Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 725-732

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.092

Molecular Detection and Characterization of *Mycoplasma* Species from Respiratory Infections of Small Ruminants in Namakkal District of Tamil Nadu

P. Ponnusamy[1*], K. Sukumar[1], A. Raja[2], S. Saravanan[1] and P. Srinivasan[3]

[1]Department of Veterinary Microbiology, [2]Education Cell, [3]Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, India

**Corresponding author*

Keywords

Mycoplasma species, *M. arginini*, *M. conjunctivae*, Small ruminants and PCR

Article Info

Accepted:
14 February 2021
Available Online:
10 March 2021

A B S T R A C T

The present study aimed to identify and characterize the *Mycoplasma* species associated with respiratory infections of small ruminants by molecular method. A total of 61 samples including nasal swabs, lung swabs and heart blood swabs were collected from 11 different sheep and goat farms in and around Namakkal district of Tamil Nadu with the history of respiratory manifestations. The molecular PCR was employed to amplify the 16S rRNA gene of *Mycoplasma* species and *M.mycoides* cluster PCR and also species specific primer of *M. capricolum* subsp. c*apri pneumoniae* (Mccp). Nasal and lung swabs of five farms were found to be positive by PCR for *Mycoplasma* genus specific 16S rRNA gene with a size of 714 bp. *Mycoplasma* genus specific positive samples were negative for *M. mycoides* cluster PCR and Mccp. The positive purified PCR amplicons from five representative samples were subjected for sequencing. Out of which, four samples from sheep were closely related with *Mycoplasma arginini* and one sample from goat was closely related with *Mycoplasma conjunctivae*. The study provides rapid detection, specificity and sensitivity compared to conventional isolation and identification methods which are time consuming, laborious and expensive.

Introduction

Respiratory diseases in small ruminants increase the production costs due to costs for treatment, high morbidity and mortality results in economic burden to the farming community. It has been reported that respiratory infections in small ruminants contribute 5.6 percent of total diseases regardless of the causative agent (Hindson and Winter, 2002). Respiratory infections of small ruminants are multifactorial in which mycoplasma is one among them (Chakraborty *et al.,* 2014). *Mycoplasma* species belong to class *Mollicutes* which are pleomorphic and vary in morphology from spherical to filament form. Their genome size is small and devoid of the cell wall (Quinn *et al.,* 2011). There are several *Mycoplasma* species associated with different disease conditions in animals as well as human beings. They are the causative agent of economically important

diseases such as pleuropneumonia in ruminants and chronic respiratory disease in poultry. Important pathogens associated with pleuropneumonia in small ruminants are *M. capricolum* subsp. *capripneumoniae*, *M.mycoides* subsp. *capri* and *M.capricolum* subsp. *Capricolum* (OIE, 2014).

Mycoplasmas are also associated with other disease conditions which include arthritis, conjunctivitis, abortions, mastitis, atypical pneumonia and keratitis in sheep and goats (Nicholas, 2002). It has been reported that mycoplasmas are found on mucosal surfaces of the various sites such as nasal cavity, conjunctiva, oropharynx, intestinal and genital tracts (Quinn *et al.,* 2011). Several species of mycoplasmas are not pathogenic and represents part of the normal flora of their host. However, *M.arginini* and *M.ovipneumoniae* are the other mycoplasmal agents frequently isolated from respiratory diseases in small ruminants which imply pathogenic potential to these organisms (Akwuobu *et al.,* 2016).

Mycoplasmas are highly fastidious bacteria that require enriched media for their growth. It is very difficult to grow by conventional techniques that are time consuming, laborious and also expensive.

Recently molecular tests based on nucleic acid detection methods are widely used such as PCR which is rapid, specific and sensitive. It is commonly used for the identification of isolates and detection of the mycoplasma organisms directly in the clinical samples (Chakraborty *et al.,* 2014).

The present study was aimed to identify and characterize the mycoplasma species from sheep and goats exhibiting respiratory manifestations in Namakkal district of Tamil Nadu, a Southern state of India. They are detected by molecular PCR followed by characterization based on nucleotide sequencing and phylogenetic analysis.

Materials and Methods

Collection of samples

A total of 61 samples representing 11 different sheep and goat farms were collected in and around the Namakkal district. Nasal swabs (n=50) were collected from sheep and goats with a history of respiratory signs including respiratory sound, coughing, serous and mucous discharge from the nostrils. Post mortem samples such as lung swabs (n=6) and heart blood swab (n=5) received from the Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal were also processed for *Mycoplasma* species detection

Detection of *Mycoplasma* species by PCR

The DNA was extracted directly from nasal swab samples by using commercial DNA extraction kit (Qiagen, Germany) as per the manufacture's instruction. Then, PCR was carried out to amplify the 16S rRNA gene of *Mycoplasma* species. In this study, a forward primer, GPO-1 (5′-ACTCCTACGGGAGGCAGCAGTA-3′) and a reverse primer, MGSO (5′-TGCACCATCTGTCACTCTGTTAACCTC-3′) were used to amplify 714 bp length of the 16S rRNA gene (Van Kuppeveld *et al.,* 1992). The PCR reactions were carried out in 20 µl volume of 10 µl of master mix (2x), 1 µl of each primer (10 pmol), 6 µl of deionized water and 2 µl of template DNA. Thermal condition for this PCR reaction was initial denaturation of 94 ˚C for 5 min followed by 94˚C for 1 min (denaturation), 55˚C for 1 min (annealing) and 72 ˚C for 1 min (elongation) for 35 cycles and final elongation of 72˚C for 5 min. The analysis of the PCR product was carried out in 1.5 percent agarose gel stained

with ethidium bromide (0.5µg/ml) and documented under the Gel documentation system.

The *Mycoplasma* genus specific positive samples were subjected to two PCR assays such as *M.mycoides* cluster PCR and *M. capricolum* subsp. *capripneumoniae* (Mccp) for diagnosis of Contagious Caprine Pleuro Pneumoniae (CCPP). The primers used in this study are given in Table 1.

Nucleotide sequencing and analysis

The purified PCR amplicons were sequenced in an automated sequencer by Sanger dideoxy sequencing method. Based on the sequencing results, contigs were constructed using BioEdit software. Assembled nucleotide sequences were subjected to BLASTn analysis. The sequences were aligned by using (ClustalW) algorithm method. The phylogenetic tree was constructed to estimate the relationship between sequences by using Neighbour Joining (NJ) algorithm using bootstrap values and distance in Mega X software. Homology searches were performed with the NCBI database and BLAST. The confirmed sequences were submitted to NCBI GenBank.

Results and Discussion

In the present study, 50 nasal swabs, six lung swabs and five heart blood swabs were processed for the detection of *Mycoplasma* species that are associated with respiratory infections of small ruminants. Out of 61 samples representing 11 farms, only five farms (45%) were found to be positive by PCR for *Mycoplasma* genus specific 16S rRNA gene with the expected fragment size of 714 bp (Figure 1).

The PCR results were in agreement with Tabata baei Qomi *et al.,* 2014. Further, *Mycoplasma* genus specific positive samples were negative for *M. mycoides* cluster PCR and *M. capricolum* subsp. *capripneumoniae* (Mccp) and hence are negative for Contagious Caprine Pleuro Pneumoniae (CCPP). The purified PCR amplicons of *Mycoplasma* genus specific positive samples from five representative samples were subjected to sequencing by Sanger's dideoxy method. Out of five samples, four samples from sheep were closely related with *Mycoplasma arginini* and one sample from goat was closely related with *Mycoplasma conjunctivae* based on NCBI BLAST search. Further, three *M. arginini* and one *M. conjunctivae* sequence were submitted to NCBI GenBank and accession numbers were obtained (MN401189, MN401231, MN401232 and MN401285). The 16S rRNA gene sequences were compared with 16S rRNA gene sequences of reference *M. arginini* and *M. conjunctivae* strains available in the GenBank. A phylogenetic tree with distance and bootstrap value was constructed based on the sequence of *M. arginini* strains of Namakkal and standard strain (Figure 2).

The 16s rRNA sequences of *M. arginini* strains of Namakkal showed 100 percent sequence identity with other sequences including ATCC sequence and NCBI reference sequences available at GenBank (Table 2).

Further, a phylogenetic tree with distance and bootstrap value was drawn based on the sequence of *M.conjunctivae* strain of Namakkal and reference strains (Figure 3). The 16s rRNA sequences of the Namakkal strain showed 99.85 percent sequence identity with other sequences including NCBI reference sequences available at GenBank (Table 3). Further evolutionary divergence between sequences of various isolates of *M.arginini* and *M.conjunctivae* were shown in Table 4 and 5 respectively.

Table.1 Primers used in this study

Name	Primer sequence	Size	Reference
***M. mycoides* cluster 16S rRNA gene**	Forward 5'-CGA AAG CGG CTT ACT GGC TTG TT-3' Reverse 5'-TTG AGA TTA GCT CCC CTT CAC AG-3'	548 bp	Bascunana *et al.*, 1994
M. capricolum subsp. Capripneumoniae (Mccp)	Forward 5'-ATC ATT TTT AAT CCC TTC AAG-3' Reverse 5'-TAC TAT GAG TAA TTA TAA TAT ATG CAA-3	316 bp	Woubit *et al.*, 2004

Table.2 Sequence identity of *M.arginini* of Namakkal strains

S. No.	Accession No.	Country	Sequence identity
1.	MK789491.1	Turkey	100%
2.	KP972459.1	Egypt	100%
3.	JQ903580.1	USA	100%
4.	HQ661825.1	South Africa	100%

Table.3 Sequence identity of *M.conjunctivae* of Namakkal strain

S.No.	Accession No.	Country	Sequence identity
1.	LR214997.1	United Kingdom	99.85%
2.	FJ226571.1	USA	99.85%
3.	NR_044781.1	Sweden	99.85%

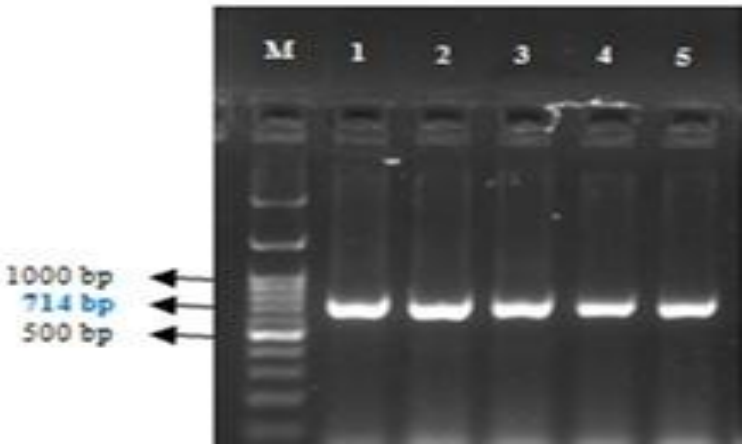
Table.4 Estimates of evolutionary divergence between sequences of *M.arginini*

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	HQ661821.1 *M.arginini* strain K1R03		0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000
2	HQ661823.1 *M.arginini* strain C1	0.000		0.000	0.000	0.000	0.000	0.000	0.002	0.000
3	HQ661825.1 *M.arginini* strain B2639	0.000	0.000		0.000	0.000	0.000	0.000	0.002	0.000
4	KP972459.1 *M.arginini* strain Dak-2/M.arg/EG014	0.000	0.000	0.000		0.000	0.000	0.000	0.002	0.000
5	MG564232.1 *M.arginini* isolate KETAW-HS	0.000	0.000	0.000	0.000		0.000	0.000	0.002	0.000
6	MG564233.1 *M.arginini* isolate SHEB-HS	0.000	0.000	0.000	0.000	0.000		0.000	0.002	0.000
7	MN401189.1 *M.arginini* Namakkal 01	0.000	0.000	0.000	0.000	0.000	0.000		0.002	0.000
8	MN401231.1 *M.arginini* Namakkal 02	0.004	0.004	0.004	0.004	0.004	0.004	0.004		0.002
9	MN401232.1 *M.arginini* Namakkal 03	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	

Table.5 Estimates of evolutionary divergence between sequences of *M.conjunctivae*

	1	2	3	4	5	6	7	8
1. NR_074135.1 *M.conjunctivae*		0.00	0.01	0.00	0.01	0.01	0.02	0.02
2. FJ226571.1 *M.conjuctivae*	0.00		0.01	0.00	0.01	0.01	0.02	0.02
3. MN401285 *M.conjuctivae* Namakkal	0.01	0.01		0.01	0.01	0.01	0.02	0.02
4. NR_044781.1 *M.conjunctivae*	0.00	0.00	0.01		0.01	0.01	0.02	0.02
5. NR_121731.1 *M.bovoculi*	0.04	0.04	0.04	0.04		0.00	0.02	0.02
6. NR_025987.1 *M.bovoculi*	0.04	0.04	0.04	0.04	0.00		0.02	0.02
7. MK789496.1 *M.ovipneumoniae*	0.09	0.09	0.10	0.09	0.10	0.10		0.00
8. MK789495.1 *M.ovipneumoniae*	0.09	0.09	0.10	0.09	0.10	0.10	0.00	

Fig.1 PCR of 16S rRNA gene of *Mycoplasma* genus



Lane M: DNA Marker (100-3,000 bp)
Lane 1, 2, 3, 4 & 5: Nasal swab samples

Fig.2 Phylogenetic tree of *Mycoplasma arginini* isolates based on 16S rRNA gene sequences

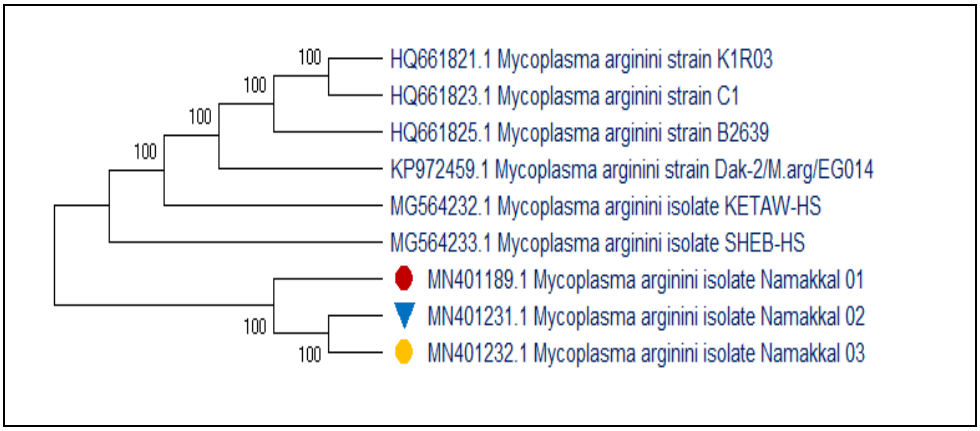
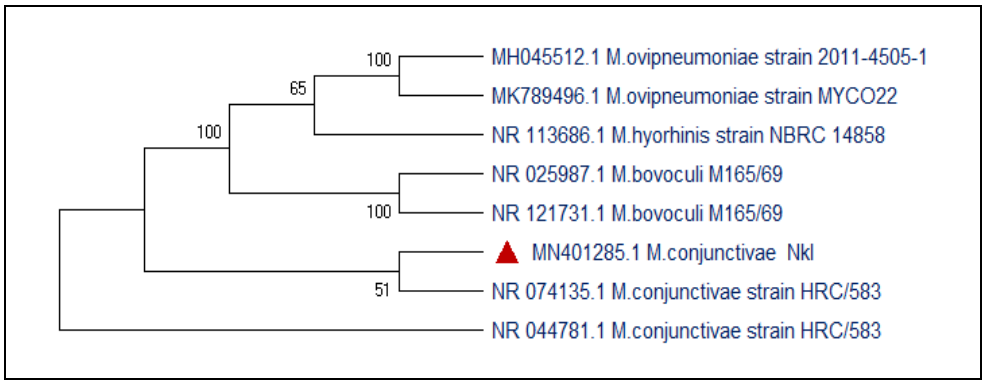


Fig.3 Phylogenetic tree of *Mycoplasma conjunctivae* isolates based on 16S gene sequences



Mycoplasma organisms are the smallest prokaryotes and cause pneumonia and other diseases such as arthritis, conjunctivitis, mastitis and abortion in small ruminants. The common species include *M. mycoides* subsp. *capri*, *Mycoplasma capricolum* subsp. *capripneumoniae*, and *M. capricolum* subsp. *capricolum* causing pneumonia or pleuropneumonia in sheep and goats on their own or combined with other microorganisms. *Mycoplasmas* inflict heavy economic losses in terms of morbidity (100%), mortality (10-100%) and loss of production performance in small ruminants. Though *M. arginini* was not considered as the primary pathogen, several reports were available indicative of its suspected pathogenic role in animal species (Akwuobu *et al.,* 2016; Azizi *et al.,* 2011; Niang *et al.,* 1999; Alley *et al.,* 1975; Valsala *et al.,* 2017).

In this study, *M. arginini* (4 farms) and *M.conjunctivae* (1 farm) were detected from sheep and goats respectively by molecular method. Our findings were following numerous reports of *M.arginini* isolation from respiratory infections and pneumonic cases of sheep and goats. Valsala *et al.,* (2017) and Ammar *et al.,* (2008) reported the prevalence of *M. arginini* from pneumonic lungs of goats and sheep. Andrea *et al.,* (2017) reported that *M. arginini* being the most frequent species detected in mycoplasma pneumonia in small ruminants. Abdel Halium *et al.,* (2019) reported the isolation of *M. arginini* suffering from respiratory infections and pneumonic lungs in high frequency rate from sheep and goats of Egypt. Whereas, *M. conjunctivae* has been associated with infectious keratoconjunctivitis outbreaks in domestic and wild caprine worldwide and was considered as the primary pathogen of this condition as suggested by Shahzad *et al.,* (2013). However, in the present study, an *M. conjunctiva was* identified in a co-infection with respiratory tract infection in goats.

In the current study, *M. arginini* and *M. conjunctivae* were detected from respiratory tract infections of small ruminants by PCR amplification of 16S rRNA gene specific for *Mycoplasma* species followed by nucleotide sequencing and phylogenetic analysis. It provides rapid detection, specificity and sensitivity compared to conventional isolation methods. The pathogenic potential of these organisms needs to be further explored in near future.

References

Abdel Halium, M. M., F. A. Salib, S. A. Marouf and Abdel Massieh, E. S. 2019. Isolation and molecular characterization of *Mycoplasma* spp. in sheep and goats in Egypt. Veterinary World. 12: 664-

670.

Akwuobu, C. A., K. F. Chah, S. I. Oboegbulem and Rabo, J. S. 2016. Pathogenicity of local isolates of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* strains in experimental West African Dwarf goats. Journal of Advanced Veterinary and Animal Research. 3: 242-251.

Alley, M. R., J. R. Quinlan and Clarke, J. K. 1975. The prevalence of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in the respiratory tract of sheep. New Zealand Veterinary Journal. 23: 137-141.

Ammar, A. M., S. I. Eissa, M. I. A. El-Hamid, H. A. Ahmed and El Aziz, A. E. E. 2008. Advanced study on mycoplasmas isolated from sheep. Zagazig Veterinary Journal. 36: 128-137.

Andrea, D. P., A. Daniela, Z. Katiuscia, M. Giuseppe and Massimo, S. 2017. *Mycoplasma pneumonia* in small rumi-nants: A ten-year long retrospective survey. Small Ruminant Research. 153: 103-106.

Azizi, S., E. Tajbakhsh, A. Rezaii, S. H. Nekouei and Namjoo, A. R. 2011. The role of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in pneumonic lungs of slaughtered sheep. Revue De Medecine Veterinaire. 162: 310-315.

Bascuriana, C. R., J. G. Mattsson, G. Bolske and Johansson, K.E. 1994. Characterization of the 16S rRNA genes from *Mycoplasma* sp. strain F38 and development of an identification system based on PCR. Journal of Bacteriology. 176: 2577-2586.

Chakraborty, S., A. Kumar, R. Tiwari, A. Rahal, Y. Malik, K. Dhama, A. Pal and Prasad, M. 2014. Advances in diagnosis of respiratory diseases of small ruminants. Veterinary Medicine Inernational. 2014: 1-16.

Elfaki, M. G., B. Abbas, O. M. Mahmoud and Kleven, S. H. 2002. Isolation and characterization of *Mycoplasma arginini* from camels (*Camelus dromedarius*) with pneumonia. Comparative Immunology, Microbiology and Infectious Diseases. 25: 49-57.

Hindson, J. C. and Winter, A. C. 2002. Respiratory disease in *Manual of Sheep Diseases*, 2nd edition (Blackwell Science, Oxford, UK), 196–209.

Niang, M., R. F. Rosenbusch, J. Lopez-Virella and Kaeberle, M. L. 1999. Differential serologic response to *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in lambs affected with chronic respiratory disease. Journal of Veterinary Diagnostic Investigation. 11: 34-40.

Nicholas, R. A. J. 2002. Improvements in the diagnosis and control of disease of small ruminants caused by Mycoplasmas. Small Ruminant Research. 45: 145-149.

OIE (2018), Contagious caprine pleuropneumonia in Terrestrial Manual, Chapter 3.7.4 *Office International des Epizooties*, 1441-1455.

Quinn, P. J., B. K. Markey, F. C. Leonard, E. S. FitzPatrick, S. Fanning and Hartigan, P.J. 2011. Veterinary Microbiology and Microbial Disease, 2nd edition (Wiley-Blackwell).

Shahzad, W., R. Munir and Rana, M. Y. 2013. Prevalence, molecular diagnosis and treatment of *Mycoplasma conjunctivae* isolated from infectious keratoconjunctivitis affected Lohi sheep maintained at Livestock Experiment Station, Bahadur nagar, Okara, Pakistan. Tropical Animal Health and Production. 45: 737–742.

Tabatabaei-Qomi, R., M. Sheykh Hasan, H. Fazaely, N. Kalhor and Ghiasi, M. 2014. Development of a PCR assay to detect Mycoplasma contamination in

cord blood hematopoietic stem cells. Iranian Journal of Microbiology. 6: 281–284.

Valsala, R., R. Rana, T. R. Arun and Singh, V.P. 2017. *Mycoplasma arginini*: high frequency involvement in goat pneumonia. Turkish Journal of Veterinary and Animal Sciences. 41: 393-399.

Van Kuppeveld, F. J., J. T. Van der Logt, A. F. Angulo, M. J. Van Zoest, W. G. Quint, H. G. Niesters, J. M. Galama and Melchers, W. J. 1992. Genus and species specific identification of *Mycoplasma*s by 16S rRNA amplification. Applied and Environmental Microbiology. 58: 2606-2615.

Woubit, S., S. Lorenzon, Peyrauda, L. Mansosilvan and Thiaucourt, F. 2004. A specific PCR for the identification of *Mycoplasma capricolum* subsp. *capripneumoniae*, the causative agent of contagious caprine pleuropneumonia (CCPP). Veterinary Microbiology. 104: 125–132.

How to cite this article:

Ponnusamy, P., K. Sukumar, A. Raja, S. Saravanan and Srinivasan, P. 2021. Molecular Detection and Characterization of *Mycoplasma* Species from Respiratory Infections of Small Ruminants in Namakkal District of Tamil Nadu. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 725-732. doi: https://doi.org/10.20546/ijcmas.2021.1003.092