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Pathomorphological studies of respiratory infections in sheep with special reference to *Mycoplasma ovipneumoniae* in and around Hassan district

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Abstract

Mycoplasma ovipneumoniae is recognized as an important pathogen associated with respiratory disease in sheep, contributing to considerable economic losses in the livestock sector. The present investigation aimed to determine the prevalence of respiratory infections in sheep from the Hassan district and adjoining regions, and to characterize the related pathological changes while confirming the involvement of *Mycoplasma ovipneumoniae* through molecular analysis. A total of one hundred sheep carcasses were examined, and tissue samples including lungs, trachea, bronchi, bronchioles, and mediastinal lymph nodes were collected for detailed gross, microscopic, and polymerase chain reaction evaluation. Respiratory lesions were observed in seventy-one animals. Molecular detection targeting the GPO3 gene of the *Mycoplasma* genus revealed infection in 32.39% of samples, and further amplification using species-specific primers for the 16S ribosomal ribonucleic acid gene confirmed *Mycoplasma ovipneumoniae* in 9.85% of cases. Prominent gross lesions in affected animals included consolidation of the lungs predominantly in cranio-ventral lobes, pulmonary congestion, edema, frothy exudate in the tracheal lumen, and mild fibrinous inflammation of the pericardium. Microscopic examination of pulmonary tissues revealed bronchopneumonia, interstitial pneumonia, and broncho-interstitial pneumonia. Additionally, fibrinous inflammation of the pericardium and depletion of lymphoid tissue in the lymph nodes, indicated systemic involvement associated with respiratory infection. The outcomes of this study confirm the circulation of *Mycoplasma ovipneumoniae* in sheep within the Hassan district and surrounding areas, highlighting its significant impact on the respiratory system and associated organs.

Keywords: *Mycoplasma ovipneumoniae*, sheep, respiratory lesions, polymerase chain reaction, pathology, Hassan district

Introduction

Livestock production is essential for global food security and rural livelihoods, contributing significantly to national economies in low- and middle-income countries (Udhayavel *et al.*, 2024) [1]. India possesses 74.26 million sheep and 148.88 million goats as per the 20th Indian Livestock Census, with Karnataka accounting for 14.88% of the national sheep population and ranking third after Telangana and Andhra Pradesh (Udhayavel *et al.*, 2024; Behl *et al.*, 2023) [1, 2].

Respiratory diseases are major constraints in sheep farming, with pneumonia causing significant morbidity, mortality, and production losses, accounting for about 10% of annual deaths in India (Maru *et al.*, 1990) [3]. Ovine pneumonia is multifactorial, involving pathogens such as *Mycoplasma ovipneumoniae*, *Pasteurella haemolytica*, Parainfluenza type-3 virus, adenovirus, and reovirus (Hazirolu *et al.*, 1996) [4]. Among these, *Mycoplasma ovipneumoniae* is increasingly recognized as a primary cause of chronic, economically important respiratory disease.

Mycoplasmas are cell wall-less bacteria causing diverse conditions in small ruminants, including mastitis, arthritis, respiratory and reproductive disorders (Yatoo *et al.*, 2018) [5]. First isolated in 1963 and now reported worldwide, *Mycoplasma ovipneumoniae* affects both clinically ill and apparently healthy animals, facilitating silent transmission (Nicholas *et al.*, 2015) [6].

Clinical signs range from mild chronic cough to severe fatal pneumonia, influenced by immunity, age, and management factors (Ayling and Nicholas, 2007) ^[7]. Economic losses arise from mortality, poor growth, reduced milk yield, and reproductive inefficiency (Alley *et al.*, 1999; Tigga *et al.*, 2014) ^[8, 9], often triggered by stressors such as transport and overcrowding (Yatoo *et al.*, 2016) ^[10].

Although culture remains the gold standard for diagnosis, it is time-consuming and technically demanding (Al-Momani *et al.*, 2006) ^[11]. PCR offers rapid, sensitive, and specific detection, making it a valuable tool for confirming *Mycoplasma ovipneumoniae* infection (Madanat *et al.*, 2001) ^[12].

Despite reports from various parts of India, detailed pathomorphological studies in Karnataka, particularly in Hassan district, are limited. Understanding the gross and microscopic lesions associated with *Mycoplasma ovipneumoniae* infection is crucial for early diagnosis and effective disease control strategies.

Therefore, the present study was undertaken with the following specific objectives

- To study the occurrence of respiratory infections of sheep in and around Hassan district,
- To diagnose *Mycoplasma ovipneumoniae* using polymerase chain reaction, and
- To record post-mortem findings and microscopic pathology of the respiratory system with special reference to *Mycoplasma ovipneumoniae* infection.

Materials and methods

The present investigation was carried out in the Department of Veterinary Pathology, Veterinary College, Hassan, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, from March 2025 to September 2025. The study aimed to determine the occurrence and pathological alterations associated with respiratory infections in sheep, with particular emphasis on *Mycoplasma ovipneumoniae* infection in and around Hassan district. The work involved gross and histopathological examination of respiratory organs and molecular confirmation of *Mycoplasma ovipneumoniae* using polymerase chain reaction.

Study area and sampling

Sheep tissue samples were collected from slaughterhouses located across eight taluks of Hassan district (Aluru, Arasikere, Arakalagudu, Beluru, Channarayapatna, Hassan, Holenarsipura, and Sakaleshpura) and from routine post-mortem examinations conducted at the Department of Veterinary Pathology, Hassan. The district lies in the south-eastern part of Karnataka and supports extensive and semi-intensive sheep farming. A total of 100 sheep carcasses were screened during the study period. Representative tissues from lungs, bronchus, bronchiole, trachea and mediastinal lymph nodes were collected. Tissues for histopathology were preserved in 10% neutral buffered formalin, whereas samples intended for molecular testing were stored at -20 °C.

Equipment, instruments and laboratory facilities

All laboratory procedures were performed using equipment available at the Veterinary College, Hassan and ICAR-National Institute of Veterinary Epidemiology and Disease

Informatics (ICAR-NIVEDI), Bengaluru. Major instruments included microtome, autoclave, hot-air oven, PCR thermocycler, micro-centrifuge, electrophoresis unit, gel documentation system, water bath, weighing balance, pH meter, incubator, microwave oven, and biosafety cabinet.

Laboratory materials and reagents

Plastic wares such as tissue collection bottles, centrifuge tubes, PCR tubes, pipettes, and micro-tips were sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Glassware including slides, coverslips, petri dishes, and measuring cylinders was cleaned in detergent, rinsed with distilled water, kept overnight, and sterilized in a hot-air oven at 160 °C for 1.5 hours. Ten% neutral buffered formalin for tissue fixation was prepared using standard formulation (formalin 40% -100 ml, distilled water -900 ml, sodium phosphate monobasic -4 g, and sodium phosphate dibasic -6.5 g).

Gross pathological examination

Systematic necropsy was conducted for respiratory organs comprising the lungs, bronchus, bronchioles, trachea along with the mediastinal lymph nodes. Gross observations regarding colour, texture, consistency, areas of consolidation, congestion, oedema, fibrin accumulation, and exudation were recorded and photo-documented. Lesions of diagnostic relevance were selected for histopathology and PCR examination.

Histopathology

Tissue samples fixed in 10% neutral buffered formalin were washed in running tap water for 2 hours and dehydrated through graded ethanol (70, 80, 90, and 100%). Cleared tissues were embedded in paraffin wax and sectioned at 5 µm thickness using a rotary microtome. Sections were de-paraffinized, rehydrated, and stained with Harris hematoxylin and eosin following the standard protocol (Suvarna *et al.*, 2018) ^[13]. Stained slides were examined under a light microscope (10×, 40× and 100× objectives) and photomicrographs were captured.

DNA extraction and polymerase chain reaction

Genomic DNA was extracted from frozen tissues by alkaline lysis method as described by Girish *et al.* (2013) ^[14]. Approximately 500 mg of homogenized tissue was treated with 0.2 N sodium hydroxide, incubated at 75 °C, and subsequently neutralized with Tris-HCl buffer. Extracted DNA was stored at -20 °C until use.

Detection of the *Mycoplasma* genus was performed using primer pair GPO3 and MGSO, while species confirmation of *Mycoplasma ovipneumoniae* was carried out using primer pair LMF1 and LMR1 targeting the 16S rRNA gene (Yadav *et al.*, 2020; Reddy *et al.*, 2023) ^[15, 16]. Polymerase chain reaction was performed in a 10 µl reaction mixture containing 5 µl of 2X PCR master mix (Hi-Media), 0.5 µl of each primer, 3 µl of template DNA, and 1 µl of nuclease-free water. Cycling conditions for GPO3 and MGSO included an initial denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension of 72 °C for 10 minutes. Species-specific amplification using LMF1 and LMR1 followed identical conditions except for an annealing temperature of 55 °C.

Gel electrophoresis and visualization

Amplified PCR products were resolved on 1.5% agarose gel prepared in 1X Tris-borate-EDTA buffer containing ethidium bromide (0.5 µg/ml). A 1 kb DNA ladder was used for molecular size estimation. Electrophoresis was carried out at 80 V for approximately 2 hours and the resulting DNA bands were visualized under a UV transilluminator using a gel documentation system. The amplicon sizes of 280 bp (*Mycoplasma* genus) and 361 bp (*Mycoplasma ovipneumoniae*) were considered confirmatory.

Results

The present study was undertaken to investigate the pathomorphological alterations associated with respiratory infections in sheep, with special emphasis on *Mycoplasma ovipneumoniae*, in and around the Hassan district. A total of 100 sheep carcasses comprising 29 males and 71 females, collected between March 2025 and September 2025, were examined in detail. The results obtained from the molecular and pathological investigations are summarized below.

Occurrence of respiratory infection

Among the 100 carcasses examined, 62 lung samples originated from slaughterhouses in and around the Hassan region and the remaining 38 samples were obtained from animals submitted for post-mortem examination to the Department of Veterinary Pathology, Veterinary College, Hassan. Gross examination revealed that 71 animals exhibited respiratory-related lesions, whereas digestive system lesions were observed in 17 animals, circulatory system lesions in 6 animals, and urinary system lesions in 6 animals (Table 1). These observations indicated that respiratory disease constituted the most common pathology in the study population.

Occurrence of *Mycoplasma ovipneumoniae* infection

Out of the 71 sheep showing respiratory-related lesions, 23 animals tested positive for the *Mycoplasma* genus by PCR. Further screening of these 23 cases with species-specific primers confirmed *Mycoplasma ovipneumoniae* in 7 animals, representing a prevalence of 32.39% for *Mycoplasma* infection and 9.85% for *Mycoplasma ovipneumoniae* among the total samples (Table 2).

Age-wise occurrence: The age-wise occurrence indicated that *Mycoplasma* infection was highest in sheep older than 12 months (43.47%; 10/23), followed by 3-6 months (30.43%; 7/23) and 6-12 months (26.08%; 6/23). Among *Mycoplasma ovipneumoniae*-positive cases, 42.85% (3/7) were between 6-12 months, while both 3-6 months and >12 months age groups accounted for 28.57% cases each (Table 3). These findings indicated a wider susceptibility across age groups with a slightly higher tendency among young and adult animals.

Breed-wise occurrence: Breed-wise analysis revealed the highest occurrence of *Mycoplasma* infection in non-descript animals (52.17%; 12/23), followed by the Hassan breed (34.78%; 8/23). Mandya (8.69%; 2/23) and Kenguri (4.34%; 1/23) recorded comparatively lower incidences. A similar pattern was observed for *Mycoplasma ovipneumoniae*, where non-descript and Hassan sheep exhibited the highest susceptibility (Table 4).

Taluka-wise occurrence

Geographical distribution of infection revealed variation across talukas of Hassan district. *Mycoplasma* infection was detected in Arasikere (20%; 4/20), Channarayapatna (20%; 2/10), Beluru (20%; 1/5), Arakalagudu (33.33%; 4/12), Hassan (25.92%; 14/54), and Holenarsipura (20%; 1/5). However, Aluru (0/2) and Sakaleshpura (0/1) recorded no infection. *Mycoplasma ovipneumoniae* infection was highest in Hassan taluka (7.4%; 4/54) and Channarayapatna (20%; 2/10), with a minor incidence in Arasikere (5%; 1/20), while other talukas recorded no positivity (Table 5).

Molecular confirmation by PCR

PCR amplification using *Mycoplasma* genus-specific primers produced a 280 bp amplicon confirming the presence of *Mycoplasma* in 23 samples (Fig. 1). Subsequent species-specific PCR using LMF1 and LMR1 primers yielded a 361 bp product confirming *Mycoplasma ovipneumoniae* in 7 samples (Figs 2a & 2b). These findings validated the role of molecular assays in accurate detection of respiratory mycoplasmosis in sheep.

Pathomorphological changes

Most affected sheep displayed reduced body weight, coarse hair coat, and nasal discharge (Fig 3). Systematic necropsy examination documented organ-wise lesions.

Trachea

Grossly, congestion of the tracheal mucosa with frothy to reddish-mucoid exudate was recorded in 78.26% (18/23) of *Mycoplasma*-positive and 85.71% (6/7) of *Mycoplasma ovipneumoniae*-positive cases (Fig 4). Histologically, epithelial sloughing, loss of cilia, goblet cell hyperplasia, lymphocytic infiltration, haemorrhages around tracheal glands and apoptotic bodies were evident (Figs 5-8).

Lungs: Multiple pneumonic patterns and accompanying lesions were observed.

Pulmonary lesion	<i>Mycoplasma</i> -positive	<i>M. ovipneumoniae</i> -positive	PCR-negative
Congestion	13%	28.57%	22.91%
Haemorrhage	26.08%	42.85%	20.83%
Pulmonary edema	17.39%	28.57%	43.75%
Atelectasis	43.47%	57.14%	45.83%
Emphysema	34.78%	57.14%	62.5%

Gross and microscopic characteristics were as follows

- **Congestion:** Dark-red firm lungs (Figs 9-10).
- **Haemorrhage:** Pinpoint to patchy discolorations; RBCs in alveolar spaces (Figs 11-12)
- **Pulmonary edema:** Wet, heavy lungs with frothy exudate (Figs 13-14).
- **Atelectasis:** Sunken deep-red areas and collapsed alveoli (Figs 15-16).
- **Emphysema:** Air-filled cavities and enlarged alveoli (Figs 17-18)

Pneumonia

Pneumonia was detected in all *Mycoplasma*-positive (23/23) and *Mycoplasma ovipneumoniae*-positive (7/7) lungs, and in 71% of total samples. Based on gross and microscopic appearance, pneumonias were categorized as

Pneumonia Type	No. of Cases
Interstitial pneumonia	24
Broncho-interstitial pneumonia	21
Bronchopneumonia	26

Interstitial pneumonia: Non-collapsed tan lungs with thickened interstitium and inflammatory infiltration (Figs 19-20).

- **Broncho-interstitial pneumonia:** Edematous congested lung with bronchiolar epithelial damage, inflammatory exudate, lymphoid hyperplasia and apoptotic cells (Figs 21-23).
- **Bronchopneumonia:** Cranio-ventral consolidation with tan-grey patches, fibrinous adhesions and heavy exudate on incision (Figs 24-27).

Mediastinal lymph nodes
Congested and edematous lymph nodes were observed in 30.43% of *Mycoplasma*-positive and 57.14% of *M. ovipneumoniae*-positive cases (Figs 28). Histologically, follicular depletion, congestion and ghost follicle formation with interfollicular eosinophilic fluid deposition were evident (Figs 29-31).

Table 1: Occurrence of lesions

Sl. No.	Type of lesion	No. of cases
01	Respiratory lesions	71
02	Digestive lesions	17
03	Circulatory lesions	06
04	Urinary lesions	06
	Total	100

Table 2: Occurrence of *Mycoplasma* and *M. ovipneumoniae* infection from March 2025 to September 2025 in and around Hassan district

Total number of cases in the study	Total no. of cases with respiratory lesion	Number of cases tested positive for <i>Mycoplasma</i> by PCR	Percentage of occurrence	Number of cases tested positive for <i>Mycoplasma ovipneumoniae</i> by PCR	Percentage of occurrence
100	71	23	32.39	7	9.85

Table 3: Age-wise occurrence of *Mycoplasma* infection (n = 23) and *Mycoplasma ovipneumoniae* infection (n = 7)

Age groups	Number of animals positive for <i>Mycoplasma</i> genus (n = 23)	Percentage	Number of animals positive for <i>Mycoplasma ovipneumoniae</i> (n = 7)	Percentage
3-6 months	7	30.43	2	28.57
6-12 months	6	26.08	3	42.85
More than 12 months	10	43.47	2	28.57

Table 4: Breed wise occurrence of *Mycoplasma* infection (n = 23) and *Mycoplasma ovipneumoniae* infection (n = 7)

Breed	Number of animals positive for <i>Mycoplasma</i> genus (n = 23)	Percentage (percent)	Number of animals positive for <i>M. ovipneumoniae</i> (n = 7)	Percentage (percent)
Non-descript	12	52.17	3	42.85
Hassan	8	34.78	3	42.85
Mandya	2	8.69	1	14.28
Kenguri	1	4.34	0	0.0

Table 5: Taluka wise occurrence of *Mycoplasma* infection (n = 23) and *M. ovipneumoniae* (n = 7)

Taluka	Total No. of animals in the study area	No. of <i>Mycoplasma</i> positive cases (n = 23)	Percentage positive (%) for <i>Mycoplasma</i>	Number of <i>M. ovipneumoniae</i> positive cases (n = 7)	Percentage positive (%) for <i>M. Ovipneumoniae</i>
Aluru	2	0	0	0	0
Arasikere	20	4	20	1	5
Arakalagudu	3	1	33.3	0	0
Beluru	5	1	20	0	0
Channarayapatna	10	2	20	2	20
Hassan	54	14	25.9	4	7.4
Holenarsipura	5	1	20	0	0
Sakaleshpura	1	0	0	0	0

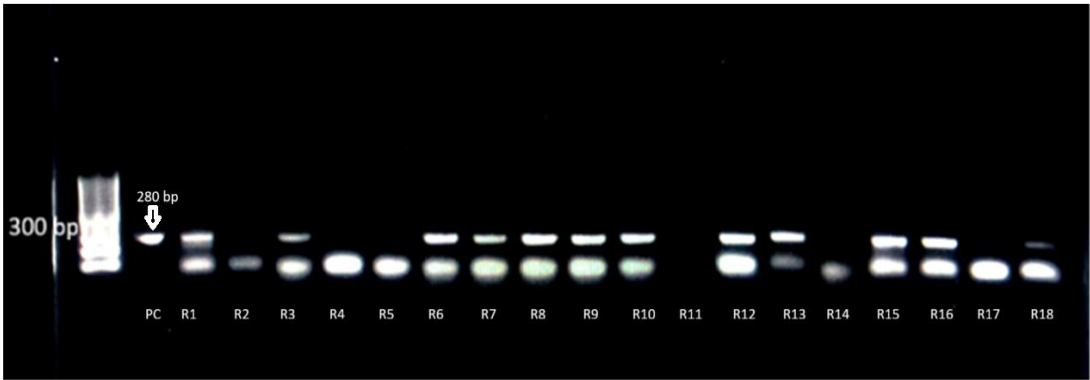


Fig 1: Gel images showing bands for the *Mycoplasma* group. 1st Lane 1 kb ladder, 2nd lane PC- positive control, 1-18 lane samples.

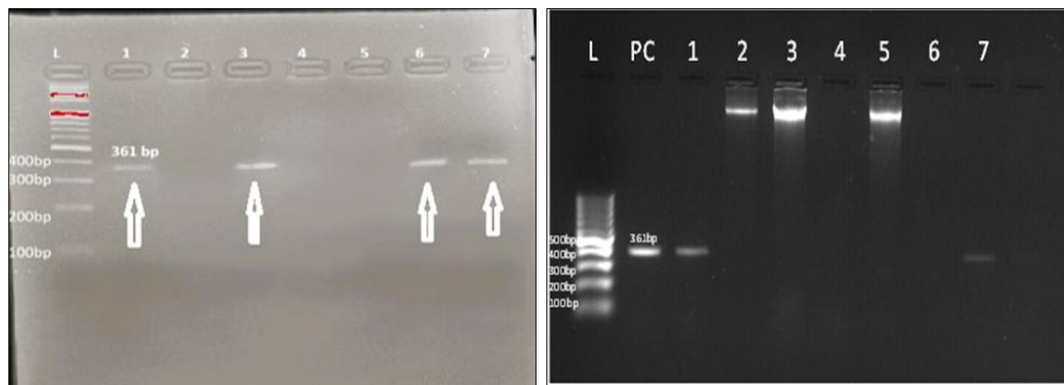


Fig 2a and 2b: Gel images showing bands for the *Mycoplasma ovipneumoniae* species specific primer LMF1 and LMR1. 1st lane 1kb ladder, 2nd lane 361 bp positive control, 1 to 7 lane samples.

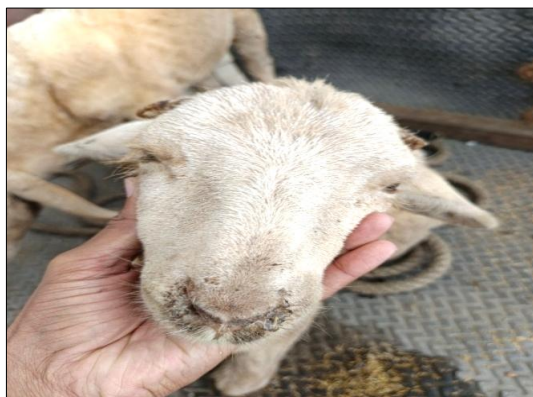


Fig 3: Sheep infected by *Mycoplasma ovipneumoniae* showing bilateral nasal discharge

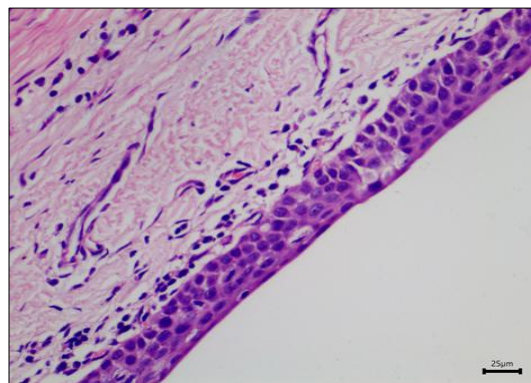


Fig 6: Section of trachea showing loss of cilia on the mucosal epithelial cells, thickening of epithelial layer, and lymphocytic infiltration in the submucosa (arrow) H&E X 400



Fig 4: Tracheal mucosa showing congestion and reddish mucoid (blood mixed) exudate in the lumen Gross

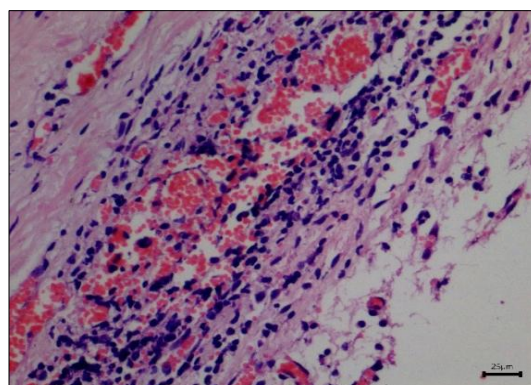


Fig 7: Section of trachea showing sloughing of the epithelial lining along with inflammatory cell infiltration and haemorrhages H&E X 400

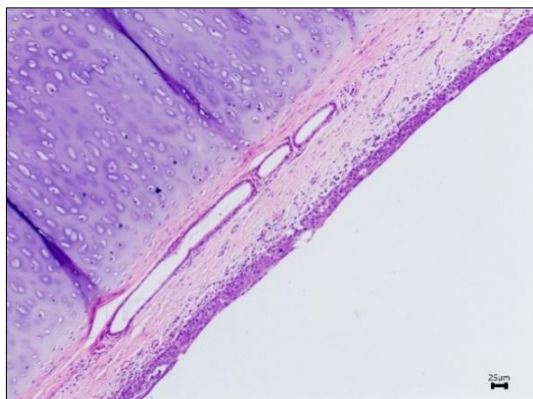


Fig 5: Section of trachea showing loss of cilia on the mucosal epithelial cells and thickening of epithelial layer H&E X 100

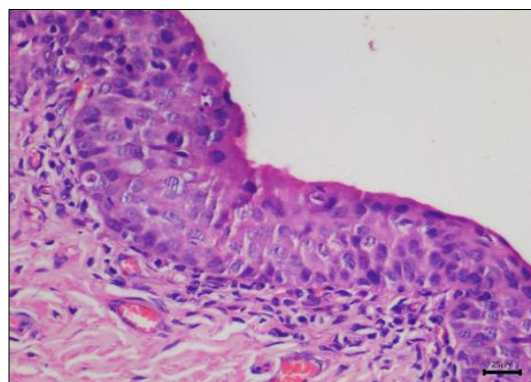


Fig 8: Section of trachea showing apoptotic bodies in the epithelial layer (arrow) H&E X 400



Fig 9: Surface view of lungs of sheep infected with *Mycoplasma ovipneumoniae* showing dark red discoloration and hardening
Gross

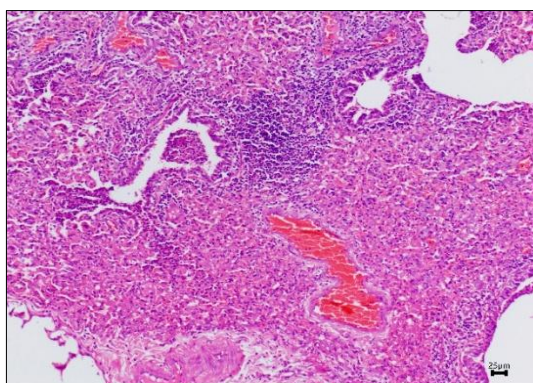


Fig 10: Section of lung showing lung parenchyma with congested blood vessels and capillaries densely packed with red blood cells
H&E X 100



Fig 11: Lungs with mycoplasmosis showing multiple pinpoint to patchy reddish discolorations due to haemorrhages on their surfaces
Gross

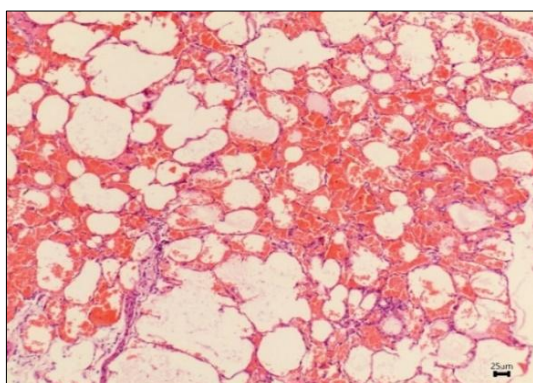


Fig 12: Section of lung showing red blood cells in the alveolar lumen and inter-alveolar space
H&E X 100



Fig 13: Edematous lung showing rib impressions on the surface
Gross

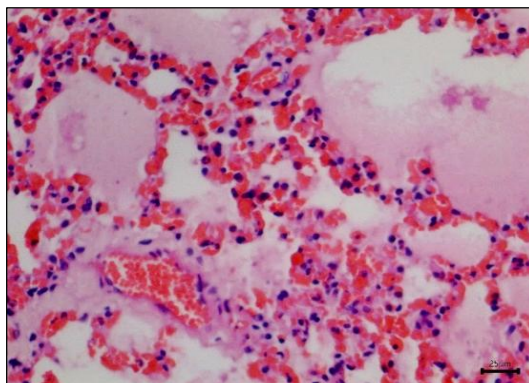


Fig 14: Section of lung showing distended alveoli with eosinophilic fluid in the lumen and significantly thickened interstitial spaces
H&E X 100



Fig 15: Lung showing areas of pulmonary atelectasis where the affected areas appeared reddish and were sunken in comparison to the surrounding normal regions (arrow)
Gross

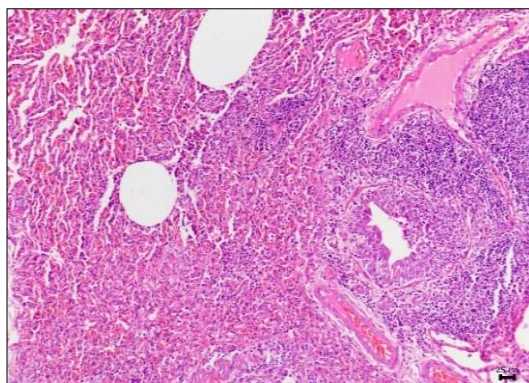


Fig 16: Section of lung showing collapsed, narrow alveoli with closely aligned, parallel walls in the atelectasis regions
H&E X 100



Fig 17: Lung showing emphysematous areas which are pale in appearance with small air-filled cavities within the normal parenchyma (arrow) Gross

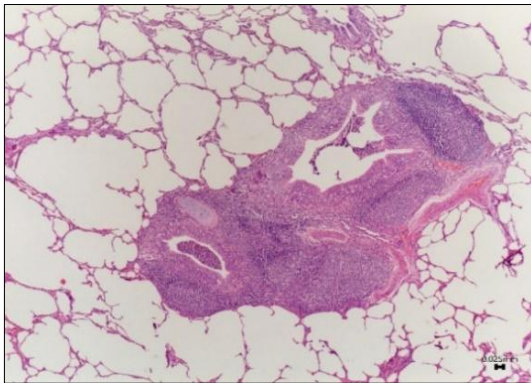


Fig 18: Section of lung showing emphysematous areas with enlarged alveoli with loss of integrity of interalveolar septa (arrows) H&E X 100



Fig 19: Lung with interstitial pneumonia showing a tan, mottled appearance with rubbery consistency Gross

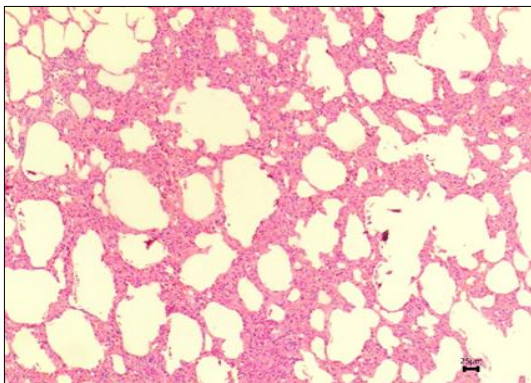


Fig 20: Section of lung showing thickened interstitial spaces due to the accumulation of eosinophilic edematous fluid, red blood cells, and inflammatory cells H&E X 100



Fig 21: Lung with broncho-interstitial pneumonia showing edema with varying degrees of congestion and patchy consolidation Gross

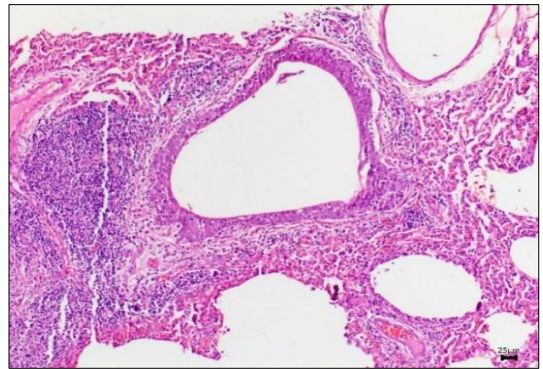


Fig 22: Section of lung with broncho-interstitial pneumonia showing inflammatory exudate in the bronchiolar lamina propria, submucosal layers and alveolar spaces. And peribronchiolar lymphoid hyperplasia (arrow) H&E X 100

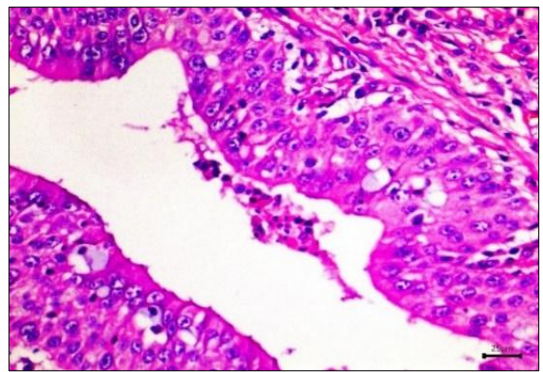


Fig 23: Section of lung showing bronchus with inflammatory exudate in the lumen, epithelial hyperplasia and vacuolar degeneration with apoptotic bodies (arrow) H&E X 400



Fig 24: Lung showing areas of consolidation in cranio-ventral portions of the both lungs which is firm, congested with a reddish-brown discoloration Gross



Fig 25: Lung showing fibrinous adhesions all over the pleural surface Gross

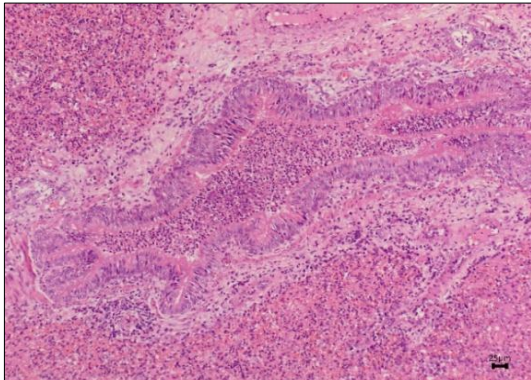


Fig 26: Section of lung showing heavily packed inflammatory exudate within the bronchus and alveolar lumen, predominantly containing neutrophils H&E X 100

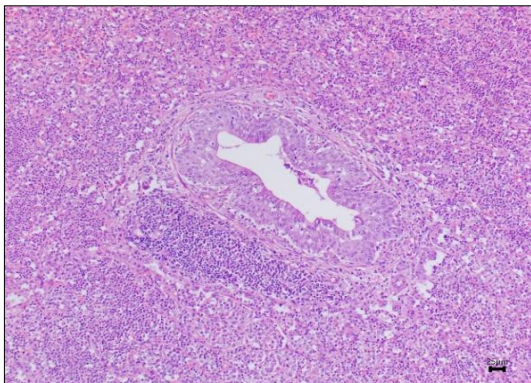


Fig 27: Section of lung showing peribronchiolar lymphoid hyperplasia, thickened bronchiolar mucosa and epithelial hyperplasia H&E X 100



Fig 28: Anterior and intermediate mediastinal lymph nodes of lamb infected with mycoplasmosis showing mild swelling, edema and congestion. Gross

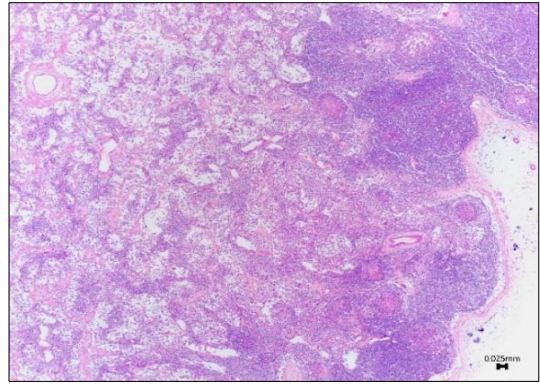


Fig 29: Section of mediastinal lymph node showing minimal to moderate lymphoid follicle depletion in cortex and medullary sinus H&E X 40

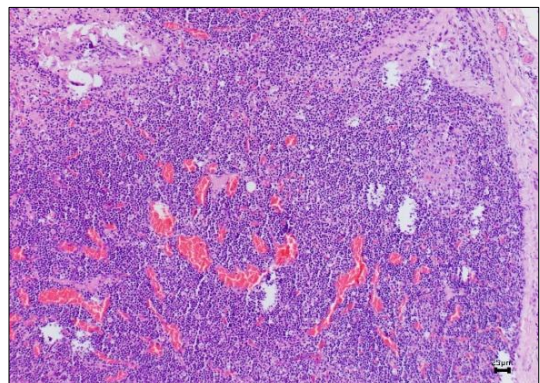


Fig 30: Section of mediastinal lymph node showing minimal lymphoid follicle depletion, interfollicular edema and congestion of blood vessels H&E X 100

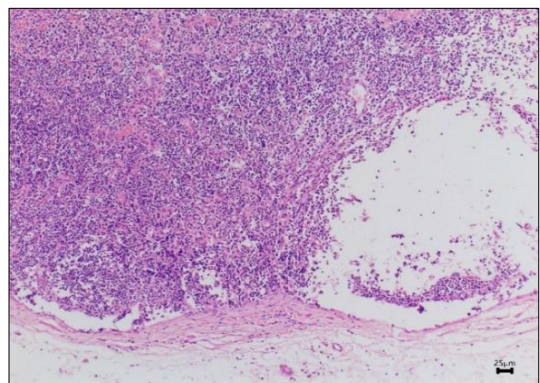


Fig 31: Section of mediastinal lymph node showing completely regressed lymphoid follicle H&E X 100

Discussion

The present study investigated gross and histopathological alterations of the respiratory system in sheep from Hassan district, with molecular confirmation of *Mycoplasma ovipneumoniae* infection using PCR. A high incidence (71%) of respiratory lesions was observed among the examined carcasses, reaffirming that respiratory disease remains a major health constraint in small ruminants. Similar observations have been reported by Kumar *et al.* (2014) ^[17] and Mekibib *et al.* (2019) ^[18], who identified respiratory disorders as leading causes of morbidity and mortality in sheep. The relatively high occurrence recorded in this study may be attributed to environmental stress, flock density, immune status, and managerial practices prevalent in the region.

Occurrence of *Mycoplasma* and *M. ovipneumoniae*

Among pneumonic lungs, 32.39% were positive for the *Mycoplasma* genus and 9.85% were confirmed as *M. ovipneumoniae*. These findings are comparable to previous reports, although inter-study variation exists. Reddy *et al.* (2023) ^[16] and El-Mashad *et al.* (2020) ^[19] reported higher species-level prevalence, possibly reflecting regional differences, host factors, or diagnostic sensitivity. Comparable genus-level prevalence has also been documented in goats (Erra, 2024) ^[20], suggesting that *Mycoplasma* infections are consistently present across small ruminant populations, though the proportion attributable specifically to *M. ovipneumoniae* varies.

The variability in species-specific detection may reflect differences in sample type, stage of infection, antimicrobial exposure, co-infections, or managemental conditions. Additionally, the multifactorial nature of respiratory disease may mask the primary role of *M. ovipneumoniae* when secondary pathogens are involved.

Age-wise occurrence: Age significantly influenced infection dynamics. General *Mycoplasma* infections were more frequent in animals above 12 months, whereas *M. ovipneumoniae* showed peak prevalence in the 6-12-month age group. This pattern supports earlier findings by Besser *et al.* (2013) ^[21], who reported higher susceptibility among post-weaning lambs, likely due to stress, immune transition, and increased exposure. The higher detection of *Mycoplasma* in adults may indicate chronic carriage and subclinical persistence, contributing to flock-level transmission.

Thus, younger sheep appear more vulnerable to active *M. ovipneumoniae*-associated pathology, while older animals may serve as reservoirs.

Breed-wise occurrence: Breed variation was evident. Non-descript sheep exhibited the highest infection rates, followed by Hassan breed, while Mandya and Kenguri showed lower prevalence. This variation likely reflects management practices, flock density, and possible genetic resilience. The higher susceptibility in non-descript animals may be associated with uncontrolled breeding and semi-intensive rearing systems. Although breed-specific studies in Karnataka are limited, these findings suggest that managemental and environmental factors may outweigh inherent breed resistance. Close-contact transmission, as demonstrated experimentally by Dassanayake *et al.* (2013) ^[22], likely contributes to higher prevalence in densely managed flocks.

Taluka-wise distribution: Geographical variation was observed across agro-climatic zones. Higher prevalence was recorded in dry and densely populated talukas such as Hassan and Arakalagudu, while no cases were detected in Aluru and Sakaleshpura, though sample size limitations must be considered. Environmental stressors, stocking density, and animal movement likely influence pathogen circulation. Chen *et al.* (2024) ^[23] emphasized that high density and ventilation deficiencies enhance *M. ovipneumoniae* transmission. The observed variability highlights the combined influence of ecological and managemental determinants in disease epidemiology.

Molecular confirmation by PCR

PCR proved to be a rapid and reliable diagnostic tool. Genus-specific amplification of the 16S rRNA gene (280 bp) identified 32.39% positivity, while species-specific amplification (361 bp) confirmed *M. ovipneumoniae* in 30.43% of genus-positive cases. These findings align with earlier molecular studies (Reddy *et al.*, 2023; Udhayavel *et al.*, 2024) ^[1, 16] and reaffirm PCR as superior to conventional culture methods, which are limited by the fastidious nature of *Mycoplasma*. The detection of *M. ovipneumoniae* in sheep from Hassan district confirms its endemic presence and underscores the importance of molecular diagnostics in routine surveillance.

Pathomorphological changes: Trachea: Grossly, congestion and mucosal exudation were observed. Histologically, epithelial sloughing, ciliary loss, lymphocytic infiltration, and goblet cell hyperplasia were evident. These lesions correspond to the organism's adherence to ciliated epithelium, leading to ciliostasis and impaired mucociliary clearance (Jones *et al.*, 1985; Lin *et al.*, 2008) ^[24, 25]. Apoptotic epithelial cells suggest oxidative stress-mediated injury, consistent with Li *et al.* (2016), who demonstrated ROS-induced apoptosis in infected airway epithelium.

Lungs: Pneumonia was the predominant lesion and was categorized as interstitial (33.8%), broncho-interstitial (29.6%), and bronchopneumonia (36.6%). These patterns are consistent with reports by Kumar *et al.* (2014) ^[17] and Daee *et al.* (2020) ^[27]. Although bronchopneumonia was common in *Mycoplasma*-positive cases, its presence in PCR-negative lungs confirms the multifactorial etiology of ovine pneumonia. Pathogenesis involves adherence of *M. ovipneumoniae* to ciliated epithelium, production of hydrogen peroxide and reactive oxygen species, oxidative stress, and epithelial apoptosis (Lin *et al.*, 2008; Li *et al.*, 2016) ^[25, 26]. Impaired mucociliary clearance facilitates secondary bacterial colonization, particularly by *Mannheimia haemolytica* (Alley *et al.*, 1999) ^[8]. Immune modulation further contributes, as the organism can suppress lymphocyte responses and resist phagocytosis (Shahzad *et al.*, 2010; Al-Kaissi and Alley, 1983) ^[28, 29]. Strain variability and recurrent colonization prolong inflammatory responses and contribute to chronic pneumonia (Bottinelli *et al.*, 2017) ^[30]. The occurrence of similar lesions in PCR-negative cases indicates involvement of other pathogens such as viral agents (PI-3, respiratory syncytial virus) and bacteria including *Pasteurella multocida* and *Mannheimia haemolytica*, emphasizing the polymicrobial nature of small ruminant respiratory disease.

Mediastinal lymph nodes

Mediastinal lymph nodes showed swelling, congestion, lymphoid depletion, and follicular atrophy, particularly in *M. ovipneumoniae*-positive cases. These findings suggest systemic immunological involvement and possible lymphoid exhaustion. Similar observations were reported by Mondal *et al.* (2004) ^[31] and Mohamed and Bader (2011) ^[32], indicating that infection extends beyond pulmonary tissues and influences regional lymphoid architecture. The study confirms that *Mycoplasma ovipneumoniae* is a significant respiratory pathogen in sheep of Hassan district, associated with multiple pneumonic patterns. Disease

occurrence is influenced by age, breed, agro-climatic factors, and management practices, with pathogenesis involving epithelial damage, oxidative stress, immune modulation, and secondary bacterial infections. However, lesions in PCR-negative cases indicate a multifactorial etiology of ovine respiratory disease. These findings emphasize the need for improved management, routine molecular diagnostics, and comprehensive disease surveillance for effective control.

Conclusion

The present study confirmed that *Mycoplasma ovipneumoniae* is one of the predominant respiratory pathogens of sheep in and around Hassan district of Karnataka. PCR targeting the 16S rRNA gene proved to be a rapid, specific, sensitive, and reliable tool for confirmatory diagnosis. Infections at both genus and species levels were associated with significant pathomorphological changes, including interstitial and broncho-interstitial pneumonia, bronchopneumonia, pulmonary congestion, edema, atelectasis, emphysema, lymphoid depletion, periportal lymphocytic infiltration, and fibrinous pericarditis. Concomitant and opportunistic infections likely aggravated the lesions, highlighting the need for further investigation. Effective control measures, regular diagnostic surveillance, and improved management practices are essential to reduce disease severity and economic losses in sheep farming.

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