Bacterial Strain Typing in a Population of Bighorn Sheep Experiencing Polymicrobial Lamb Pneumonia

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ABSTRACT: We recently developed an assay for multilocus sequence typing of bighorn sheep respiratory pathogens using multiplex polymerase chain reaction (PCR), amplicon sequencing, and a custom bioinformatics pipeline. The assay specifically assesses *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mycoplasma ovipneumoniae*, as well as providing a broad analysis of bacterial composition through a 16S rRNA analysis. We used this assay to analyze 30 post-mortem tissue samples, acquired over 14 years, from a population of bighorn sheep in southeastern Colorado (RBS 9). Chronic respiratory disease and lamb mortality in this herd prompted removal of a sub-herd in 2013, but the population was considered stable from 2013–2022 despite the persistence of respiratory pathogens in the adult population. During the summers of 2022 and 2023, we observed heavy lamb mortality in 1 sub-herd due to polymicrobial bronchopneumonia. We pursued bacterial strain typing to help understand what changed in the population that might explain the re-appearance of lamb pneumonia. Lung tissues from the lambs with pneumonia were predominated by a single strain each of *M. ovipneumoniae*, *B. trehalosi*, and *P. multocida*. Prior to 2022, in the absence of observable lamb pneumonia, multiple strains of *M. ovipneumoniae* and *P. multocida* were circulating in the bighorn population. The strains of *M. ovipneumoniae* and *P. multocida* identified in the lamb pneumonia cases were present in the population since at least 2016 and 2010 respectively. Only 1 strain of *B. trehalosi* was detected in the bighorn population prior to the observation of lamb pneumonia, while a different *B. trehalosi* strain was found in the dead lambs. The introduction of a novel *B. trehalosi* strain was the most significant microbial change in the population that we identified associated with the observation of lamb pneumonia in 2022/2023. Continued strain typing work will provide context for further interpretations.

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KEYWORDS: *Bibersteinia trehalosi*, bioinformatics, bighorn sheep, Colorado, *Mannheimia haemolytica*, multilocus sequence typing, *Mycoplasma ovipneumoniae*, *Pasteurella multocida*, pathogen, polymerase chain reaction (PCR), polymicrobial lamb pneumonia.