Development and Utility of a Gene Transcription Panel for Desert Bighorn Sheep (*Ovis canadensis nelsoni*)

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EXPANDED ABSTRACT Respiratory disease is a key factor impacting the success of the ongoing conservation and recovery of wild sheep populations (WAFWA 2017). Although the primary pathogens involved in the bighorn sheep pneumonia complex have been identified, the wide variability in herd response following infection is not well understood (Cassirer et al. 2018). The response of populations infected with *Mycoplasma ovipneumoniae* has been variable, from minimal to extensive herd mortality followed by years to decades of either poor lamb recruitment or little expression of disease and minimal impact on lamb survival (Coggins and Mathews 1992, Jorgenson et al. 1997, Cassirer et al. 2018). This variation is thought to be caused by differences in pathogen virulence, intrinsic or extrinsic factors that impact individual or herd immunity, including lungworm (*Protostrongylus* spp.) or mite (*Psoroptes ovis*) infections, malnutrition, inbreeding, harsh weather conditions, or stress associated with overcrowding (Risenhoover et al. 1988, Bailey 1990, Jones and Worley 1994, Monello et al. 2001). Although substantial management strategies have been implemented, they have been ineffective in halting the spread of the epizootic (Cassirer et al. 2018).

Traditional approaches to bighorn respiratory disease research have focused mainly on the role that pathogens play in the respiratory disease complex. The contribution of environmental variation to animal immunity and infections is largely unknown; current health evaluations and diagnostics for desert bighorn sheep provide limited information on the overall health of the animal and almost no information on the potential contributing risk-factors inherent in the habitat. This lack of diagnostic information makes it difficult to identify specific environmental conditions and stressors potentially linking variable herd responses to the spillover of such pathogens as *M. ovipneumoniae* in desert bighorn sheep (*Ovis canadensis nelsoni*) herds in Nevada.

Gene-based diagnostics such as gene transcription provide an innovative, minimally-invasive tool that improves our understanding of the health of desert bighorn sheep populations. The advantage of using gene transcript analysis in desert bighorn sheep diagnostics lies in the capacity to measure physiologic responses (acute or chronic) of an individual to environmental stressors. The earliest observable signs of health impairment are altered levels of gene transcripts, evident prior to clinical manifestation (McLoughlin et al. 2006). By concurrent evaluation of transcript levels for genes representative of multiple internal systems, it is possible to measure a physiological response of individuals as well as populations to environmental stressors like pathogens, nutritional deficiency, or contaminants. Consequently, application of quantitative gene transcript analysis technology will provide an invaluable addition to current approaches for monitoring indications of potential health impairment (McLoughlin et al. 2006). Stressor-specific analyses of gene transcription profiles can inform management actions that may mitigate stressor impacts and improve bighorn sheep recovery.

We developed real-time PCR assays for 14 genes of interest and two reference genes. These have been validated on desert bighorn sheep samples randomly selected from populations experiencing differing extrinsic and intrinsic pressures; these included the Muddy and River Mountains, Pintwater Range, and Bare Mountain populations (Figure 1). Genes of interest represent immunological and physiological systems critical to responses to stressors (inflammation, cell signaling, apoptosis, detoxification, antiviral, antibacterial, and general stress).

We analyzed gene transcription data using multivariate, nonparametric, multi-dimensional scaling analysis (NMDS) in conjunction with cluster analysis for statistical and graphical representation of individual bighorn sheep clustered by similarity in transcription and not by pre-defined groups. Statistical comparisons of individuals grouped by clusters were made using similarity profile permutation (SIMPROF) to test for significance among a priori, unstructured clusters of samples (R Development Core Team 2012). We then used principal components analysis (default stats package; R 2.8.1, R Development Core Team 2012) to determine primary genes driving cluster separation.

Individuals within a wildlife population comprise a range of physiological states. As such, clusters designated by NMDS analysis (Figure 2) included individuals across populations, indicating some similar physiologic responses. Cluster 3 comprised most sheep from the Muddy Mountains, the designated reference population (based on historic lack of M. ovipneumoniae). Thus, cluster 3 should represent animals whose physiologic responses are similar to those in the reference population; indeed, cluster 3 is comprised of sheep from all populations, a reflection of the natural occurrence of relatively “physiologically normal” individuals from the populations sampled. Cluster 1 is comprised of sheep from the River Mountains and Pintwater Range with transcript profiles representing increased (relative to other clusters) anti-viral and inflammatory responses and decreased anti-inflammatory responses. The latter has been linked with the ability of Mycobacterium to evade immune responses (Redford et al. 2011). Cluster 2 comprised sheep mostly from the Pintwater Range, with an additional two from the Muddy Mountains and one from the Bare Mountains. These sheep were characterized by high levels of heat shock protein 70 (HSP70), which has been implicated in exposure to a number of stressors (Iwama et al. 1999, Tsan and Gao 2004).

Cluster 4 comprised sheep mostly from the Pintwater Range. This cluster characterized the most divergent transcript profiles among the clusters, with multiple gene implications of physiological response to hydrocarbons or dioxin-like substances and virus.

The results of our study demonstrate that establishment of gene transcript profiles in peripheral blood samples has the potential to contribute towards an understanding of disease dynamics in desert bighorn sheep, and towards a management regime effective at mitigating the effects of *M. ovipneumoniae* by incorporating both immunological and ecological context.

Biennial Symposium of the Northern Wild Sheep and Goat Council 21:2-6; 2018

KEYWORDS *Mycoplasma ovipneumoniae*; desert bighorn sheep; *Ovis canadensis nelsoni*; gene transcription; immune function.

LITERATURE CITED

Bailey, J. A. 1990. Management of Rocky Mountain bighorn sheep herds in Colorado. Special report/Colorado Division of Wildlife (USA).

Cassirer, E. F., Manlove, K. R., Almberg, E. S., Kamath, P. L., Cox, M., Wolff, P., et al. 2018. Pneumonia in bighorn sheep: Risk and resilience. *Journal of Wildlife Management* 82:32-45.

Coggins, V. L., and Matthews, P. 1992. Lamb survival and herd status of the Lostine bighorn herd following a *Pasteurella* die-off. *Proceedings of the Biennial Symposium of the Northern Wild Sheep and Goat Council* 8:147-54.

Iwama, G. K., Mathilakath, M. V., Forsyth, R. B., and Ackerman, P. A., 1999, Heat shock proteins and physiological stress in fish. *American Zoology* 39:901-9.

Jones, L.C., and Worley, D.E. 1994. Evaluation of lungworm, nutrition, and predation as factors limiting recovery of the Stillwater bighorn sheep herd, Montana. Biennial Symposium of the Northern Wild Sheep and Goat Council 9:25-34.

Jorgenson, J. T., Festa-Bianchet, M., Gaillard, J. M., and Wishart, W. D. 1997. Effects of age, sex, disease, and density on survival of bighorn sheep. *Ecology* 78:1019-32.

McLoughlin, K., Turteltaub, K., Bankaitis-Davis, D., Gerren, R., Siconolfi, L., Storm, K., et al. 2006. Limited dynamic range of immune response gene expression observed in healthy blood donors using RT-PCR. *Molecular Medicine* 12:185-95.

Monello, R. J., Murray, D. L., and Cassirer, E. F. 2001. Ecological correlates of pneumonia epizootics in bighorn sheep herds. *Canadian Journal of Zoology* 79:1423-32.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org/

Redford, P. S., Murray, P. J., and O'garra, A. 2011. The role of IL-10 in immune regulation during M. tuberculosis infection. *Mucosal immunology* 4:261-70.

Risenhoover, K. L., Bailey, J. A., and Wakelyn, L. A. 1988. Assessing the Rocky Mountain bighorn sheep management problem. *Wildlife Society Bulletin (1973-2006)* 16:346-52.

Tsan, M. F., and Gao, B. 2004. Cytokine function of heat shock proteins. *American Journal of Physiology-Cell Physiology* 286:C739-C744.

Western Association of Fish and Wildlife Agencies Wild Sheep Working Group (WAFWA). 2017. Adaptive wild sheep disease management venture (DMV) strategy. https://www.wafwa.org/committees___groups/wild_sheep_working_group/disease_management_venture/

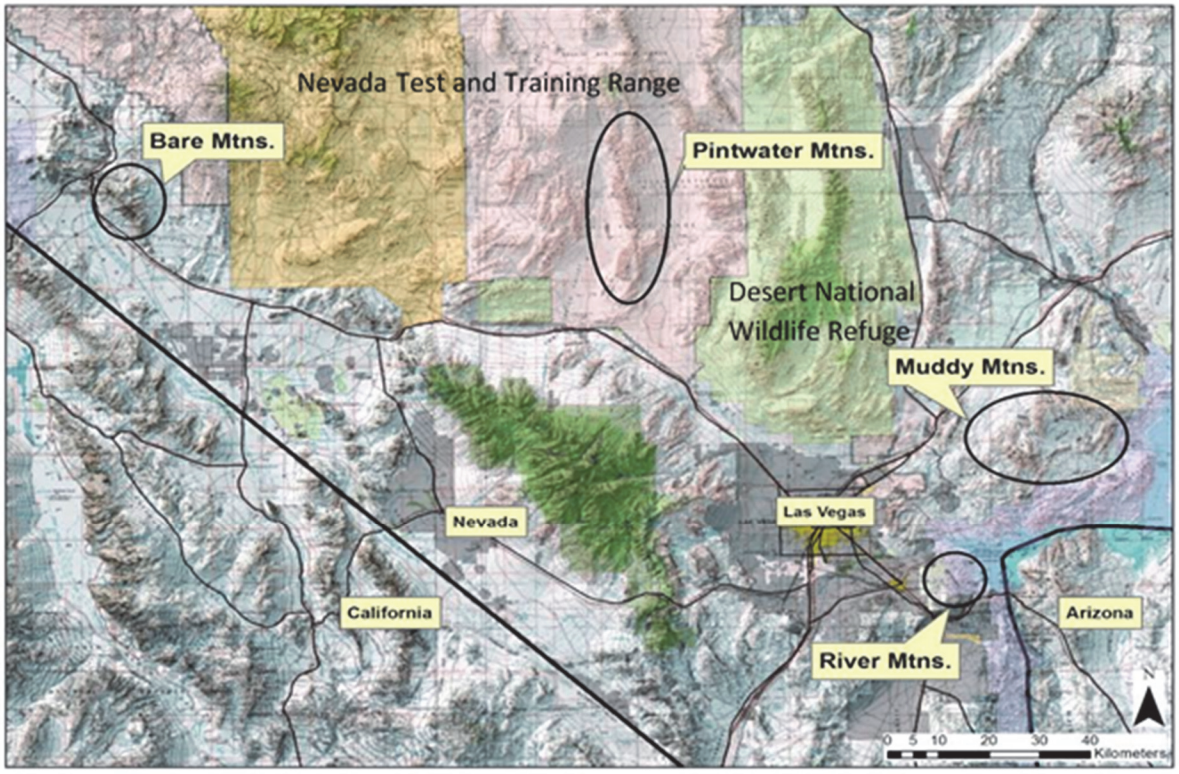

Figure 1. Map showing location of the mountain ranges, Pintwater Range, Muddy Mountains, River Mountains, and Bare Mountain Range, Nevada, where desert bighorn sheep were captured and blood samples were obtained for development of the RNA transcription panel.

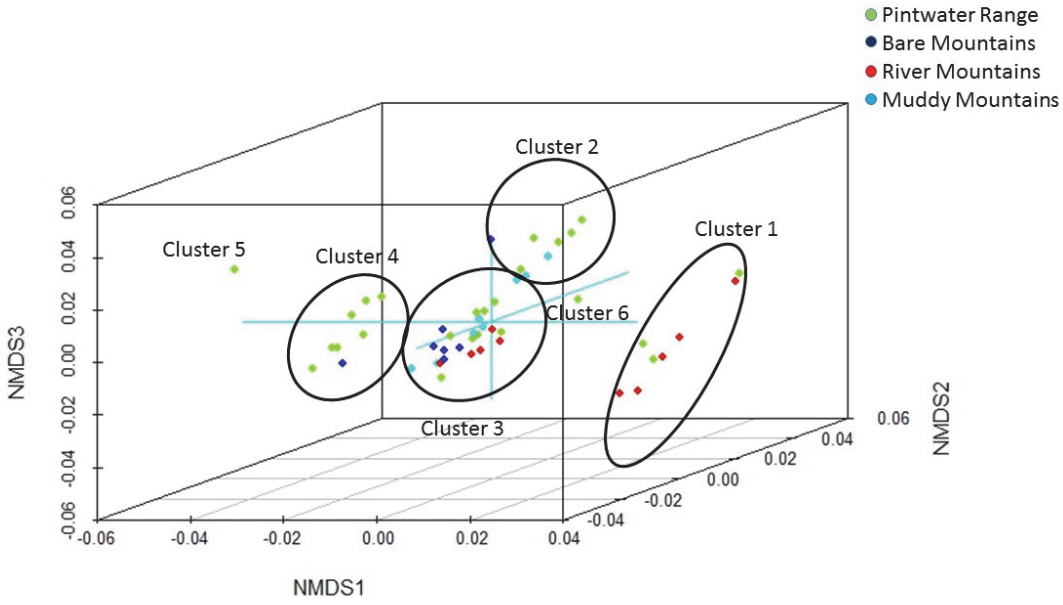

Figure 2. Multivariate, nonparametric, multi-dimensional scaling (NMDS) of gene transcription profiles of bighorn sheep sampled from four different populations (Muddy Mountains, River Mountains, Bare Mountains, Pintwater Range). Significant clusters are identified; SIMPROF, R 2.8.1, R Development Core Team 2012.