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Molecular detection of *Mycoplasma ovipneumoniae* in ovine pneumonia and it's role as a promoter of *Mannheimia haemolytica* co-infection

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Abstract

Mycoplasma ovipneumoniae is primarily a respiratory pathogen in sheep. It causes atypical pneumonia and predisposes animals to more severe secondary bacterial infections. This study investigated the presence of *M. ovipneumoniae* in sheep showing respiratory affections using Polymerase Chain Reaction (PCR). Also, an association with *Mannheimia haemolytica* is assessed, as a co-infection. DNA extraction from 164 lung tissue samples of pneumonia affected sheep was done and screened by PCR with *M. ovipneumoniae* specific primers. Nine samples were detected with *M. ovipneumoniae* of which five of the samples were also positive to *M. haemolytica*. When the PCR data is compared with the post mortem findings, it is interesting to note that, co-infected cases showed more extensive lung damage with pulmonary consolidation and fibrinous pleuritic compared to those infected with *M. ovipneumoniae* alone, demonstrating a synergistic effect. These findings suggest that *M. ovipneumoniae* acts as a promoter pathogen which enables colonization and pathogenesis of secondary bacteria that lead to severe form of pneumonia. This study suggests that an early molecular detection of *M. ovipneumoniae* might play a crucial role in timely prevention and treatment of respiratory disease outbreak in sheep flocks there by preventing economic losses to the sheep farmers.

Keywords: *Mycoplasma ovipneumoniae*, PCR detection, *Mannheimia haemolytica*, co-infection, Ovine pneumonia

Introduction

Among the health challenges in sheep, respiratory diseases are the most significant. High morbidity, reduced productivity and economic losses are the virtues of respiratory diseases in sheep worldwide. Half of the mortality in small ruminants can be attributed to pneumonia alone and *M. ovipneumoniae* is a recognized leading species that cause atypical pneumonia in sheep (Browning *et al.*, 2010; Lacasta *et al.*, 2019) ^[1, 2]. In general, the organism harbours in the upper respiratory tract and is often detected in both diseased and apparently healthy sheep (Lin *et al.*, 2008; Weiser *et al.*, 2012) ^[3, 4]. Nevertheless, under stress conditions or in co-infections, the organism shows evident pathogenicity predisposing the lung to secondary bacterial invasion.

It has been reported that *M. ovipneumoniae* impairs mucociliary clearance mechanism of the respiratory tract by inducing ciliostasis and epithelial damage. Mucociliary clearance mechanism is the defence mechanism of the respiratory tract. When this line of defence is impeded, colonization of the opportunistic bacteria, especially *Mannheimia haemolytica*, resulting in aggressive fibrinous pneumonia and thereby increased mortality (Dassanayake *et al.*, 2010; Bottinelli *et al.*, 2017) ^[5, 6]. These synergistic interactions of *M. ovipneumoniae* categorizes itself as a promoter pathogen in ovine respiratory disease complexes than merely being a commensal.

Diagnosing *Mycoplasma* through conventional culture methods is laborious and technically demanding since it is a fastidious organism which requires enriched medium with prolonged incubation periods (Nicholas *et al.*, 2008) ^[7].

In contrast, detection of *M. ovipneumoniae* through PCR tends to be more specific, sensitive and rapid; which makes it an indispensable tool in epidemiological surveys and outbreak investigations (Noll *et al.* 2022) [8].

Since, *M. ovipneumoniae* poses itself as both primary respiratory pathogen and as a promoter for secondary bacterial infections, molecular detection of the organism is an obligation for timely prevention and rapid treatment of pneumonia outbreaks in sheep flocks. Taking into consideration the facts put forth, the present study was carried out to investigate the presence of *M. ovipneumoniae* in pneumonic sheep as well as to assess its association *M. haemolytica* as a co-infection, using PCR.

Materials and Methods

The present study was conducted in sheep flocks in and around Proddatur, Rayalaseema region, Andhra Pradesh. Sheep showing clinical signs of respiratory disease like nasal discharge, cough, pyrexia and respiratory distress were taken into consideration. Post mortem was conducted in sheep died of respiratory illness and the samples were preserved. Pneumonic lung tissues (N=164) were collected aseptically, during necropsy from sheep that died of respiratory disease. Of each sample 25 mg was preserved at -20 °C until further processing.

Genomic DNA was extracted from lung tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. Molecular detection of *M. ovipneumoniae* was carried out by PCR using specific primers LMF1 and LMR1 (Table 1). Amplification was

performed in thermal cycler as per the conditions given in Table 2. PCR products thus generated were analysed for *M. ovipneumoniae* specific bands if any, using 1.2% sub-merged agarose gel electrophoresis with ethidium bromide and visualized under UV light (Figure 1).

The presence of *Mannheimia haemolytica* in the same sample set of lung samples has been pre-tested and reported earlier by our group (Pavan *et al.*, 2025) [9]. In the present study, the published PCR results of our previous work were utilized to determine the proportion of *M. haemolytica* among *Mycoplasma ovipneumoniae*-positive cases.

Results

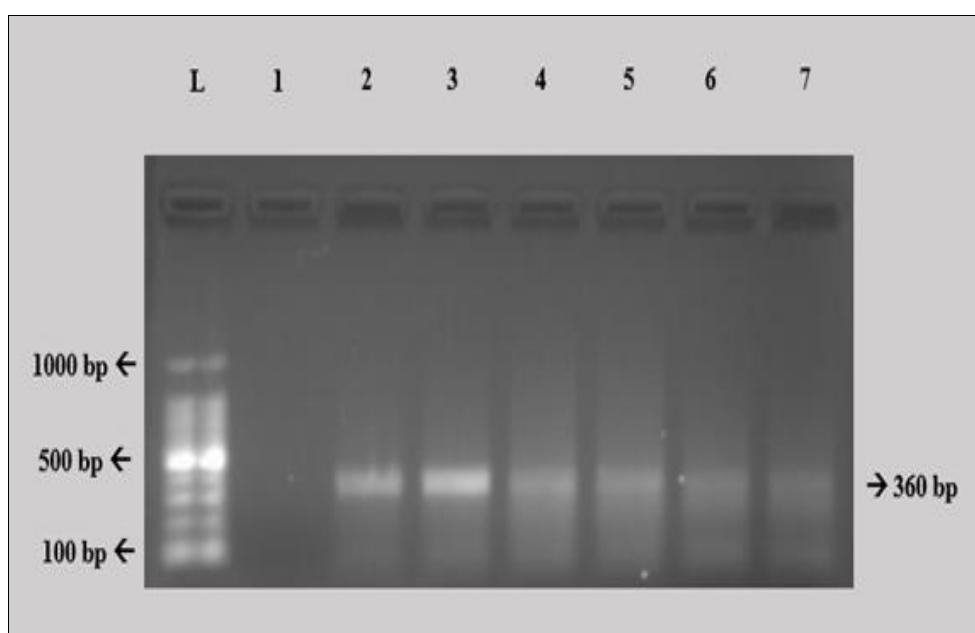
Mycoplasma ovipneumoniae was detected in 9 samples (5.48%) out of 164 pneumonic lung samples by PCR, yielding the expected 360 bp amplicon (Figure 1). When examined under post mortem, lungs positive for *M. ovipneumoniae* showed varied degrees of consolidation and inflammation. In cases where the organism was detected alone (N=4), gross lesions were noted with mild to moderate catarrhal pneumonia, congestion and patchy consolidation. Of the nine positive cases, five (55.55%) were also reported to be positive for *Mannheimia haemolytica* based on our earlier study (Pavan *et al.*, 2025) [9] and these co-infected lungs under post mortem showed severe form of fibrinous bronchopneumonia, pleural adhesions and expanded consolidation when compared to *M. ovipneumoniae*-only cases. The overall prevalence of *M. ovipneumoniae* in sheep with pneumonic lungs under the study was 5.48% with a severe form lesions observed in co-infected cases compared to single infections.

Table 1: Primers used for the detection of *Mycoplasma ovipneumoniae* isolates

Gene name	Primer sequence (5'-3')	Amplicon size(bp)	Reference
LMF1	5'-TGAACGGAATATGTTAGCTT-3'	360	McAuliffe <i>et al.</i> , (2013) [10]
LMR1	5'-GACTTCATCCTGCACTCTGT-3'		

Table 2: PCR conditions for identification of *Mycoplasma ovipneumoniae*

Gene	Initial Denaturation (°C/min)	Denaturation (°C/sec)	Annealing (°C/sec)	Initial Extension (°C/sec)	Final Extension (°C/min)	No. of Cycles	Product size (bp)
LMF	94 /5min	94/60sec	56/ 45 sec	72/60 sec	72/5min	35	360



Lane L-100 bp DNA ladder; Lanes 3, 4, 5, 6, 7-Samples positive for *M. ovipneumoniae*; Lane 1-Negative control; Lane 2-Positive control

Fig 1: PCR amplification of 360 bp specific for *M. ovipneumoniae* using primer LMF1/LMR1

Table 3: Prevalence of *Mycoplasma ovipneumoniae* and co-infections in sheep lung samples (N=164)

Pathogen detected	Number of positive cases	Percentage (%)
<i>Mycoplasma ovipneumoniae</i> only	4	2.44
<i>M. ovipneumoniae</i> + <i>Mannheimia haemolytica</i>	5	3.05
Total <i>M. ovipneumoniae</i> positive	9	5.48

Discussion

Presence of *Mycoplasma ovipneumoniae* was established in sheep lungs effected with pneumonia by PCR in the present study and also demonstrated its association with *Mannheimia haemolytica* through the use of previously published PCR data from the same samples. These findings accentuate the importance of *M. ovipneumoniae* not only as a primary respiratory pathogen but also as a promoter of secondary bacterial infections which lead to more severe clinical consequences. The occurrence of *M. ovipneumoniae* in the present study (5.48%) is consistent with earlier reports where detection rates varied depending upon geography, management and diagnostic methods. Lin *et al.*, (2008) [3] reported extensive existence of *M. ovipneumoniae* in sheep flock and is found in apparently healthy sheep too, substantiating its role in pneumonia outbreaks. Weiser *et al.*, (2012) [4] documented its presence in Bighorn sheep populations in the United States and was strongly linked to pneumonia-associated deaths.

Our results also strongly support the established notion that *M. ovipneumoniae* is the predisposing cause for the bacterial invasion in the respiratory tract. Browning *et al.*, (2010) [1] put forth its abilities to cause ciliostasis and epithelial damage which impairs mucociliary clearance and there by facilitating colonization of opportunistic pathogens. In this present work of ours, 55.55% of the lung samples that were positive for *M. ovipneumoniae* were also reported positive for *M. hemolytica* in our earlier study (Pavan *et al.*, 2025) [9]. This co-occurrence of both the organisms is in accordance with the findings of Dassanayake *et al.* (2010) [5], who demonstrated experimentally that *M. ovipneumoniae* infection predisposes bighorn sheep to fatal *M. hemolytica* pneumonia. Further it was confirmed by the observations noted by Bottinelli *et al.* (2017) [6] in natural outbreaks that dual infections lead to more severe pathological affections when compared to infection by single organisms, proving the synergism between the organisms.

The pathological lesions noted in the co-infected cases in our study viz., marked fibrinous bronchopneumonia, pleural adhesions and extended consolidation are the proof of the promoter role of *M. ovipneumoniae*. In contrast, in sheep infected with only *M. ovipneumoniae*, we could observe milder catarrhal bronchopneumonia. This pattern of response to these infections clearly demonstrate that *M. ovipneumoniae* initiates respiratory compromise that augments a favourable environment for colonization and proliferation of *M. hemolytica*, thereby amplifying disease severity.

As far as detection of *M. ovipneumoniae* is concerned, conventional culture-based techniques are often inadequate as this organism is fastidious in nature with slow growth (Nicholas *et al.*, 2008) [7]. Hence in this study we used PCR as a detection technique which is a rapid, specific and sensitive alternative as suggested by the observations of Noll *et al.* (2022) [8]. Rapid tests like PCR to identify the causative pathogen is crucial for timely intervention in outbreak scenarios and the ability to detect *M. ovipneumoniae* directly from the tissue samples offers significant diagnostic edge.

Conclusion

This study demonstrates that *M. ovipneumoniae* is crucial with its dual role in ovine respiratory disease viz., as a primary cause of atypical pneumonia and as a facilitator for severe secondary bacterial infections. This highlights the need of incorporating surveillance on *M. ovipneumoniae* into routine flock health management and to design stringent preventive strategies that address both mycoplasmal and bacterial respiratory pathogens.

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Conflict of Interest

Not available

Financial Support

Not available

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