that WDFW conduct a parallel study that will increase our understanding of the effects TAHD has on elk vital rates.

RESEARCH GOALS AND OBJECTIVES

The work we are proposing here is needed so WDFW can develop management strategies that are informed by sound, objective science. Those management decisions will be vetted with the HDTAG and the HDPWG. Our primary research goals are to quantify how TAHD affects the survival, pregnancy rates, productivity, and nutritional condition of adult female elk. Our specific study objectives include:

1. *Estimate the effects of TAHD on survival of adult (≥ 2 years old) female elk.*
2. *Determine cause-specific mortality rates for adult female elk that have TAHD.*
3. *Estimate the effects of TAHD on the pregnancy rates of adult female elk.*
4. *Estimate the effects of TAHD on elk productivity (i.e., survivorship of calves).*
5. *Estimate the effects of TAHD on the level of condition (i.e., % IFBF) that hunter-harvested adult female elk are able to achieve in autumn.*

STUDY AREA

This study will occur within the range of the MSH (WDFW 2006) and Willapa Hills (WDFW 2014) elk herds (Figure 2). We will request biological samples from hunters throughout both herd areas (see **Body Condition** below), but will limit our survival analysis and any other components of our study that are associated with radio-collared elk (e.g., captures, calf-at-heel observations, etc.), to Game Management Units (GMUs) 520 (Winston), 522 (Loo-wit), 524 (Margaret), 550 (Coweeman), and 556 (Toutle). Collectively, these GMUs represent

the core range of the MSH herd and the study area of McCorquodale et al. (2014) (Figure 2). There are several reasons why we chose to restrict our radio-collared elk to this core area, rather than radio-collar elk throughout each herd area or in all areas where TAHD has been observed.

First, we are interested in radio-collaring elk that have TAHD. Both the public (Figure 2) and WDFW biologists report frequent observations of limping elk within these five GMUs, which suggests TAHD is observed more frequently on the landscape and we are likely to have a higher probability of encountering elk affected by TAHD during our captures.

Second, the primary objective of our survival analysis is to determine whether TAHD directly affects the survival of adult female elk. Many factors that vary spatially and have the potential to affect elk survival (e.g., predator density, hunter density, climate, etc.) may also interact with TAHD. Thus, it is important that we define a study area where we can reasonably control for indirect effects by assuming all other mortality factors are relatively constant in the sample population. A larger study area (and consequently range of values for the above-mentioned variables) will undoubtedly result in increased stochastic variation in the data independent of TAHD.

Finally, unless TAHD has a large effect on survival (i.e., probability of survival declines by >10%), logistical constraints make it difficult to maintain the sample sizes (≥100 radio-marked animals) we would need to detect an effect of TAHD on survival (Winterstein et al. 2001). Given the findings reported by McCorquodale et al. (2014) for the 16 cow elk that had TAHD, we anticipate an effect size of <5%, which would require upwards of 200 or more radio-collared elk (Ilai Keren, WDFW, unpublished data). Therefore, our approach is to focus on estimating survival of adult female elk that are affected by TAHD compared with an internal control and the results of McCorquodale et al. (2014) (for elk that were not affected by TAHD), which will meet our primary objective despite being more limited in its scope of inference.

Mount St. Helens Elk Herd Area

Physiography and Climate.—Most of the MSH elk herd area is located in the western Cascades, where mountainous terrain is the most common topographic feature. However, the western and northern portions of the herd area consist of rolling foothills and level, to mostly level, terrain along the major drainages and the Interstate Highway 5 corridor. Elevation ranges from approximately sea level to 12,307 feet at Mt. Adams. Most of the MSH herd area is part of

the Southern Washington Cascade Province, but the westernmost portion is part of the Puget Trough Province (Franklin and Dyrness 1973). The climate of the study area is Pacific maritime, with cool, wet winters and relatively dry summers. Annual precipitation ranges 63–157 inches, with most of the annual precipitation falling between October and April. Winter snowfall is common, varies considerably across years, and persists for much of the winter at higher elevations.

Plant Communities.—Franklin and Dyrness (1973) described three major forest zones in the MSH herd area, including the lower elevation Western Hemlock (*Tsuga heterophylla*), the mid elevation Pacific Silver Fir (*Abies amabilis*), and the high elevation Mountain Hemlock (*T.mertensiana*) zones. Franklin and Dyrness (1973) list a variety of plant communities and associations for each of the major zones, which reflects differences in soil type, elevation, aspect, and slope. Douglas fir (*Pseudotsuga menziesii*) is a naturally occurring co-dominant tree in the western hemlock zone, which industrial forest companies typically promote because of its high commercial value and fast-growing characteristics. Timber harvest on industrial forestlands and some state lands has historically been by clear-cutting. Forest management has produced a distinctive and extensive mosaic of recent clear-cuts and second growth stands of various ages.

Population Status.—The eruption of Mount St. Helens substantially reduced herd size in 1980, but elk quickly recolonized the affected area and the herd increased in size soon after. As habitat succession advanced and peaked in the 1990s, episodic winter mortality events began to occur, which indicated the MSH herd was well above the ecological carrying capacity of the herd area. Recent management objectives have been associated with reducing herd size by approximately 30% (McCorquodale et al. 2014). WDFW's current management objective is to promote population stability.

Willapa Hills Elk Herd Area

Physiography and Climate.—Topography in the Willapa Hills elk herd area ranges from level to rolling along major drainages and in the northern portion of the herd area, to mountainous in the interior regions of the Willapa Hills. Elevation ranges from sea level to just over 3,000 feet. Annual precipitation averages >80 inches with the majority occurring as rainfall from October through April. Snowfall events are rare with average annual accumulations of less

than 2.5 inches. Average temperatures range from a high of 70°F in July to a low of 35°F in February.

Plant Communities.— Most of the Willapa Hills elk herd area falls within one of two major plant communities described by Franklin and Dyrness (1973). Coastal sites below 500 feet in elevation often are part of the Sitka spruce (*Picea sitchensis*) zone, whereas the majority of the area is within the western hemlock (*Tsuga heterophylla*) zone. Both of these plant communities are very productive and have been heavily logged. Following removal of the climax tree species most suitable sites were replanted with Douglas fir (*Pseudotsuga menzieseii*), a species that may be dominant or co-dominant on unlogged timber stands in the western hemlock zone. On lower elevation mesic sites, western red cedar (*Thuja plicata*) is often an important component of the tree layer. Common deciduous trees include vine maple (*Acer circinatum*), big-leaf maple (*Acer macrophyllum*), and red alder (*Alnus rubra*).

Population Status.—The Willapa Hills elk herd expanded its range and increased in size when industrial timber management practices in the herd area peaked during the 1950s, but harvest data indicates population size has remained relatively stable for the past decade. WDFW's current management objectives are to promote a stable to increasing population (WDFW 2014).

METHODS AND STUDY DESIGN

Capture and Marking

We will capture adult (≥ 2 years old) female elk via aerial darting from a Bell 206B Jet Ranger helicopter in late-February. We will immobilize elk using carfentanil (3mg) and xylazine (50 mg). We will blindfold elk to minimize stress during handling, administer clostridium vaccine and analgesic (flunixin meglumine) injections, and treat the dart wound. We will mark each captured elk using a colored and numbered ear-tag and a mortality-sensitive, GPS (Global Positioning System)-equipped radio-collar. We will remove an upper canine tooth to determine age using microhistological analysis of cementum annuli (Hamlin et al. 2000; Matson's Laboratory, Milltown, MT). Finally, we will antagonize immobilants by administering naltrexone (300 mg) and tolazoline (700 mg).

Radio-collars

We will employ a GPS-enabled radio-collar that is manufactured by VECTRONIC Aerospace (Berlin, Germany) and uses the GLOBALSTAR satellite network to attempt a GPS fix every 13 hours. This periodic schedule will approximate two locations per day and allow fixes to cycle through time of day. Locations will be stored daily on-board the collar and via the GLOBALSTAR satellite network. Radio-collars will also be equipped with a mortality sensor and use the GLOBALSTAR satellite network to send an alert via email or mobile phone when the mortality sensor is activated. This functionality will allow for quick detection of mortality events and assist with correctly identifying proximate causes of mortality. Radio-collars will also be equipped with a mortality-sensitive VHF (Very High Frequency) beacon to assist with collar recovery and with survival monitoring in the event of GPS failure. The VHF beacon will only transmit from 0800–1600 (Pacific Standard Time), which will more than double the collar's battery life to ca. 4 years.

Body Condition

We will determine late-winter body condition [i.e., % ingesta-free body fat (IFBF)] during captures by having an experienced observer use a portable ultrasound to measure maximum subcutaneous rump fat thickness (MAXFAT) and determine a rump body condition score (rBCS) following the procedures of Cook et al. (2001*a*). We will use estimates of MAXFAT and rBCS to estimate %IFBF at time of capture following the procedures of Cook et al. (2010). We will also measure each elk's chest girth to estimate body mass following the procedures of Cook et al. (2003). We will determine lactation status for each elk we capture by expressing milk from the udder; the presence of milk indicates nursing within 3–11 days (Flook 1970), and thus survival of a calf to that point in time.

We will also estimate autumn body condition of hunter-killed female elk using modified Kistner scores (Kistner et al. 1980) following the procedures of Cook et al. (2001*b*). This method of estimating %IFBF consists of using reference photos to visually score the level of fat deposition that is associated with the heart, pericardium, and kidneys and then applying those values in a predictive equation that estimates %IFBF (Cook et al. 2001*b*). We will solicit these organs from all antlerless permit holders within the MSH and Willapa Hills elk herd areas. We

will also request that hunters submit teeth from harvested elk so we can determine age via examination of cementum annuli (Hamlin et al. 2000; Matson's Laboratory, Milltown, MT). Lastly, we will have hunters determine lactation status and note whether harvested elk had TAHD. We will request that hunters submit photos of each hoof so we can confirm TAHD presence, but do not anticipate every hunter will respond to this request.

McCorquodale et al. (2014) requested organ samples from 3,170 hunters that were issued antlerless permits 2009-2011 and 2,096 of those hunters harvested an elk, of which 364 submitted organ samples that could be used to predict %IFBF (i.e., they submitted ≥2 of the necessary organs). This suggests that 17% of hunters that harvest an elk will respond to our request for biological samples. We anticipate there will be approximately 200 and ≥300 antlerless elk harvested annually in the Willapa Hills and MSH elk herd areas, respectively. Consequently, we will make a formal request for biological samples with the expectation we will receive ≥30 usable samples from the Willapa Hills elk herd area and ≥45 samples from the MSH elk herd area during each year of our study. We will use generalized linear models (GLM; Quinn and Kenough 2002) to identify effects of covariates on autumn body condition of elk. Covariates we are likely to consider in our analysis are TAHD presence, date of harvest, GMU, year, and indices of environmental conditions (e.g., summer-autumn precipitation).

Survival Analysis

We will initiate captures in February 2015 with the goal of capturing and marking 80 adult female elk (60 affected, 20 non-affected). We will conduct subsequent captures in 2016, 2017, and 2018 to replace collars that we censor from our analysis (see below), or to replace study animals that have died. We will censor any elk that dies (regardless of proximate cause) within 30 days of being captured because we will be unable to rule out capture-related stress as a factor that contributed to mortality (Beringer et al. 1996). We will capture elk before spring green-up so we anticipate some elk will die before the start of a new biological year (i.e., May 1). Thus, we will conduct captures with the primary goal of maintaining a sample size of 80 elk at the time of captures, and ≥70 radio-collared elk at the start of each biological year. Regardless of disease status, we will not euthanize any radio-collared elk except in situations where we determine that death is imminent.

We will estimate annual survival rates for radio-collared elk using known-fate models and maximum likelihood methods implemented in Program MARK (White and Burnham 1999). We will develop an a priori set of candidate models that include combinations of covariates we believe are biologically plausible and would be reasonably expected to affect survival (Johnson and Omland 2004). Covariates that we are likely to consider are age, year, GMU, TAHD status, and indices of environmental conditions (e.g., winter severity index). We will use an information-theoretic approach based on Akaike's Information Criterion corrected for small sample size (ΔAIC_c) and impose the rule of parsimony to select the model that best describes survival (Burnham and Anderson 2002).

Although our primary intent will be to estimate the survival of elk affected by TAHD, we will also radio-collar 20 elk that are presumably unaffected by TAHD to serve as a control group for survival rates in the sample population, independent of TAHD status. McCorquodale et al. (2014) estimated annual survival of adult females was 52% in 2012, compared to 85% for all other years, and the observed decline in survival was strongly correlated with unusually severe winter conditions. If a similar weather event were to occur during the duration of our study, and our sample only included elk affected by TAHD, we would have no way of knowing if elk without TAHD were similarly affected by severe weather, which could lead to an incorrect interpretation of our results. We will complete a separate survival analysis for unaffected elk using the known-fate model procedures described above. The imbalance in sample sizes between TAHD and the healthy group will reduce statistical power for that contrast, and we anticipate our estimates of survival for elk not affected by TAHD will have low levels of precision, which will limit our ability to detect covariates that influence survival (Winterstein et al. 2001). However, a sample size of 20 should be large enough to detect a decrease in survival that is similar in magnitude to that observed by McCorquodale et al. (2014).

Cause Specific Mortality

If TAHD predisposes affected elk to mortality, it will be important to understand which proximate causes of mortality (i.e., predation, starvation, etc.) are elevated. In general, it seems plausible that elk affected by TAHD are most likely to die of starvation, but that is unknown. We will attempt to investigate all deaths of radio-collared elk within 24 hours of receiving a message that a mortality event has occurred. We will perform a field necropsy on all mortalities

to determine proximate cause of death. In some cases, we may collect samples that will be analyzed in a lab setting to assist with determining proximate cause of mortality. We will classify proximate causes of mortality as predation, human-caused (legal harvest, wounding loss, poaching), accidents, starvation, infectious disease (aside from TAHD), other, or undetermined. We will calculate annual cause-specific mortality rates for affected and non-affected radio-collared elk using the approach of Heisey and Fuller (1985).

Pregnancy Rate

We will use two methods to determine pregnancy status at time of capture. When qualified and experienced personnel are available, we will determine pregnancy status via ultrasonography, and if not available, we will determine pregnancy status via analysis of Pregnancy–Specific Protein B (PSPB) in serum samples collected during each capture (Noyes et al. 1997). Because of our unbalanced study design and our plan to capture the minimum number of elk required to maintain a sample size of 80 elk, 2016-2018, it is unlikely we will have sample sizes that allow for yearly comparisons. Instead, we will compare pregnancy rates at capture between groups, using data pooled over the length of the study.

We will compare pregnancy proportions between affected and non-affected elk using the Fisher Exact Test (Zar 2010). We may also use logistic regression (Hosmer and Lemeshow 1989) to model the dichotomous outcome of pregnancy (pregnant or not pregnant) as a function of TAHD status and other covariates (e.g., age).

Productivity

We define productivity as the early survivorship of calves. We will determine the effects of TAHD on elk productivity using two approaches: 1) by estimating calf-at-heel ratios from radio-collared elk and 2) by using lactation rates of hunter harvested elk as surrogates of calf survival. We will employ both approaches in 2015 and then assess how well each method worked and whether we will continue to employ these methods during subsequent years of this study.

Calf-at-heel ratios.—We will monitor radio-collared elk during the month of August and first week of September to determine which cows have successfully produced a calf. Elk productivity is most commonly assessed in September (WDFW 2008), but we will conduct our monitoring efforts in August to avoid conflicts with established hunting seasons. Based on the

findings of McCorquodale et al. (2014), we anticipate that ≤70% of the females we capture will be pregnant, which would result in 50–55 elk that would need to be monitored in 2015 to determine whether or not they have a calf-at-heel. We anticipate that number will increase to 70 or more elk during 2016-2018, because most radio-collared elk will be carry-overs from captures in preceding years and thus, pregnancy status will be unknown.

In most instances, we will only classify a cow as having a calf-at-heel when we are able to obtain visual confirmation of a calf successfully nursing from the radio-collared cow. However, there may be times when this level of confirmation is not required. For example, if we observed a radio-collared cow with a calf and no other elk are observed or otherwise detected (e.g., other elk are heard vocalizing, but not seen) for an extended period, we would still feel comfortable classifying this elk as having a calf-at-heel. Calves will be 3-4 months old at the beginning of August and may only have 4-5 short nursing bouts throughout the day (Hudson et al. 2002). In addition, elk are likely to be in habitats that are not conducive to obtaining visual observations, except during early morning and early evening hours. Consequently, this approach of assessing calf survival has the potential to be very time consuming, especially since the VHF beacon of our collars will only be functional from 0800–1600 (PST). To minimize the amount of time and resources needed to implement this approach, we will only require one confirmation of a calf-at-heel for each radio-collared elk and we will allow that confirmation to occur any time throughout the monitoring period.

We will use logistic regression (Hosmer and Lemeshow 1989) to identify covariates that influence the likelihood of a radio-collared elk being observed with a calf in August. Covariates we are likely to consider are age, TAHD status, year, and indices of environmental conditions (e.g., summer-autumn precipitation). We may also utilize other covariates such as group size and habitat as weights on the precision of the calf-at-heel observation.

Lactation rates of hunter-harvested elk.—The methods we will employ to generate lactation and TAHD status data from hunter-harvested elk are described above (see **Body Condition**). We will use logistic regression to identify covariates that influence lactation rates of hunter harvested elk as described for the calf-at-heel method. Covariates we are likely to consider are age, TAHD status, year, and herd area. We do not anticipate we will have a large enough sample size to make GMU level comparisons or to include GMU as a covariate in our logistic models.

WORK PLAN AND BUDGET

WORK PLAN for FY 2015 (July 2014–June 2015)												
Task	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Develop study proposal												
Order radio-collars												
Order capture supplies												
Obtain approval from necessary landowners to conduct captures (e.g., timber companies, USFS)												
Conduct captures and deploy radio-collars												
Monitor survival and movements												
Develop strategy for requesting biological samples from hunter-harvested elk												
Develop strategy for conducting calf-at-heel surveys												
Order supplies needed for collecting biological samples from hunter-harvested elk												
WORK PLAN for FYs 2016–2019 (July 2015–June 2019)												
Task	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Monitor survival and movements												
Conduct calf-at-heel surveys												
Collect biological samples from hunter-harvested elk												
Order replacement radio-collars and capture supplies as needed												
Conduct captures and deploy radio-collars												
Generate modified Kistner scores for organs collected from hunter-harvested elk												
Write annual progress report (FY 2016-2018)												
Final Report will be completed June–August 2019												

BUDGET						
Budget Item	**FY 2015**	**FY 2016**	**FY 2017**	**FY 2018**	**FY 2019**	**Total**
GPS radio-collars and associated GPS location data	$108,000[a]	$7,500[b]	$7,500	$7,500	--	$130,500
Capture supplies (drugs, darts, syringes, etc.)	$20,000[c]	$6,500	$6,500	$6,500	--	$39,500
Helicopter charter for captures	$60,000	$19,000	$19,000	$19,000	--	$117,000
Supplies for collecting biological samples from hunter-harvested elk	--	$5,000	$5,000	$5,000	$5,000	$20,000
Cementum annuli analysis		$1,000	$1,000	$1,000	$1,000	$4,000
Miscellaneous supplies	$3,000	$3,000	$3,000	$3,000	$3,000	$15,000
Total	**$191,000**	**$42,000**	**$42,000**	**$42,000**	**$9,000**	**$326,000**

[a] GPS locations were purchased in advance–i.e. this includes the cost of 2 locations/day for 80 collars over 4 years.
[b] Assumes we will have to replace 10 collars that have failed, were destroyed, or we have lost contact with. This includes the activation charge. Data cost is not associated with a specific collar, so we will not have to account for costs associated with GPS locations.
[c] n = 80 elk in FY 2015 and 25 elk in FY 2016–2018.

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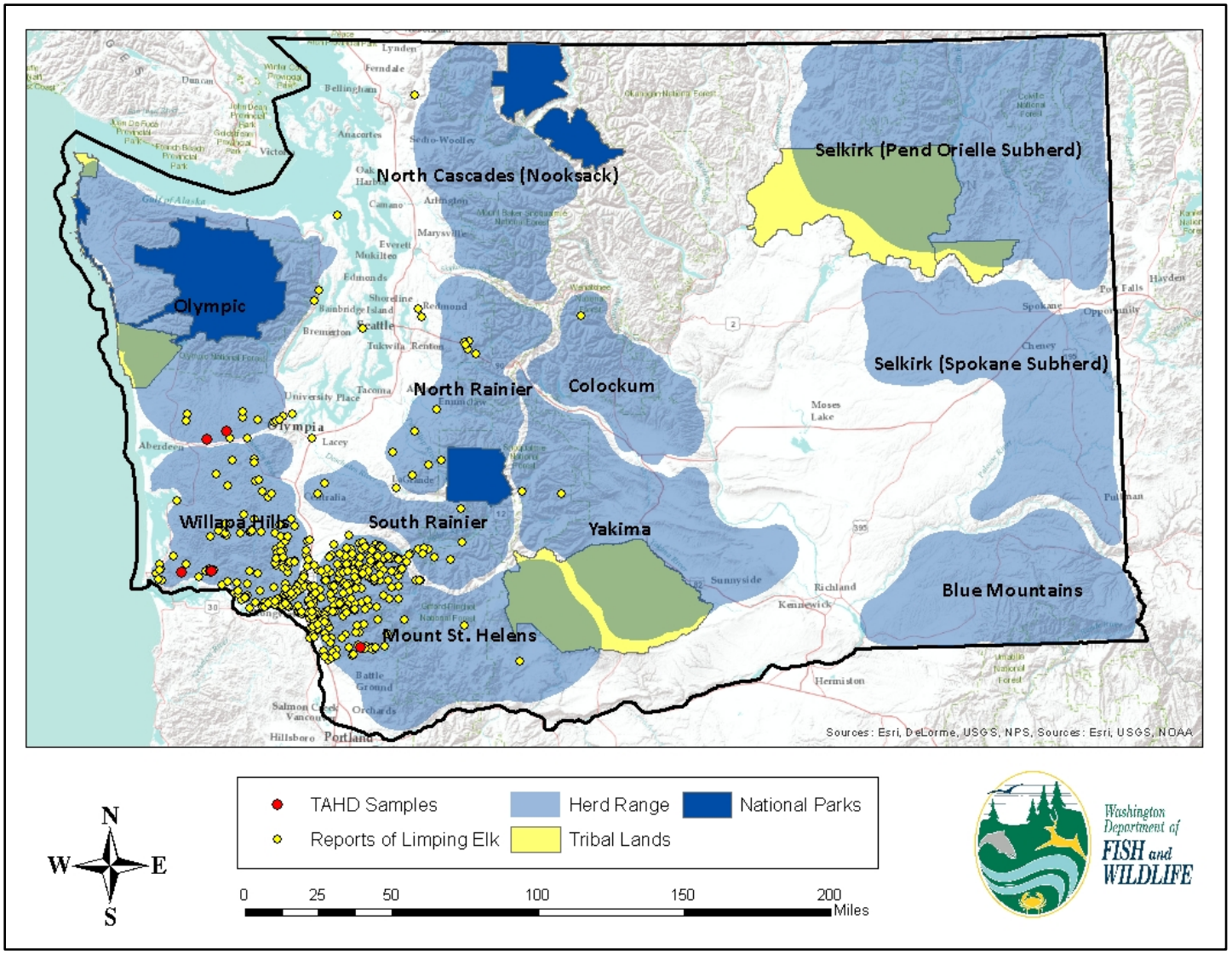

Figure 1. Map depicting the generalized range of the 10 elk herds formally identified by the Washington Department of Fish and Wildlife (WDFW), the location of reports received by WDFW of limping elk, and the location of biological samples that were collected and clinically diagnosed as treponeme associated hoof disease (TAHD samples).

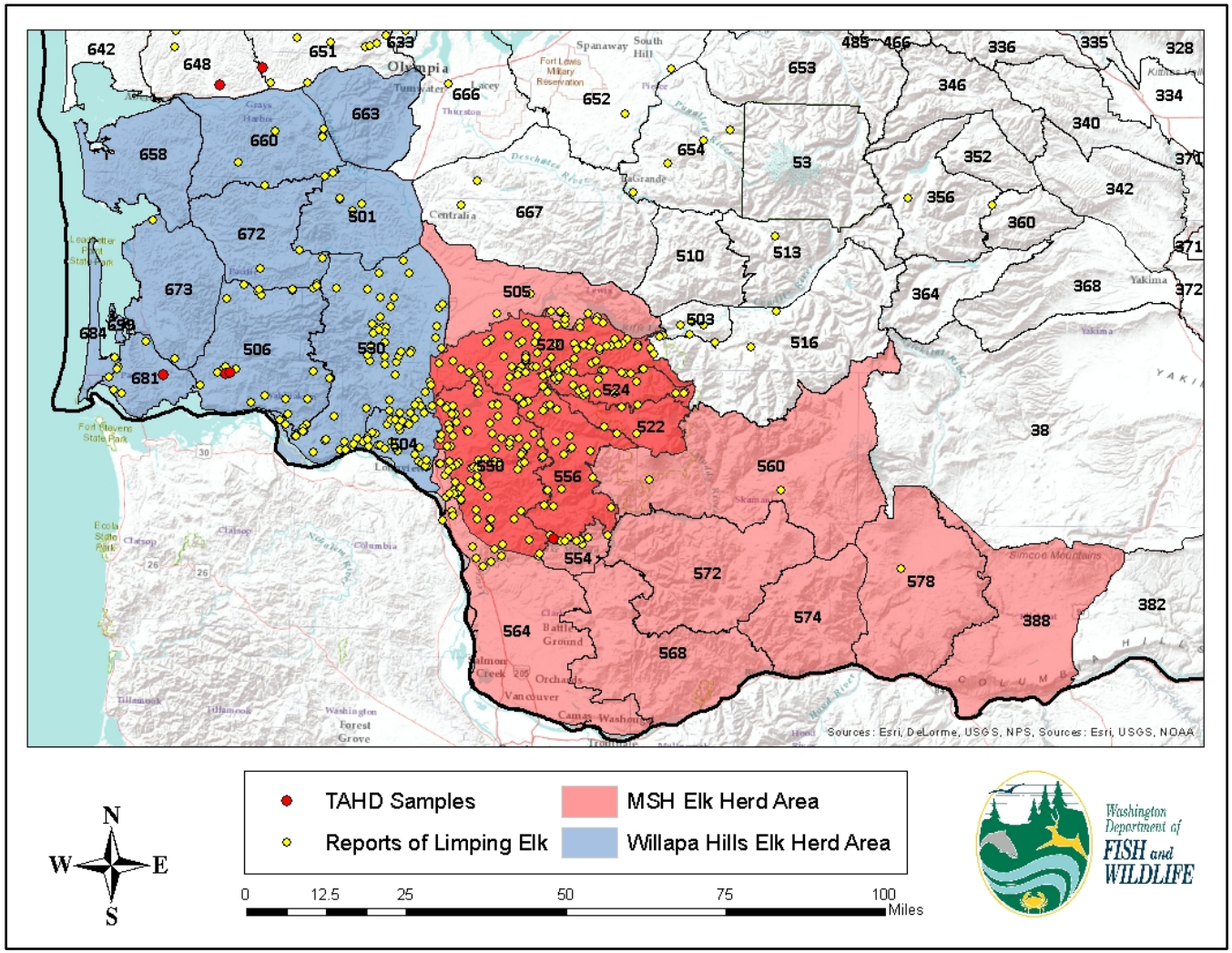

Figure 2. Map depicting the Game Management Units (GMUs) that comprise the Willapa Hills (blue) and Mount St. Helens (MSH; red) elk herd areas, the location of reports received by the Washington Department of Fish and Wildlife of limping elk, and the location of biological samples that were collected and clinically diagnosed as treponeme associated hoof disease (TAHD samples). GMUs within the MSH elk herd area that are shaded darker red represent the study area of McCorquodale et al. (2014) and our proposed study area.