

Short Communication

Study on Mycoplasmal pneumonia at the Ziaran abattoir

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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, Ionas *et al* 1991, Daniel *et al* 2006). *Mycoplasmas* have specific attachment among the mucociliary system of the bronchiols (Cheville 1983). *M. capricolum 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. arginini* were isolated in 5 goat herds (20%) (C. De la Fe *et al* 2005). This study describes isolation and histopathology of *Mycoplasma 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 - fall 2006). Totally 282

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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 μ m and stained with Haematoxylin & Eosin. Microbiology. 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 CTT 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 μ l containing 2.5 μ l of 10 X PCR buffer, 4 μ l of 25 mM MgCl₂, 0.75 μ l of 10 mM dNTPs, 0.15+0.15 μ l each primer, 0.1 U Taq DNA

polymerase. Consequently 17.35 μ l 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 pneumonic 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 pneumonic cases in 4 sheep and 2 goats (Figure 1).

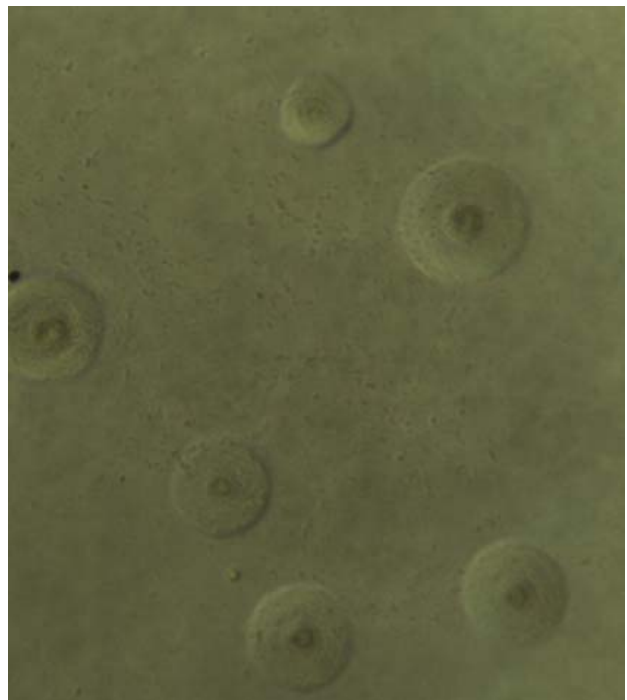


Figure 1. Showing fried egg shaped colonies on the surface of an agar plate.

All the cultures had been analyzed positive results in the PCR in their genus before they were attempted to identify the strains of *Mycoplasma*. In

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).

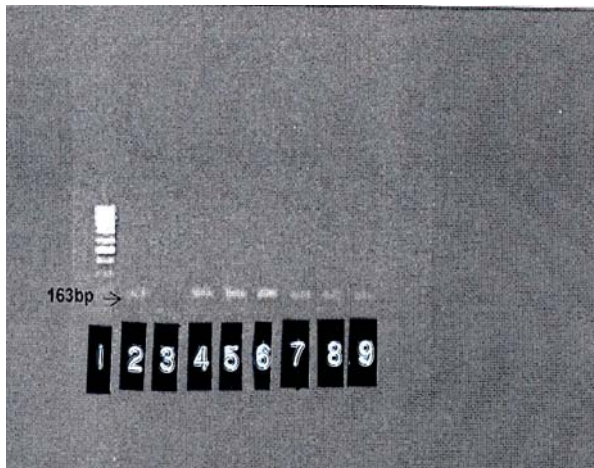


Figure 2. Agarose gel electrophoresis of PCR products, *Lane 1:* DNA ladder 100bp. *Lane 2:* DNA control positive. *Lane 3:* DNA control negative. *Lane 4:* DNA extracted from goat sample (no.583). *Lane 5:* DNA extracted from goat sample (no.584). *Lane 6:* DNA extracted from sheep sample (no.149).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

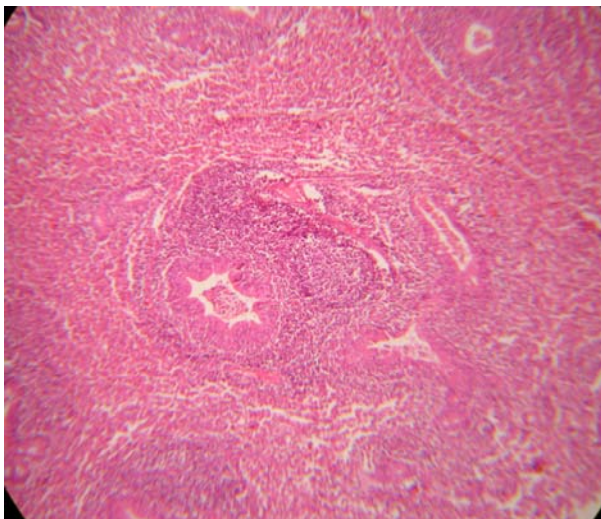


Figure 3. showing pre-bronchial lymphoid cell infiltration in lung tissue , No.279 H&E 10X.

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.

condition	normal		infected		total	
	No.	%	NO.	%	NO.	%
sheep	9872	97.46	257	2.54	10129	83.25
goat	2014	98.78	25	1.22	20.39	16.75
total	11886	97.68	282	2.32	12168	100

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.

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References

- Bölske, G., Mattsson, J.G., Bascunana, C.R., Bergstrom, K., Wesonga, H. and Jöhansson, K.E. (1996). Diagnosis of contagious caprine pleuropneumonia by detection and identification of *Mycoplasma subsp. capripneumoniae* by PCR and restriction enzyme analysis. *Journal Clinical Microbiology* 34 (4) :785-791.
- Bölske, G., Msami, H., Humlesjö, N.E., Erno, H. and Jönsson, L. (1988). Mycoplasma capricolum in an outbreak of Polyarthritits and Pneumonia in Goats *Acta Veteinary Scandinavica* 29 (3-4): 331-338.
- C. De la Fe Assuncao, P., Antunes, T., Rosales, R.S. and Poveda, J.B. (2005). Microbiological survey for *Mycoplasma spp.* in a contagious agalactia endemic area. *The Veterinary Journal* 170: 257-259.
- Cheville N.F.(1983). *Cell Pathology* (2nd edn.), Pp: 539. The Iowa State University Press, Ame, Iowa, USA..
- Daniel, J.A., Held, J.E., Brake, D.G., Wulf, D.M. and Epperson, W.B. (2006). Evaluation of the prevalence and onset of lung lesions and their impact on growth of Lambs. *American Journal of Veterinary Research* 67 (5): 890-894.
- East, N.E., DaMassa, A.J., Logan, L.L., Brooks, D.L. and McGowan, B. (1983). Milkborne outbreak of mycoplasma mycoides subspecies mycoides infection in a commercial goat dairy. *Journal of American Veterinary Medicine Association* 182 (1):1338-1341.
- Erami, M., Hashemi, S.J., Pourbakhsh, S.A., Shahsavandi, S. Mohammadi, S. Shoshtari, A.H. and Jahanshiri, Z. (2007). Application of PCR on detection of aflatoxinogenic molds. *Archive of Razi Institute* 62 (2): 95-100.
- Hirsh, D.C. and Chung Zee, Y. (1999). *Veterinary Microbiology* (1st edn.). Pp :165-172. Blackwell, Science Inc. USA.
- Ionas, G., Clarke, J.K. and Marshall, R.B. (1991). The isolation of multiple strains of mycoplasma ovipneumoniae from individual pneumonic sheep lungs. *Veterinary Microbiology* 29: 349-360 .
- McMartin, D.A., MacOwan, K.J. and Swift, LL. (1980). A century of classical contagious pleuropneumonia : from original description to aetiology. *British Veterinary Journal* 136: 507-515.
- Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994). *Clinical Veterinary Microbiology*. Pp.:320-326. Wolf Publishing, Mosby Co. U.S.A.
- Ros Bascunana, C., Mattsson, J.G., Bolske, G. and Erik Johansson, K. (1994). Characterization of the 16S rRNA Genes from Mycoplasma sp. Strain F38 and development of an identification system based on PCR, *Journal of Bacteriology* 176 (9):.2577-2586.
- Stalheim, H.V. (1983). Mycoplasmal respiratory diseases of ruminants: A review and update. *Journal of American Veterinary Medicine Association* 182 (4): 404-406.
- Stewart, D.A. (1970). A computer system for processing inspection data for animals slaughtered for human consumption in abattoir in Northern Ireland. *Record of Agricultural Research (Belfast)* 18: 117-124.