Diagnosis of *Mycoplasma ovipneumoniae* in Bighorn Sheep

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Abstract: Respiratory disease continues to cause very significant morbidity and mortality in wild populations of Bighorn sheep (*Ovis canadensis*). Although fatal pneumonia is typically associated with various species of *Pasteurella*, *Bibersteinia*, and *Mannheimia*, the role of these agents in the initiation and/or the spread of disease is unclear. Epidemiologic data implicate domestic sheep (*Ovis aries*) as the direct or indirect source of the contagious component of Bighorn sheep pneumonia. Recent studies have strongly correlated *Mycoplasma ovipneumoniae*, an organism endemic in domestic sheep, with respiratory disease outbreaks in Bighorn sheep. An obstacle to testing the hypothesis that *M. ovipneumoniae* is a primary pathogen of epidemic bighorn respiratory disease is the difficulty in accurately detecting this organism and differentiating it from other mycoplasmas. This presentation will review the optimal sampling, laboratory testing, and strain typing methods for detecting *M. ovipneumoniae*.

Mycoplasmas are bacteria that are common commensals of the mucous membranes of most vertebrates. While most are non-pathogenic, others can be primary or opportunistic pathogens and cause significant diseases. Multiple mycoplasma species are often present in an animal, requiring that any mycoplasma isolates be accurately speciated. Mycoplasmas differ from other bacteria in that they lack cell walls, and are thus sensitive to desiccation and osmotic changes and do not persist well outside of their hosts. They are also very small with limited genetic coding potential and metabolic capabilities, and thus require a large number of nutrients be provided from their environment. These physical and biochemical properties of mycoplasmas mean that special care is needed to maintain optimal viability during transport the laboratory, and that once in the laboratory mycoplasmas are highly fastidious, slow and often very difficult to grow, and exhibit limited biochemical reactivity that can be used to identify/speciate them.

For the detection of *M. ovipneumoniae*, in live sheep nasal swabs are more rewarding than oropharyngeal swab samples (both can be collected and pooled as one sample). In clinically affected sheep at necropsy, tissue samples or exudates from sites with lesions, including the lungs, middle ear canals, and paranasal sinuses are preferable. Although swabs can be used, tissues or exudates (several grams or milliliters, respectively) are preferable from necropsied animals. Swabs made of wood and/or cotton should be avoided as these can be inhibitory to mycoplasma. In our experience, the ideal transport media appears to be one of the growth broths normally used for mycoplasma culture. Alternatively, transport systems specifically formulated for mycoplasma can be used. The least desirable, although these often work, are the standard bacterial transport systems used for detection of *Pasteurella*. In all cases, samples should be kept cool and shipped to laboratory as soon as possible – optimally, by the next day.

M. ovipneumoniae is particularly challenging to isolate and identify in the laboratory. While *M. ovipneumoniae* will grow relatively well in a number of mycoplasma growth broths, they often do not grow on agars of the same formulations. This has created a problem since the typical indicator of a culture being designated positive or negative is visible growth on the agar plate – thus, this procedure will give a high percentage of false negatives. For this reason, WADDL is now detecting growth of *M. ovipneumoniae* by performing PCR on the broth cultures. This method is giving much more consistent results. At this time, PCR for *M. ovipneumoniae* done directly on clinical samples (i.e. without initial broth culture amplification) has not been validated and is not offered by WADDL. Another consideration when interpreting *M. ovipneumoniae* culture results is there often are multiple mycoplasma species present in the sample and that grow in the broth, but only the non-*M. ovipneumoniae* isolates grow on the agar plates. In our experience with domestic and Bighorn sheep, the most common such mycoplasma is *Mycoplasma arginini*. Thus, isolates from agar plates, for example to be used to bank isolates or for other studies, need to be re-isolated and verified as *M. ovipneumoniae*.

Serologic testing for antibodies to *M. ovipneumoniae* can also be used to detect infections based upon host response to infection. Serological testing has advantages over agent detection by PCR in that chronic, previous infection status can be identified even in the absence of active shedding of bacteria. A disadvantage of serologic detection of *M. ovipneumoniae* is that an antibody response may take 7-10 days after infection to be at a high enough level to detect and early infections can be missed. Several antibody tests have been developed by WADDL, including an Indirect Hemagglutination Test (IHA) and a Competitive ELISA (cELISA). The IHA test used initially has now been discontinued due to difficulties with test standardization and performance. Although the IHA appeared to give accurate results for negative and high-titer positive tests, there was less accuracy with low titer positive test results. The IHA test was replaced with the cELISA in the Spring of 2010. Development and initial validation of the new *M. ovipneumoniae* cELISA is now complete, and appears to be much more reliable. Despite the improved methods for detecting *M. ovipneumoniae* antibody, it is still very important to realize that the current antibody testing is validated only for detecting infection in herds, not individual animals. For example, antibody testing should not be used to assess the status of individual animals, and the status of a herd should be interpreted with caution if it only contains a single positive animal.

Strain typing of *M. ovipneumoniae* isolates from Bighorn sheep is possible and can potentially provide important epidemiologic information about the origins and transmission dynamics within and between animals. A number of molecular methods have been used for typing isolates from domestic sheep, and can successfully discriminate isolates from different origins and within affected flocks of sheep. These same methods can also likely be used for Bighorn sheep isolates and provide important epidemiologic information about the origins and transmission dynamics within and between animals. The method currently being evaluated by Washington State University is sequencing of the intergenic spacer region of the ribosomal gene complex. Initial results indicate that this method has good discriminatory power for *M. ovipneumoniae* isolates, and also has the advantage that it does not require isolation and extensive amplification of isolates.

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