The Pharma Innovation Journal 2023; SP-12(9): 785-790

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277-7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.23**
TPI 2023; SP-12(9): 785-790

www.thepharmajournal.com
Received: 01-06-2023
Accepted: 05-07-2023

**Gurusrinath Reddy M**
PG scholar,
Department of Microbiology,
CVSc, Tirupati, Andhra Pradesh, India

**Vijayalakshmi S**
Associate professor,
Dept of Microbiology,
CVSc, Tirupati, Andhra Pradesh, India

**Raniprameela D**
Professor and Head,
State Level Diagnostic Laboratory, Tirupati, Andhra Pradesh, India

**Sailaja N**
Professor,
Department of Pathology.
CVSc, Tirupati, Andhra Pradesh, India

**Corresponding Author:**
**Vijayalakshmi S**
Associate professor,
Dept of Microbiology,
CVSc, Tirupati, Andhra Pradesh, India

# Studies on *Mycoplasma ovipneumoniae* infection and its associated bacterial pathogens in pneumonic sheep

**Gurusrinath Reddy M, Vijayalakshmi S, Raniprameela D and Sailaja N**

**Abstract**
In the present study a total of 148 lung tissues from sheep with gross pathological lesions like congestion, consolidation and hepatization were collected in a micro centrifuge tube containing PPLO broth during postmortem examination and slaughter. Out of 148 lung tissues, 52 samples were found to be positive for genus *Mycoplasma* yielding 280 bp product. Out of 52 *Mycoplasma* positive samples 53.84% (28/52) lung tissues were found to be positive for *Mycoplasma ovipneumoniae*. Cultural and biochemical isolation studies of 28 lung swabs from *Mycoplasma ovipneumoniae* positive samples on selective media *viz*., blood agar, brain heart infusion agar, Macconkey agar, mannitol salt agar revealed *Pasteurella multocida* 7/28(25%), *Mannheimia haemolytica* 5/28(17.85%), *E. coli* 9/28(32.14%), *Klebsiella* 6/28(21.4%), *S. aureus* 10/28(35.71%) and *Streptococcus* spp 7/28(25%).

**Keywords:** *Mycoplasma ovipneumoniae*, bacterial pathogens, pneumonic sheep

## 1. Introduction
Pneumonia is one of the most common disease of sheep, in all animal husbandry conditions, throughout the world. The etiology of pneumonia in lambs is extremely complex and relates to synergistic effects of both management practices and infectious agents. Though a wide variety of microorganisms have been recovered from the respiratory tract of pneumonic sheep, important pathological agents include bacteria like *Mycoplasma ovipneumoniae, Mannheimia haemolytica, Pasteurella multocida, Klebsiella pneumonia* and *Streptococcus spp* and viruses like the *Maedi visna-virus, Para-influenza type 3 virus* (PI-3), *Adenoviruses* and *Respiratory syncytial virus* (Khan and Mumtaz *et al.*, 1993) [9].

Pneumonia caused by *Mycoplasma ovipneumoniae* is of prime importance in sheep. *Mycoplasma ovipneumoniae* causes atypical non progressive proliferative pneumonia in lambs and is frequently isolated from lung, trachea, nose and occasionally from eyes of sheep with pneumonia and found in respiratory tract of healthy sheep (DaMasa *et al*., 1992) [2]. The main clinical signs of atypical pneumonia include fever, pyrexia, hyperthermia, weight loss, coughing and purulent nasal discharges (Dassanayake *et al*., 2010) [8]. Atypical pneumonia is characterized grossly by irregular and clearly demarcated areas of consolidation with red-brown discoloration in the cranioventral lobes of lungs. Microscopically, it is characterized by lymphoid proliferation around airways, bronchiolar epithelial hyperplasia, and neutrophil accumulation in airspaces (Lindstrom *et al*., 2018) [15]. *Mycoplasma ovipneumoniae* was mainly transmitted via respiratory tract through close contact between animals and the lambs were infected within a few days of birth (Lindstrom *et al.* 2018) [15]. *M. ovipneumoniae* induces the loss of mucociliary defense of the respiratory tract and facilitates the rapid proliferation and descent of *M. haemolytica* and other upper respiratory pathogens into the lower respiratory tract and induces fatal bronchopneumonia.

## 2. Materials and Methods
### 2.1 Collection of samples
Lung tissues and lung swabs were collected during necropsy and from dead sheep at slaughter houses. A total of 148 lung tissues were collected from dead sheep showing gross pathological lesions of congestion, consolidation and hepatization in a micro centrifuge tube containing PPLO broth and brought to the laboratory over ice maintaining cold chain. The samples were then immediately incubated at 37 °C in $CO_2$ incubator maintaining 5% $CO_2$ for 48 h. The other set of samples are collected in 10% formalin for further histopathological examination.

A total of 148 lung swabs were collected in duplicates and one set was transfers into a sterile test tube containing BHI broth and another set in nutrient broth and was brought to the laboratory immediately and incubated at 37 °C.

### 2.2 DNA extraction from lung tissues

Extraction of DNA from lung tissues was carried out as per the method described by Liu *et al*. (2001) [16]. Approximately 2 gm of tissues were taken from each sample and homogenized by adding 2 ml of TE buffer in mortar and pestle. The homogenized suspension was allowed to settle in micro centrifuge tubes for 10 min. The supernatant was collected and centrifuged at 10,000 rpm for 10 min. The pellet was dissolved in 100µl of TE buffer and then boiled for 10 min. Immediately, the samples were chilled by keeping on ice. After cooling, the lysate was centrifuged at 10,000 rpm for 2 min. The supernatant containing DNA was collected and stored at -20 ºC until further use.

### 2.3 Detection of 16S rRNA gene of genus *Mycoplasma* and *Mycoplasma ovipneumoniae*

The oligonucleotide primers were obtained from Sigma Aldrich India Pvt. Ltd., Bangalore. Details of the primer sequence are enlisted in Table 1. For PCR amplification, 5 µl of DNA was added to 20µl reaction mixture containing 200µM of dNTPs, 0.2µM of each primer, 1.875 mM of $MgCl_2$, and 1.25 U of Taq DNA polymerase in 1×PCR buffer. PCR conditions for *Mycoplasma* included the initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30s, annealing at 56 °C for 30s, extension at 72 °C for 30s, and the final extension at 72 °C for 10 min. PCR conditions for the *Mycoplasma pneumoniae* comprised initial denaturation at 94 °C for 5 min, followed by 30 cycles consisted of denaturation at 94 °C for 30s, annealing at 55 °C for 30s, extension at 72 °C for 30 , and the final extension at 72 °C for 7 min. The amplified PCR products (5 µl) were separated in agarose gel (1.5% w/v) stained with ethidium bromide (0.5 µg/ml) by running in horizontal submarine electrophoresis unit using 1×TAE as running buffer and examined under the gel documentation system.

### 2.4 Isolation and identification of bacterial pathogens associated with *Mycoplasma ovipneumoniae*

The lung swabs collected from lung tissues which were found to be positive for *Mycoplasma ovipneumoniae* were further processed for isolation of bacteria. The lung swabs collected in BHI broth were further processed for isolation of *Pasteurella multocida* and *Mannheimia haemolytica*. Similarly, the lung swabs collected in nutrient broth were further processed for isolation of other bacterium causing respiratory infections in sheep.

#### 2.4.1 Isolation of *Pasteurella multocida* and *Mannheimia haemolytica* from lung swabs

Following incubation of lung swabs in BHI broth at 37 °c for 24 h, the sample subsequently cultured on blood agar. The inoculated plates were incubated aerobically for 24-48 h at 37°c, the plates were examined for bacterial growth and the colonies were examined for colony morphology, grams staining and biochemical test like oxidase test, catalase test, nitrate reduction and indole test.

##### 2.4.1.1 DNA extraction from blood agar grown bacterial culture

Extraction of DNA from phenotypically characterized *Pasteurella multocida* and *Mannheimia haemolytica* colonies on blood agar/BHI was carried out as per the method described by Ozbey *et al*. (2004) [19]. Briefly a loopful of bacterial culture was taken from identified colony with sterile inoculation loop and resuspended in 500µl of sterile PBS. The samples were subjected for centrifugation at 10,000 rpm for 1 min at room temperature. Cell pellet was washed twice in 1ml of sterile PBS and resuspended in final volume of 100µl of PBS. The cell suspension was boiled at 95°c for 10 min in water bath and immediately chilled on ice. After cooling the lysate was centrifuged at 13000 rpm for 2 min in a refrigerated centrifuge. The sample was used as template for PCR.

##### 2.4.1.2 Detection of 16S rRNA gene of *Pasteurella multocida* and *Mannheimia haemolytica*

The oligonucleotide primers were obtained from Sigma Aldrich India Pvt. Ltd., Bangalore. Details of the primer sequence are enlisted in Table 1. For PCR amplification, 5 µl of DNA was added to 20 µl reaction mixture containing 200 µM of dNTPs, 0.2 µM of each primer, 1.875 mM of $MgCl_2$, and 1.25 U of Taq DNA polymerase in 1×PCR buffer. PCR conditions for *Pasteurella multocida* included the initial denaturation at 94 °C for 3 min, followed by 30 cycles consisted of denaturation at 94 °C for 30 s, annealing at 57 °C for 40 s, extension at 72 °C for 60 s, and the final extension at 72 °C for 10 min. PCR conditions for the *Mannheimia haemolytica* comprised initial denaturation at 95 °C for 3 min, followed by 30 cycles consisted of denaturation at 95 °C for 60 s, annealing at 48 °C for 60 s, extension at 72 °C for 30 s, and the final extension at 72 °C for 5 min. The amplified PCR products (5 µl) were separated in agarose gel (1.5% w/v) stained with ethidium bromide (0.5 µg/ml) by running in horizontal submarine electrophoresis unit using 1×TAE as running buffer and examined under the gel documentation system.

**Table 1:** Primers used for detection of Pathogens in the study

| Primers | Primer Name | Nucleotide Sequence | Amplicon Size |
|---|---|---|---|
| *Mycoplasma* genus specific primer (Cetinkya *et al*., 2009) [6] | GPO3F | 5'-TGGGGAGCAAACAGGATTAGATACC-3' | 280 bp |
| | MGSO | 5'-TGCACCATCTGTCACTCTGTTAACCTC-3' | |
| *Mycoplasma ovipneumoniae* (McAuliffe *et al*., 2003) [17] | LMF1 | 5'-TGAACGGAATATGTTAGCTT-3' | 361 bp |
| | LMR1 | 5'-GACTTCATCCTGCACTCTGT-3' | |
| *Pasteurella multocida* (Townsend *etal*. 1998) | KMT1T7 | 5'-ATCCGCTATTTACCCAGTGG-3' | 460 |
| | KMT1SP6 | 5'-GCTGTAAACGAACTCGCCAC-3' | |
| *Mannheimia haemolytica* (Hawari *et al*.2008) | PHSSA F | TTCACATCTTCATCCTC | 325 |
| | PHSSA R | TTTTCATCCTCTTCGTC | |

### 2.4.2 Isolation of other bacteria from lung swabs

The lung swabs collected in nutrient broth were incubated at 37 °C for 24 h. The broth culture was streaked on nutrient agar, Mac Conkey agar, eoisin methylene blue agar, Edwards medium and Mannitol salt agar and incubate at 37 °C for 24 hrs. single colonies were identified based on grams staining, colony morphology and biochemical test like catalase test, oxidase test, Indoe test, methyl red test, voges proskauer test and citrate utilization test.

## 3. Results

### 3.1 Molecular detection of Mycoplasma and *Mycoplasma ovipneumoniae*

Out of 148 lung tissue DNA samples screened, 52 samples were found to be positive for genus *Mycoplasma* yielding 280 bp product (fig.1). The samples positive for genus *Mycoplasma* by 16S*r*RNA PCR were subjected to PCR for detecting 16S *r*RNA gene of *Mycoplasma ovipneumoniae*. Out of 52 lung tissue DNA samples 28(53.8%) were found positive for *Mycoplasma ovipneumoniae* yielding 361 bp product (fig.2).

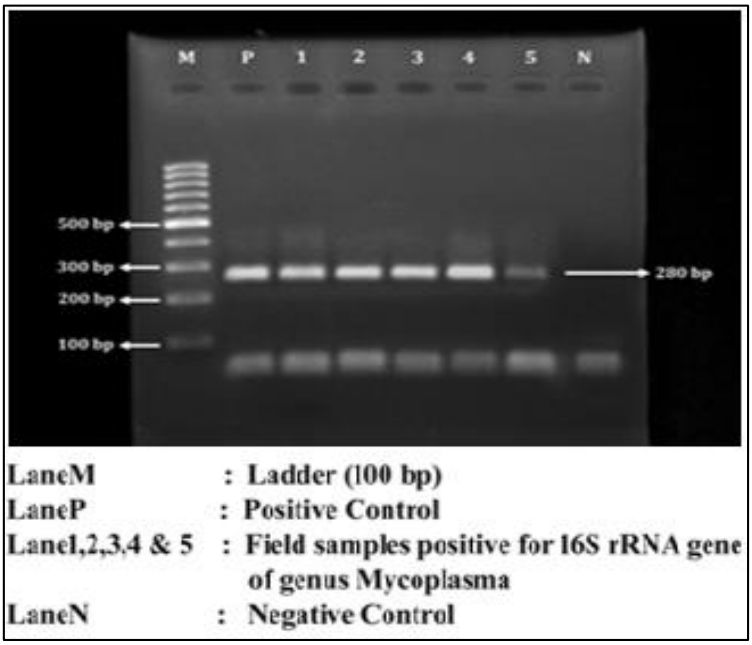


**Fig 1:** Amplification of 16S rRNA gene of genus Mycoplasma from clinical cases of respiratory infections in sheep

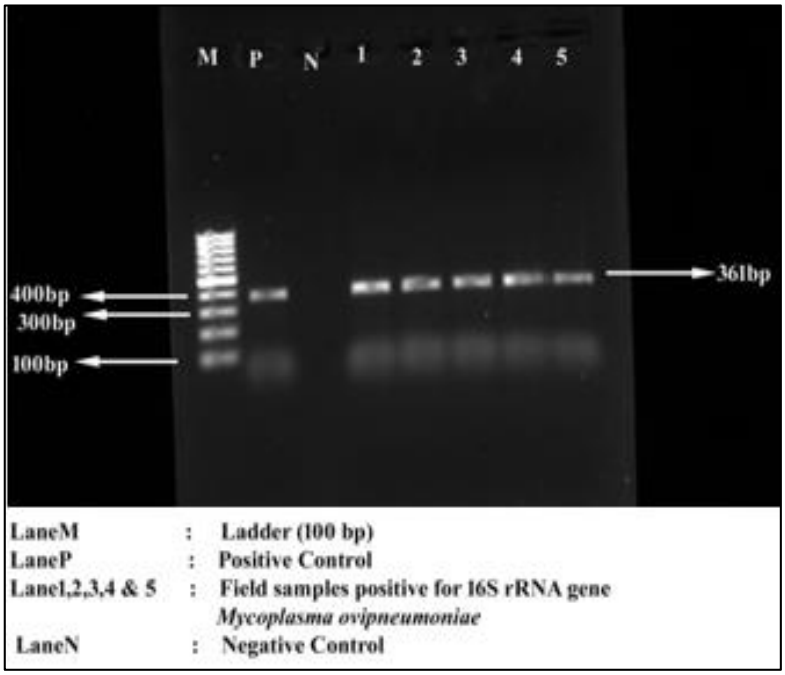


**Fig 2:** Amplification of 16S rRNA gene of genus *Mycoplasma ovipneumoniae* from clinical cases of respiratory infections in sheep

**3.2 Preliminary isolation and identification of bacteria associated with *Mycoplasma ovipneumoniae* in pneumonic lung:** The 28 lung swabs collected from lung tissues which were positive for *Mycoplasma ovipneumoniae* were subjected for preliminary isolation of associated bacteria. Out of which 7(25%) *Pasteurella multocida*, 5(17.85%) *Mannheimia haemolytica*, 9(32.14%) *Escherichia coli*, 6(21.4%) *Klebsiella spp*, 10(35.71%) *Staphylococcus aureus and* 7(25%) *Streptococcus spp* samples were positive.

### 3.3 Cultural characteristics

Out of 28 lung swabs processed for isolation of *Pasteurella multocida*, 7(25%) isolates were recovered. The isolates produced small, greyish, opaque, circular translucent and non haemolytic colonies on blood agar. On BHI, small, white, dew drop like, circular translucent colonies and there was absence of growth on Mac conkey agar**.** *Mannheimia haemolytica*, 5(17.85%) isolates have been recovered in pure cultures. The isolates produced small, greyish, rough, and hemolytic colonies on blood agar. On BHI, small, white, dew drop like, circular translucent colonies and on Mac conkey agar, small, dew drop, pink pinpoint like colonies were observed**.** *E. coli* produced pink colonies on Mac conkey agar and metallic sheen on EMB agar. *Klebsiella* produced mucoid pink colonies on Mac conkey agar. *Staphylococcus* produced yellow-colored colonies on mannitol salt agar. *Streptococci* produced beta hemolysis on blood agar.

### 3.4 Biochemical identification

All the seven isolates of *Pasteurella multocida* showed positive reaction to oxidase, catalase, nitrate reduction and indole production, and negative for MR, VP, citrate utilization and urease tests. On TSI the organism produced yellow slant and yellow butt without production of gas and $H_2S$. All the 5 isolates of *Mannheimia haemolytica* gave positive reaction to oxidase, catalase, nitrate reduction and negative for indole production, MR, VP, citrate utilization and urease tests. On TSI the organism produced red slant and yellow butt without production of gas and $H_2S$.

### 3.5 Molecular confirmation of pure cultures of *P. multocida* and *M. haemolytica*

Phenotypically positive isolates of *P. multocida* and *M. haemolytica* were further confirmed by amplifying KMT1 gene and PHSSA gene with PCR Yeilding 460bp (fig.3) and 325bp (fig.4) respectively.

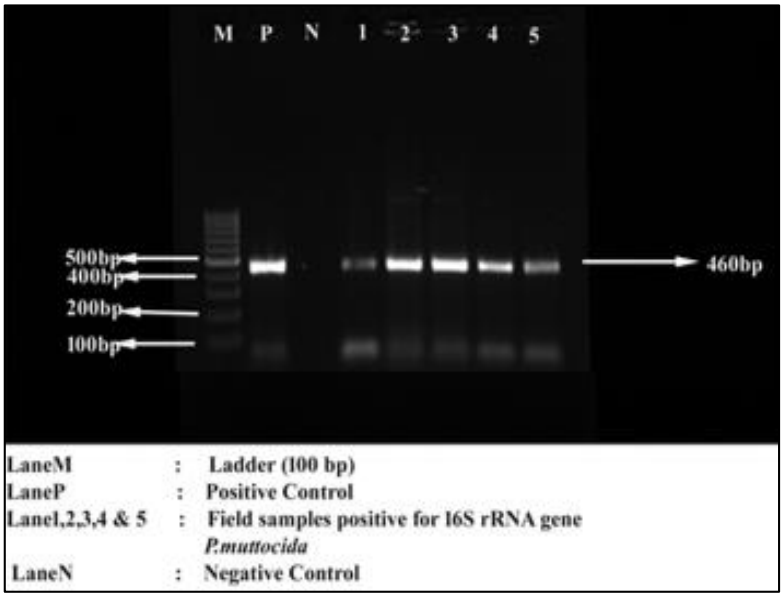


**Fig 3:** PCR Amplification of 460 bp product specific for *P. multocida*

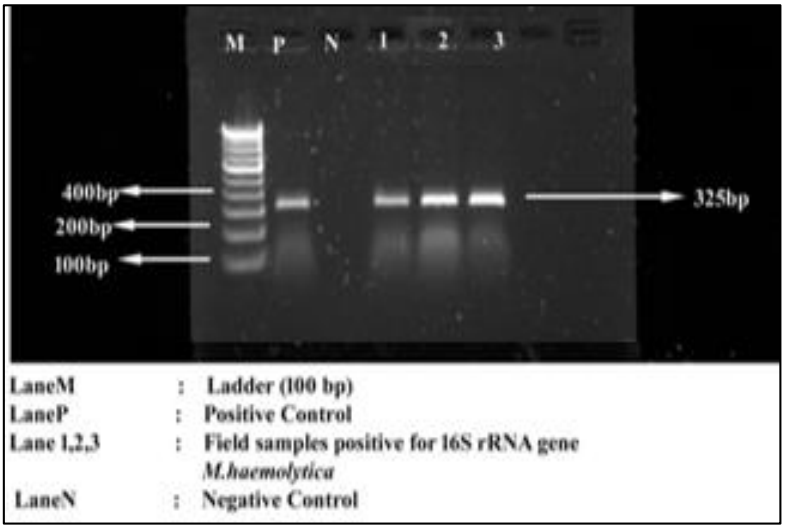


**Fig 4:** PCR Amplification of 325 bp product specific for *M. haemolytica*

## 4. Discussion

The overall occurrence of *Mycoplasma* in sheep lung tissues in the present study was found to be 35.13 percent (52/148). The occurrence of *Mycoplasma* in respiratory infections of sheep in the present study indicated highest rate of incidence 57.14 percent in lambs aged between 0-6 months followed by 28.9 in hoggets aged 6-12 months, 13.96 percent in adult sheep. Abdel helium (2019) [1] and Elshafay (2016) [11]

reported that the occurrence of *Mycoplasma* was more frequent in young animals than adults which is in accordance with the present study. This high incidence rate of *Mycoplasma* in pneumonic cases of sheep in the present study and in others studies indicates a carrier status and difficulty in controlling the disease. The occurrence of *Mycoplasma ovipneumoniae* in *Mycoplasma* positive sheep lung tissues was found to be 53.84 percent (28/52) based on the PCR results. This results are in accordance with 46.71 percent (64/137) of McAuliffe *et al*. (2003) [17] from UK, 44.4 percent of Adehan *et al.,* (2006) [2] from Nigeria and 44.4 percent by Kilik *et al*., (2013) [14]. On the contrary low prevalence was reported by Cheng *et al.* (2015) from China reported 28.8 percent (52/180) and Elnaker *et al*. (2017) [10] from Egypt reported 22.22 percent. Lindstrom *et al*. (2018) [15] reported that 82.92 percent (34/41) which is very high compared to present studies. The occurrence of *Mycoplasma pneumoniae* in lambs 0-6 months, 6-12 months and above one year age was found to be 35.7%, 30.76% and 15.7% respectively. Alley *et al*. (1975) [3] from Newzealand and Ionas *et al*. (1991) [13] reported the incidence of 79 percent and 64 percent in lambs less than six months of age. Elnaker *et al*. (2017) [10] reported that the highest rate of death due to respiratory signs was noticed in lambs aged about 8 months (38.3%) than that of the lambs aged about two months (25.97%). On the contrary Kilic *et al*. (2013) [14] reported the occurrence of *Mycoplasma ovipneumoniae* 37.5 percent in lambs followed by 45 percent in adult sheep. *Pasteurella multocida* is a gram negative bacteria causes respiratory manifestations in all most all animal species. In the present study 25 percent of *Pasteurella multocida* infections are associated with *Mycoplasma ovipneumoniae* infections. Besser *et al*. (2008) [4] reported predominance of *Pasteurella multocida* in Montana lambs from US During the study 17.85 percent of *Mannheimia haemolytica* infections are associated with *Mycoplasma pneumoniae* infections. But higher prevalence of *Mycoplasma pneumoniae* in association with *Mannheimia haemolytica* was reported by Rifatbegovic *et al.* (2011) [20] 60 percent, Lindstrom *et al*. (2018) [15] 63.41 percent, Tauni *et al.* (2017) [22] 63 percent, Sheehan *et al*. (2007) [21] 90 percent and Besser *et al.* (2008) [4] 57 percent of sheep lungs tissues. In the present study a very low percentage (17.85%) of *Mannheimia haemolytica* in association with *Mycoplasma pneumoniae* was reported compared to other studies. Attempts were made for the isolation of associated bacteria in causing pneumonia in the lung tissues. In the present study 10/28 (35.7%) *Staphylococcus aureus*, 9/28 (32.14%) *E. coli*, 6/28 (21.4%) *Klebsiella pneumonia and 7/28(25%) Streptococcus species* were isolated.

Elnaker *et al*. (2017) [10] reported bacterial isolation of *Staphylococcus aureus* (76.9%), *Escherichia coli* (23.07) and *Klebsiella* (46.1%) associated with *Mycoplasma pneumoniae*. Similarly Nicholas *et al*. (2008b) in their studies reported that *M. ovipneumoniae infection* with secondary bacterial infection includes *P. multocida*, *M. haemolytica*, *Staphylococcus* and *streptococcus* infections. Rifatbegovic *et al.* (2011) [20] reported the occurrence of *Streptococcus* spp in *Mycoplasma* o*vipneumoniae* cases.

## 5. Conclusion

*M. ovipneumoniae* was found to be strongly associated with pneumonia in sheep. *M. ovipneumoniae* was detected in 53.8 percent *Mycoplasma* positive pneumonic lungs suggesting important role in forming complexity of pneumonia. *M. ovipneumoniae* occurred predominantly associated with *Pasteurella multocida* compared to *Mannheimia haemolytica* whereas *Staphylococcus, Streptococcus species, Klebsiellla and E. coli* found to be other pathogens associated with *M. ovipneumoniae.* The present study also suggested that *M. ovipneumoniae* may acts as sole Pneumonic pathogen.

## 6. References


1. Abdel Halium MM, Salib FA, Marouf SA, Abdel Massieh ES. Isolation and molecular characterization of *Mycoplasma* spp. in sheep and goats in Egypt, Veterinary World. 2019;12(5):664-670.
2. Adehan RK, Ajuwape ATP, Adetosoye AI, Alaka OO. Characterization of *Mycoplasmas* isolated from pneumonic lungs of sheep and goats. Small Ruminant Research. 2006;63:44-49.
3. Alley MR, Quinlan JR, Clarke JK. The prevalence of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in the respiratory tract of sheep. New Zealand Veterinary Journal. 1975 ;23:137-41.
4. Besser TE, Cassirer EF, Potter KA, Vanderschalie J, Fischer A, Knowles DP, *et al*. Association of *Mycoplasma ovipneumoniae* infection with population-limiting respiratory disease in free-ranging Rocky Mountains bighorn sheep (*Ovis canadensis* canadensis). Journal of Clinical Microbiology. 2008;46:423-430.
5. Cheng C, Jun Q, Qingling M, Zhengxiang H, Yu M, Xuepeng C, *et al.* Serological and molecular survey of sheep infected with *Mycoplasma ovipneumoniae* in Xinjiang, China. Tropical Animal Health and Production. 2015;47(8):1641-1647.
6. Çetinkaya B, Kalin R, Karahan M, Atil E, Manso Silván L, Thiaucourt F. Detection of contagious caprine pleuropneumonia in East Turkey. Review of Scientific Technology. 2009;28:1037-1044.
7. Damasa AJ, Wakenell P, Broos DL. Mycoplasmas of goats and sheep. Journal of Veterinary Diagnostic Investigation. 1992;4:101-113.
8. Dassanayake RP, Shanthalingam S, Herndon CN, Subramaniam R, Lawrence PK, Bavananthasivam J, *et al. Mycoplasma ovipneumoniae* can predispose bighorn sheep to fatal *Mannheimia haemolytica* pneumonia. Veterinary Microbiology. 2010;145:354-359.
9. Khan, Ahmad M. Respiratory infection of lambs with *Mycoplasma ovipneumoniae* Retrospective Theses and Dissertations; c1993. p. 10244.
10. Elnaker YF, Ibrahim NA, Diab MS. Microbiologial and Epidemiological Studies on Sheep and Goat Deaths in New Valley Governorates, Alexander Journal of Veterinary Sciences. 2017;55(2):28-35.
11. Elshafay DY, Nermin A, Yasser FE, Emad EY. Isolation and Molecular Characterization of *Mycolplasma arginini* Isolated from Sheep and Goats with Respiratory Manifestation in Dakahilia Governorate. 3rd Conference of the Scientific Research Institute of Animal Society. Vol. 3. Modern Trends Health for the Advancement of Animal Health, Egypt, Giza; c2016.
12. Hawari AD, Hassawi DS, Sweiss M. Isolation and identification of *Mannheimia haemolytica* and *Pasteurella multocida* in sheep and goats using biochemical tests and random amplified polymorphic DNA (RAPD) analysis. J Biol. Sci. 2008;8(7):1251-1254.
13. Ionas G, Clarke JK, Marshall RB. The isolation of multiple strains of *Mycoplasma ovipneumoniae* from

individual pneumonic sheep lungs. Vet. Microbial. 1991;29:349-360.
14. Kilic A, Kalendar H, Eroksuz H, Muz A, Tasdemir B. Identification by culture, PCR, and immunohistochemistry of Mycoplasmas and their molecular typing in sheep and lamb lungs with pneumonia in Eastern Turkey. Tropical Animal Health and production. 2013;45:1525-1531.
15. Lindstrom L, Tauni FA, Vargmar K. Bronchopneumonia in Swedish lambs study of pathological changes and bacteriological agents. Acta Veterinaria Scandinavica. 2018;60:54.
16. Liu T, Garcia M, Levisohn S, Yogev D, Kleven S. Molecular variability of the adhesion-encoding gene pvp A among *Mycoplasma gallisepticum* strains and its application in diagnosis. Journal of Clinical Microbiology. 2001;39:1882-1888.
17. McAuliffe L, Hatchell FM, Ayling RD, King AIM, Nicholas RAJ. Detection of *Mycoplasma ovipneumoniae* in Pasteurella-Vaccinated sheep flocks with respiratory disease in England. Veterinary Record. 2003;153:687-688.
18. Nicholas RAJ, Aying RD, Loria GR. Ovine mycoplasmal infections. Small Ruminant Research. 2008b;76:92-98.
19. Ozbey G, Kilic A, Ertasand HB, Muz A. Random amplified polymorphic DNA (RAPD) analys of *Pasteurella multocida* and *Manheimia haemolytica* strains isolated from cattle, sheep and goats. Veterinární Medicíne. 2004;49:65-69.
20. Rifatbegovic M, Maksimovic Z, Hulaj B. *Mycoplasma ovipneumoniae* associated with severe respiratory disease in goats. Veterinary Record; c2011. p. 168-565a.
21. Sheehan M, Casidy JP, Brady J, Ball H, Doherty ML, Quinn PJ, *et al*. An aetiopathological study of chronic bronchopneumonia in lambs in Ireland. The Veterinary Journal. 2007;173:630-637.
22. Tauni FA. Association of Mycoplasma ovipneumoniae Infection with Respiratory Disease in Swedish Sheep, Faculty of Veterinary Medicine and Animal Science, Department of Biomedical Sciences and Veterinary Public Health; c2017. https://stud.epsilon.slu.se/10145/1/asp_tauni_f_20170424.pdf
23. Townsend KM, Frost AJ, Lee CW, Papadimitriou JM, Dawkins HJ. Development of PCR assays for species- and type-specific identification of *Pasteurella multocida* isolates. Pubmd.gov. 1998;36(4):1000-1096. https://www.ncbi.nlm.nih.gov/pubmed/9542944