Diagnostic Strengths of Strain Typing Bacteria During Surveillance and Bighorn Sheep Die-offs

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ABSTRACT: The etiology of bighorn sheep respiratory disease is often questioned when the suspected bacterial pathogen is found in both healthy and diseased individuals. Several approaches have been employed to investigate pathogens at a sub-species level, in attempt to understand virulence differences. Examples identifying genetic differences include detecting the presence or absence of certain genes such as leukotoxin (Shanthalingam et al. 2013) and measuring relatedness through multilocus sequence typing (MLST; Kamath et al. 2019). Historically, functional assessments of bacterial isolates using biochemical testing have been utilized to identify associations between biogroups and die-offs (Wolfe et al. 2010). However, most bighorn sheep disease surveillance programs focus on identifying the prevalence of common bacterial pathogens. This routinely involves bacterial culture and PCR. Recently, mass spectrometry (MALDI) has been introduced into diagnostic workflows following bacterial culture to increase accuracy of species identification. MALDI measures protein expression and generates a unique protein 'fingerprint' for each bacterial isolate. This allows characterization of the bacteria beyond 'species', to a sub-species level called 'biotype'. To better understand diagnostic abilities of MALDI, we performed whole genome sequencing (to recover Leukotoxin and MLST) and MALDI biotyping on *Mannheimia spp.* isolates cultured from around Wyoming. Samples were obtained through routine surveillance and disease sampling during the Laramie Peak Die-off in 2020 and 2021. Here we introduce MALDI biotyping and review the strengths and weaknesses of strain-typing methods to help inform respiratory disease management efforts.

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