Molecular detection and serology of *Mannheimia / Bibersteinia / Pasteurella* in the lungs of pneumonic bighorn sheep.

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Abstract: Pneumonia caused by members of the Family *Pasteurellaceae* has played a significant role in the decline of free ranging bighorn sheep (*Ovis canadensis*, BHS) populations in North America. *Mannheimia haemolytica* consistently causes fatal bronchopneumonia in BHS under experimental conditions. However, *Biberstenia trehalosi* and *Pasteurella multocida* have been isolated more frequently than *M. haemolytica* from pneumonic lungs of BHS. This has led to the misconception that *M. haemolytica* may not be the primary bacterial pathogen of this deadly disease in BHS. A recent study by us has revealed that *B. trehalosi* and *P. multocida* can outgrow and inhibit *M. haemolytica* growth. The objective of this study was to detect the presence of *M. haemolytica* in the pneumonic lungs of BHS that died in the recent outbreaks in Western United States. We obtained pneumonic lung tissue of BHS from three States. Since *M. haemolytica* was not isolated from the great majority of these specimens by culture-dependent methods, we developed a culture-independent method for the detection of *M. haemolytica*. Total genomic DNA from lesional tissues was extracted and species-specific PCR assay was performed. This assay detected the presence of *M. haemolytica* in cases where the culture-dependent methods failed to detect this organism. We have also developed a multiplex PCR assay to detect *M. haemolytica*, *B. trehalosi* and *P. multocida* simultaneously. The leukotoxin (Lkt) produced by *M. haemolytica* is the primary virulence factor of this organism. Lkt-neutralizing antibody titer in the BHS is an indicator of infection of these BHS with Lkt-positive *M. haemolytica.* Therefore we also determined the Lkt-neutralizing antibody titer of serum samples from diseased BHS by MTT dye reduction cytotoxicity inhibition assay. Serum titers of most of the animals were between 1:200 and 1:800. These results indicate the involvement of *M. haemolytica* in bronchopneumonia in free-ranging BHS.

Biennial Symposium of the Northern Wild Sheep and Goat Council 17:68; 2010

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