RESEARCH ARTICLE

Molecular Detection of *Mycoplasma ovipneumoniae* from Respiratory and Non-Respiratory Infections of Goats in and around Tirupati Region

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Abstract

Mycoplasmosis is a significant contagious disease that adversely impacts the economy of small ruminant farming. *Mycoplasma ovipneumoniae* is one of the major etiological agents associated with atypical pleuropneumonia and keratoconjunctivitis in goats. In the current study, a total of 238 samples (128 nasal swabs, 39 conjunctival swabs, 15 mastitis milk, 5 arthritis fluids, 45 lung tissues and 6 pleural fluids) were collected from affected goats and slaughter houses in and around Tirupati region (India). Molecular detection was done by genus-specific and species-specific PCR targeting the 16S *r*RNA gene, yielding 270 bp and 361 bp products, respectively. The overall incidence of genus *Mycoplasma* and *M. ovipneumoniae* was 61.76% and 19.32%, respectively. Phylogenetic analysis of the present isolate TPTMOVI L1, clustered with clade formed by goat nasal swab isolates from Andhra Pradesh (MK 138543.1 and MK182761.1) and segregated into distinct cluster with sheep lung isolate from USA (PQ 631135.1). The isolate showed genetic relatedness with goat isolates from Andhra Pradesh and USA. Notably, the detection of *M. ovipneumoniae* from conjunctival swabs confirms its involvement in ocular infections, particularly keratoconjunctivitis, in addition to respiratory disease. Higher detection rates were observed in nasal swabs and lung tissue samples, indicating the predominance of respiratory involvement, while ocular positivity highlights the multifactorial clinical presentation of the infection.

Key words: Goats, Incidence, Molecular Detection, *Mycoplasma ovipneumoniae*, Phylogenetic analysis.

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Introduction

Mycoplasma species are distributed globally and are responsible for serious diseases affecting goats, including contagious caprine pleuropneumonia, contagious agalactiae, pneumonia, arthritis, mastitis, conjunctivitis, and meningitis (Chakraborty *et al*., 2014; Kumar *et al*., 2014; Jain *et al*., 2015; Parker *et al*., 2018). *Mycoplasma ovipneumoniae* is known to cause chronic, non-progressive, atypical pneumonia in sheep, goats and bighorn sheep. It also serves as a predisposing factor for pneumoniae causing bacterial or viral infections in goats (Ayling *et al*., 2007; Nicholas *et al*., 2008). *Mycoplasma ovipneumoniae* may be more prevalent in environment with high temperatures and low relative humidity (Fernández *et al*., 2016; Mousa *et al*., 2021).

Mycoplasma ovipneumoniae is primarily spread through the respiratory tract due to close contact between animals (Lindstrom *et al*., 2018). Halium *et al.* (2019) noted respiratory symptoms, fever ranging between 40-42°C accompanied by depression, and nasal and ocular discharges that were mucoid, mucopurulent, or purulent in nature. Furthermore, when the animals' chests were auscultated, they made coughing and abnormal sounds. Maksimovic *et al.* (2017) indicated that under conditions of stress, the severity of this infection increases, resulting in consolidated lung lesions, acute fibrinous pneumonia, pleurisy, and pulmonary

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abscesses leading to death. Halium *et al.* (2019) examined lungs, tracheas, and tracheal bifurcation with several pathological abnormalities, like edema and congestion with red and grey hepatization, haemorrhages in trachea and in tracheal bifurcations, and varying degrees of pleuritis. This study reports the molecular detection, phylogenetic analysis and incidence of *Mycoplasma ovipneumoniae* from respiratory and non-respiratory infections of goats in and around Tirupati region of India.

Materials and Methods

A total of 1545 goats were examined for respiratory, conjunctivitis, mastitis, and arthritis infections from different villages in and around Tirupati region and a total of 238 clinical samples were collected. Out of 238 clinical samples, 128 animals were found to have respiratory symptoms like nasal discharges (mucopurulent to purulent, Fig. 1), coughing, sneezing. Similarly, 39 animals exhibited conjunctivitis symptoms like reddening of eyes and ocular discharges. Fifteen animals showed symptoms of mastitis like swelling of udder and change in consistency of milk, and 5 animals exhibited symptoms of arthritis like limping, swelling of the carpel joint and pain on palpation. A total of 45 lung tissues with congestion and consolidations, and 6 pleural fluids were collected from slaughter houses and postmortem examinations (Fig. 2). Clinical samples were inoculated into PPLO broth and incubated anaerobically for 7-10 days in CO_2 incubator, maintaining 5% CO_2.

DNA Extraction from PPLO Broth

The inoculated PPLO broth tubes with nasal swabs, conjunctival swabs, mastitis milk, arthritis fluid and pleural fluids were examined for colour change and turbidity. DNA extraction was done from these broth tubes separately. The DNA extraction procedure was carried out as per the method described by Liu *et al.* (2001). After incubation, the clinical samples in micro centrifuge tubes were gently vortexed to ensure the clinical material from the swab was adequately collected, and later the swab was discarded. The samples were then subjected to centrifugation for 10 min at 13,200 x g using a refrigerated centrifuge. The resultant cell pellet was washed twice with 1 mL of PBS, and subsequently, 100 µL of PBS was added to the pellet. The cell suspension was then boiled at 95°C for 10 min in a water bath and immediately subjected for snap chilling. After snap chilling the lysate was centrifuged at 13,200 x g for approximately 2 min in a refrigerated centrifuge. A volume of 2 µL from the supernatant was utilized as the template for PCR.

DNA Extraction from Lung Tissues

DNA extraction from lung tissues was performed according to the standard protocol mentioned in QIAamp DNA Mini Kit. Cat No. 51304.

PCR for Detection of 16S *r*RNA of *Mycoplasma* and *Mycoplasma ovipneumoniae*

Detection of the 16S rRNA gene of genus *Mycoplasma* and species *Mycoplasma ovipneumoniae* was done by PCR according to the procedures described by Junqueira *et al.* (2020) and McAuliffe *et al.* (2003), respectively. Details of primer sequences and PCR conditions used are listed in Table 1 and 2.

Results and Discussion

Clinical samples from goats suspected of *Mycoplasma* were inoculated into PPLO broth and after an incubation of 7-10 days, the positive samples exhibited slight turbidity and

Table 1: Primers for genus *Mycoplasma* and *Mycoplasma ovipneumoniae*

S. No	Primers	Gene	Primer Name	Nucleotide Sequence	Amplicon Size	References
1	Genus *Mycoplasma*	16 S *r*RNA	GPO3	5′ GGG AGC AAA CAG GAT TAGATA CCC T 3′	270 bp	Junqueira *et al.* (2020)
			MGSO	5′TGC ACC ATC TGT CAC TCT GTT AAC CT 3′		
2	*Mycoplasma ovipneumoniae*	16 S *r*RNA	LMF1	5′TGA ACG GAA TAT GTT AGC TT 3′	361 bp	McAuliffe *et al.* (2003)
			LMR1	5′GAC TTC ATC CTG CAC TCT GT 3′		

Table 2: Cyclic conditions for amplification

S. No	Step	Genus *Mycoplasma*			*M. ovipneumoniae*		
		Temperature (°C)	Duration	Cycles	Temperature (°C)	Duration	Cycles
1	Initial denaturation	94	4 min	1	94	5 min	1
2	Denaturation	94	30 sec	35	94	30 sec	30
3	Annealing	56	30 sec		55	30 sec	
4	Extension	72	30 sec		72	30 sec	
5	Final extension	72	10 min	1	72	7 min	1

Fig. 1: Goats showing mucopurulent nasal discharges

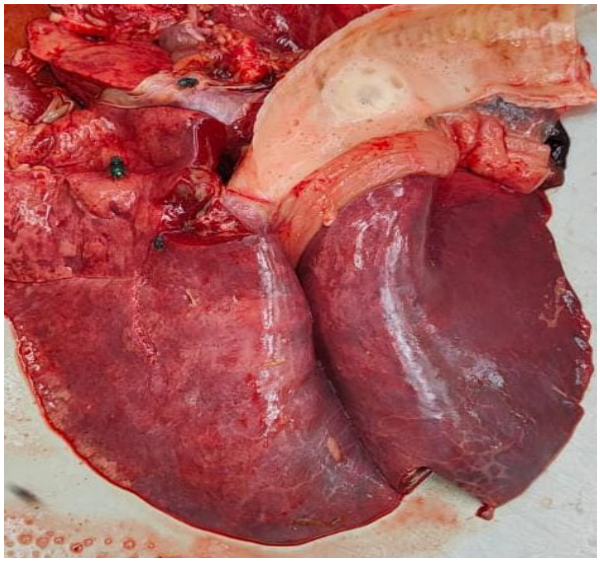

Fig. 2: Congestion and consolidation of goat lung tissue

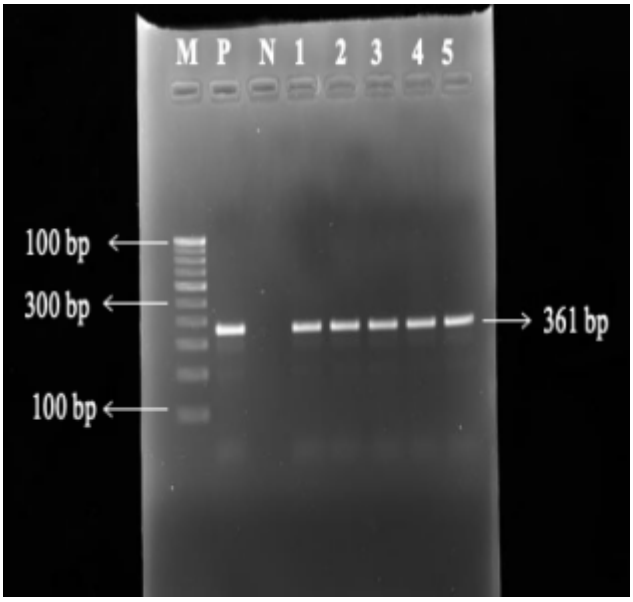

Fig. 3: Amplification of 16S *r*RNA gene of *Mycoplasma ovipneumoniae.* Lane M: Ladder 100 bp, Lane P: Positive control, Lane N: Negative control, Lane 1-3: Nasal swabs positive for 16S *r*RNA gene of *M. ovipneumoniae,* Lane 4: Lung tissue positive for 16S *r*RNA gene of *M. ovipneumoniae,* Lane 5: Conjunctival swab positive for 16S *r*RNA gene of *M. ovipneumoniae.*

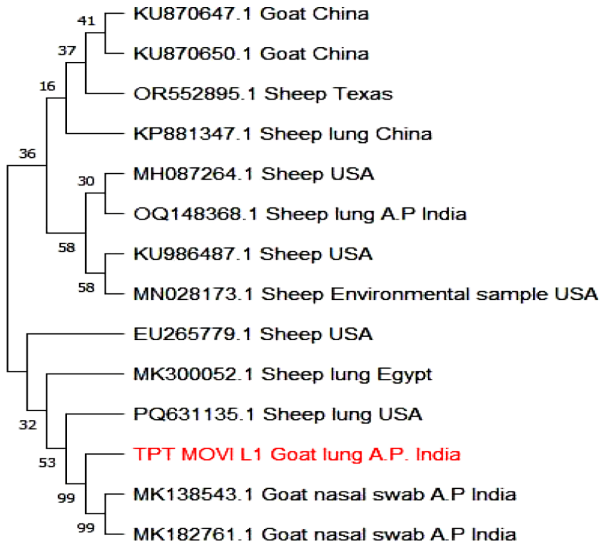

Fig. 4: Phylogenetic analysis of *Mycoplasma ovipneumoniae* with other reference sequences in NCBI database. Phylogenetic tree was constructed using MEGA 11 version 11.0 software by Maximum likelihood with 1000 bootstrap replicates using Kimura 2-parameter model

a colour change of the broth to yellow, suggesting the multiplication of *Mycoplasma* organisms. The growth was mostly observed at the bottom of the broth tubes, and upon slight agitation, a whirlpool like formation was noted. Similar conditions for *Mycoplasma* growth have been documented in previous studies (Harasawa *et al*., 2004; Ezzi *et al*., 2007; Gharaibeh and Al-Roussan, 2008). Following the incubation period, positive samples exhibited a yellow colour change with mild turbidity in the broth medium.

In the present study, out of 238 goat clinical samples collected (128 nasal swabs, 39 conjunctival swabs, 45 lung tissues, 6 pleural fluids, 15 mastitis milk and 5 arthritis fluids), 147 culture DNA positive samples (68 nasal swabs, 30 conjunctival swabs, 38 lung tissues, 4 pleural fluids, 5 mastitis milk and 2 arthritis fluids) tested were positive for the genus *Mycoplasma* yielding 270 bp product. The present study reported that the overall incidence of the genus *Mycoplasma* in goats was 61.76%. Yadav *et al.* (2020) from Andhra Pradesh

and Kumar *et al.* (2011) from Gujarat, reported the incidence of genus *Mycoplasma* as 29.6% and 8.35% in goats, which was less compared to our study, while Santhiya *et al.* (2021) from Kerala and Deepika *et al.* (2023) from Andhra Pradesh, reported an incidence of 55.33% and 55.91% of the genus *Mycoplasma,* which is in accordance with our study. Awan *et al.* (2009) from Pakistan noted the incidence of the genus *Mycoplasma* as 56.7% in goats, which was also in accordance with our finding.

Out of 147 genus *Mycoplasma* positive samples, 46 (26 nasal swabs, 8 conjunctival swabs, and 12 lung tissues) were found to be positive for *Mycoplasma ovipneumoniae* yielding 361 bp product (Fig. 3). The overall incidence of *Mycoplasma ovipneumoniae* was found to be 19.32%. This is consistent with the finding of 17.2% by Deepika *et al.* (2023) from Andhra Pradesh and 17.33% by Karthik *et al.* (2023) from Karnataka in goats. Ongor *et al.* (2011) utilizing genus specific and species-specific PCR identified 11% (75/692) of the samples from goats nasal discharges as *Mycoplasma* spp., and 8.1% (56/692) as *M. ovipneumoniae*.

For confirmation of *M. ovipneumoniae*, the PCR product from one representative lung tissue (TPTMOVI L1) sample was sequenced, the obtained nucleotide sequence was verified by NCBI BLAST and showed 97.68% homology with *Mycoplasma ovipneumoniae* strains. Phylogenetic analysis of this isolate TPTMOVI L1, clustered with clade formed by goat nasal swab isolates from Andhra Pradesh (MK 138543.1 and MK182761.1) and segregated into distinct cluster with sheep lung isolate from USA (PQ 631135.1). The isolate showed genetic relatedness with goat isolates from Andhra Pradesh and USA (Fig. 4). The occurrence of *Mycoplasma ovipneumoniae* was not detected in culture DNA positive mastitis milk and arthritis fluid samples. The occurrence of *M. ovipneumoniae* in conjunctivitis-infected goats is confirmed by PCR for the first time in Andhra Pradesh.

Conclusion

The results of our study indicate the significance of *M. ovipneumoniae* in respiratory and conjunctival infections. The increased incidence of *M. ovipneumoniae* was due to poor hygiene practices. One of the specific findings in our study was the detection of *Mycoplasma ovipneumoniae* in conjunctivitis cases of Mycoplasmosis in goats. The findings of this study demonstrate the widespread occurrence of *Mycoplasma ovipneumoniae* in goats of the Tirupati region and emphasize its important role in both respiratory and ocular infections. The results highlight the significance of molecular diagnostic approaches for the early and accurate detection of the pathogen from respiratory and ocular samples. Early diagnosis is essential for effective disease management and control strategies, thereby contributing to reduced production losses and improved health management in goat populations.

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