Enhanced Bacterial Pathogen Detection via Improved Sample Collection and Laboratory Diagnostics

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ABSTRACT Culture and isolation of the common bacterial pathogens responsible for pneumonia in bighorn sheep (*Ovis canadensis*) can be difficult. Our laboratory increased diagnostic sensitivity for these pathogens by integrating polymerase chain reaction (PCR) into our laboratory regimen and improving field-sampling techniques. We used published PCR protocols to screen all the bacterial growth from culture plates for *Mannheimia* and *Bibersteinia* spp. leukotoxins, followed by *Mannheimia* spp. specific leukotoxin, and finally a PCR to detect *M. haemolytica*. The addition of these PCRs to our standard culture protocol resulted in the detection of 29% more leukotoxin positive *Mannheimia* spp. (including *M. haemolytica*) than by gross identification of bacterial colonies on Columbia Blood Agar (CBA) or Columbia Selective Agar (CSA). In addition, we optimized our sample collection techniques in the field to ensure microbial viability and recovery. Optimization steps included multiple swabs from the tonsillar crypts and immediate inoculation of CBA or CSA plates. Culture plates were placed in a mobile incubator held at 37° C with 10% CO_2. Phenotypic colonies were removed and recultured every 24 hours until delivery to the laboratory. These improvements in field and laboratory techniques have increased our ability to detect potential pathogens in bighorn sheep populations.

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