ADF&G Wildlife Health and Disease Surveillance Program
Interpreting MLST data for strain-typing of *Mycoplasma ovipneumoniae* sequence segments
October 1, 2020

The purpose of this brief is to explain and document what Alaska Department of Fish and Game (ADF&G) has received from the Washington Animal Disease Diagnostic Laboratory (WADDL; Pullman, WA) regarding MLST (Multi-Locus Sequence Typing or "strain-typing"), and how we have interpreted those data based on published literature and feedback from Dr. Besser to date (29 Sept 2020).

Once the detection of bacteria in a sample has been identified by Polymerase chain reaction (PCR), it can be further characterized by using additional PCR tests and sequencing the resulting products. This MLST approach involves picking a small number (often 5-7) of the "housekeeping" genes (genes required for basic cell function) spread across the genome to evaluate potential genetic differences.

For *Mycoplasma ovipneumoniae* (M. ovi) this includes 4 loci (short segments of genome first developed by Cassirer et al. in 2017, to differentiate and describe strains of M. ovi and is currently used by WADDL):

- **LM**: (~345 base pairs) a region in the 16S rRNA gene that is highly conserved. This is the same region that is used by some PCR tests to identify Mycoplasmas including M. ovi.
- **IGS**: (~400 base pairs) intergenic space. More variable than the 16S rRNA region, but also used to identify Mycoplasmas including M. ovi.
- **rpoB:** (~565 base pairs) a protein
- **gyrB:** (~400 base pairs) a protein

Numerous alleles (alternative forms of a gene) have been detected within each of the four loci (84 in LM, 271 in IGS, 204 in rpoB, and 338 in gyrB), and undoubtedly many more remain to be discovered. For the MLST these four genes are combined ("concatenated") into one string of base pairs. This allows for a longer string of nucleotides for comparison, and a better ability to differentiate strains than looking only at one short fragment. The MLST sequences are not contiguous in the genome. Because the sequences are generated separately using different primers (depending on which PCR method was used), there is a chance that the sequences are not always from the same organism if multiple organisms or strains are present in a sample. In practice, evidence to support that this is a usual or common problem has not been identified.

Currently this MLST protocol is what is being used for strain typing by WADDL. Using this protocol, Kamath (2019) describes M. ovi to be very diverse, including hundreds of strain types. From the Kamath paper**,** strains that differed by no more than 4 base pairs were considered to be the same strain (99.8% identity). At this time, "strains" in M. ovi are used for basic identification and determination of relationships between organisms and not determining pathogenicity (whether a strain causes disease).

MLST was applied to M. ovi detected in 11 Dall's sheep and 15 caribou samples collected from 2004-2019 across Alaska. Full 4-locus MLST data was obtained from 11 Dall's sheep and 12 caribou, identifying all as a single M. ovi strain type (<5 differing bases detected). Partial MLST data (1-3 loci successfully sequenced) were produced from 3 additional caribou. All the partial MLST data were identical to the corresponding loci in the 4-locus MLST. Therefore, no evidence supporting any different M. ovi strain types within these wildlife species was found. These results are consistent with transmission of a single

strain type among and between these Alaska wildlife populations. This strain is more genetically similar to domestic sheep M. ovi strains than to domestic goat M. ovi strains as defined by Kamath et al. 2019; however, currently available MLST data cannot identify the source of this strain.

MLST was applied to M. ovi from two domestic sheep (one residing in Alaska and one exotic import) and nine domestic goats. Complete MLST data could be obtained only from the two domestic sheep and two of the domestic goats, all of which differed from each other and from the strain found in Alaska wildlife. Partial MLST data was produced in the other seven goats, five producing 3-locus sequences and two producing 2-locus sequences. These partial MLST data were sufficient to indicate the presence of at least six additional M. ovi strains in Alaska domestic animals, which all again differed from the strain found in Alaska wildlife. [1]

The exotic import was a Corsican sheep that was illegally imported to Alaska from Tennessee. The animal was dead on arrival at Fairbanks International Airport in 2009 and was diagnosed with multifactorial bacterial bronchopneumonia[2]. In 2020, two laboratories detected the presence of M. ovi in archived frozen lung tissue from the animal. This animal provides an example of the important difference between strain typing using multiple loci and sequencing one locus. While strains may appear similar at one locus, that similarity may not be present when looking at a longer sequence of base pairs. Comparing the imported Corsican sheep to the M. ovi identified in AK wildlife:

- Comparing each fragment separately
    - LM: 100% identity
    - IGS: 98.25% identity (5 base pair changes)
    - rpoB: 95.91% identity (21 base pair changes)
    - gyrB: 94.5% identity (22 base pair changes
- Concatenated sequences: 96.83% identity

In summary, these data identify the presence of a single M. ovi strain in Dall's sheep and caribou populations that differed from all M. ovi strains detected in domestic sheep and goats. This illustrates that additional work is needed to understand M. ovi in wildlife in Alaska.

Literature Cited

Cassirer, E. F., K. R. Manlove, R. K. Plowright, and T. E. Besser. 2017. Evidence for strain-specific immunity to pneumonia in bighorn sheep. The Journal of Wildlife Management **81**:133-143.

Kamath, P. L., K. Manlove, E. F. Cassirer, P. C. Cross, and T. E. Besser. 2019. Genetic structure of *Mycoplasma ovipneumoniae* informs pathogen spillover dynamics between domestic and wild Caprinae in the western United States. Scientific Reports **9**:15318.

[1] The domestic sheep and goats were tested in a blinded study by DEC and included with permission (R. Gerlach, 28Sep2020). These data cannot be reproduced or published without express permission.

[2] This animal was necropsied by ADF&G at the direction of the State Veterinarian and USDA/VS.