**RESEARCH ARTICLE**
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2021.52.3.267-271

# Molecular test for detection of *Mycoplasma ovipneumoniae* associated with respiratory tract infection from goats in north and central parts of Kerala*

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Citation: Santhiya, P., Surya Sankar, Mini, M., Siju Joseph and Venkatachalapathy, T.R. 2021. Molecular test for detection of Mycoplasma ovipneumoniae associated with respiratory tract infection from goats in north and central parts of Kerala. *J. Vet. Anim. Sci.* **52**(3): 267-271.
DOI: https://doi.org/10.51966/jvas.2021.52.3.267-271

*Received: 23.01.2021* *Accepted: 01.03.2021* *Published: 30.09.2021*

**Abstract**

*Mycoplasmal pneumonia is an important contagious disease that significantly affects the economy of small ruminant farming worldwide and Mycoplasma ovipneumoniae (M. ovipneumoniae) is one of the major aetiological agents associated with pleuropneumonia in goats. It is considered as a serious epidemic disease of goats due to its huge economic impact and hence, rapid and early diagnosis of the disease is warranted. Clinical mycoplasmosis often lacks pathognomonic signs, so definitive diagnosis of the disease is quite burdensome. Polymerase chain reaction (PCR) test has been proven to be a specific and sensitive technique for the early diagnosis of mycoplasmosis. The present study highlights the detection of M. ovipneumoniae employing PCR test in 150 nasal swab samples collected from goats with symptoms of respiratory tract infection from five districts of Kerala. Results revealed that, out of 150 samples, 83 (55.33 per cent) were positive in 16S rRNA Mycoplasma genus specific PCR test. Among the 83 genus positive samples, 68 samples (45.33 per cent of total 150 samples) were positive in M. ovipneumoniae specific PCR test.*

**Keywords:** *Goats, polymerase chain reaction (PCR), Mycoplasma ovipneumoniae*

In India, goat farming is one of the major sources of income for small scale farmers. Among the various infectious diseases, the one caused by *Mycoplasma* causes significant economic losses to goat industry. It is one of the *Office International des Epizooties* (OIE) listed notifiable diseases, which affects international trade and is responsible for major constraint in world economy (OIE, 2008). *Mycoplasma* produces various disease manifestations such as pneumonia,

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conjunctivitis, arthritis and mastitis (Nicholas, 2002). Several *Mycoplasma* species are identified to be of pathogenic significance in goats worldwide (Nicholas *et al.,* 2008). Among these, *M. ovipneumoniae* is well thought-out as the cause of non-progressive pneumonia and was first identified in Australia in 1972 (Ongor *et al.,* 2011). In goats, primary infection with *M. ovipneumoniae* may facilitates invasion by other bacteria such as *Mannheimia haemolytica*, which may enhance the pathological process (McAuliffe *et al.,* 2003). Along with other pathogenic mycoplasmas in the same animal, *M. ovipneumoniae* might cause variation in the morbidity and mortality of the disease in the same animal (Halium *et al.,* 2019).

Diagnosis of *M. ovipneumoniae* based on clinical and post-mortem lesions will not give confirmatory diagnosis because symptoms can be shared by other clinically important infections. *Mycoplasma* is a highly fastidious organism and is very difficult to isolate on artificial medium. Nowadays, molecular techniques such as PCR test employing specific oligonucleotide primers offer the rapid and specific detection of *M. ovipneumoniae*.

In India, studies on caprine mycoplasmosis mainly focused on contagious caprine pleuropneumonia and contagious agalactia (Kumar *et al.,* 2011; Reji *et al.,* 2018). On the other hand, only few studies concerning *M. ovipneumoniae* have been conducted (Jana *et al.,* 2005; Reji, 2018) and therefore, there is paucity of information regarding the infections caused by this organism.

In the present study, we report the molecular detection of *M. ovipneumoniae* from goats with respiratory tract infection during a period of 14 months from central and north parts of Kerala.

## Materials and methods

### *Collection of samples*

A total of 150 nasal swabs were collected from goats with respiratory infection from different parts of Kerala. Sterile cotton swabs were pre-wetted in phosphate buffered saline (PBS) and inserted deep into the nasal passage. The swabs were then placed back in PBS and snapped off the handle. The samples collected were transported immediately to the laboratory under cold conditions and subjected to direct detection of *M. ovipneumoniae* employing PCR.

### *DNA extraction*

Deoxyribonucleic acid (DNA) was extracted from the nasal swabs using Hi Pura multi sample DNA extraction kit (HiMedia, India) and stored in elution buffer at -20˚C till use. Concentration and 260/280 OD value of the extracted DNA were checked by Nanodrop 2000 (Thermo Scientific).

### *Polymerase chain reaction (PCR) assay*

Two different PCR assays were used to identify the organism up to the species level. Initially all the DNA samples were subjected to their reactivity with *16S rRNA* genus specific primers and later with *M. ovipneumoniae* specific primers.

The PCR amplification was carried out in a volume of 12.5 µL reactions in 200 µL capacity PCR tubes containing 1.25 µL molecular biology grade nuclease free water, 6.25 µL 2X PCR master mix, one microlitre (10 p*M*) of each of the forward and reverse primers and three microlitre of DNA (10 ng/µL). One negative control without template DNA was included to monitor any contamination. The contents of the tubes were mixed gently, spun briefly and the tubes were placed in an automatic thermal cycler for amplification.

### *Identification of 16S rRNA gene specific to genus Mycoplasma*

*Mycoplasma* genus specific PCR was performed using the *16S rRNA* of *Mycoplasma* specific primers GPO3F (5' TGG GGA GCA AAC AGG ATT AGA TAC C3') and MGSO (5' TGC ACC ATC TGT CAC TCT GTT AAC CTC3') for an expected amplified product of 280 bp (Botes *et al.,* 2005). The conditions used in the PCR test are given in table 1.

**Table 1.** PCR conditions for amplification of *16S rRNA* gene specific to genus *Mycoplasma*

| Step | Temperature | Time | No. of cycles |
|---|---|---|---|
| Initial denaturation | 94°C | 2 min. | 1 |
| Denaturation | 94°C | 15 sec. | 35 |
| Annealing | 59.3°C | 15 sec. | |
| Extension | 72°C | 15 sec. | |
| Final extension | 72°C | 5 min. | 1 |

***Identification of M. ovipneumoniae***

The PCR was performed using the *M. ovipneumoniae* specific primers MOVPF (5' GTT GGT GGC AAA AGT CAC TAG 3') and MOVPR (5' CTT GAC ATC ACT GTT TCG CTG 3') for an expected amplified product of 418 bp (Halium *et al.,* 2019). The conditions used in the PCR test are given in table 2.

***Submarine agarose gel electrophoresis***

Amplified PCR products were resolved in one per cent agarose gel in 1X TBE buffer. Five microlitre of the PCR product was loaded into the wells. A 100 base pair DNA ladder (SRL) was also run alongside the samples to ascertain the size of the amplified products. Electrophoresis was carried out at 50 V and 16 mA until the dye migrated two-third of length of the gel. The gel was visualised under UV transilluminator and the results were documented in a gel documentation system (Bio-Rad).

**Results and discussion**

As per OIE (2008), nasal swab containing clinical material from live goats showing respiratory signs was the sample of choice for the diagnosis of *Mycoplasma*. Hence, the same was collected from ailing goats with clinical signs suggestive of mycoplasmosis. Similar procedure was also followed by Reji *et al.* (2018).

The nasal swabs were collected from a total of 150 goats showing respiratory tract infection. Out of which, 83 were positive for *16S rRNA* genus specific PCR (Fig. 1). The genus positive samples were then subjected to a gradient PCR with *M. ovipnuemoniae* specific primers to find out the optimised annealing temperature for further PCR. A nasal swab collected from Thrissur produced an amplicon size of approximately 418 bp at the annealing temperatures of 59.9°C, 61.5°C and 62.4°C. Of these, 61.5C for one minute was selected as the optimum. The above mentioned sample was taken as the positive control. Among 83 samples that are positive for *Mycoplasma* genus, 68 found to be positive for *M. ovipneumoniae* (Fig. 2). Similar observations were made by Ongor *et al.* (2011) in goats, who detected *M. ovipneumoniae* directly from nasal swabs employing PCR.

Clinical symptoms and post-mortem lesions even though aid in the diagnosis of mycoplasmosis in field conditions, the definitive diagnosis cannot be made solely on these findings because symptoms and lesions can be shared by other clinically similar infections. Sampling requires expertise and culture, isolation and identification by biochemical tests

**Table 2.** PCR conditions for amplification of *M. ovipneumoniae* specific PCR

| Step | Temperature | Time | No. of cycles |
|---|---|---|---|
| Initial denaturation | 94°C | 1 min. | 1 |
| Denaturation | 94°C | 1 min. | 35 |
| Annealing | 61.5°C | 1 min. | |
| Extension | 72°C | 2 min. | |
| Final extension | 72°C | 5 min. | 1 |


**Fig. 1.** Agarose gel electrophoresis of *Mycoplasma* genus specific PCR products

Lane M: 100 bp DNA ladder
Lane 1: Positive control
Lane 2: Negative control
Lane 3, 4, 5, 6, 7, 8 and 9: Positive samples
Lane 10 and 11: Negative samples

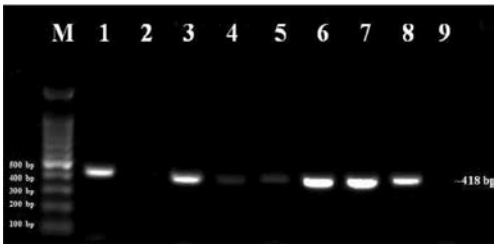


**Fig. 2.** Agarose gel electrophoresis of *Mycoplasma ovipneumoniae* specific PCR products

Lane M: 100bp DNA ladder
Lane 1: Positive control
Lane 2: Negative control
Lane 3, 4, 5, 6, 7 and 8: Positive samples
Lane 9: Negative sample

require specialised laboratory, infrastructure and costly media, hence rarely practiced (Thiaucourt and Bolske, 1996). Meanwhile, it was reported that reduced viability and fastidious nature of *Mycoplasma* in clinical samples could also be affecting the culture results (Bolske *et al.,* 1996). As per Woubit *et al.* (2004), cross reactions with other *Mycoplasma* species were the main limitation of the serological techniques. Molecular techniques like polymerase chain reaction (PCR) being highly specific and sensitive, enables the rapid detection of *M. ovipneumoniae* in samples containing multiple *Mycoplasma* species. Hence, PCR test proved effective both at field and laboratory level diagnosis (OIE, 2008; Halium *et al.,* 2019).

The results of the present study suggested that *M. ovipneumoniae* is the main organism associated with respiratory tract infection in goats in Kerala. In another study carried out in the same region by Reji (2018), documented that *M. ovipneumoniae* was the predominant organism associated with respiratory infection in goats, followed by *M. conjunctivae* and *M. agalactiae.* Respiratory disease due to *M. ovipneumoniae* has been reported in India in a few studies (Sikdar and Uppal, 1986; Jana *et al.,* 2005). In a recent study carried out in Egypt, a high frequency of *M. ovipneumoniae* was detected using PCR in goats showing respiratory symptoms such as coughing and nasal discharge (Halium *et al.,* 2019). Apart from domesticated goats and sheep, *M. ovipneumoniae* also affects wild ruminants. In Washington, Highland *et al.* (2018), detected *M. ovipneumoniae* in nasal swab taken from mule deer employing PCR.

## Conclusion

More number of samples needs to be tested from wide geographical area by molecular epidemiological studies to study the role of *M. ovipneumoniae* in pleuropneumonic cases. Nucleotide sequencing and phylogenetic analysis are required to identify the prevalent strains so as to develop suitable protocol for effective control and prevention of the disease in the state.

## Acknowledgement

The authors are thankful to Kerala Veterinary and Animal Sciences University for providing all facilities for the completion of this work.

## Conflict of interest

The authors declare that they have no conflict of interest.

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