Wyoming Wild Sheep Foundation—Bighorn sheep Rampage Article 2019

Grant In Aid Project: Genotyping of Mannheimia sp. isolates from three Bighorn sheep herd units in Wyoming using MALDI-TOF mass spectrometry

Funding Cycle: 1/2018-12/2019

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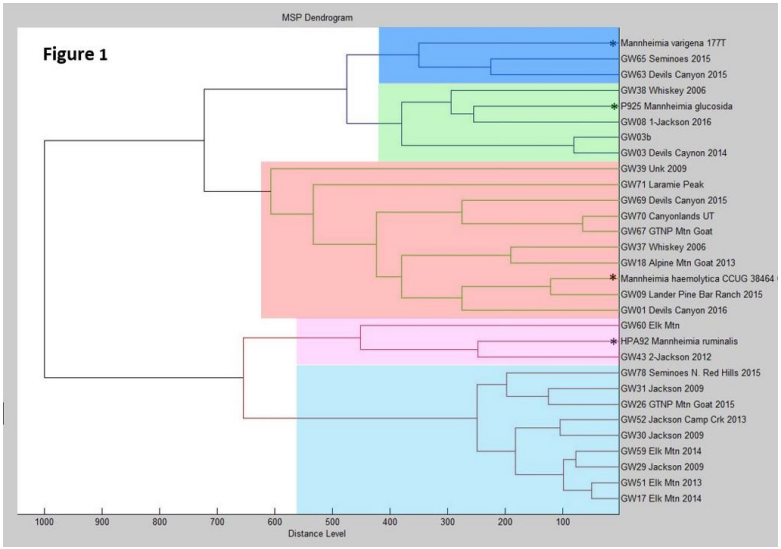
Problem: Previous bacterial pathogens identified from bighorn sheep herd units across Wyoming, may have been mis-characterized due to limitations of laboratory methods (ie traditional bacterial culture techniques)

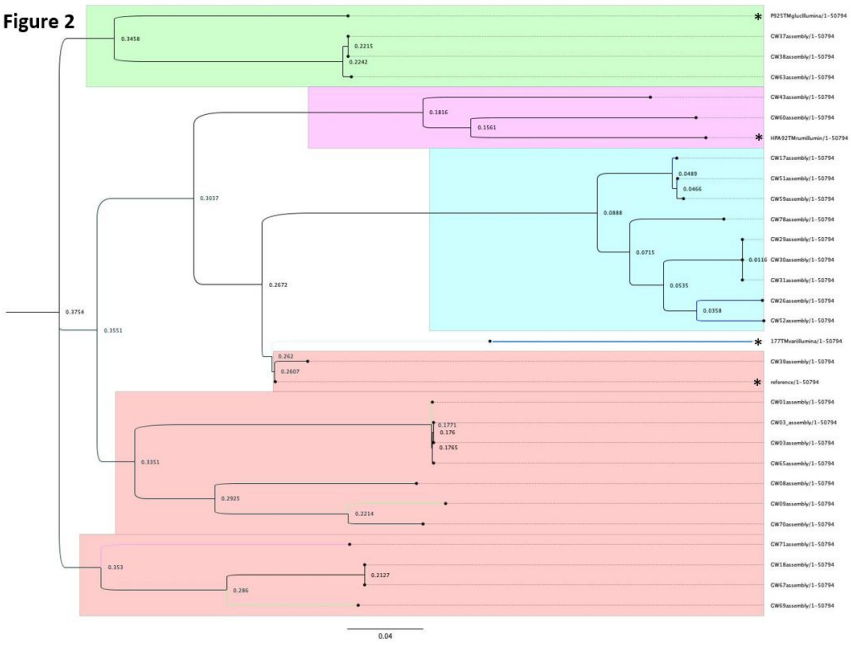
Goal: Working in collaboration with the Wyoming Game and Fish Wildlife Health Laboratory, and utilizing bacterial isolates they have saved over the years; we will utilize new technology to provide better identification to these bacterial pathogens. We will start by characterizing the *Mannheimia* sp. using mass spectrometry and whole genome sequencing. These are both relatively new techniques that will be used to provide the most exact identification of our isolates. Ultimately this data will be used to 1) build a bighorn sheep bacterial respiratory database to make better diagnoses from future isolates, 2) determine if these isolates are associated with decreases in herd health.

Progress: We have confirmed the viability of 92 *Mannheimia* sp. isolates by growing them in culture (which is more than double the number of our original project!). All 92 have undergone mass spectrometry to obtain their unique protein fingerprint. These fingerprints can be used to determine how closely each isolate resembles others. As an example, in **Figure 1**, we show 23 bighorn sheep isolates, and compare them to known *Mannheimia varigena, Mannheimia ruminalis, Mannheimia glucosida* and *Mannheimia haemolytica* spectra profiles (indicated by asterices). The known isolates were gifted to us by Dr. Henrick Christensen and imported from Denmark. The five main clusters that have emerged are color coded as *M. varigena (dark blue), M. glucosida* (green), *M. haemolytica* (red), *M. ruminalis* (purple), and *M. granulomatis* (light blue).



To obtain the maximum amount of genetic information from each isolate, we performed whole genome sequencing using two different methods. The first method was performed in collaboration with the Wyoming Public Health Department, and 79 (of 92) *Mannheimia* sp. isolates have been completed. The second method is being performed in collaboration with Virginia Tech, and 62 isolates have been completed. We are currently combining all the data from the two different methods, and have assembled the complete genomes from 23 of the isolates. Since we have entire genomes' worth of data, we are able to extract information to compare certain genes across isolates (ie whether the leukotoxin gene is present). Additionally, we can compare the relatedness of these isolates using multiple techniques: whole genome data, mass spectrometry data, and individual genes (*16S* and *rpoB)*. In **Figure 2**, using the same isolates as shown in Figure 1,five main clusters emerge and are color coded as *M. glucosida* (green), *M. ruminalis* (purple), *M. granulomatis* (blue), *M. varigena* (dark blue line), and *M. haemolytica* (red). Again the reference isolates are noted by asterices.

Figure 2



In **Table 1** we compare the different techniques in their species determination in the *Mannheimia* genus. Our ultimate goal is to combine these techniques (sequencing and mass spectrometry) and provide more resolution of *Mannheimia* species identification as we continue our analyses.

Table 1: Preliminary data comparison of 23 bighorn sheep isolate identification based on mass spectrometry (protein) vs. sequencing (DNA).

***Mannheimia* species**	**Mass spectrometry**	***rpoB* and *16S* sequencing**	**Whole Genome sequencing**
M. haemolytica (red)	7	6	10
M. glucosida (green)	4	6	3
M. ruminalis (purple)	2	3	2
M. varigena (dark blue)	2	5	0
M. granulomatis (blue)	8	0	8
Either *M. varigena* or *granulomatis*	0	3	0

Future direction:

- Continue to analyze the whole genome sequencing data of all 92 *Mannheimia* sp. isolates.

- Whole genome sequence comparison. Currently, a good software package has not been developed so we are working on the cutting edge of technology with our collaborators at the National Center for Genomic Research in New Mexico!

- Combine mass spectrometry and whole genome sequencing data to fully resolve *Mannheimia* species.

- Continue this same process with other bacterial pathogens in bighorn sheep pneumonia including; *Pasteurella multocida, Bibersteinia trehalosis, and Mycoplasma ovipneumoniae.*