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GIA Form

1 message

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ksondger@uwyo.edu, cander98@uwyo.edu, <mary.wood@wyo.gov> Wed, May 1, 2019 at 3:58 PM
To: info@wyomingwildsheep.org, skilpatrick@wyomingwildsheep.org, joni@wyomingwildsheep.org

Project Details:
Project Title: What is the Respiratory Microbiome of Wild Sheep?
Project Type: Research
Affiliate: University of Wyoming & Wyoming Game and Fish
Project Location: Laramie, WY
Project Description: Respiratory disease is a persistent threat to the distribution and abundance of bighorn sheep across the Rocky Mountain West. Respiratory disease of bighorn sheep remains one of the most thoroughly studied, but least understood diseases affecting wildlife, but researchers are progressively overcoming the many misleading facets of respiratory disease and its etiology. Namely, the advancement of DNA detection assays (PCR), rather than culture-based methods, have improved the accuracy of evaluating prevalence rates of potential pathogens in bighorn sheep herds 1. The manifestation of respiratory disease has been commonly reported to be a result of an introduction of a novel pathogen, or pathogenic strains, typically causing acute pneumonia epizootics and recurring lamb die-offs 2–4. Disease can progress into a chronic form with varying degrees of morbidity and mortality; individuals that survive the initial disease can continue to shed the pathogens to newborn lambs, and previously unexposed individuals 2,5. Because pathogen prevalence rates are inconsistent, and diseased herds can have varying morbidity and mortality rates, researchers are still debating over which bacterial pathogen is the primary cause, and which bacteria are associated with secondary infections 2,3. Regardless, most researchers agree respiratory disease in bighorn sheep is polymicrobial. Just as bacteria can be the cause of disease, some (probiotics) help to maintain the health of the animal. For example, many species of Lactobacillus have been documented to inhibit the growth of pathogenic Pasteurellaceae in cattle 6. While studying particular pathogens remains important, new research on polymicrobial diseases in humans and livestock have associated the proportions of commensal bacteria to pathogens with disease virulence, morbidity, and mortality 7,8. Understanding the consortium of microbes (or microbiome) and their relationships in the upper respiratory tract of bighorn sheep, may help to determine what leads to respiratory disease outbreaks. Microbiome investigations only recently has become feasible with the reduced cost and advancement in DNA sequencing This study proposes to investigate respiratory microbiomes of free-ranging and captive sheep in Wyoming, Nebraska, and Alaska with varying herd health status. 2018-2019 winter captures for disease surveillance focused on three populations in northwest Wyoming; Whiskey Mountain (n=41 nasal, n=41tonsil), Jackson (n=26 nasal, n=26 tonsil), and Absaroka (n=15 nasal, n=15 tonsil), as these herds have different recovery rates from past pneumonia outbreaks (Fig. 1 & 2). Additionally, Nebraska's two populations were sampled, Pine Ridge (n=27 nasal, n=27 tonsil) and Wildcat Hills (n=20 nasal, n=20 tonsil), as the Pine Ridge population has had acute lamb die-offs since a pneumonia epizootic in 2014, while the neighboring Wildcat Hills shows continued population growth (Fig. 1 & 2). We have received nasal swabs (n=22) from a population of clinically healthy Dall sheep in Alaska. These samples will serve as the baseline microbiome data for Dall sheep and provide us with an understanding of sheep microbiomes previously unexposed to pathogens. Disease surveillance is currently planned for these Wyoming, Nebraska, and Alaska bighorn sheep populations for winter of 2019-2020. Additionally, nasal (n=10) and tonsil (n=14) swabs have been collected from captive sheep at Tom Thorne and Beth Williams Wildlife Research Center. Seven pregnant ewes and five yearlings will be undergoing a paired-longitudinal study with antimicrobial supplementation to determine if it is feasible to clear Mycoplasma ovipneumoniae infection. This provides an opportunity to evaluate microbiomes in a wildlife population over time, with only one variable (antibiotic therapy). We will collect both nasal and tonsil samples from both the ewes and yearlings, a minimum of three times a year.
Project Problem: Our main objective is to determine how the microbiome composition (abundance, numbers, and type) differs between different populations of bighorn sheep, and what composition characteristics are associated with respiratory disease. Current bacterial surveillance in bighorn sheep requires both nasal and tonsil samples be collected from individual sheep. We will compare microbiome composition between nasal and tonsil samples from individual animals. If there is no significant difference in microbiome composition, then we can recommend to wildlife managers that only the less invasive nasal sample is necessary during routine surveillance of bighorn sheep. Furthermore, we can evaluate the effect of antibiotics, such as enrofloxacin and tulathromycin, on the respiratory microbiome of captive sheep that are chronic shedders of bacterial pathogens.
Problem Solution: We have collected 161 nasal swabs and 143 tonsil swabs from different bighorn sheep herds in captive and free-ranging populations in Wyoming, Nebraska, and Alaska (Table 1). We will extract the DNA from swabs and prepare libraries for sequencing at the University of Minnesota. Raw data will be downloaded onto Teton (University of Wyoming Supercomputer), filtered and de-noised, then assigned into operational taxonomic units (OTU; a proxy for species) per swab using QIIME2. OTUs are used to calculate bacterial proportions at selected hierarchical levels (i.e. order, family, genus, etc). Phylogenetic trees will be created using the OTUs to determine alpha diversity using quantitative and qualitative measures between samples (Figure 3). Diversity metrics such as a weighted Unifrac will also be computed for beta diversity that allows quantitative measure of community dissimilarity between samples (Figure 4). Outputs will be converted into figures using R. Following the bioinformatics analyses, we will use individual health assessment data and determine how differences in nasal/tonsil microbiomes are associated with herd health. We hope to be able to identify the exact species and genotypes of respiratory pathogens (unpublished data) that may be more strongly associated with decreased herd health. This could ultimately enable sampling of bighorn sheep prior to transport, characterize the bacterial population genotype, and allow us to predict successful herd unit introductions. This project provides a novel opportunity to assess microbiome composition in a group of known pathogen shedding animals throughout the course of antibiotic therapy. It also allows comparison between different geographic populations of bighorn sheep (Wyoming vs. Nebraska), and different species of sheep (Ovis dalli dalli vs. Ovis canadensis). We expect to see a difference in nasal microbiome between Dall sheep and bighorn sheep. This study will provide

baseline data for future Dall sheep microbiome studies. Secondly, we expect that the microbiome composition will differ significantly between pre- and post-antibiotic therapy samples. By analyzing the microbiome composition in a controlled study, we will be able to determine the efficacy of antibiotics on the pathogen load, particularly Mycoplasma ovipneumoniae, on captive animals known to be chronic shedders. Lastly, we expect to see differences in microbiome composition between herds in Wyoming, Nebraska, and Alaska. Preliminary data indicates differences in the diversity of microbiome between herd units (Figure 3B). By increasing sample size and adding herd health data, we will be able to determine how microbiome composition effects herd health. This can be used to develop effective disease mitigation practices, disease screenings, and outbreak recovery strategies.

Biography Of Applicant:
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Current Member: No

Cost To Be Funded
Equipment.......$0......................................$$0
Services...........$16,000...$$4,000
Publishing........$2,000.....................................$$0
Monitoring......$0...$$0
Supplies...........DNA extraction: $3,000 Gloves/Tubes/Buffer/96 well plates/expendables: $4,000 Total: $7,000.......$$5,000
Other.............$7,000...$$0
Totals $............$32,000..$$9,000

Other Organizations:
Organization 1: Wyoming Game & Fish ($5,000)
Organization 2: Alaska Dept of Fish and Game ($4,000)
Organization 3:
Organization 4:

Media Contacts:
Media 1: Laramie Live
Media 2: Laramie Boomerang
Media 3: Wyoming NPR
Media 4: Casper Star Tribune

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2 attachments

Wyoming Wild Sheep Foundation Appendix.pdf
792K

ADFG Letter of Support.pdf
50K