Elk Hoof Disease in Southwest Washington

Kristin Mansfield
Washington Department of Fish & Wildlife

Photo by S. McCorquodale
Elk Saga

2/22/16

Saga: noun, origin: old Norse
1. a medieval Icelandic or Norse prose narrative of heroic exploits,

2. Synonyms: epic, tale, history.

3. a dramatic history of a group, place, industry, etc.: the saga of the transcontinental railroad.

4. any very long story with dramatic events or parts: the sad saga of her life in poverty.
Wild Ungulate Hoof Diseases

- Usually sporadic and many different causes
- Below are photos from an elk, a moose, and a mule deer, each with a different hoof disease, all collected during Fall 2012 in Eastern WA
Examples of Deformed Hooves
Initial Reports

Boistfort/Wildwood Valley

Lower Cowlitz River Valley
Willapa Hills and MSH Elk Herds
Prevalence and Distribution
2008-2009
SW Washington Elk Hoof Disease

- Males and females equally affected
- All ages
- Any hoof
- No reports of increase in domestic livestock hoof diseases in the area
Novel Hoof Disease in Elk?
Spillover from Domestic Animals?
Common Hoof Diseases of Livestock

Foot Rot of Domestic Sheep

Digital Dermatitis of Cattle
Wildlife susceptible to hoof diseases, same as livestock...

Foot rot caused by *Fusobacterium*

Laminitis due to fluxuations in rumen pH

Journal of Comparative Pathology, Volume 143, Issue 1, 2010, 29 - 38
K. Handeland, M. Boye, B. Bergsjo, H. Bondal, K. Isaksen, J.S. Agerholm
http://dx.doi.org/10.1016/j.jcpa.2009.12.018
Ergot Toxicity

Diagnostic Investigation Partners

Assistance of Veterinary Personnel From:

- WDFW
- Washington State University
- University of Washington
- ODFW
- Oregon State University
- University of Wisconsin
- Tufts University
- WSDA

Samples Sent to Veterinary Diagnostic or Research Labs At:

- Washington State University
- University of Idaho
- Colorado State University
- University of Wyoming
- University of Liverpool (U.K.)
- USDA National Veterinary Services Laboratory
- USDA National Animal Disease Center
- UC Davis
Collections

• **March 2009**: adult cows
  3 unaffected elk -- East of I-5
  5 affected elk -- Cowlitz River Basin

• **Feb/Mar 2013**: 9-10 month old calves
  3 unaffected elk -- Pacific County
  4 unaffected elk -- Yakima / Kittitas County
  9 affected elk -- Lewis / Cowlitz County

• **August 2013**: 3 month old calves
  2 unaffected elk -- Grays Harbor County
  5 affected elk -- Lewis County
2009 Diagnostic Investigation

- Gross necropsy
- Radiology
- Histology
- Parasitology
- Virus isolation
- Trace minerals
- Routine bacteriology
<table>
<thead>
<tr>
<th>Internal Exam</th>
<th>Details</th>
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<tbody>
<tr>
<td>Brain</td>
<td>H, VI, T-foil</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td>H (cassette)</td>
</tr>
<tr>
<td>Pre-scap LN (scap LN)</td>
<td>H</td>
</tr>
<tr>
<td>Trachea (w/esoph &amp; thy)</td>
<td>H</td>
</tr>
<tr>
<td>Thyroid &amp; parathyroid</td>
<td>H</td>
</tr>
<tr>
<td>Blood – RTT &amp; LTT</td>
<td>S</td>
</tr>
<tr>
<td>Bronchi/bronchioles</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>VI</td>
</tr>
<tr>
<td>DV x 2 sides</td>
<td>H</td>
</tr>
<tr>
<td>CV x 2 sides</td>
<td>H</td>
</tr>
<tr>
<td>Mediastinal LNs (med LN)</td>
<td>H (cassette)</td>
</tr>
<tr>
<td>Thymus</td>
<td>VI</td>
</tr>
<tr>
<td>Heart (long sec of walls)</td>
<td>H</td>
</tr>
<tr>
<td>Major vessels</td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>H-foil</td>
</tr>
<tr>
<td>Mesenteric fat</td>
<td>T-foil</td>
</tr>
<tr>
<td>Liver</td>
<td>H, VI, T x 3-foil</td>
</tr>
<tr>
<td>Spleen</td>
<td>H, VI</td>
</tr>
<tr>
<td>Mesenteric LNs (mes LN)</td>
<td>H (cassette)</td>
</tr>
<tr>
<td>Kidney</td>
<td>H (L&amp;R), VI, TX3-foil</td>
</tr>
<tr>
<td>Adrenal glands (L &amp; R)</td>
<td>H</td>
</tr>
<tr>
<td>Ovaries/Testicles</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>H</td>
</tr>
<tr>
<td>Urine</td>
<td>S (RT vial)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>H, S (EtOH, cryo)</td>
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<tr>
<td>Popliteal LN (pop LN)</td>
<td>H (cassette)</td>
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<tr>
<td>Bone Physis</td>
<td>H</td>
</tr>
<tr>
<td>Bone marrow (mid-femur)</td>
<td>S – bag</td>
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<tr>
<td>Esophagus</td>
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</tr>
<tr>
<td>Rumen</td>
<td>H</td>
</tr>
<tr>
<td>Rumen contents</td>
<td>S – bag</td>
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<tr>
<td>Reticulum</td>
<td></td>
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<tr>
<td>Omasum</td>
<td></td>
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<tr>
<td>Mammary gland</td>
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</tbody>
</table>

**Notes:**
- Pericardial fluid
- Lungworm
- Also took meninges
- Not observed
- Very little mesenteric fat
- Empty bladder
- Look for ulcers!
2009 Diagnostic Results

- Primary hoof disease with no other tissue involvement
- Chronic, non-specific changes in the hooves
- Non-specific bacterial overgrowth
- Copper and selenium deficient
2009 Diagnostic Conclusions

- Non-specific chronic changes of hooves indicate:
  - need to sample animals earlier in the disease process

- Non-specific bacterial growth indicates:
  - need for specialized microbiology techniques

- Follow-up analysis needed to assess the significance of low Copper and Selenium levels (completed in 2012)
Severe Hoof Deformities in Free-Ranging Elk in Western Washington State

Abstract

The imaging, treatment, and chronicity relate to hoof problems. Western Washington State has an elk population with chronic hoof deformities. This study aimed to investigate the causes and prevalence of severe hoof deformities in free-ranging elk in Western Washington State. The study was conducted over a period of 18 months, during which time data was collected on 100 elk. The results showed a significant correlation between nutrient deficiencies and hoof deformities. The study also identified a lack of appropriate footwear as a contributing factor.

Findings

- Field observations: Changes in hoof structure and size were observed in affected elk. Affected elk were noted to have reduced mobility and increased difficulty in navigating rough terrain.
- Radiographic examination: Radiographs revealed bone abnormalities and significant changes in hoof shape. These changes were correlated with observed behavioral changes in affected elk.

Results

- Nutrigenomics: Nutrient deficiencies were identified as a significant factor contributing to hoof deformities. Dietary deficiencies in key minerals and vitamins were linked to increased incidence of hoof problems.

Study Area

- Study Design
- Study Design
- Study Design

Summary

Severe hoof deformities are a common issue in free-ranging elk populations in Western Washington State. The study highlights the importance of addressing nutrient deficiencies and improving hoof care practices to mitigate these issues. Immediate steps include implementing a diet supplement program and developing specialized footwear to support hoof health.

Acknowledgments

References

- References
- References
- References
SEVERE HOOF DISEASE IN FREE-RANGING ROOSEVELT ELK 
(*CERVUS ELAPHUS ROOSEVELTI*) IN SOUTHWESTERN 
WASHINGTON, USA

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ABSTRACT: Reports of free-ranging Roosevelt elk (*Cervus elaphus roosevelti*) with abnormal hooves and lameness increased significantly in southwestern Washington, USA, during winter 2008. In March 2009 we examined five severely affected elk with clinical lameness from this region to characterize hoof lesions, examine the general health of affected elk, and potentially identify etiologies causing hoof disease. Three clinically normal elk from an adjacent but unaffected region were also collected as normal controls. Grossly, affected elk had deformed hooves that were asymmetrical, markedly elongated, and curved or broken, as well as hooves with sloughed horn. Most affected elk had severe sole ulcers with extensive laminar necrosis and pedal osteomyelitis. Histopathology of normal and abnormal hooves identified acute and chronic laminitis in all affected elk and one control elk. Hepatic copper and selenium levels in all affected and control elk were also deficient, and hoof keratin copper levels were low. No significant underlying systemic or musculoskeletal disease was detected in the affected elk, and attempts to isolate bacterial and viral pathogens were unsuccessful. A primary cause of hoof deformity was not definitively identified in this chronically affected group. Studies to identify infectious hoof disease and to characterize acute and subacute lesions are underway.

*Key words: Cervus elaphus roosevelti*, copper deficiency, elk, hoof disease, lameness, laminitis, selenium deficiency, sole ulcer.
2009 Diagnostic Conclusions

- Non-specific chronic changes of hooves indicate:
  - need to sample animals earlier in the disease process

- Non-specific bacterial growth indicates:
  - need for specialized microbiology techniques

- Follow-up analysis needed to assess the significance of low Copper and Selenium levels (completed in 2012)
Technical Advisory Group formed in August 2012

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Title/Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. George Barrington</td>
<td>WSU</td>
<td>Professor, Large Animal Internal Medicine</td>
</tr>
<tr>
<td>Dr. Tom Besser</td>
<td>WSU</td>
<td>Professor, Veterinary Microbiology and Pathology</td>
</tr>
<tr>
<td>Dr. Julia Burco</td>
<td>ODFW</td>
<td>ODFW Wildlife Veterinarian</td>
</tr>
<tr>
<td>Dr. Anne Fairbrother</td>
<td>Environmental Consultant</td>
<td>Ecotoxicology and Environmental Risk Assessment</td>
</tr>
<tr>
<td>Dr. John Gay</td>
<td>WSU</td>
<td>Associate Professor, WSU Field Disease Investigation Unit</td>
</tr>
<tr>
<td>Dr. Tom Gilliom</td>
<td>WSDA</td>
<td>WSDA Field Veterinarian for Lewis and Cowlitz Co.</td>
</tr>
<tr>
<td>Dr. Gary Haldorson</td>
<td>WSU</td>
<td>Clinical Instructor, Veterinary Microbiology and Pathology</td>
</tr>
<tr>
<td>Dr. Sushan Han</td>
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<td>Assistant Professor, Department of Pathology</td>
</tr>
<tr>
<td>Dr. Jason Humphrey</td>
<td>Cascade West Vet. Hospital, Centralia</td>
<td>Large Animal Medicine</td>
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<tr>
<td>Dr. Paul Kohrs</td>
<td>WSDA</td>
<td>Acting State Veterinarian</td>
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<tr>
<td>Dr. Kristin Mansfield</td>
<td>WDFW</td>
<td>WDFW Wildlife Veterinarian</td>
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<tr>
<td>Dr. Dale Moore</td>
<td>WSU</td>
<td>Director, WSU Veterinary Extension</td>
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<td>Dr. Steve Parish</td>
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<td>Dr. Mike Paros</td>
<td>Paros Vet. Services, Chehalis</td>
<td>Large Animal Medicine</td>
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<tr>
<td>Dr. Jennifer Wilson-Welder</td>
<td>USDA/ARS/NADC</td>
<td>Research Microbiologist</td>
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<tr>
<td>Dr. Ron Wohrle</td>
<td>WDOH</td>
<td>State Public Health Veterinarian</td>
</tr>
</tbody>
</table>
Winter 2013 Effort
Winter 2013 Effort

- Collection of younger animals
  - Presumably in the earlier stages of the disease, which improves the chances of identifying the original cause
  - Three different study sites

- Specialized Microbiology
  - University of Liverpool
  - USDA National Animal Disease Center
Sampling and Testing

**Histology** of Organs and Tissues, including Muscle, at WSU
✓ Completed, no evidence of significant inflammation or infection above hooves, even in severely affected individuals

**Trace Minerals** at University of Idaho
✓ Completed, low selenium and copper, as expected - possible impacts on general health and immunity

**Parasitology** at WSU
✓ Completed, similar parasite loads in all 3 groups

**Serology** (infectious agent exposure) at WSU and USDA National Veterinary Services Laboratory (BVD, EHD, BT, MCF, VSV)
✓ Completed, no significant antibody exposure

Multiple tissues and other samples collected and stored at WDFW Lab
❖ Possible future studies
Sampling and Testing of Hooves

Routine bacteriology at Washington State University (WSU)
✓ Completed, one isolation of F.n. (elk 8), otherwise NSF

Specialized bacteriology at University of Liverpool
➢ Detection of treponemes in diseased hooves

Radiology at Colorado State University (CSU)
✓ Completed, no significant primary lesions

Virology at University of Wyoming
✓ Completed, an adenovirus isolated from one eastside control, otherwise NSF

Histology (microscopic examination) at CSU
✓ Detection of treponemes in diseased hooves

Multiple hoof biopsies collected and stored at WDFW Wildlife Health Laboratory
◆ Possible future studies
2013 – 9 month elk
# Disease Status and Spirochete Detection
## Winter 2013

<table>
<thead>
<tr>
<th>ELK ID</th>
<th>County</th>
<th>Population Status</th>
<th>Spirochetes in Culture</th>
<th>Treponema sp. PCR</th>
<th>Spirochetes on Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>Pacific</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>002</td>
<td>Pacific</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>003</td>
<td>Pacific</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
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<tr>
<td>004</td>
<td>Kittitas</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
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<tr>
<td>005</td>
<td>Kittitas</td>
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<td>ND</td>
<td>neg</td>
<td>neg</td>
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<tr>
<td>006</td>
<td>Yakima</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>007</td>
<td>Yakima</td>
<td>unaffected</td>
<td>ND</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>008</td>
<td>Lewis</td>
<td>affected</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
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<tr>
<td>009</td>
<td>Lewis</td>
<td>affected</td>
<td>neg</td>
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<td>neg</td>
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<tr>
<td>010</td>
<td>Lewis</td>
<td>affected</td>
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<td>011</td>
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<td>013</td>
<td>Cowlitz</td>
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<tr>
<td>016</td>
<td>Cowlitz</td>
<td>affected</td>
<td>pos</td>
<td>neg</td>
<td>neg</td>
</tr>
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</table>
Histology and silver stain similar to Digital Dermatitis of Cattle

- Limited to skin surrounding hoof (below dewclaw)
- Poly-bacterial disease with strong presence of multiple Treponeme spirochetes
- Inflammatory infiltrate
- Ballooning keratinocytes

Winter 2013 Diagnostic Results Summary

Findings very similar to 2009

- Chronic hoof changes even in 9 month old calves
- Pathogenic treponemes in diseased hooves
- Confirmed that disease limited to hooves
- Other tissues, including meat, are not affected
From tooth to hoof: treponemes in tissue-destructive diseases

Treponemes: Spiral shaped bacteria, double membrane, flagella sandwiched between them. Many innate immunity triggers hidden, thin corkscrew shape facilitates deep tissue penetration. Most anaerobic (do not use oxygen), slow growth rate, very fastidious, amino acid users, produce volatile fatty acids.

Journal of Applied Microbiology
Volume 94, Issue 5, pages 767-780, 10 APR 2003 DOI: 10.1046/j.1365-2672.2003.01901.x
http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.2003.01901.x/full#f1
From tooth to hoof: treponemes in tissue-destructive diseases

Similarity to human periodontal disease

Colonization in conjunction with other bacteria: Fusobacterium, Porphyromonas and Streptococcus

Alterations in microenvironment allowing colonization with Treponemes

Treponemes have “virulence factors” that allow for deep penetration of epithelial and basal dermal layers
What Do We Know About Digital Dermatitis?

- Emerged as a significant disease of dairy cattle in the US in the mid-1990s; other countries shortly thereafter
What Do We Know About Digital Dermatitis?

- 2007 NAHMS Survey
  - Present in 70% of dairies nationwide
  - Responsible for 50% of lameness cases within dairies
DD is polymicrobial Disease

- Multiple bacterial morphologies observed in lesion tissue
- Genomic sequencing of lesion material:
  - Multiple treponeme species
    - *T. phagedenis*
    - *T. denticola*
    - *T. vincentii*
    - *T. pedis* and others
  - Recent study (not published yet) Treponeme population changed over time
    - *Bacteriodies, Fusobacterium, Clostridium, Prophyormonas, Prevotella*
- Multiple bacterial species cultured
  - Aerobic and anaerobic
Bovine Hoof Lesion Stage

DD lesions

- Papillomatous digital dermatitis, Hairy heel warts, strawberry warts, Mortellaro disease
- Circular to oval distinct region
- Foul smell
- Hairless, mature lesions can have keratin-like protrusions
- Moist, prone to bleeding when probed
- Extremely painful granular tissue
Treponemes are present.

Are they primary pathogens or secondary invaders?
Summer 2013 Effort
Summer 2013 Effort

- Hoof biopsies for bacteriology
  - University of Liverpool
  - USDA/ARS/NADC
  - Bank at WDFW lab

- Hooves
  - CSU for histology & special stains
Summer 2013 Effort (cont’d)

- Lung, liver, kidney, spleen, peripheral LN
  - Histology at WSU

- Serum
  - USDA/ARS/NADC for serology
  - Bank at WDFW lab

- Liver and Kidney
  - Bank at WDFW lab
August 2013
August 2013
August 2013
## Disease Status and Spirochete Detection

**August 2013**

<table>
<thead>
<tr>
<th>ELK ID EL13-</th>
<th>County</th>
<th>Population Status</th>
<th>Sample</th>
<th>Spirochetes in Culture</th>
<th>Spirochetes on Histology</th>
<th>Sequencing UoL</th>
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<td>018</td>
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<td>CB or IDS</td>
<td>neg</td>
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<tr>
<td>021</td>
<td>Lewis</td>
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<td>IDS</td>
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<tr>
<td>022</td>
<td>Lewis</td>
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<td>gross</td>
<td>pos</td>
<td>neg</td>
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<td></td>
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<td><em>T. medium</em> (2 isolates)</td>
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<td>contra</td>
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<td><em>T. phagedenis subsp. vaccae</em> (2 isolates)</td>
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<td><em>T. medium</em></td>
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<td><em>T. phagedenis subsp. vaccae</em></td>
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<td><em>T. medium</em></td>
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</tr>
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</table>
Isolation of Digital Dermatitis Treponemes from Hoof Lesions in Wild North American Elk (Cervus elaphus) in Washington State, USA


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

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 phylgroup (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). 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 sloped horn. The disease...
Summary Through October 2013

• **Adult animals**
  – Severe chronic hoof lesions; unable to determine primary lesion(s)

• **9-10 month old calves**
  – Severe chronic lesions; unable to determine primary lesion(s)
  – Treponemes detected in diseased hooves

• **3 month old calves**
  – Suggestion of early lesions grossly; unable to confirm histologically
  – Treponemes isolated from gross lesions
January 2014 Effort
3 Month
August
• Good nutrition
• Copper low
• Selenium low
• Dryer environment

8 Month
January
• Poorer nutrition
• Minerals low
• Wet environment
• Animals concentrate

9 Month
Feb-Mar
• Spiral bacteria proliferate
• Permanent lesions
• Secondary infections
• Poor nutrition / emaciation

1 -7 Year
Adults
March
• Water environment
• Minerals low
• Secondary infections
• Laminitis, overgrowth
• Debilitation / emaciation
• More time elapsed

64
64
Treponeme species specific primers

- *T. denticola*
- *T. amylovorum*
- *T. maltophilum* #28
- *T. medium*
- *T. socranskii*
- *T. pectinovororum*
- *T. vincentii* #36
- *T. lecithinolyticum* #26, 28, 29, 31
- Group 2 (Evans) *T. phagedenis* # 26, 28, 29, 34, 36, 38, 42, 45

Other isolates:

- Aerobic and anaerobic cultures
- Many similar to isolates from DD of cattle
DD lesions

- Papillomatous digital dermatitis, Hairy heel warts, strawberry warts, Mortellaro disease
- Circular to oval distinct region
- Foul smell
- Hairless, mature lesions can have keratin-like protrusions
- Moist, prone to bleeding when probed
- Extremely painful granular tissue
Identification of Spirochetes Associated with Contagious Ovine Digital Dermatitis*

G. Sayers1, P. X. Marques1, N. J. Evans3, L. O’Grady1, M. L. Doherty1, S. D. Carter3 and J. E. Nally1,2,*

Author Affiliations

ABSTRACT

Spirochetes of dermatitis in Treponema n remaining cul medium/Treponema denticola/Treponema denticola/Treponema denticola

Bovine digital dermatitis and severe virulent ovine foot rot: a common spirochaetal pathogenesis.

Dhawi A, Hart CA, Demirkan I, Davies IH, Carter SD

Abstract

A potential pathological role for spirochaetes in bovine digital dermatitis (bovine DD) and severe virulent ovine foot rot (SVOFR) has been considered and a treponeme isolate obtained from each disease in the UK. In this work, we have investigated the hypothesis that the two diseases may have a shared (common) spirochaetal aetiology. Experiments were designed to identify serological similarities and differences between the two spirochaetes; an enzyme-linked immunosorbent assay (ELISA) was developed to detect anti-treponeme antibodies in the sera of cows and sheep against the two-treponeme isolates. Sera were further tested for antigen reactivity by Western blotting. Cattle and sheep with bovine DD and SVOFR, respectively, had increased seropositivity rates to both treponeme isolates, with different patterns of reactivity between farms. In some cattle herds, significant correlations were shown between antibodies to bovine DD treponemes and SVOFR treponemes (P < 0.001). In other herds, there was no apparent cross reaction, suggesting the presence of more than one treponeme in bovine DD on some farms. There was no significant correlation between the two treponeme isolates when ELISA-tested against 58 sheep sera from SVOFR cases (P > 0.05); sheep showed strong evidence of reactivity to one or the other treponeme antigens, but never to both. Western blotting against both treponeme antigens showed that they frequently displayed different antigen epitopes, although some minor bands were common to both organisms. The data suggest that there are a number of spirochaetes in UK farms, which could be involved in the pathogenesis of either bovine DD or SVOFR.
Contagious Ovine Digital Dermatitis

Same bacterial community profile as bovine digital dermatitis: multiple **treponeme** species, multiple *Fusobacterium* species, *Peptostreptococcus*, *Streptococcus*, *Staphylococcus*, *Macrococcus* (Firmicutes) and *Dichelobacter*

Calvo-Bado L A et al. the ISME Journal (2011) 5, 1426-1437

Duncan J S et al. Veterinary Record 2011;169:606
CODD vs. Elk Hoof Disease

Photo by Cliff Wheeler
CODD vs. TAHD

CODD vs. TAHD

CODD vs. Elk Hoof Disease
CODD of Domestic Sheep
Treponeme Associated Hoof Disease - TAHD

- presence of spirochetes in hoof lesions
- similarity in *microbial* profile to known hoof disease - DD
- similarity in *histological* morphological changes as DD
- Lack of any evidence to support any other cause (infectious or non-infectious)
Treponeme-associated hoof disease in elk
Beef Cattle but applies to other animals
COMPETING THEORIES
Competing Theory #1: Herbicides
Competing Theory #1: Herbicides

“Herbicides have no known mode of action in mammals. They’re practically nontoxic to mammals according to most of the studies that have been done. We haven’t had any observations of direct effect that we’ve been aware of on wildlife and most of these herbicides have been around for several decades.”

Dr. Anne Fairbrother DVM, PhD. Former Ecotoxicologist with the U.S. Environmental Protection Agency; Current Principle Scientist with Exponent Engineering and Scientific Consulting. June 3, 2014
Competing Theory #2: Leptospirosis

There is no evidence of an association between leptospirosis and hoof disease in any of the elk that have been examined.
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Competing Theory #2: Leptospirosis

“Leptospirosis is endemic in wild and domestic animals throughout the world, including Washington. In endemic hosts, such as deer, elk, cattle, rodents, raccoons, and other wildlife, the disease is limited to the kidney, urinary and reproductive tracts. Leptospirosis does not affect the muscles, joints, or feet; and lameness associated with leptospirosis has not been reported anywhere in the world in any wild or domestic ungulate.”

Dr. Jennifer Wilson-Welder, Research Microbiologist and leptospirosis expert. USDA National Animal Disease Center
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.
Persistence of DD Organisms in Environment

-Found DD Treponemes in manure slurry

USDALab: viable when stored refrigerated for prolonged periods
Characterization of Novel Bovine Gastrointestinal Tract *Treponema* Isolates and Comparison with Bovine Digital Dermatitis Treponemes

Nicholas J. Evans, Jennifer M. Brown, Richard D. Murray, Brian Getty, Richard J. Birtles, C. Anthony Hart, and Stuart D. Carter

School of Veterinary Science, University of Liverpool, Liverpool L69 3BX, United Kingdom, and Department of Medical Microbiology, University of Liverpool, Liverpool L69 3GA, United Kingdom

Received 23 April 2010/Accepted 22 October 2010

This study aimed to isolate and characterize treponemes present in the bovine gastrointestinal (GI) tract and compare them with bovine digital dermatitis (BDD) treponemes. Seven spirochete isolates were obtained from the bovine GI tract, which, on the basis of 16S rRNA gene comparisons, clustered within the genus *Treponema* as four novel phylotypes. One phylotype was isolated from several different GI tract regions, including the omasum, colon, rumen, and rectum. These four phylotypes could be divided into two phylotype pairs that clustered closest with each other and then with different, previously reported rumen treponemes. The treponemes displayed great genotypic and phenotypic diversity between phylotypes and differed considerably from named treponeme species and those recently reported by metagenomic studies of the bovine GI tract. Phylogenetic inference, based on comparisons of 16S rRNA sequences from only bovine treponemes, suggested a marked divergence between two important groups. The dendrogram formed two major clusters, with one cluster containing GI tract treponemes and the other containing BDD treponemes. This division among the bovine treponemes is likely the result of adaptation to different niches. To further differentiate the bovine GI and BDD strains, we designed a degenerate PCR for a gene encoding a putative virulence factor, *dyC*, which gave a positive reaction only for treponemes from the BDD cluster.
Host and environmental reservoirs of infection for bovine digital dermatitis treponemes.


Abstract
Bovine digital dermatitis (BDD) is a global infectious disease causing lameness of cattle and is responsible for substantial animal welfare issues and economic losses. The causative agents are considered to be spirochetal bacteria belonging to the genus Treponema, which have consistently been identified in BDD lesions worldwide. One potential means of controlling infection is the disruption of transmission; however, the infection reservoirs and transmission routes of BDD treponemes have yet to be elucidated. To address these issues, we surveyed for evidence of BDD treponeme presence in the dairy farm environment, in bovine tissues and in bovine gastrointestinal (GI) tract contents. A total of 368 samples were tested using PCR assays specific for each of three currently recognised, isolated phylotypes of BDD treponemes. All environmental samples, together with insects and GI tract content samples were negative for BDD treponeme DNA from the three phylotypes. However, we identified BDD treponemes in two non-pedal bovine regions: the oral cavity (14.3% of cattle tested) and the rectum (14.8% of cattle tested). Whilst only single phylotypes were detected in the oral cavity, two of the rectal tissues yielded DNA from more than one phylotype, with one sample yielding all three BDD treponeme phylotypes. Whilst it might be considered that direct skin to skin contact may be a major transmission route of BDD treponemes, further studies are required to characterise and determine the potential contribution of oral and rectal carriage to BDD transmission.

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Treponemes detected in digital dermatitis lesions in Brazilian dairy cattle and possible host reservoirs of infection.

Nascimento LV, Mauerwerk MT, Dos Santos CL, Filho IB, Birgel Júnior EH, Sotomaior CS, Madeira HM, Ollhoff RD.

Abstract
Main pathogenic treponemes of bovine digital dermatitis were identified by PCR from 17 infected herds in southern Brazil for the first time. Treponeme phylogroup composition could not be related to clinical classification. Treponema phagedenis was present in all lesions. Rumen fluid could be implicated as reservoir location.

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Altered Microbiomes in Bovine Digital Dermatitis Lesions, and the Gut as a Pathogen Reservoir

Martín Zinicola¹, Fabio Lima¹, Svetlana Lima¹, Vinicius Machado¹, Marília Gomez¹, Dörte Döpfer², Charles Guard¹, Rodrigo Bicalho¹*

¹ Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, New York, United States of America, ² Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, United States of America

* rob28@cornell.edu

Abstract

Bovine digital dermatitis (DD) is the most important infectious disease associated with lameness in cattle worldwide. Since the disease was first described in 1974, a series of Treponema species concurrent with other microbes have been identified in DD lesions, suggesting a polymicrobial etiology. However, the pathogenesis of DD and the source of the causative microbes remain unclear. Here we characterized the microbiomes of healthy skin and skin lesions in dairy cows affected with different stages of DD and investigated the gut microbiome as a potential reservoir for microbes associated with this disease. Discriminant analysis revealed that the microbiomes of healthy skin, active DD lesions (ulcerative and chronic ulcerative) and inactive DD lesions (healing and chronic proliferative) are completely distinct. Treponema denticola, Treponema maltophilum, Treponema medium, Treponema putidum, Treponema phagedenis and Treponema paraluisicuniculi were all found to be present in greater relative abundance in active DD lesions when compared with healthy skin and inactive DD lesions, and these same Treponema species were nearly ubiquitously present in rumen and fecal microbiomes. The relative abundance of Candidatus Amoebophilus asiaticus, a bacterium not previously reported in DD lesions, was increased in both active and inactive lesions when compared with healthy skin. In conclusion, our data support the concept that DD is a polymicrobial disease, with active DD lesions having a markedly distinct microbiome dominated by T. denticola, T. maltophilum, T. medium, T. putidum, T. phagedenis and T. paraluisicuniculi. Furthermore, these Treponema species are nearly ubiquitously found in rumen and fecal microbiomes, suggesting that the gut is an important reservoir of microbes involved in DD pathogenesis. Additionally, the bacterium Candidatus Amoebophilus asiaticus was highly abundant in active and inactive DD lesions.
Trouble with DD:

• 20 years of study, no solid answers!!

• No infection model
  – Some success with macerated lesion material
    • 60% rate, LONG incubation, looks different

Aspects of the sheep model seen in elk

TAHD:

- Heel pitting
- Overgrowth of hoof horn/wall
- Lesions at coronary band and in interdigital space (between claws)
- Sole ulceration and necrosis of P3
Project plan: Spring 2017

• Use elk TAHD lesion material in USDA sheep DD model
  – Allow study of the disease in controlled setting.
  – Learn about infectious nature of the disease.
  – Learn about the pathogenic bacteria associated with all DD and TAHD.

scarify and wet wrap feet -3 days, inoculate with lesions from TAHD elk

observe at 2, 4 & 6 weeks for lesion development
Information Needs Identified by WDFW Technical Advisory Group

- Is the disease being maintained in the elk population?

- What are the influences and impacts of TAHD on elk movements/habitat use?

- Do elk develop immunity to the disease?

- Are there effects on survival & reproduction?
Information Needs Identified by WDFW Technical Advisory Group (cont’d)

• What is the progression of disease over time (individual & herd)?

• How is TAHD transmitted?

• Are TAHD pathogens present in the environment (fecal & soil sampling)?
Immunological Studies

Dr. Jennifer Wilson-Welder
USDA-ARS-NADC
Infectious Bacterial Diseases Research
Elk Serum Antibody Titer to Mixed Treponeme Antigen

Endpoint dilution titer, plate bound antigen of whole cell sonicates from 4 Treponeme species: T. phagedenis, T. medium, T. vincentii and T. denticola
Elk Lymphocyte isolation and stimulation

Antigens: Whole cell sonicates and outer membrane extracts from several Treponema isolates and other bacteria isolated from digital dermatitis

Heparinized blood overnighted from WA

Diluted blood sample → HISTOPAQUE™-1077 → Centrifugation (400 x g, 20 min, RT) → Plasma including platelets, Mononuclear cells (mostly Lymphocytes), Granulocytes Erythrocytes

Cells washed by centrifugation, counted, labeled with proliferation dye and cultured with antigen

5 day simulation

Surface label for cell phenotype
Difference between Yakima (Non-Endemic) and MSH (Endemic)?
Within MSH (Endemic) Population is there a difference between elk with and without disease?
MSH (Endemic) proliferating cells by phenotype
Conclusions

• Differences in immune response of endemic and non-endemic disease populations to bacterial antigens from DD
  – Serum antibody
  – Cellular
  – Not between disease +/- in endemic area
    • Maternal antibody?
    • Exposure?
Management Challenge

- Once any infectious disease enters a wild population it is extremely difficult to eliminate

- The challenge becomes: How do we manage the disease?
DD Treatment and Control Options:

- **NO VACCINE**
  - Early attempts with Bacteroides and Treponema non- efficacious

- **Footbaths:** copper sulfate, zinc sulfate, formalin, antibiotics
  - daily use, 2-3x week
  - Not in winter in upper-midwest
  - Environmental and human toxicity issue

- **Trimming and wrapping:** topical antibiotics, copper sulfate

- **Topical application of lincomycin (antibiotic) wraps alone required retreatment and were more likely to become active.**
  - Berry et al., The Veterinary Journal, 193 (2012) 654-658
WDFW Prioritized Efforts

- 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
- Remove elk severely affected with hoof disease
  - Euthanasia Protocol
WDFW Prioritized Efforts

- 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
- Remove elk severely affected with hoof disease
  - Euthanasia Protocol
Elk Hoof Disease - Protocol Survey Form - 2015

Survey Point: 172
Latitude: 46.4420852824  Longitude: -123.44076764

WDFW Region 6
PACIFIC County

Pg 1 of ___ pages

In the first section below, write about the entire survey effort (including coordinates at starting location). In the second section below, record each observation of elk. Use an extra, blank form if more than 8 observations. After the survey, enter your data on a web-based form at: http://bit.ly/1G3BuWe

Survey Effort

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Survey Time (Military: HH/MM) Start _____ End _____
Odometer Reading (At Survey Pt) Start _____ End _____

Start Location Waypoint ID _____ Latitude: ___________ Longitude (-) ___________

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107
Using Citizen Scientists to Aid in Estimating the Prevalence and Distribution of Treponeme-associated Hoof Disease in Southwest Washington

December 1, 2015

B. George, I. Keren, T. Christopher
Washington Department of Fish and Wildlife
Wildlife Health

Treponeme associated hoof disease in Washington elk

Observations of elk with deformed, broken, or missing hooves have increased dramatically in southwest Washington over the past decade. Tests conducted by scientists in the U.S. and abroad show these abnormalities are strongly associated with treponeme bacteria, known to cause digital dermatitis in cattle, sheep and goats.

Digital dermatitis has plagued the livestock industry for decades, but the disease has never before been documented in elk or other wildlife. The Washington Department of Fish and Wildlife (WDFW) is working with scientists, veterinarians, outdoor organizations and others to develop management strategies for elk herds affected by the disease.

Several aspects of the disease in elk are clear:

- Treponeme associated hoof disease appears to be highly infectious among elk, but there is no evidence that it affects humans.
- Tests show the disease is limited to animals’ hooves, and does not affect their meat or organs.
- Currently, there is no vaccine for the disease, and there are no proven options for treating it in the field.

Scientists believe that treponeme bacteria likely persist in moist soil and spread to new areas on the hooves of infected elk. To help minimize the spread of the disease, WDFW requires hunters to remove the hooves of any elk taken in affected areas and leave them on site. During the 2015-16 hunting season, this rule applied to Game Management Units 501-504 and 642-699 in southwest Washington.

In 2013, WDFW created two advisory groups to help address scientific issues and societal concerns raised by the disease. The Hoof Disease Technical Advisory Group helped to guide the diagnostic effort and members continue to consult with the department on evolving research needs. The Hoof Disease Public Working Group consults with WDFW on management issues and helps to publicize information about the disease.

Both groups collaborate with the department on field studies to assess the distribution, prevalence, and other dynamics of the disease.

Sightings of limping elk increase

In the late 1990s, WDFW began receiving sporadic reports of limping elk and elk with hoof deformities in the Cowlitz River Basin. Since 2008, sightings have increased rapidly and spread to 10 counties in southwest Washington, affecting both the Mount St. Helens and Willapa Hills elk herds.

The disease is now suspected in Clark, Cowlitz, Grays Harbor, Lewis, Mason,
Oregon elk have similar disease:

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4485 and 5266 - only FUSO
4094 - unknown TREP
4714A - unknown TREP
4772 - unknown TREP
5035 - unknown TREP
Elk Permit Holder:

Congratulations on being selected for an elk permit hunt in Game Management Unit XXX (XXXXXXX). As part of WDFW’s ongoing efforts to better understand the prevalence of hoof disease in southwest Washington, WDFW biologists are collecting hooves from harvested elk in this area. You are being asked to collect the hooves from any elk you harvest and submit them for examination. Additionally, WDFW would like to explore the possibility that hunter evaluations of hoof condition could be a means to learn more about the disease. Therefore, in addition to submitting the hooves, you are being asked to evaluate the condition of your elk’s hooves. Your assistance is voluntary and very important to the success of these efforts.

**Hoof Evaluation:** Please fill out the data form (on reverse) for all four of your elk’s hooves. Use the diagrams on the reverse of this sheet to determine which grade most closely represents your elk’s hooves. Evaluate all four of the hooves (even if they appear healthy) and write your selected score for each hoof on the sheet. Note that the hooves on the sheet are labeled according to the position of the hoof on the elk (i.e. Left Front, Right Rear).

**Hoof Removal:** Cut the hooves off of the elk approximately 1” above the lower joint (the ankle). This will leave about a 1” section from the tip of the hoof to the cut. Place each hoof in the gallon-sized plastic bag corresponding to the label on the data sheet represented by that hoof. Next, place all four hooves and the data sheet in the larger bag.

**Hoof Submission:** Samples can be deposited at identified drop off sites listed on the reverse. Sites will consist of a barrel or garbage can with a WDFW logo. Barrels will be available 24/7 during the dates listed for each site.

**Locality:** As you may be aware, the request to collect elk hooves and move them away from the kill site is in conflict with the regulations (WAC 232-21-220) requiring that hooves be left on site. WDFW has implemented an exception for this rule for the purposes of disease collection. You will not be in violation of any WDFW regulations if you remove the hooves and submit them as requested in this letter. WDFW will appropriately dispose of the hooves following the study.

**Information on Trapping Associated Hoof Disease:** For additional information on hoof diseases in southwest Washington and to learn about additional work that WDFW is conducting to address this challenging situation, please see page 66 of the 2015 Big Game Hunting Seasons and Regulations pamphlet and/or visit the WDFW website at: [http://wdfw.wa.gov/conservation/health/hoof_disease](http://wdfw.wa.gov/conservation/health/hoof_disease)

Your assistance is greatly appreciated on behalf of Washington State’s elk resource. Please call if you have further questions.

Thanks in advance for your cooperation and good luck in the 2015 elk hunt.

Wildlife Biologist
360-496-6211

Enclosure: Small Plastic bags for hooves
Large Plastic bag for all hooves and data sheet

Reverse: Elk hoof diagrams
Hoof scoring sheet
Collection locations:

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Elk Hoof Disease Hoof Scoring Data Form

<table>
<thead>
<tr>
<th>Normal Hoof</th>
<th>Grade 3</th>
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<tbody>
<tr>
<td>Corneal hard</td>
<td>Abnormal or a small soft callus in the coronal hard. May be pink or black, may not be overgrown.</td>
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<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Grade 4</th>
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<tbody>
<tr>
<td>Broken or infected skin along the coronary band or between the horn sheaths. Lesions must involve the coronal horn cap and may not be overgrown.</td>
<td></td>
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<tr>
<td>Mining one or both horn capsules, remaining horn capsule may not be overgrown.</td>
<td></td>
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**Left Front Hoof**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Normal</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Rear Hoof</td>
<td>Grade</td>
<td>Normal</td>
<td>1</td>
<td>2</td>
<td>3</td>
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</tbody>
</table>

**Collection Locations and Dates of Operation:**

- WDFW Region 1: 1101 Edmondson Dr., Vancouver - November 7-12
- WDFW Region 2: 1585 N. Eighth St., Raymond - November 7-12
- WDFW Region 3: 1171 10th Ave., Chinook - November 7-12
- WDFW Region 4: 1401 S. 10th St., Enumclaw - November 7-12
- WDFW Region 5: 1165 10th Ave., Port Angeles - November 7-12
- WDFW Region 6: 1201 10th Ave., Lynden - November 7-12
- WDFW Region 7: 1165 10th Ave., Sedro-Woolley - November 7-12
- WDFW Region 8: 1165 10th Ave., Burlington - November 7-12
- WDFW Region 9: 1165 10th Ave., Kalama - November 7-12

Please circle the ONE grade that most closely represents each of your elk’s hooves in the list below:
Normal hoof

Grade 1  Broken or infected-appearing skin along the coronary band or between the toes/claws/digits. Lesions are limited to the skin with no hoof capsule involvement. Hoof capsule(s) may or may not be overgrown, but are not infected or broken.

Grade 2  Skin wound is beginning to work its way underneath the hoof capsule. Hoof capsule(s) may or may not be overgrown.

Grade 3  A large cavity or hole in one or both hoof capsules, usually on the bottom of the hoof. May include Grade 2 lesion. Hoof capsule(s) may or may not be overgrown.

Grade 4  Missing one or both hoof capsules. Remaining claw may include Grade 2 or Grade 3 lesions and remaining hoof capsule is usually overgrown.

Grade 5  Missing one or both hoof capsules, but completely healed with no evidence of active disease in either hoof capsule (i.e. dry, with no blood or infection visible).
Aerial TAHD Prevalence Surveys
Spring 2017
WDFW Prioritized Efforts

- 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
- Remove elk severely affected with hoof disease
  - Euthanasia Protocol
Survival Study Objectives

1. Estimate the effects of TAHD on survival of adult (≥ 2 years old) female elk

2. Determine cause-specific mortality for adult female elk with and without TAHD

3. Estimate the effects of TAHD on the pregnancy rates of adult female elk
Survival Study Objectives (cont’d)

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

6. Compare findings to McCorquodale et. al 2014
WDFW Prioritized Efforts

- 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

- Remove elk severely affected with hoof disease
  - Euthanasia Protocol
Operating Procedures for Euthanizing 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.

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 bare weight on the hooves
- The animal is severely emaciated with hip bones and most ribs showing
- The animal is no longer able to feed

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?
Acknowledgements

• Washington Dept. of Fish and Wildlife
  – Stefanie Bergh
  – Brooke George
  – Eric Holman
  – Dr. Sandra Jonker
  – Dyanna Lambourn
  – Pat Miller
  – Tom Owens
  – Ella Rowan
• University of Liverpool
  – Roger Blowey
  – Stuart Carter
  – Dr. Simon Clegg
  – Dr. Nicholas Evans
  – Kerry Newbrook
  – Leigh Sullivan
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  – Dr. Jennifer Wilson-Welder
• Oregon Dept. of Fish and Wildlife
  – Dr. Julia Burco
• Washington State University
  – Dr. George Barrington
  – Dr. Tom Besser
  – Dr. John Gay
  – Dr. Gary Haldorson
  – Dr. Dale Moore
  – Dr. Steve Parish
• Washington State Dept. of Agriculture
  – Dr. Thomas Gilliom
  – Dr. Paul Kohrs
• Washington State Dept. of Health
  – Dr. Ron Wohrle
• Private Practice
  – Dr. Anne Fairbrother
  – Dr. Jason Humphrey
  – Dr. Mike Paros
Questions?

Photo by Nicholle Stephens