Advancing Diagnostic Precision through MALDI-TOF and Whole Genome Sequencing

CHRISTOPHER A.W. MACGLOVER, *Department of Veterinary Sciences, University of Wyoming, Laramie, WY, 82070, USA; cmacglov@uwyo.edu*
JESSICA JENNINGS-GAINES, *Wildlife Health Laboratory, Wyoming Game and Fish Department, Laramie, WY, 82070, USA*
HALLY KILLION, *Wyoming State Veterinary Laboratory, Laramie, WY, 82070, USA*
KERRY S. SONDGEROTH, *Department of Veterinary Sciences, University of Wyoming, Laramie, WY, 82070, USA*

ABSTRACT: We performed a comprehensive retrospective study to discern the diagnostic capabilities of MALDI-TOF mass spectrometry to strain-type bacterial pathogens implicated in bighorn sheep respiratory disease. This investigation sought to elucidate the etiology of acute die-off events by examining 81 *Mannheimia* spp. isolates through advanced MALDI-TOF strain typing functions and validated with whole genome sequencing. Analysis of *Mannheimia* spp. isolates, including *Mannheimia haemolytica*, *M. glucosida*, *M. ruminalis*, and *M. varigena*, revealed substantial misidentification when solely utilizing the standard manufacturer MALDI-TOF database, particularly concerning *Mannheimia glucosida*, which was frequently misidentified as *Mannheimia haemolytica*. However, the addition of type-strain profiles to the standard MALDI-TOF database corrected the inaccuracies, demonstrating the critical role of a comprehensive reference database, now available for routine bacterial identification at the Wyoming State Veterinary Laboratory. After correctly classifying the isolates using whole genome sequencing, Mannheimia haemolytica was only isolated in bighorn sheep during acute all-age die-offs (1993 Shell Canyon, WY; 2008 Rattlesnake Butte, CO; 2020–2021 Laramie Peak, WY; 2022–2023 Devil's Canyon, WY). The study also identified a novel *Mannheimia* species frequently present in asymptomatic bighorn sheep. These findings underscore the limitations of the current manufacturers MALDI-TOF databases in accurately diagnosing pathogenic agents, emphasizing the need for updated and expanded databases to support more accurate species identification obtained from bighorn sheep. Presently, it is recommended to use sequencing, either whole genome or MLST phylogenetics, for accurate differentiation of *Mannheimia* species, but we have shown that it can be used to inform MALDI-TOF for more robust bacterial identification with the ultimate goal of informing and optimizing management and conservation strategies.

Biennial Symposium of the Northern Wild Sheep and Goat Council 24:10; 2024

KEYWORDS: bacterial pathogens, bighorn sheep, Colorado, MALDI-TOF mass spectrometry, *Mannheimia haemolytica*, *Mannheimia glucosida*, *Mannheimia ruminalis*, *Mannheimia varigena*, strain typing, Wyoming.