DOI: 10.7589/2013-07-163 *Journal of Wildlife Diseases*, 50(2), 2014, pp. 259–270

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.

INTRODUCTION

Hoof disease in wild and captive cervids has been described worldwide, and etiologies include infectious diseases such as epizootic hemorrhagic disease and infectious pododermatitis (Sleeman et al. 2009; Handeland et al. 2010), noninfectious diseases such as mineral deficiencies and endophyte toxicity (Flynn et al. 1977; Handeland and Vikren 2005), and chronic laminitis (Gray et al. 2001; Clauss et al. 2009), which is generally related to nutritional imbalance. In many cases, hoof disease in wild cervids may be multifactorial and influenced by diet, behavior, habitat use, range quality, and population genetics (Lavin et al. 2004).

Sporadic reports of free-ranging deer, elk, and moose with hoof or leg abnormalities and lameness are received yearly throughout Washington. The majority of reports are isolated cases for which a variety of causes are diagnosed (Washington Department of Fish and Wildlife [WDFW] unpubl. data). During the winter of 2008, reports of free-ranging Roosevelt elk (*Cervus elaphus roosevelti*) with abnormal hooves and lameness increased significantly in southwestern (SW) Washington, and reports have continued to increase in geographic distribution since that time (WDFW unpubl. data). We undertook an investigation in March of 2009 to characterize hoof lesions, identify possible etiologies, and evaluate elk for underlying diseases. Five clinically lame elk with abnormal hooves were euthanized and examined. Affected elk were from herds within the Cowlitz River Basin region of SW Washington, an area from which the majority of hoof disease had been reported. Additionally, three clinically normal elk from a nonaffected herd outside of this study area were euthanized and examined. Here we provide clinical and pathologic findings and characterize chronic hoof

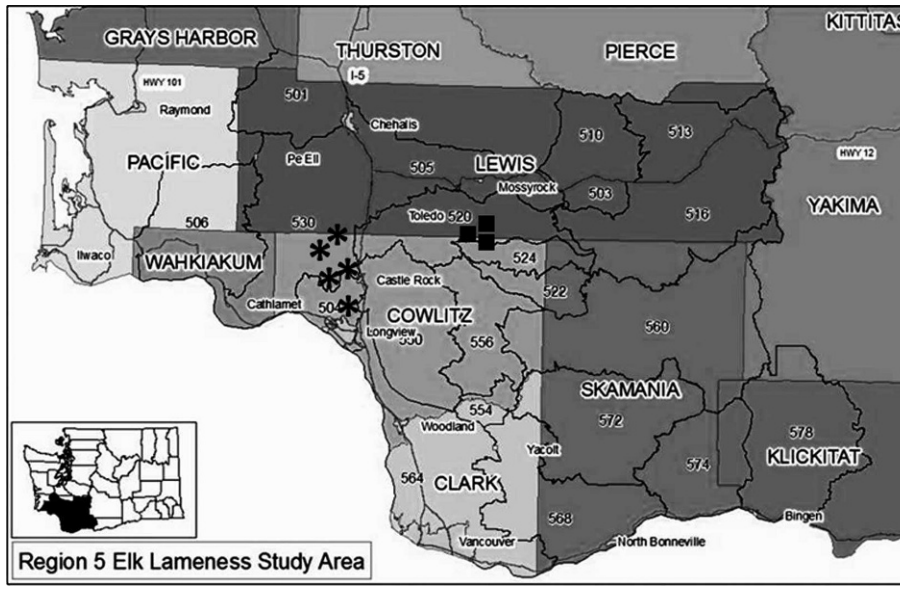

FIGURE 1. Cowlitz River Basin, Washington State, USA, study area with the euthanasia location of five Roosevelt elk (*Cervus elaphus roosevelti*) with hoof lesions noted by asterisks (*). Affected elk represented three herds with several affected cohorts in each. The capture locations of three grossly normal Roosevelt control elk are noted by squares (■). Control elk were from a nonaffected herd immediately east of the study area.

disease in a population of free-ranging Roosevelt elk of SW Washington.

MATERIALS AND METHODS

Study area

Elk were collected in March 2009 on private lands in SW Washington. The study area extended from Chehalis, Washington (46°39′43.92″N, 122°57′46.08″W) on the northern border to Longview, Washington (46°8′17.88″N, 122°56′12.84″W) on the southern border, including areas both west and east of Interstate Highway 5 (Fig. 1). The study site was comprised of a mixture of cultivated pasture, shared by domestic cattle (*Bos taurus*), sheep (*Ovis aries*), and horses (*Equus ferus caballus*), interspersed with large regions of native lowland maritime coniferous forest and managed timberlands composed of Douglas fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western redcedar (*Thuja plicata*) (Franklin and Dyrness 1973).

Necropsy

Five adult female elk with visible hoof abnormalities and lameness from within the study area (study elk) and three adult female elk with grossly normal hooves from outside of the study area (control elk) were killed via gunshot and immediately necropsied in the field. Field necropsies were performed on each elk (Table 1) and the following fresh specimens were collected sterilely and maintained chilled on wet ice until processing in the laboratory: lung, liver, kidney, spleen, mesenteric lymph node, ileum, coronary band, feces, serum, whole blood, and all four distal limbs removed at the level of the carpus or tarsus. Representative specimens of all viscera were collected for histopathology and preserved at least 24 hr in 10% neutral-buffered formalin prior to processing. In pregnant elk, the fetus was necropsied and representative tissues including distal limbs were collected, with fresh and formalin-preserved tissues handled similarly to those of the adults. An upper canine tooth was removed from each adult elk for aging via cementum annuli analysis (Matson's Lab LLC, Milltown, Montana, USA) (Hamlin et al. 2000). Fresh specimens were processed at either Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA), Washington Animal Disease and Diagnostic Laboratory (WADDL, Pullman, Washington, USA), or Colorado State University Diagnostic Medicine Center (CSUDMC, Fort Collins, Colorado, USA).

TABLE 1. Summary of gross lesions from affected study site Roosevelt elk (*Cervus elaphus roosevelti*) and normal control elk harvested from southwestern Washington. Measurements of hooves (cm) provided for each limb and significant visceral lesions.

Elk No.	Age (yr)	Status and origin[a]	Left fore		Right fore		Left hind		Right hind		Other gross lesions
			Medial (cm)	Lateral (cm)	Medial (cm)	Lateral (cm)	Medial (cm)	Lateral (cm)	Medial (cm)	Lateral (cm)	
1	3	NP/NL, study site	8.2[b]	10.5	9.9	7.8[b]	9.0	9.1	9.4	9.0	Pulmonary abscesses, lung worms, left front valgus deformity
2	4	NP/L, study site	10.6	10.4	10.5	10.3	5.5[c]	14.5	19.6[d]	15.5	Lung worms, serous atrophy of marrow
3	5	P/NL, study site	9.5	9.0	10.0	9.3	14.0[d]	15.0	8.8	8.4	Serous atrophy of marrow
4	7	P/L, control	9.3	9.3	9.4	9.5	9.5	9.3	9.4	9.6	None
5	2	P/NL, control	8.6	8.6	8.4	8.8	8.5	8.6	8.8	8.6	None
6	5	NP/NL, control	8.9	9.1	8.5	8.4	8.5	8.5	8.4	8.4	None
7	<1	NP/NL, study site	9.9	11.1	9.4	9.0	11.6[d]	9.2	6.4[b]	11.6[d]	None
8	2	NP/NL, study site	8.5[d]	8.7	10.2	9.5	9.2	8.5	8.1	8.0	None

[a] NP = nonpregnant; NL = nonlactating; L = lactating; P = pregnant.

[b] Sloughed hoof wall.

[c] Broken hoof.

[d] Sole ulcer.

Radiology and histopathology

Paired abnormal limbs from three study elk and paired normal limbs from two control elk were radiographed at Washington State University Veterinary Teaching Hospital. Digital images of anterior-posterior and lateral views were obtained and were interpreted by a board-certified veterinary radiologist.

Histopathology was performed at WADDL on all formalin-fixed tissues by routine methods (Lillie 1965). Each hoof was sagittally sectioned toe to heel on a commercial band saw to an approximate thickness of 5 mm and fixed in 10% neutral buffered formalin for 24–48 hr. Formalin-fixed sections were then decalcified in a solution of 5% formic acid and 37% formalin (Spencer and Bancroft 1980) for 24–48 hr. Sections were then dekeratinized using a paste solution of potassium thioglycolate, calcium hydroxide, and sodium hydroxide (Nair®, Nair Industries, Marietta, Georgia, USA). Sections were packed in paste and wrapped with plastic film until keratin was pliable (approximately 3 wk), routinely removing and replacing the paste every 4 days (Bancroft and Gamble 1980, with modifications). Once pliable, sections were paraffin embedded, sectioned 7–10 μm, and stained with hematoxylin and eosin for microscopic examination. Replicate sections of slides were Gram stained with Brown and Hopp's as described (Luna 1968) and silver stained for spirochete bacteria (Steiner's) and fungi (Grocott's methenamine silver) (Crookham and Dapson 1991). All prepared slides were examined by light microscopy by two board-certified anatomic pathologists who were blinded to the gross findings.

Laboratory testing

Virus isolation: For each elk, fresh lung, liver, spleen, kidney, mesenteric lymph node, ileum, and coronary band were pooled, and for each fetus, fresh liver, spleen, kidney, and hooves were pooled. Tissue pools were chilled on wet ice for approximately 48 hr before arrival in the laboratory. Once in the laboratory, specimens were chilled at 4 C and cultured for virus isolation (Wyoming State Veterinary Laboratory). Tissue cultures included bovine embryonic testicle (Schmidt and Lenneth 1964), white-tailed deer (*Odocoileus virginianus*) umbilical endothelial cells (Howerth and Stallknecht 1995), and commercially available bovine cardiopulmonary arterial endothelium (CPAE, No. CCL 209, ATCC, Rockville, Maryland, USA), using methods for each culture as previously described.

Serology: Whole blood was collected from the heart, aorta, or vena cava at necropsy. Serum was separated, stored at −20 C, and later thawed for analysis of antibody titers to

viruses known to be associated with vascular disease and hoof or coronary band lesions at WADDL. Specifically, antibodies to epizootic hemorrhagic disease viruses (EHDVs) were measured by agarose gel immunodiffusion assay (Pearson and Jochim 1979) and antibodies to bluetongue virus (BTV) were measured by a competitive enzyme-linked immunosorbent assay commercially available kit (Veterinary Medical Research and Development, Pullman, Washington, USA) performed per the manufacturer's instructions. A cutoff inhibition value of 50% was used to determine positive results. Bovine viral diarrhea virus (BVDV) type I was analyzed by virus neutralization (Hill 1977).

Parasitology: Feces were collected at necropsy and stored at 4 C until processed and examined for intestinal parasite ova using the Wisconsin sugar flotation method, lung worm larvae (*Dictyocaulus* sp.) using the Baermann technique (Baermann 1917), and liver fluke ova (*Fasciola* sp. or *Fascioloides* sp.) using sedimentation (Zajac and Conboy 2006). Sample preparation and light microscopy were overseen by a parasitologist at WADDL.

Bacteriology: Fresh sections of coronary band, hoof wall, heel bulb, and full-thickness skin from the interdigital space were pooled for individual elk, chilled at 4 C, and cultured by streaking on trypticase soy agar with 5% sheep blood agar plates, MacConkey's agar plates, and Columbia blood agar for aerobic culture and anaerobic blood agar plates for anaerobes. Hoof wall lesions from each elk were cultured similarly, but separately. Aerobic plates were incubated 48–72 hr under atmospheric conditions at 37 C, and Columbia blood agar was incubated similarly under 5% CO_2. Anaerobic plates were cultured under anaerobic conditions at 37 C. Anaerobic culture isolates were sequenced by PCR using 16s rDNA primers (Drancourt et al. 2000) and the species identified. All samples were processed at WADDL.

Hepatic trace mineral analysis: Five grams of fresh liver was collected from each elk, chilled at 4 C, and analyzed for copper, selenium, cobalt, zinc, manganese, and molybdenum levels by inductively coupled plasma mass spectrometry (Anderson 1996) at WADDL. Results were reported on a wet weight basis and compared to values reported for cattle (Kincaid 1999), California tule elk (*Cervus canadensis nannodes*; Johnson et al. 2007), and Rocky Mountain elk (*Cervus elaphus nelsoni*; Zaugg and Kinsel 1997).

Hoof keratin copper analysis: One normal and one abnormal hoof from each of three study elk and two normal hooves from each of two control elk were selected for analysis. Approximately 1 cm^3 of keratin from the dorsal hoof wall was collected 2 cm distal to the coronary band. Hoof wall samples were frozen at −20 C until analysis at CSUDMC. Analysis was performed by digestion of the hoof specimen and flame atomic absorption spectroscopy as previously described for analysis of hair, with some modifications (Helrich 1990). Briefly, fresh hoof wall without lamina was washed and approximately 1 g of hoof was dried, ashed at 600 C, and dissolved in 10 mL of 3.6 N HNO_3 for spectroscopy. Results were compared to values reported for free-ranging Alaskan moose (*Alces alces gigas*; Flynn et al. 1977) and domestic cattle (Hidiroglou and Williams 1986; Sugg et al. 1996).

RESULTS

Necropsy

Gross hoof lesions and ancillary visceral lesions are summarized in Table 1. Elk had a spectrum of gross hoof lesions, which occurred in medial or lateral hooves of affected limbs, and paired forelimbs and hindlimbs, but rarely involved both forelimbs and hindlimbs concurrently. Hoof lesions included overgrowth of the hoof wall and elongation of the toe (5/5 elk) that varied from moderate (11.1 cm; Fig. 2B) to severe (19.6 cm) with twisting deformation (Fig. 2C, D); complete sloughage of the hoof wall (3/5 elk; Fig. 2C, D) with secondary osteomyelitis and septic arthritis (2/5); hoof wall defects including ulcers, horizontal ridges, and vertical clefts (3/5 elk); full-thickness sole ulcers with laminar necrosis and osteomyelitis of P3 (4/5 elk; Fig. 3A–D); and heel bulb ulceration and hyperplasia (3/5 elk; Fig. 2E). Three control elk had no gross hoof lesions (Fig. 2A). Significant gross visceral lesions were detected only in study elk and included serous atrophy of bone marrow fat and generalized emaciation (2/5 elk); mild pulmonary nematodiasis (*Dictyocaulus* sp.; 2/5 elk); and small pulmonary abscesses (1/5 elk). Gross visceral lesions and emaciation were not detected in control elk.

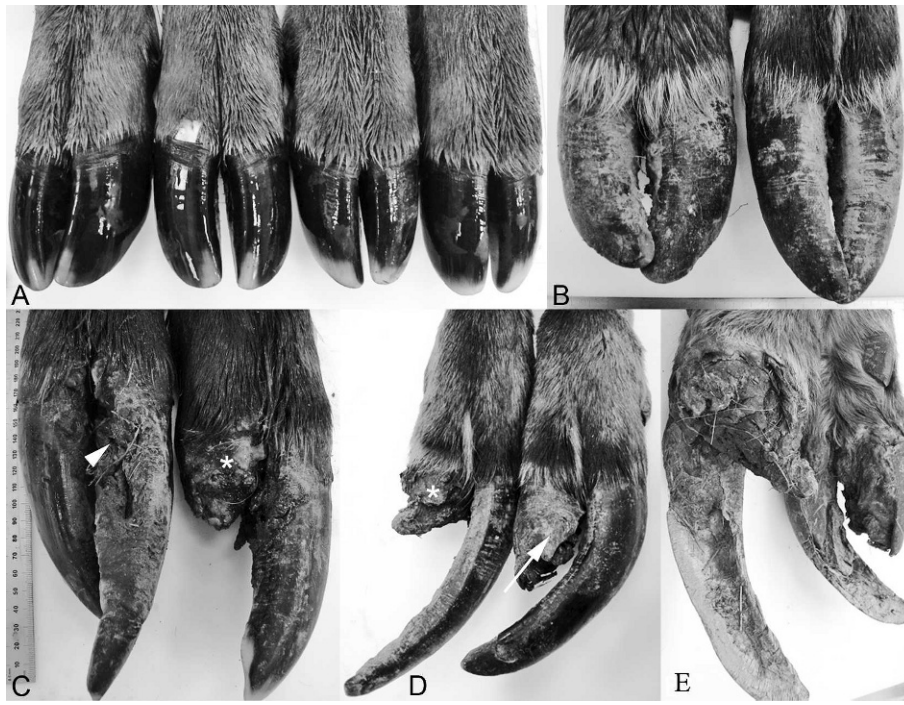

FIGURE 2. Hooves from clinically normal and clinically lame Roosevelt elk (*Cervus elaphus roosevelti*). (A) Hooves from clinically normal control elk 5. (B) Forelimbs from lame study elk 8, which had moderate overgrowth and scissoring of the hooves and clinical lameness. (C) Hindlimbs from lame study elk 7 with severe overgrowth and deformity of the hooves, sloughing of the hoof wall at the coronet band (asterisk), and dissecting laminitis from necrotizing sole ulceration (arrowhead). (D) Forelimbs from lame study elk 1 with severe overgrowth of hooves and sloughed (asterisk) and broken hooves (arrow). (E) Palmar surface of the forelimbs from lame study elk 1 showing hyperkeratosis and ulceration of the heel bulbs. Numbers on rule at left are at 10-mm intervals.

Radiology and histopathology

Hoof and fetlock radiographs of three study elk were performed, and findings included P3 osteomyelitis in sloughed, broken, and ulcerated hooves (2/3 elk; Fig. 4A) with septic arthritis of the distal interphalangeal joint (1/3 elk) and mild pedal osteitis of intact elongated hooves (2/3 elk; Fig. 4B, C). No bone abnormalities were noted in two radiographed control elk.

Detailed histologic examination was performed on all study and control elk, including full-sagittal sections of hooves; incisional biopsies of the coronary band, interdigital space, and heel bulb; and representative incisional biopsies of all viscera. Histologic findings in the limbs of the study elk were moderate to severe lymphoplasmacytic perivasculitis of the hoof lamina (Fig. 5B; 5/5 elk) and of the dermis of the heel bulb and coronary band (5/5 elk); suppurative, necrotizing laminitis with vasculitis and arteriolar thrombosis (Fig. 5C) in broken, sloughed, and ulcerated hooves (5/5 elk); mild to severe chronic hypertrophic arteriosclerosis with vacuolar degeneration of the tunica media and adventitial fibrosis (Fig. 5D; 5/5 elk); and normal parallel keratin tubules of the hoof wall and sole (5/5 elk). Histologic findings in the limbs of control elk included moderate laminar perivasculitis and arteriosclerosis (1/3 elk) and no histologic hoof lesions (Fig. 5A; 2/3 elk). Special stains (GMS, Gram, Steiner's) of sagittal sections of study elk hooves, coronary band, interdigital space, and heel

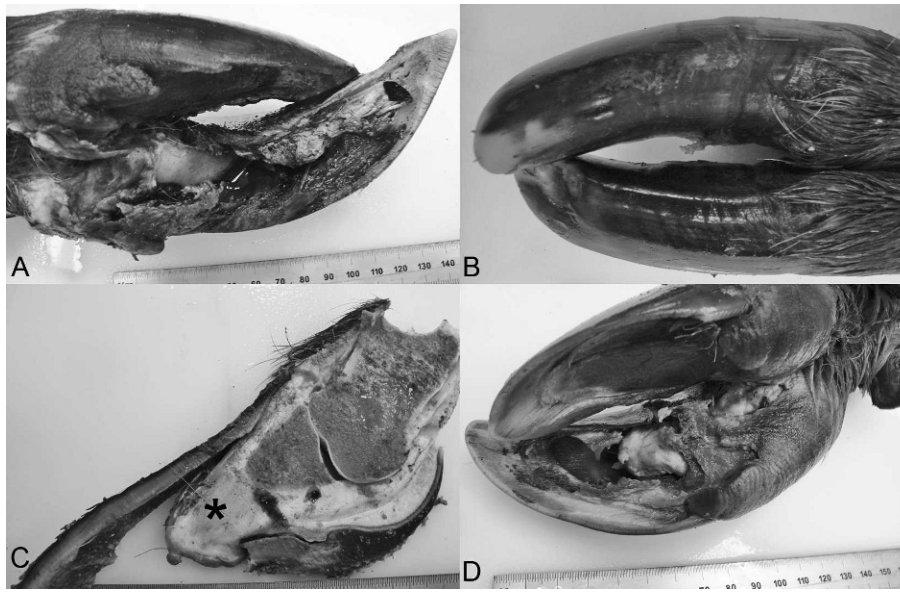

FIGURE 3. Gross photographs of sole ulcers from clinically lame Roosevelt elk (*Cervus elaphus roosevelti*) euthanized in the study area. (A) Plantar surface of the right hind lateral hoof from lame study elk 7 showing severe sole ulceration and marked overgrowth and scissoring of the hooves bilaterally. (B) Sagittal cross section of the right hind lateral hoof from lame study elk 7 (image A) showing severe sole ulceration and diffuse laminar necrosis with osteomyelitis of the third phalanx (P3) and granulation tissue (asterisk). (C) Dorsal surface of the left forelimb from lame study elk 8 showing moderate overgrowth of the hooves, but grossly normal hoof walls. (D) Palmar surface of the same left forelimb (image C) shows severe sole ulceration and laminar necrosis with extensive granulation tissue surrounding P3.

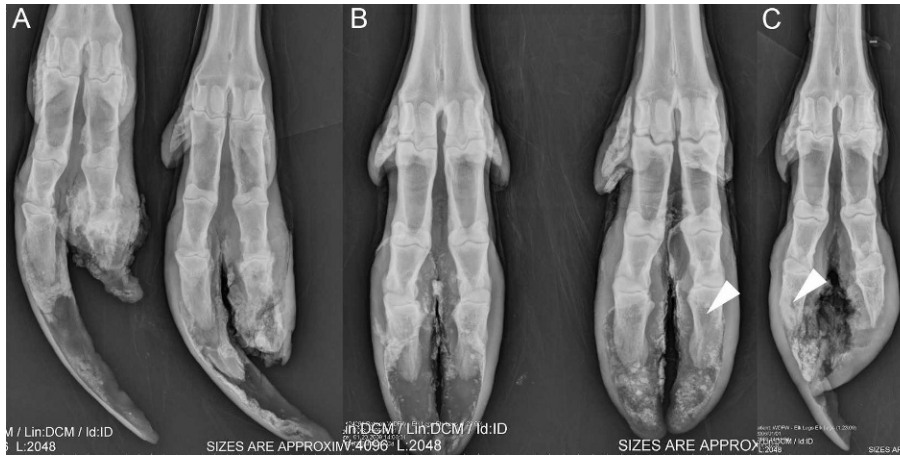

FIGURE 4. Anterior-posterior radiographs of three clinically lame Roosevelt elk (*Cervus elaphus roosevelti*) from the study site. (A) Forelimbs from elk 1 showing osteomyelitis and interphalangeal osteoarthritis associated with broken and sloughed hooves, but minimal bony changes on intact hooves despite severe overgrowth of the hoof wall. (B) Forelimbs from lame elk 8 showing minimal bony changes but osteomyelitis of the third phalanx (P3) of the left front medial toe (arrowhead) associated with sole ulceration. (C) Left hindlimb from elk 3 with markedly overgrown hooves and minimal bony lesions but osteomyelitis of P3 of the medial hoof (arrowhead) associated with a sole ulcer.

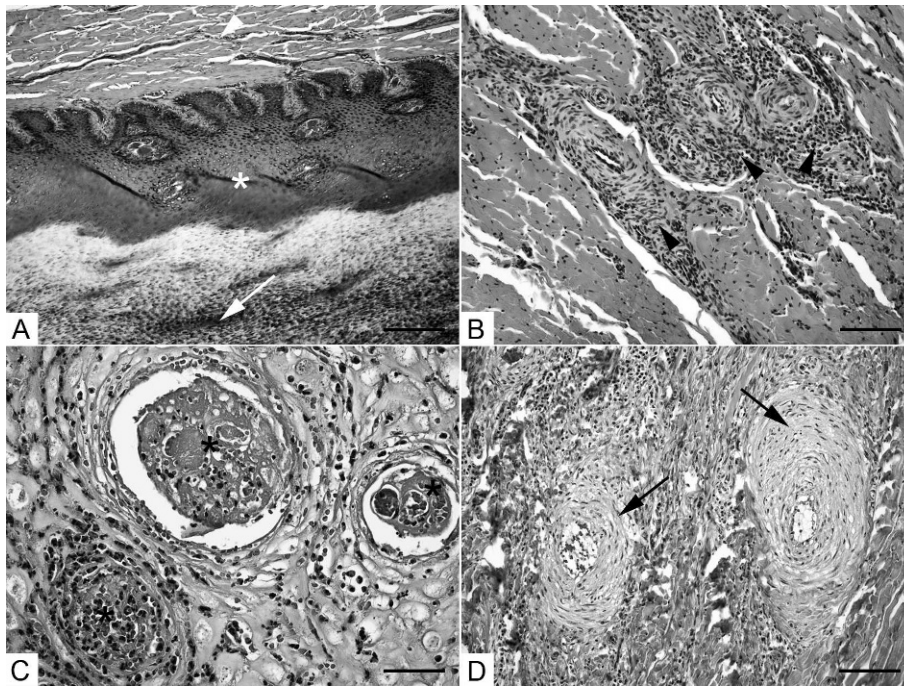

FIGURE 5. Photomicrographs of hooves from Roosevelt elk (*Cervus elaphus roosevelti*) that were clinically normal or lame. (A) Normal lamina (white arrowhead), laminar epithelium (white asterisk), and hoof wall (white arrow) from a clinically and grossly normal control elk 5. H&E stain. 40×. Bar=500 μm. (B) Marked lymphoplasmacytic laminar perivasculitis (black arrowheads) from lame study elk 1. Similar chronic lesions are noted in all five study elk and one clinically normal control elk. H&E stain. 100×. Bar=100 μm. (C) Suppurative laminar vasculitis and thrombosis (black asterisks) from lame study elk 2 consistent with acute necrotizing laminitis in association with a broken hoof. Similar lesions are noted in three of five study elk and no control elk. H&E stain. 200×. Bar=25 μm. (D) Chronic arteriosclerosis of laminar blood vessels (black arrows) from lame study elk 3. Note intimal hypertrophy, medial smooth muscle proliferation and vacuolar degeneration, and extensive fibrosis of the tunica adventitia. Similar lesions are noted in all five study elk and one control elk. H&E stain. 100×. Bar=100 μm.

bulb showed only a superficial mixture of gram-negative and -positive noninvasive bacteria, which was often not directly associated with inflammation.

Histologic examination of viscera showed several minor spontaneous lesions in study and control elk. Lesions included moderate hepatic portal and bridging fibrosis (3/8 elk); mild lymphocytic perivasculitis of the meninges, brain, heart, or gastrointestinal tract (4/8 elk); mild nonsuppurative myocardial degeneration (1/8 elk); mild glomerulonephritis (1/8 elk); mild lymphoplasmacytic renal interstitial nephritis (4/8 elk); chronic small abscesses in the lungs, tongue, or kidney (3/8 elk); and moderate numbers of skeletal muscle sarcocysts (*Sarcocystis* sp.) not associated with inflammation (8/8 elk).

Laboratory testing

Virus isolation from fresh tissues of all study and control elk and three fetuses was negative, and no elk had detectable antibody to EHDV, BTV, or BVDV type I. Parasitologic examination of fresh feces from four study elk and two control elk was performed. All elk had low numbers of some or all of the following parasites: *Dictyocaulus* sp., *Trichuris* sp., *Capillaria* sp., *Strongylus* sp., and coccidia. Bacterial culture of the interdigital space, coronary band, heel bulb, and hoof lesions from four study elk and two control elk yielded

mixed aerobic and anaerobic cultures consistent with overgrowth of contaminants. Anaerobic cultures grew colonies that were identified by 16s rDNA PCR and sequencing as opportunistic environmental pathogens *Helcococcus ovis* and *Anaerovorax* sp. (2/4 study elk). Hepatic trace mineral concentrations (Table 2) showed copper deficiency in study elk (4/5 elk, range 3.1–9.6 μg/g), and control elk (3/3 elk, range 2.5–6.0 μg/g), as well as selenium deficiency in study elk (5/5 elk, range 0.056–0.41 μg/g) and control elk (3/3 elk, range 0.11–0.12 μg/g). Hepatic cobalt was elevated in study elk (3/5 elk, range 0.091–0.12 μg/g) but normal in control elk (3/3 elk). No elevated or deficient levels of hepatic zinc, manganese, molybdenum, chromium, nickel, arsenic, cadmium, or lead were detected in any of the study or control elk (Table 2). Copper levels of hoof keratin were determined for three study elk and two control elk (Table 2). In study elk, both abnormal and normal hooves from each animal were examined and keratin copper levels ranged from 0.87 to 3.15 parts per million dry weight, similar to copper levels in normal hooves from control elk.

DISCUSSION

Hoof disease in herds of free-ranging Roosevelt elk has been a growing concern in SW Washington in the last 4 yr, with the number of affected elk and distribution of affected herds steadily increasing. After detailed gross and histologic evaluation of five affected elk from the Cowlitz River Basin study area, no underlying systemic or bone related disease was detected, indicating hoof disease is likely a primary lesion. Elk had moderately (10 cm) to severely (19.6 cm) elongated and deformed hooves with histologically normal horn. Accelerated hoof growth, resulting in abnormally long hooves, has been well described in wild and domestic ruminants and is associated with chronic hoof inflammation from various causes, including chronic laminitis (Boosman et al. 1991; Singh et al. 1992; Gray et al. 2001) and infection with contagious hoof disease bacteria (Handeland et al. 2010). Hoof elongation can be exacerbated by disuse from pain and lack of normal mobility and wear. Most study elk had severe sole ulcers at the heel-sole junction with laminar necrosis in at least one hoof. Sole ulcers occurred in severely elongated hooves as well as relatively normal-length hooves, which may indicate that ulceration is a primary lesion with secondary horn overgrowth accelerated by chronic inflammation and lack of weight bearing. Sole ulcers are common sequelae to chronic laminitis in domestic ruminants and are thought to be due to vascular compromise of the corium, leading to laxity in the suspensory apparatus of P3 and compressive damage of the digital pad and sole by P3. Concurrently, chronic inflammation results in production of weak hoof wall and sole keratin, which can culminate in ulceration of the sole at the heel-sole junction (Lischer and Ossent 2001; van Amstel and Shearer 2006). Three study elk had sloughed or broken hooves with scarring of the coronary bands and necrosis of the dermal corium. Sloughing of the hoof wall in ruminants is most commonly associated with severe laminitis, where prolonged vascular compromise leads to laminar necrosis and shearing and detachment of the dermal corium and hoof wall (Thoefner et al. 2005). This may have been exacerbated by mechanical torque caused by extraordinarily long hooves. Histologically, all study elk and one control elk had prominent laminar perivascular inflammation and hypertrophic arteriosclerosis with thrombosis, lesions classically described in chronic laminitis of domestic ruminants. These lesions indicate that hoof deformity is at least ultimately associated with chronic laminitis. Unfortunately, it is unclear whether laminitis is the primary cause of hoof disease or secondary to chronic hoof deformity and inflammation.

TABLE 2. Summary of trace mineral analysis of fresh liver and hooves from study site and control Roosevelt elk (*Cervus elaphus roosevelti*).[a]

Mineral	Elk No., origin								Reference values		
	1, study	2, study	3, study	4, control	5, control	6, control	7, study	8, study	Elk[b]	Bovine	Alaskan moose[c]
Copper (μg/g)[d]	9.6	20	7.9	3.8	6.0	2.5	3.1	8.7	20–120	20–120	N/A
Copper hoof (PPM DW)	N/D	Abn: 3.15; Nor: 1.49	N/D	Nor: 1.68; Nor: 2.06	Nor: 2.27; Nor: 0.87	N/D	Abn: 2.77; Nor: 2.66	Abn: 1.11; Nor: 2.27	N/A	4.03–4.5[e]	5.3–5.8
Selenium (μg/g)	0.41	0.31	0.05	0.11	0.12	0.12	0.19	0.12	0.45–0.75	0.25–1.4	N/A
Cobalt (μg/g)	0.092	0.083	0.05	0.065	0.062	0.064	0.12	0.091	N/A	0.03–0.08	N/A
Zinc (μg/g)	28	77	37	34	29	27	30	42	23–80	23–80	N/A
Manganese	4.2	2.9	3.9	4.3	3.8	3.3	4.1	3.7	2–6	2–6	N/A
Molybdenum (μg/g)	1.3	1.1	1.2	1.1	1.0	1.0	1.3	1.3	0.14–1.4	0.14–1.4	N/A

[a] PPM DW = parts per million dry weight; N/D = not done; Abn = abnormal hoof; Nor = normal hoof; N/A = not available.

[b] Elk normal parameters adapted from references established for California tule elk (*Cervus canadensis nannodes*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) (Zaugg and Kinsel 1997; Johnson et al. 2007).

[c] Alaskan moose (*Alces alces gigas*) hoof copper parameters derived from Flynn et al. (1977).

[d] Hepatic copper.

[e] Bovine (*Bos taurus*) hoof copper normal parameters derived from Hidiroglou et al. (1986) and Sugg et al. (1996).

Study elk had limited soft tissue lesions involving the interdigital skin, heel bulb, and coronary band, including those with severely deformed hooves. These regions are key sites for both early and advanced infectious hoof disease lesions as described in many species of domestic and nondomestic ruminants (Wani and Samanta 2005; Belloy et al. 2007; Bennett et al. 2009; Evans et al. 2009). Though soft tissue lesions were not consistently detected, the five study elk had chronic disease, and earlier lesions may have been no longer apparent. Importantly, infectious hoof diseases that are well described in domestic animals may present differently in this nondomestic species. We performed aerobic and anaerobic bacterial culture of hoof lesions and soft tissues, and no pathogenic bacteria or fungi were isolated. However, the interval of tissue collection in the field and processing in the laboratory was prolonged and suboptimal for important fastidious aerobes and spirochetes such as *Dichelobacter nodosus*, *Fusobacterium necrophorum,* and *Treponema* sp., respectively. Additionally, these organisms, even under optimal conditions, require specialized growth media and conditions that were not utilized in this study. Attempts to evaluate younger animals with more acute lesions are underway, and fresh tissues are being collected into appropriate medium and refrigeration in the field for optimal culture and PCR.

Virus isolation and serology of study elk did not identify an underlying viral etiology and showed lack of serum antibody and, therefore, exposure to viruses that could potentially cause vascular and hoof-related disease, specifically BTV, EHDV, and BVDV I. Mineral analysis of liver showed that all elk were generally selenium deficient and all but one study elk were copper deficient (Hidiroglou and Williams 1986; Zaugg and Kinsel 1997; Johnson et al. 2007). Copper levels in hoof wall of all elk were below limits previously established for wild Alaskan moose (Flynn et al. 1977) and domestic cattle (Sugg et al. 1996). Copper is a vital component of keratin, and deficiency may lead to abnormal sulfur cross-linking, resulting in defective hoof keratin, but generally also defective antlers and hair coat (Gogan et al. 1989; Johnson et al. 2007), which was not identified in this study or in affected elk in this region in general. Copper deficiency in cattle is associated with increased incidence of infectious hoof disease, heel cracks, and sole ulcers thought to be due to weak, fragile hoof and sole keratin (Tomlinson et al. 2004). A population of Alaskan moose was described with lameness and severe overgrowth of the hooves (Flynn et al. 1977), and the cause of this lesion, called "slipper foot," was speculated to be copper deficiency. The role of dietary copper deficiency in hoof deformity, laminitis, and sole ulcers in the Cowlitz River Basin Roosevelt elk has not been determined, though elk in this region have historically cohabited with low dietary selenium and copper without previous outbreaks of hoof disease. Studies in moose and domestic cattle, however, would suggest copper deficiency may be an important predisposing factor, or at least an additional complication, to hoof disease in this population. Future studies will continue to elucidate the role of copper in this disease process, as well as to examine the biotin status of these elk. Biotin is an important component of normal hoof, skin, and hair development in most species and is associated with degenerative and necrotic hoof disease in states of deficiency, particularly reported in swine (Misir and Blair 1986).

Much remains to be learned about the influence of environmental factors on hoof disease in these wild elk. Factors of interest include forage contamination with toxic endophytes, specifically ergot and fescue toxicity, the latter of which was anecdotally described in domestic cattle in this region in the 1980s (C. Gay unpubl.). Endophytic toxins are capable of causing

digital vascular compromise leading to laminitis and distal gangrenous devitalization of the hooves and limbs (Handeland and Vikøren 2005). Diagnosis of toxic endophytes requires detailed range studies, which will be incorporated in future projects. Additionally, basic understanding of herd dynamics in affected regions will be essential, including knowledge of foraging behavior, nutrition, daily movements, migration, habitat usage, and interaction with domestic livestock. As SW Washington is a very wet maritime habitat, the roles of humidity and climate change also need to be considered. The winter of 2007–2008, when cases of hoof disease were first noted, was a historically wet year with remarkable rainfall and areas of flooding, which may have concentrated both wildlife and domestic livestock, allowing for transmission of contagious hoof diseases. Importantly, no hoof disease has been reported in domestic livestock by area veterinarians and landowners.

In conclusion, adult Roosevelt elk in SW Washington with chronic and severe lameness had severely overgrown and deformed hooves with sole ulcers and sloughed hoof walls. Study elk and one control elk had laminar vascular lesions consistent with chronic laminitis. Affected and control elk were copper and selenium deficient. Initial screening did not identify an underlying viral or infectious bacterial pathogen. However, an infectious etiology seems plausible to explain rapid and continued spread of hoof disease. Studies to identify and characterize acute hoof lesions and to optimize conditions for bacterial isolation are necessary and underway. Investigation of habitat use, principal diet, and behavior may be necessary to completely understand the dynamics and influence of these factors on the pathogenesis of hoof disease in this population of free-ranging Roosevelt elk.

ACKNOWLEDGMENTS

A special thank you to Gary J. Haldorson and the late Lindsay Oaks at Washington Animal Disease and Diagnostic Laboratory (WADDL) for additional review of histopathology and bacteriology, respectively; Pat Miller, Annemarie Prince, Ella Rowan, Eric Holman, Scott Shroeder, and Ted Holden of Washington Department of Fish and Wildlife for field assistance and sample handling; Tom Besser and Dan Bradway from WADDL for bacterial PCR; Patricia Talcott from WADDL and Dwayne Hamar from Colorado State University Veterinary Diagnostic Laboratory for hepatic trace element and heavy metal levels and hoof copper analysis, respectively; Michael Garner (Northwest Zoopath); Richard Whittington (University of Sydney, Australia), and Steve Parish (Washington State University College of Veterinary Medicine) for consult and valued opinions; and Jay Oaks for technical assistance with figures. This research was funded by the Washington Department of Fish and Wildlife with minor contributions from Colorado State University Diagnostic Medicine Center.

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Submitted for publication 12 July 2013.
Accepted 9 October 2013.