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Genetic sequencing using 16S rRNA for pathogen identification in retropharyngeal lymph nodes from wild elk

David J. Wilson, Department of Animal, Dairy and Veterinary Sciences, Utah State University, P.O. Box 6338, Logan, UT 84341, USA *David.Wilson@usu.edu*
Jacqueline P. Kurz, Department of Animal, Dairy and Veterinary Sciences, Utah State University, P.O. Box 6338, Logan, UT 84341, USA

Abstract: Cervical region lymph nodes collected by hunters from 43 wild hunter-harvested elk (*Cervus elaphus*) in Utah were submitted to the Utah Veterinary Diagnostic Laboratory during fall 2009. We evaluated these lymph nodes as specimens for identification of bacterial pathogens using 16S rRNA genetic sequencing. Thirty-seven bacterial species were identified; each was found in 2 to 30 individual elk. Many common ruminant livestock pathogens were identified in elk; pathogens previously reported in elk were *Pasteurella multocida* and *Streptococcus* spp. Cervical region lymph nodes harvested from wild ruminants appear to be acceptable samples for genetic sequencing of bacteria.

Key words: Bacteria, elk, genetic sequencing, lymph nodes, pathogens, 16S rRNA

Free-ranging elk (*Cervus elaphus*) inhabit the Intermountain West region of the United States. Reports of bacterial pathogens that have been found in elk are primarily from surveys of farmed elk, which are more readily captured and tested than wild elk. Farmed elk also have different behavior and movement than free-ranging elk, so the pathogens found in the 2 populations may differ. Pathogens reported in elk include *Pasteurella multocida* (Rhyan et al. 1997) and *Streptococcus* spp. (Hattel et al. 2007). Some elk carry infectious diseases of importance to livestock (McCorquodale and DiGiacomo 1985, Brook et al. 2013). Brucellosis, caused by *Brucella abortus*, a zoonotic disease that causes reproductive failure in both elk and cattle (*Bos taurus*), is the most common disease reported to be transmissible from wild elk to livestock; most scientific literature regarding disease common to elk and livestock focuses on brucellosis epidemiology and diagnosis (Rhyan et al. 2013).

The Utah Veterinary Diagnostic Laboratory (UVDL) receives cervical region lymph nodes, primarily retropharyngeal lymph nodes, from approximately 100 wild elk harvested by hunters each year throughout the United States, mainly from the Intermountain West region of Utah, Idaho, and Colorado. These lymph nodes are tested for chronic wasting disease (CWD), a transmissible spongiform encephalopathy (Williams 2005.) We investigated the potential usefulness of such tissues for diagnosing other disease etiologic agents of elk because of the established widespread sampling of cervical region lymph nodes from elk by hunters and custom slaughter meat processors. The objective was to test for pathogens, using16S rRNA sequencing, including those of recognized importance in elk or livestock species, particularly ruminants, in lymph node tissue.

Methods

Retropharyngeal lymph nodes were submitted for CWD testing to the UVDL from wild elk harvested by hunters throughout the United States, mainly from Utah, Idaho, and Colorado during the 2009 hunting season. Lymph nodes from 43 elk harvested within Utah only in areas far from livestock farming (within elk populations that were observed by Utah Division of Wildlife Resources to stay away from farms throughout the year) were selected for detection of bacteria using genetic sequencing. Selection of these animals as the sampling population allowed detection of bacterial species present within free-ranging elk that were unlikely to have contracted them from contact with domestic livestock species.

Lymph nodes were hunter-harvested; therefore, asepsis and storage conditions prior

Table 1. Bacteria detected with 100% homology to known bacteria (16s rRNA method) in elk lymph nodes. Only those detected in more than a single animal are included.

Bacteria	No. elk[a]	% of elk
Carnobacterium spp.	30	70
Serratia liquefaciens	29	67
Psychrobacter spp.	22	51
Carnobacterium divergens	17	40
Pseudomonas spp.	15	35
Pantoea agglomerans	12	28
Pseudomonas syringae	12	28
Pasteurella multocida	9	21
Propionibacterium acnes	9	21
Janthinobacterium lividum	8	19
Arthrobacter citreus	6	14
Erysipelothrix rhusiopathiae	6	14
Clostridium algidicarnis	5	12
Psychrobacter glacincola	5	12
Acinetobacter spp.	4	9
Morganella morganii	4	9
Stenotrophomonas rhizophila	4	9
Acidovorax spp.	3	7
Ralstonia spp.	3	7
Delftia spp.	2	5
Proteus vulgaris	2	5
Staphylococcus hominis	2	5
Streptococcus uberis	2	5
Tiedjeia arctica	2	5

[a] Total of 43 elk were tested

to arrival at the UVDL were unknown. After sections were removed for CWD testing at the UVDL, the remaining portions of the lymph nodes from the 43 elk were trimmed aseptically to harvest samples only from within the lymph nodes. After opening the surface of the lymph nodes with a scalpel, other instruments were used to collect tissue from inside the lymph nodes to minimize contamination. However, the possibility of organisms growing within the organs due to spoilage of the lymph nodes before arrival to the laboratory could not be entirely excluded. The samples were stored frozen at -20°C for 2 years. After thawing, the lymph nodes were tested using genetic sequencing by 16S rRNA gene sequencing at the Center for Integrated Biosystems (CIB) at Utah State University. Genomic DNA extraction was performed at the CIB, and the Sequencer FLX System (454 Life Sciences, a Roche company, Branford, Connecticut, USA) was used for all 3 gene sequencing and bacterial identification methods. Final diagnosis of the bacteria found in each elk's lymph node samples was based upon highest percent gene sequence match (homology) with known bacteria, and the resultant best match ID. Results are reported only for pathogens found in >1 elk, with 99% to 100% homology according to 16S rRNA sequencing.

Results

Bacteria identified from elk lymph nodes with 100% homology (16S rRNA) to known bacterial species (Table 1) included *Serratia liquefaciens* (67% of elk), which can cause mastitis in dairy cattle (Bowman et al. 1986) and also contributes to the pathogenesis of screwworm (*Cochliomyia hominivorax*) infestation in cattle (Chaudhury et al. 2010); *Psychrobacter* spp. (51%), which has been found on teat ends and in milk of mastitic dairy cattle (Braem et al. 2012); and *Pseudomonas* spp. (35%), an environmental bacteria that causes mastitis in cattle (Wilson et al. 1997, Atulya et al. 2014), sheep (*Ovis aries*), and goats (*Capra aegagrus hircus*; Leitner and Krifucks 2007). *Pantoea agglomerans* (28%) and *Propionibacterium acnes* (21%), which have both been isolated from conjunctivae of cattle with bovine keratoconjunctivitis ("pinkeye"; Hare et al. 2008), were also found. *Pasteurella multocida* (21%) has been isolated from the lung of a wild elk with purulent bronchopneumonia (Rhyan et al. 1997) and is an important respiratory pathogen of cattle, sheep, goats, swine (*Sus scrofa*), bison (*Bison bison*), chickens (*Gallus gallus domesticus*), turkeys (*Meleagris gallopavo*), and other domestic species (Arumugam et al. 2011).

Erysipelothrix rhusiopathiae (14%) is the causative agent of erysipelas, a costly disease complex that affects humans and several species of animals and birds. Manifestations of erysipelas include dermal infarctions (diamond skin disease), endocarditis, arthritis, nephritis, and septicemia (Brooke and Riley 1999).

Streptococcus uberis (5%) is a major mastitis pathogen in cattle (Wilson et al. 1997, Lundberg et al. 2014) and sheep (Guerreiro et al. 2013), and has also been isolated from goat mastitis cases (Al-Graibawi et al. 1986). *Streptococcus* spp. have been isolated from cases of pneumonia causing death of farmed elk (Hattel et al. 2007).

Bacteria isolated with 99% to 99.9% homology to known strains included *Moellerella wisconsensis* (23% of elk; 99.1% homology), also reported from the lung of a goat that was among several goats that died from respiratory disease (Casalinuovo and Musarella 2009). *Acinetobacter lwoffii* (9% of elk; 99.1% homology) has been isolated from the conjunctiva of cattle with bovine keratoconjunctivitis (Hare et al. 2008). *Enterobacter cloacae* (3% of elk; 99.7% homology) is a cause of coliform mastitis in dairy cattle (Nam et al. 2009). The remaining bacteria identified were from environmental sources including soil, plants, water, or fish. Complete data for all bacteria identified, including in only 1 elk or of lower homology, are available on request.

Discussion

Retropharyngeal lymph nodes harvested from wild elk by hunters were useful tissue samples for genomic testing for bacteria. A number of the bacteria isolated are recognized as pathogens of ruminants, including domestic livestock species. Because of the large number of lymph nodes collected for CWD sampling every year, and resultant familiarity of hunters with harvesting these organs, they will likely be a practical sample for disease surveillance purposes. Pathogens detected in the elk lymph nodes that were previously reported in elk were *Pasteurella multocida*, reported in free-ranging elk, and *Streptococcus* spp., reported in farmed elk (Rhyan et al. 1997, Hattel et al. 2007). In contrast, 2 other pathogens, each found in 1 hunter-harvested wild elk at necropsy in a previous study (Rhyan et al. 1997), *Brucella abortus* and *Trueperella pyogenes* (formerly *Actinomyces pyogenes*, then *Arcanobacterium pyogenes*), were not detected in any elk in this study. Those studies both tested all major organs, not lymph nodes.

A recent study provides evidence that contact between free-ranging elk and domestic cattle may occur frequently. Rancher observations and telemetric collars (181 elk had collars) were both used to study 7 wild elk herds with an estimated population of approximately 4,000 animals in southwestern Alberta, Canada. Telemetry identified 59% of 241 cattle pastures as used by elk, including 79% of those that ranchers also observed elk using, and 37% of pastures that ranchers never observed elk using. Direct contact between elk and cattle was observed by 73% of ranchers, with reports that the highest contact between elk and cattle occurred during January and February (Pruvot et al. 2014).

There was no way to determine whether the bacteria detected in the cervical region lymph nodes were causing pathology in other organs of the same animals at the same time. Cervical region lymph node isolates need to be compared to isolates from other body sites at necropsy of elk in future studies. In addition, evidence of pathology in major organs of wild ruminants needs to be evaluated in association with the pathogens isolated from both cervical lymph nodes and those organ systems.

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David J. Wilson (photo unavailable) is a veterinarian and the epidemiologist at the Utah Veterinary Diagnostic Laboratory. He is also a dairy extension veterinarian at Utah State University working primarily with mastitis, udder health, and infectious diseases of dairy cattle and goats.

Jacqueline P. Kurz (photo unavailable) is a veterinarian completing a residency in veterinary pathology at the Utah Veterinary Diagnostic Laboratory and pursuing a Ph.D. degree at Utah State University. The main focus of her research is the genetic basis of mastitis resistance in dairy cattle.