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Maria E. Harvey  
*Iowa State University*

Ricardo F. Rosenbusch  
*Iowa State University*

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Determining Preferred Methods of Detection and Genomic Fingerprinting for *Mycoplasma ovipneumoniae*

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Maria E. Harvey, veterinary student; Dr. Ricardo F. Rosenbusch, professor, department of veterinary microbiology and preventative medicine

**Summary and Implications**

*Mycoplasma ovipneumoniae* infection is a problem among lambs raised in indoor confinement operations. This study looked at different *M. ovipneumoniae* culture and identification techniques to determine preferred methods of detection and genomic fingerprinting. The results indicated that SP4 media and the Hum4 primer AP-PCR system were best for culturing and fingerprinting *M. ovipneumoniae*. Using these methods will allow for studies on transmission of *M. ovipneumoniae* in barn raised lambs.

**Introduction**

*Mycoplasma ovipneumoniae* infections are a common problem among barn housed lambs less than one year in age. *M. ovipneumoniae* colonizes the mucosal surfaces of the respiratory tract resulting in a non-productive cough. This can lead to immnosupression, poor growth, and in severe cases rectal prolapse. The rectal prolapse is especially a problem in lambs with very shortly docked tails as is the custom for youth competitions.

**Materials and Methods**

**Media Study**

Five lambs were sampled from a central Iowa flock for *Mycoplasma ovipneumoniae* by taking a sample from the pharyngeal tonsil area. A sterile cotton swab was guided with an oral speculum to the area of the pharyngeal tonsil region. The swabs were placed in a sterile 15mL test tube and transported on ice from the farm to the laboratory. The swabs were then removed from the test tube using sterile forceps and gently rolled onto SP4 media and Friis media. Both media are selective for *Mycoplasma spp.* growth and contained antibiotics to suppress growth of contaminants. The plates were incubated at 37°C with 5% carbon dioxide for four days. The plates were then examined for the presence of *M. ovipneumoniae* by looking for the characteristic colony morphology of a center-less fried egg appearance.

**Primer Study**

Samples from the pharyngeal tonsil area were obtained from flocks in the central Iowa area using the method described in the media study. The samples were plated on Mycoplasma selective agar and incubated as described above. *M. ovipneumoniae* colonies were then picked from the plates and placed into Mycoplasma selective broth and incubated for two days at 37°C with 5% carbon dioxide. The broth was then passed through a 0.45 μ nitrocellulose filter, re-plated on Mycoplasma selective agar and incubated as described in the media study. Two *M. ovipneumoniae* colonies were picked from each animal sampled, placed in Mycoplasma selective broth and incubated for two days. The *M. ovipneumoniae* DNA was then extracted from the culture pellet and prepared for AP-PCR analysis. The DNA was either amplified with the Hum4 primer or the REP1 and REP2 primer pair. The cycling conditions for the Hum4 primer were as follows: Lid at 105°C, 1x 94°C for 2 minutes, 40x (94°C for 30 seconds, 37°C for 60 seconds, 72°C for 90 seconds), 1x 72°C for 4 minutes, pause at 4°C. The cycling conditions for the REP1 and REP2 primers were as follows: Lid at 105°C, 1x 94°C for 2 minutes, 35x (94°C for 30 seconds, 40°C for 90 seconds, 72°C for 120 seconds), 1x 72°C for 2 minutes, pause at 4°C. The PCR samples were then electrophoresed on a 1.5% agarose gel for one hour at 140 volts. The gel was stained using ethidium bromide and photographed.

**Results**

**Media Study**

A total of thirty six samples from five lambs were plated with eighteen samples on SP4 and eighteen samples on Friis media. Twenty-four of the plates were positive for *Mycoplasma spp.* growth and seventeen plates swabbed contained *M. ovipneumoniae* growth. Twelve of the plates with *M. ovipneumoniae* growth were plates with SP4 media, indicating that this media allowed better recovery of this mycoplasma.

**Primer Study**

The REP1 and REP2 primers never produced good quality detectable bands. When the gel was maximally loaded with the REP1 and REP2 PCR products, weak bands were observed.
bands were detected, but they were of poor quality for computer analysis. The gels loaded with the Hum4 primer product gave good quality bands that could be analyzed using computer software. Depending on the colony lineage analyzed nine to twenty bands were produced by Hum4 amplification.

Figure 1. Gel created from *M. ovipneumoniae* DNA amplified using AP-PCR with the Hum4 primer.

**Discussion and Conclusion**

The results of the media trial have demonstrated a preferred media to be used when attempting to culture *Mycoplasma ovipneumoniae*. All the sheep sampled were positive for *M. ovipneumoniae*. All sheep sampled had at least one *M. ovipneumoniae* positive sample on SP4\textsuperscript{3+} media, but not all *M. ovipneumoniae* positive sheep had samples grow on the Friis\textsuperscript{3+} media. This would indicate that SP4\textsuperscript{3+} media is the better choice of media to use when trying to culture *Mycoplasma ovipneumoniae*.

The results of the primer trial favored the use of the Hum4 primer over the REP1 and REP2 primers when performing AP-PCR on DNA extracted from *Mycoplasma ovipneumoniae*. There were nine to twenty bands produced after gel electrophoresis using the Hum4 primer and these bands were strong enough to be analyzed using computer software. There were relatively few bands produced after gel electrophoresis using the REP1 and REP2 primers and these bands were too weak to be easily analyzed using computer software.

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