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

A.S. Leaflet R2140

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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 $SP4^{3+}$ 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. Samples from the pharyngeal tonsilar area of lambs from flocks in the central Iowa area were obtained and used to determine if there was any difference in growth of *M. ovipneumoniae* on two different Mycoplasma selective media, $SP4^{3+}$ and modified $Friis^{3+}$. Both media contain three bacterial inhibitors, cefobid, bacitracin, and thallium acetate. *M. ovipneumoniae* DNA was extracted from the culture samples and used to determine a preferred primer to use when amplifying the DNA for AP-PCR fingerprinting.

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 37ºC with 5% carbon dioxide. The broth was then passed through a .45µ 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 105ºC, 1x 94ºC for 2 minutes, 40x (94ºC for 30 seconds, 37ºC for 60 seconds, 72ºC for 90 seconds), 1x 72ºC for 4 minutes, pause at 4ºC. The cycling conditions for the REP1 and REP2 primers were as follows: Lid at 105ºC, 1x 94ºC for 2 minutes, 35x (94ºC for 30 seconds, 40ºC for 90 seconds, 72ºC for 120 seconds), 1x 72ºC for 2 minutes, pause at 4ºC. The PCR samples were then electorphoresed on a 1.5% argarose gel for one hour at 140 volts. The gel was stained using ethidium bromide and photographed.

Results

Media Study

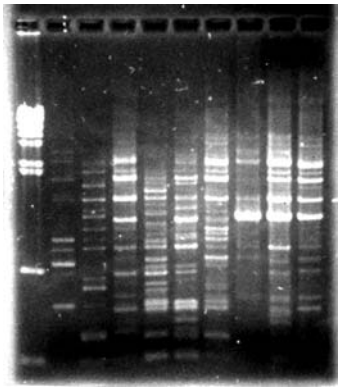
A total of thirty six samples from five lambs were plated with eighteen samples on $SP4^{3+}$ and eighteen samples on $Friis^{3+}$. Twenty-four of the plates were positive for *Mycoplasma spp*. growth and seventeen plates swabbed contained *M. ovipneumoniae* growth. Twelve of the plates with *M. ovipneumoniae* growth were plates with $SP4^{3+}$ 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 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 $SP4^{3+}$ media, but not all *M. ovipneumoniae* positive sheep had samples grow on the $Friis^{3+}$ media. This would indicate that $SP4^{3+}$ 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.

Acknowledgement

This study was supported in part with a grant from the Iowa Livestock Health Advisory Council.