Short Communication

Study on Mycoplasmal pneumonia at the Ziaran abattoir

Ezzi1*, A., Pourbakhsh2, S.A. Moradi bidhendi3, S.
1. Department of Pathology, Razi Vaccine and Serum Research Institute, Karaj, Iran
2. Mycoplasma Reference Laboratories, Razi Vaccine and Serum Research Institute, Karaj, Iran
3. Department of Bacteriology, Razi Vaccine and Serum Research Institute, Karaj, Iran

ABSTRACT

In a survey of pneumonia due to Mycoplasma 282 out of 12168 ovine and caprine lung condemnation were collected (2.32%). Mycoplasma spp. has been isolated from pneumonic cases in 4 sheep and 2 goats. PCR studies were confirmed the genus of Mycoplasma although attempting for identification of strains M. mycoides, M. capricolum/caprine pleuropneumonia and M. arginin were in failure. The lesions initially showed raised consolidation at the right cranio-ventral lobes. Histopathological observations revealed purulent interstitial pneumonia & bronchitis (33.33%) purulent bronchopneumonia (33.33%) purulent fibrinous pneumonia (16.6%) and progressive pneumonia (16.6%). This approach has the potential to allow the recognition of genus of Mycoplasma as a primary factor for inducing pneumonia.

Keywords: Pneumonia, Broncho pneumonia, Mycoplasma spp., PCR

INTRODUCTION

Mycoplasma respiratory disease has a special place in veterinary medicine (Stalheim 1983). Sheep and goats are an important production animals for milk and meat. Economic losses associated with the disease are often the result of a complex interaction between infection, poor management and environment condition. Lung tissue condemnation data has been reported in many abattoirs (Stewart 1970, Jonas et al 1991, Daniel et al 2006). Mycoplasmas have specific attachment among the mucociliary system of the bronchiols (Cheville 1983). M. capriculum subsp. Capripneumonia is the causative agent of caprine pleuropneumonia (CCPP) affects goats as high as 100 with mortality of 70% (McMartin et al 1980) showing extensive cross-reactions to mycoplasma strain F38 (Bolske et al 1988). M. mycoides and M. arginin were isolated in 5 goat herds (20%) (C. De la Fe et al 2005). This study describes isolation and histopathology of Mycoplasmal pneumonia in sheep and goats during one year (2004-2005) at the Ziaran abattoir.

MATERIALS AND METHODS

10129 sheep and 2039 goats were inspected at the Ziaran abattoir (100 km far from Tehran) weekly within the year (fall 2005 - fall2006). Totally 282
lung tissue condemnation showed consolidation which were collected and transferred to the Pathology department at the Razi institute.

**Pathology.** Lung tissue for histological examination were fixed in 10% formalin. Sections were cut at 5 µm and stained with Haematoxylin & Eosin. Samples were collected from lungs for *Mycoplasma* isolation. Mycoplasma cultured on PPLO Broth Media 3 to 7 days incubating at 37°C and then across the surface of an agar plate before colonies were apparent. The positive colonies detected every 48 hours. After 72 hours the first positive cases were confirmed. (Hirsh & Chung Zee 1999). DNA extraction. 1 ml of each sample was transferred to Eppendorf tube and extracted DNA in accordance with Erami *et al* 2007.

**Polymerase Chain Reaction (PCR).** Studies were carried out on DNA from samples of *Mycoplasma*. Three published primer sets were used for the specific detection of genus and strains of M. mycoides, capricolum/caprine pleuropneumonia, M. arginini (East *et al* 1983, Ros Bascunana *et al* 1994, Bolske 1996). In genus as follow: (Biotech. Co.) F: 5'-GCT GCG GTG AAT ACG TTC T-3' R: 5'-TCC CCA CGT TCT CGT AGG G-3'. The 163 bp fragments were amplified. In strains of mycoides/capricolum /pleuropneumonia (CCPP) as follow: (Isogen Co.). MmF: 5'-CGA AAG CGG CTT ACT GGC TTG TT -3'. MmR: 5'-TTG AGA TTA GCT CCC CT CAC AG-3'. The 548 bp fragments were amplified. Visualization of amplified products was done by UV illumination after electrophoresis on 1% agarose gel and ethidium bromide staining. In strain of arginini as follow: (Isogen Co.) MAGF 5'-GCA TGG AAT CGC ATG ATT CCT-3'. GP4R:5'-GGT GTT CTT CCT TAT ATC TAC GC-3'. The 545 bp fragments were amplified. PCR was performed in 25 µl containing 2.5 µl of 10 X PCR buffer, 4 µl of 25 mM MgCl₂, 0.75 µl of 10 mM dNTPs, 0.15+0.15 µl each primer, 0.1 U Taq DNA polymerase. Consequently 17.35 µl of extracted DNA as template was carried out. Visualization of amplified products was done by UV illumination after electrophoresis on 1% agarose gel and ethidium bromide staining.

**RESULTS AND DISCUSSION**

282 out of 12168 ovine and caprine lung condemnation collected from the Ziaran abattoir weekly within the year 2005-2006 (2.32%). Grossly the pneumatic lesions were involved mostly at the right cranial lobes except in one case which observed in 3 lobes (right and left cranial and left caudal). *Mycoplasma spp.* has been isolated and showing as a fried egg shaped colonies from pneumatic cases in 4 sheep and 2 goats (Figure 1).

![Figure 1. Showing fried egg shaped colonies on the surface of an agar plate.](image)
fact 6 bands related to 6 samples included lanes 4, 5 (goats), lanes 6, 7, 8 and 9 (sheep) proved to be positive although the lane 9 (no.389) is weaker than others (Figure 2).

Figure 2. Agarose gel electrophoresis of PCR products. Lane 1: DNA ladder 100bp. Lane 2: DNA control positive. Lane 3: DNA control negative. Lane 4: DNA extracted from goat sample (no.583). Lane 5: DNA extracted from goat sample (no.584). Lane 6: DNA extracted from sheep sample (no.149). Lane 7: DNA extracted from sheep sample (no.279). Lane 8: DNA extracted from sheep sample (no.347). Lane 9: DNA extracted from sheep sample (no.389).

The identification of strains of M. capriculum, M. mycoides, M. arginini and F38 by using their primers was failed. In fact detecting with the other strains such as M.agalactiae and M. putrefaciens for goats and M.agalactiae and M.ovipneumonia for sheep needs their primers in further studies in future time. Histopathological observations revealed obliteration of bronchiols, bronchial hyperplasia, interstitial pneumonia with purulent bronchopneumonia and fibrin deposition within the alveolar septa in goats and pre- bronchial lymphoid cell infiltration (Figure 3).

Table 1. Relative frequency distribution of affected cases to pneumonia at the Ziaran abattoir.

<table>
<thead>
<tr>
<th>condition</th>
<th>normal</th>
<th>infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>sheep</td>
<td>9872</td>
<td>97.46</td>
</tr>
<tr>
<td>goat</td>
<td>2014</td>
<td>98.78</td>
</tr>
<tr>
<td>total</td>
<td>11886</td>
<td>97.68</td>
</tr>
</tbody>
</table>

Bronchitis/bronchiolitis and intra-lobular fibroblast in sheep were also prominent. Consequently, purulent interstitial pneumonia and bronchitis (33.33%) purulent bronchopneumonia (33.33%) purulent fibrinous pneumonia (16.6%) and progressive pneumonia (16.6%). Relative frequency of pneumonia in sheep comparable with goats was 2.54% versus 1.22% (Table 1). The Respiratory System is sites of infection of Mycoplasma spp. They are capable of destroying the cilia of the epithelial cells of bronchials and then predisposing to secondary bacterial invasion such as Pasteurella spp. (Quinn et al 1994) .As far as our knowledge the major important microscopic lesion for studying mycoplasmosis is pre-bronchial lymphoid cell infiltration as well as purulent bronchopneumonia which are in accordance with Bolske et al 1988 although the authors had not seen the former lesion in goats.
Acknowledgment

The authors gratefully thank Ms. Eramie for technical assistance at the Mycoplasma Reference Laboratory, Razi Vaccine and Serum Research Institute.

References


