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# GIA Form

1 message

**ksondger@uwyo.edu,** <HEdwar@uwyo.edu> Fri, Oct 27, 2017 at 3:47 PM
To: info@wyomingwildsheep.org

**Project Details:**
Project Title: Genotyping of Mannheimia sp. isolates from three Bighorn Sheep herds in Wyoming using MALDI-TOF mass spectrometry
Project Type: Research and Education
Affiliate: University of Wyoming and Wyoming Game & Fish
Project Location: Laramie, WY
Project Description: : The Wyoming Game and Fish Wildlife Health Laboratory has monitored five bighorn sheep herds for respiratory pathogens since 2011. During this time, multiple types of bacteria associated with respiratory disease have been isolated. Additional surveillance of body condition and overall herd health herd in association with respiratory pathogens has focused on three herds during the past three years (2015-2017). The pathogens isolated from the Absaroka, Jackson, and Whiskey Mountain herds include Mycoplasma ovipneumoniae, Mannheimia sp., Bibersteinia trehalosi, and Pasteurella multocida (see Figure 1). These pathogens have been identified by routine culture and PCR techniques. While bacterial culture has been the “traditional” way of identifying bacteria based on their biochemical profiles, newer techniques have recently become available and affordable. These techniques include using mass spectrometry to identify bacteria based on their protein fingerprints, and sequencing to identify bacteria based on their DNA. Together these techniques are able to characterize bacteria in ways traditional culture techniques could not. One concern is that traditional bacterial culture techniques may have mis-identified bacteria. For example, bacteria classified as Mannheimia haemolytica, may actually be Mannheimia glucosida or Mannheimia ruminalis based on beta-hemolysis and fermentation of different laboratory sugars (L-arabinose, D-sorbitol, D-xylose, maltose, and dextrin). We are interested in re-characterizing bacterial isolates based on mass spectrometry and sequencing. Further classification is also possible, as some bacterial species may have different biotypes or genotypes that are more strongly associated with pneumonia. For example, Mannheimia hemolytica genotype 2 isolates are predominately associated with the lungs in cattle pneumonia (Loy and Clawson, 2017). While we are interested in re-characterizing all the respiratory disease associated bacteria that have been isolated from these three herds, we propose to start with Mannheimia sp.
Project Problem: Mannheimia sp. has been previously isolated from the Absaroka, Jackson, and Whiskey Mountain herds; we want to determine the species and genotypes of Mannheimia spp. that are being isolated using mass spectrometry and DNA sequencing. If differences are found, we would like to correlate these findings with herd health. Being able to accurately identify pathogenic biotypes is vital when comparing overall health in different herd units. It will also be important when discerning habitat, body condition, and pathogenic biotypes and how they may synergistically influence herd health.
Problem Solution: The bacteria isolates have previously been collected by the Wyoming Game and Fish Wildlife Health Laboratory. From 2012-2017, approximately 35 Mannheimia sp. isolates have been collected and saved at -20°C (See Table 1). We propose to analyze these isolates using mass spectrometry (via Bruker MALDI-TOF), which generates a unique protein fingerprint (or spectrum) for each. These fingerprints can be compared using ClinProTool 3.0 software (Bruker), and assigned into genotypes (See Figure 1). The DNA from these isolates will also be extracted, sequenced (16S ribosomes), and analyzed to determine if the genotypes assigned correlate with genetic differences. This information will provide additional characterization of the bacteria being isolated from these herds, that has not previously been done. After re-characterizing all the bacterial pathogens (Mannheimia, Bibersteinia, Pasteurella), we hope to be able to identify the exact species and genotypes 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.

**Biography Of Applicant:**
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Current Member: No

**Cost To Be Funded**

Equipment.....0.....$0
Services.....Grad student tuition $6000.....$0
Publishing.....$2000.....$0
Monitoring.....0.....$0
Supplies.....Sequencing 35 x $200 ea = $7000 MALDI-TOF reagents $1000 =$1000 Agar plates/ other culture reagents = $1000 Total = $9000.....$0
Other.....Undergraduate salary $1000.....$0
Totals $.....18,000.....$

**Other Organizations:**

Organization 1: Morris Animal Foundation (submission due Nov. 15, request $150,000)
Organization 2:
Organization 3:
Organization 4:

**Media Contacts:**

Media 1: Laramie Boomerang
Media 2:
Media 3:
Media 4:

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**2 attachments**

**WWSF_Mannheimia genotypes_Figures_Sondgeroth 2017.pdf**
429K

**WWSF Collaborative Support.pdf**
920K