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Pathological characterization of lung lesions and molecular detection of *Mycoplasma spp*. in slaughtered goats

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ABSTRACT: Mycoplasmosis is a chronic respiratory disease of goat causing huge economic loss to the farmers. A slaughter house based study was carried out from December 2023 to March2024 to characterize the gross, histopathological lesions in lungs and also to confirm the role of Mycoplasma spp. by PCR in slaughtered goats in Puducherry, India. Out of 350 carcasses examined, pneumonic lesions were recorded in 104 cases accounting for 29.7 %. All the affected animals in the present study were above 1 year of age. Sex wise lesions were recorded more in males (60.5%, 63/104) than females (39.4% 41/104). Breed wise, the occurrence of lung lesions were: salem black (28.8%, 30/104), kodiaadu (26.9%, 28/104) and non-descript (44.2%, 46/104). Gross examination revealed emphysematous, edematous and grey to red areas of consolidation with exudation from the cut surface. Representative tissue samples were collected and fixed in 10% formalin and processed by routine paraffin embedding techniques. Histopathological examination of affected lung tissue showed extensive area of haemorrhage, pleural thickening, bronchus associated lymphoid tissue [BALT] hyperplasia, septal thickening, epithelial hyperplasia and mononuclear infiltration. From histopathologically confirmed cases, six lung samples were selected for PCR targeting the 16S rRNA gene specific for Mycoplasma spp. Four samples (66.66%) yielded the expected 425 bp amplicon. PCR-positive product subjected to sequencing (Accession number: PV498626) and phylogenetic analysis revealed close similarity to Mycoplasma capricolum subsp. capripneumoniae strains. This study confirms the involvement of Mycoplasma spp. in caprine pneumonia and highlights the utility of molecular tools such as PCR and sequencing in the definitive diagnosis and epidemiological tracking of caprine respiratory infections.

Keyword: Goat; Lung; Histopathology, PCR, Sequencing.

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INTRODUCTION

Among various disease affecting goat herds, respiratory illness is considered to be most common. Mycoplasmosis is a chronic respiratory disease of goat that causes great economic loss to the farmers (Mousa *et al*., 2021).In domesticated ruminants such as sheep, goats, and cattle, 40 different species of Mycoplasma are found.

The World Organization for Animal Health has identified Contagious bovine pleuropneumonia caused by*Mycoplasma mycoides subsp. Mycoides*, contagious caprine pleuropneumoniae by *Mycoplasma capricolum subsp. Capripneumoniae*, Infectious agalactia by several Mycoplasma species are considered to have a high economic impact (Pavone *et al*., 2023). Mastitis, conjunctivitis, pneumonia, and arthritis represent some of the clinical expressions of Mycoplasma spp. infection in ruminants (Minion, 2002; Chazel *et al*., 2010).The disease is manifested as acute, subacute and chronic forms of pneumonic lesions and is often associated with high infertility, indirect economic losses and less mortality (Adehan *et al*., 2006).

Mycoplasma is probably widespread in nature, but due to their poor growth on synthetic media *in vitro*, they have likely not been readily isolated. It is a highly selective microbe that needs exceptionally specific media to grow *in vitro*. Its lowered ability to synthesize the macromolecules required for growth reflects their divergent evolution from other bacteria (Halium *et al*., 2019; Nicholas *et al*., 2008). Although recent advancements aids in identification of specific Mycoplasma species, PCR remains the most effective and quickest method for identifying the species-specific Mycoplasma (Awan *et al*., 2012). The present communication reports the occurrence of Mycoplasmosis in slaughtered goats by pathological findings and molecular diagnosis.

MATERIALS AND METHODS

Study design

The present study was conducted from December, 2023 to March, 2024 for a period of 4 months. Total of 350 goats of different sex with age of 1 year were screened for respiratory illness in the slaughter houses in Puducherry, India.

Histopathological examination

Lung tissue samples from selected cases were fixed in 10% neutral buffered formalin followed by routine paraffin embedding technique and serially sectioned with a microtome at 4-micron thickness, and stained with Hematoxylin and Eosin for observation of histopathological changes.

Molecular diagnosis

Representative lung tissue samples with gross lesions were collected and stored at -20°C.DNA extraction was performed with the GF-1 Tissue DNA Extraction Kit (Vivantis), according to the manufacturer's instructions. The PCR reaction was performed in a volume of 50 µL, including 25 µL My Taq Red Mix, 2×, 1 µL from each primer (20 mM of each), DNA template 200 ng, and completed with sterile water up to 50 µL. Common primer 16S RNA gene and specific primers (16-23 S intergenic spacers) were used for molecular detection of Mycoplasma species. The primer sequence and PCR cycle conditions for molecular diagnosis of Mycoplasmosis is represented in Table 1 (Alberti *et al*., 2006).

DNA Extraction and PCR Amplification

Genomic DNA was extracted from lung tissues using a standard phenol-chloroform method. The 16S ribosomal RNA gene was amplified using universal Mycoplasma-specific primers forward (5′-ggggttgtacttggttga-3′) and Reverse (5′-ggcgatcacctctgactg-3′). PCR reactions were carried out under standard cycling conditions optimized for the target gene.

Table 1. Primers sequence, PCR cycling conditions for molecular detection of *Mycoplasma species*

Strain	Primer sequence	Fragment size (bp)	Primary denaturation	30-35 cycle	
				Secondary denaturation	Annealing
Mycoplasma species (16 s rRNA)	F: **GGGGTTGTACTTGGTTGA** R: **GGCGATCACCTCTGACTG**	425	94°C 5 min	95°C 1 min	55°C 45 s

Sequencing and Analysis

PCR products were purified and sequenced using Sanger dideoxy sequencing. The obtained sequence was screened for chimeras using the software Mega and the resulting partial 16S rRNA gene sequence (425 bp) was deposited in GenBank under accession number PV498626.1.

RESULTS

Gross findings

Out of 350 goats screened, 104 goats showed respiratory illness, nasal discharge, pale mucous membrane (Fig.1 and 2). In such cases, lesions of pneumonia (Fig.3) characterised by emphysema, edema and red to grey areas of consolidation were recorded accounting for 29.7%.Sex wise pneumonic lesions (Fig. 4) were recorded more in males (60.5% 63/104) than females (39.4% 41/104). Breed wise occurrence of lung lesions (Fig. 5) were more in non-descript (44.2% 46/104 followed by Salem black (28.8% 30/104) and Kodiaadu (26.9% 28/104).

Histopathological findings

Prominent findings included thickening of pleural, characterised by deposition of fibrin and infiltration of inflammatory cells (Fig.6), pulmonary edema characterised by the accumulation of fluid within the alveoli and alveolar interstitial space and bronchus-associated lymphoid hyperplasia (BALT) hyperplasia were the other changes observed (Fig.8). Additionally, haemorrhage (Fig.8), catarrhal bronchiolitis and infiltration by inflammatory cells were observed (Fig.9).

Figure 1. Pale pink conjuctival mucous membrane.

Figure 2. Mucus discharge from the nostrils.

Figure 3. Lungs showing Pneumonic changes in apical and middle lobes.

Molecular identification

Out of 350 goats screened, 104 had pneumonic lesions from which 6 samples were subjected to PCR

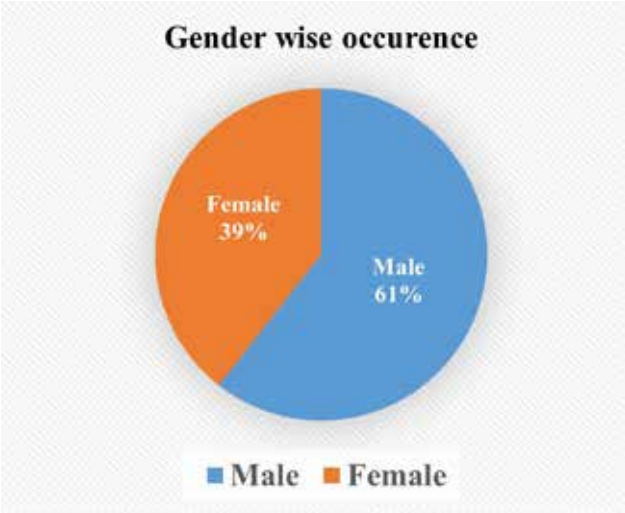

Figure 4. Gender wise occurrence of pnuemonic lesion.

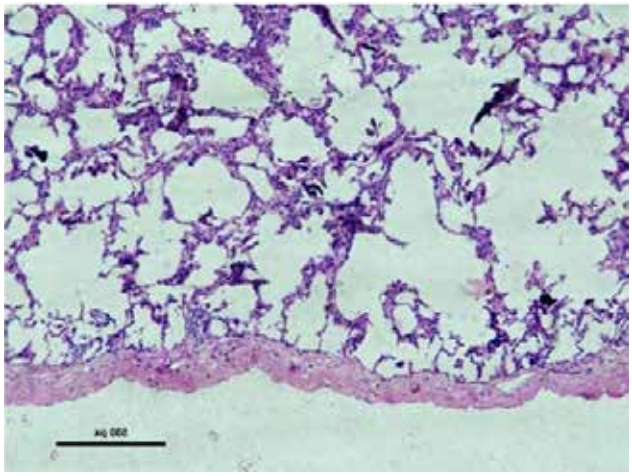

Figure 6. Lung showing Thickening of pleura (arrow) H&Ex100.

targeting the 16S rRNA specific for *Mycoplasma spp*. and 4 yielded an expected amplicon size of 425bp (66.66%) samples confirming the role of Mycoplasma spp. (Fig.10). The nucleotide variations and similarities at 16S rRNA gene region specific for the Mycoplasma sp. are depicted in Table 2. Variations among the species/ subspecies were detected at 12 positions with respect to our Indian isolate (PV498626).

The 425 bp fragment of the 16S rRNA gene of Mycoplasma capricolum subsp. capripneumoniae was successfully amplified and sequenced from goat lung tissue. BLAST analysis showed that the query sequence (PV498626) had maximal identity of 99.53% with the other known M. capricolum subsp. capripneumoniae sequences around the world in the NCBI gene Bank. The phylogenetic analysis also authenticated that the study sequence clustered with other M. capricolum subsp. Capripneumoniae sequences around the world in the NCBI gene Bank. (Fig. 11).

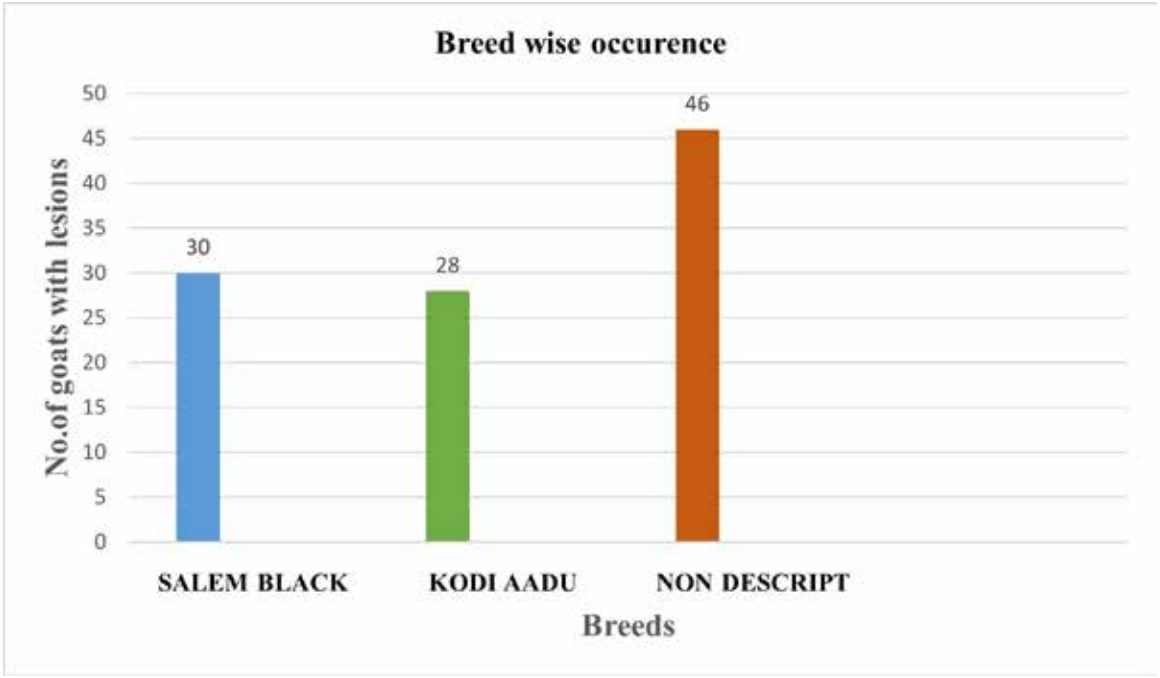

Figure 5. Breed wise occurrence of pneumonic lesions.

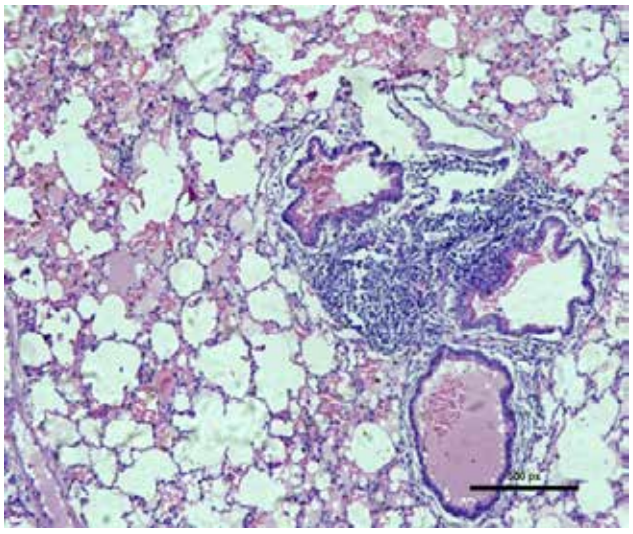

Figure 7. Lung showing Pulmonary edema with BALT hyperplasia (arrow) H&Ex100.

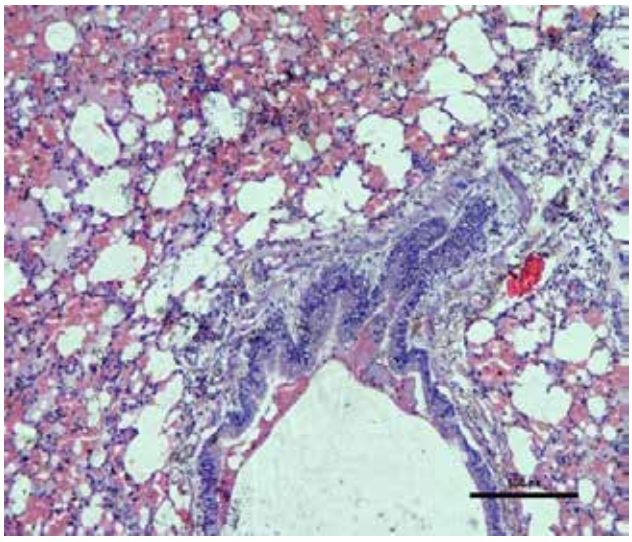

Figure 9. Lung showing Presence of cattarahal exudate (arrow) in bronchiole H&E x100.

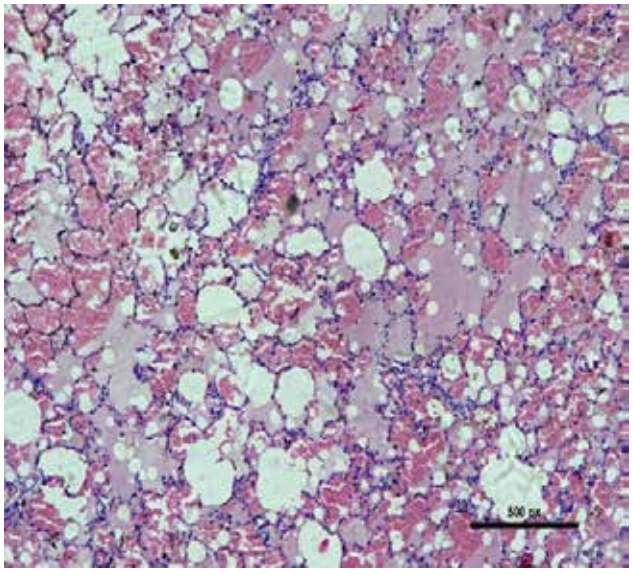

Figure 8. Lung showing interstitial Haemorrhages and edema H&Ex100.

DISCUSSION

Respiratory infections represent a significant challenge in small ruminant population leading to substantial economic losses globally. These losses are mainly associated with decreased productivity in terms of milk and meat which are crucial commodities in many agricultural economies. Among the myriad etiological agents responsible for causing respiratory illness, *Mycoplasma sp*. stands out as highly pathogenic organism (El-Deeb *et al*., 2017). This organism is responsible for causing chronic respiratory conditions which often go unnoticed until clinical signs become evident thereby causing huge economic loss by reducedmilk yield and increased mortality (Yatoo *et al*., 2018). Other serious problems associated with *Mycoplasma* infection are contagious caprine pleuropneumonia, conjunctivitis, arthritis, mastitis, and respiratory distress (Sheik *et al*., 2016). In small ruminants *M. ovipneumoniae* and *M. arginini* are frequently isolated from pneumonic lesions (Valsal *et al*., 2017).

Gross findings of the present study were in accordance with earlier reports of Yatoo and Kanwar (2016).Key histological changes, such as extensive hemorrhagic pneumonia, edema, bronchus-associated lymphoid tissue (BALT) hyperplasia, and pleural thickening, indicate a chronic and aggressive infection process. Citti and Blanchard,(2013) reported that BALT hyperplasia, in particular, reflects a persistent immune response and is often observed in response to chronic infections. This hyperplasia is likely driven by the pathogen's evasion mechanisms, which include antigenic variation and suppression of host immune defences leading to prolonged inflammation and lymphoid activation within the bronchial walls. Adehan *et al*. (2006) noted that the majority of alveoli and bronchioles contained a combination of neutrophils and macrophages, while other alveoli were filled with edema fluid. In the current study, edema was the predominant feature.

One of the significant challenges in diagnosing *Mycoplasma* infections is the difficulty of culturing these organisms. *Mycoplasma* is fastidious pathogens that require specific growth conditions,

Table 2. Nucleotide variations and similarities between different Mycoplasma species.

Nucleotide position at 16S ribosomal RNA rrnB operon of Mycoplasma & their variations & similarities within species/subspecies level differentiation	**72**	**82**	**109**	**123**	**137**	**170**	**253**	**298**	**317**	**318**	**384**	**389**
CP014346.1 Mycoplasma mycoides subsp. mycoides France 2016	T	C	A	A	A	G	T	G	C	G	G	C
MK192912.1 Mycoplasma mycoides subsp. mycoides Pakistan 2017	A	C	A	A	A	G	T	G	C	G	G	C
U26049.1 Mycoplasma mycoides subsp. mycoides Sweden 1999	C	C	A	A	A	G	T	G	C	G	G	C
DQ157864.1 Mycoplasma capricolum subsp. capricolum Slovenia 2005	C	C	A	A	A	G	T	G	C	G	G	C
CP101903.1 Mycoplasma capricolum subsp. capricolum China 2023	C	C	A	A	A	G	T	G	C	G	G	C
KP718739.1 Mycoplasma capricolum subsp. capricolum Germany 2015	C	C	A	A	A	G	T	G	C	G	G	C
CP006959.1 Mycoplasma capricolum subsp. capripneumoniae China 2015	C	C	A	A	A	G	C	A	C	G	G	T
AF009843.1 Mycoplasma capricolum subsp. capripneumoniae Sweden 1998	C	T	C	A	A	G	T	G	T	G	A	C
CP041703.1 Mycoplasma capricolum subsp. capripneumoniae UAE 2020	C	T	C	A	A	G	T	G	T	A	A	C
PV498626 Isolate India 2024	C	T	C	T	T	T	T	G	T	G	A	C

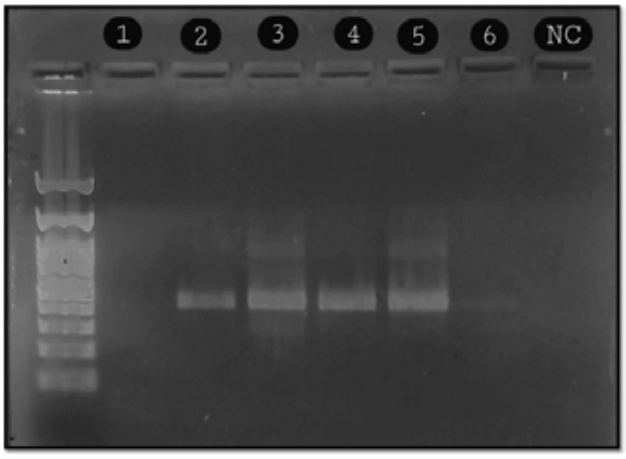

Figure 10. Agarose gel electrophoresis of PCR products showing a distinct band at ~425 bp.

including complex media supplemented with sterols, and they often exhibit slow growth rates. Additionally, contamination with other bacterial flora can interfere with successful isolation. Even when culture is achieved, the process is labor-intensive and time-consuming, which delays the diagnosis and initiation of appropriate treatment. As a result, molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have gained prominence in detecting *Mycoplasma* infections (Noll *et al*.,2022).The superior accuracy of PCR not only aids in early and precise detection but also facilitates the implementation of timely and targeted therapeutic interventions, thereby minimizing economic losses (Razin *et al*.,1998).In the present study,6/104 randomly selected lung samples subjected to PCR targeting the 16s rRNA specific for *Mycoplasma spp* showed a positive result for Mycoplasma in four cases (66.6%).

Molecular detection and phylogenetic analysis confirmed the presence of Mycoplasma capricolum subsp. capripneumoniae in goat lung tissue. The use of 16S rRNA gene sequencing provided specific and reliable identification.

The phylogenetic clustering with known strains of the same subspecies supports the diagnostic value of this molecular approach. The observed genetic similarity with globally reported strains highlights the conserved nature of the 16S rRNA region in Mycoplasma and underlines its utility in epidemiological studies.

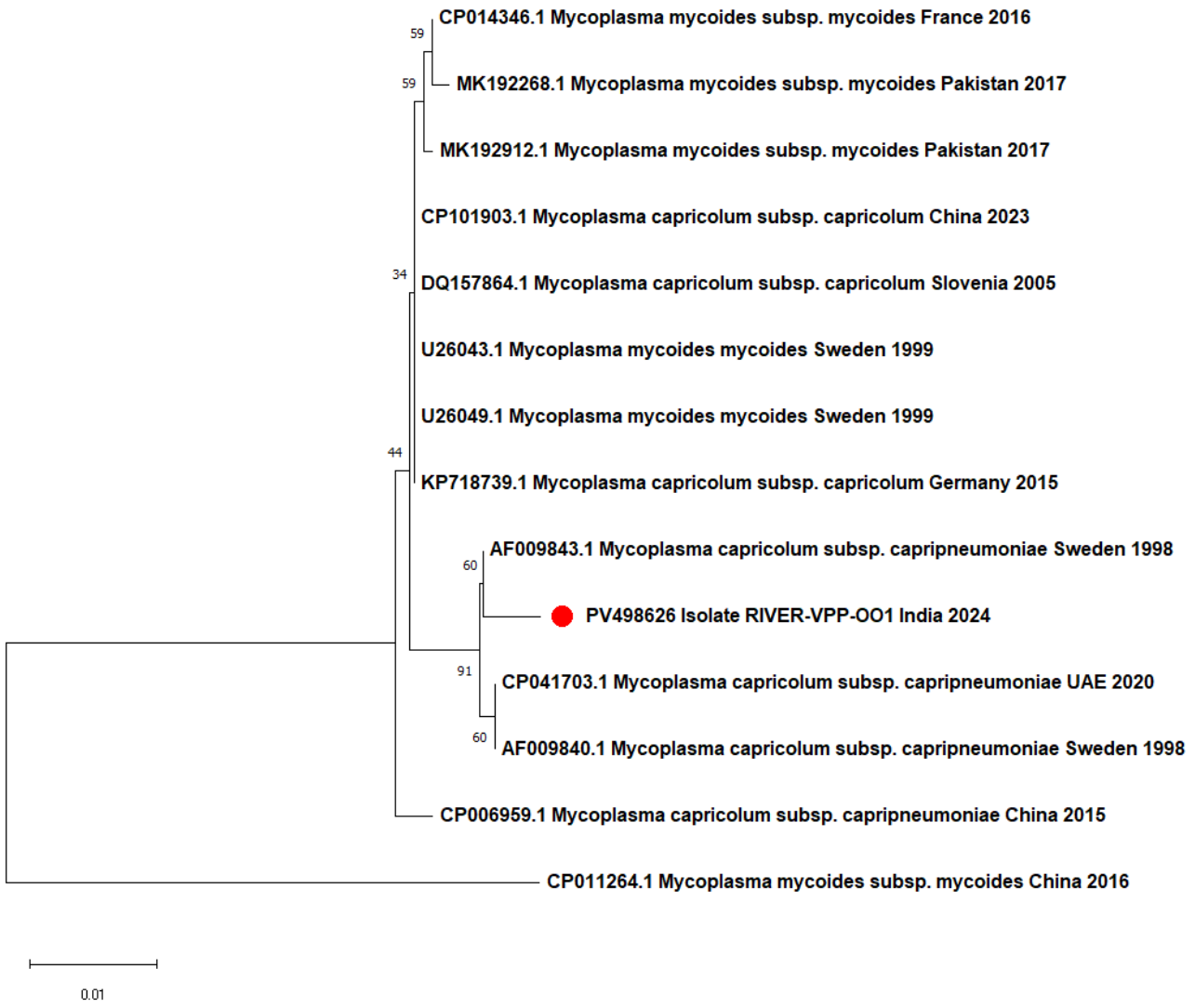

Figure 11. Phylogenetic tree based on partial 16S rRNA gene sequences showing the relationship of the amplified Mycoplasma capricolum subsp. capripneumoniae sequence with other Mycoplasma species.

Several studies have documented the prevalence of *Mycoplasma* infections in goat populations across different regions, highlighting its widespread impact. The study by Rehman *et al*. (2022) reported sero-molecular evidence of *Mycoplasma capricolum* subsp. *capripneumoniae* in goats from southern Pakistan, confirming its endemic nature. In a study from Maharashtra, India, a high incidence of *Mycoplasma capricolum* subsp. *capripneumoniae* was confirmed in goats through pathological and molecular diagnosis (Chaygude *et al*., 2023).

These findings underscore the endemic nature of *Mycoplasma* infections in various geographical regions. Although, the higher positivity in the present study could be attributed to the sample size and sampling bias, the prevalence rate of 66.6% observed in this study is notably high, indicating a significant burden of Mycoplasma infections in the goat population of Puducherry. Effective control measures, including routine health monitoring, molecular diagnostics, and stringent biosecurity practices, are crucial to curbing the transmission of Mycoplasma. These measures will not only improve herd health but also minimize the economic losses associated with decreased productivity and mortality.

CONCLUSION

Mycoplasmosis in goat population represents a significant health challenge affecting both welfare of animals and economic productivity. The study highlights the effectiveness of 16S rRNA gene-based

PCR and sequencing in the identification of Mycoplasma capricolum subsp. capripneumoniae from goat lung tissue. It underscores the importance of early detection and accurate diagnosis of Mycoplasma infections. The study indicating higher incidence (66.6%) of Myoplasmosis in goats of Puducherry underscoring the importance of robust surveillance and control measures to mitigate the disease transmission.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the author(s). This work was supported by the Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, India

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Not Applicable

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