The Pharma Innovation Journal 2018; 7(12): 21-23

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(12): 21-23

www.thepharmajournal.com
Received: 16-10-2018
Accepted: 18-11-2018

RM Reji
MV. Sc. Scholar, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

BK Mani
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

S Sankar
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

V Thirupathy
Professor and Head, Livestock Research Station, Thiruvizhamkunnu, Kerala Veterinary and Animal Sciences, Pookode, Wayanad, Kerala, India

PS Reshma
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

N AIswarya
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

TS Akkara
Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Correspondence
RM Reji
MV. Sc. Scholar, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Isolation and identification of *Mycoplasma agalactiae* associated with respiratory tract infection in goats: A case study

RM Reji, M Mini, BK Mani, S Sankar, V Thirupathy, PS Reshma, N AIswarya and TS Akkara

Abstract
Respiratory tract infections are the major cause of economic loss in goats. *Mycoplasma agalactiae* acts as an important etiological agent in mastitis, arthritis, kerato-conjuctivitis, pleuropneumonia and septicaemia syndrome (MAKePS). This paper reports a case study regarding the outbreak of respiratory disease associated with *M. agalactiae* in a herd of goats in Thrissur district, Kerala. The goats were presented with symptoms of respiratory distress, conjunctivitis, lethargy and reduced production. Nasal swabs were collected and dispensed in modified Hayfick's broth without crystal violet. After incubation at 37°C for about 72 h., DNA was isolated from the broth and subjected to *Mycoplasma* genus specific PCR, which revealed amplicons of 280 bp confirming the genus. Also, the solid medium which was inoculated with filtered drops of incubated broth presented typical fried egg like colonies after five days of incubation and the isolates were identified as *Mycoplasma* by PCR. The species was confirmed as *M. agalactiae* by nucleotide sequencing and this is the first report documenting the detection and isolation of *M. agalactiae* from goats of Kerala.

Keywords: Mycoplasma agalactiae, MAKePS, Modified Hayflick's broth

1. Introduction
Goats are highly susceptible to a number of *Mycoplasma* spp., particularly *Mycoplasma capricolum* subsp. *capripneumoniae* and other mycoplasmas of the *Mycoplasma mycoides* cluster (Nicholas, 2002) [1]. Among the various diseases, MAKePS syndrome is a significant disease and is characterised by mastitis, arthritis, kerato-conjuctivitis, pleuropneumonia and septicaemia (Thiaucourt and Bolske, 1996) [2]. The members of *Mycoplasma* implicated in MAKePS syndrome are *M. mycoides* subsp. *mycoides* Large Colony (MmmLC), *M. mycoides* subsp. *capri* (Mmc), *M. capricolum* subsp. *capricolum* (Mcc), *M. putrefaciens* (Mp) and *M. agalactiae* (Kumar *et al*., 2011) [3]. The infection due to *M. agalactiae* is also known as contagious agalactia (Bergonier and poumarat, 1996) [4]. The incubation period of infection caused by *M. agalactiae* in goats and sheep is one to eight weeks (Azevedo *et al*., 2006) [5]. Since *M. agalactiae* has been isolated from nasal secretions, faecal samples, milk, and aborted foetus, it is believed that the primary site of predilection is the mucosa of respiratory tract, small intestine, and alveoli of mammary glands, respectively, depending upon the respiratory, oral, and mammary routes of infection. Common clinical symptoms associated with the infection include fever, anorexia, lethargy, and unwillingness to follow the herd, followed by clinical symptoms depending upon the involvement of various organs such as mammary glands, lungs, genitalia, joints, and conjunctiva (Kumar *et al*., 2014) [6]. This study documents the investigation of an outbreak of respiratory disease associated with *M. agalactiae* in a herd of goats.

2. Materials and methods
Respiratory disease was reported in a herd of goats in Thrissur district. The goats were presented with symptoms of depression, lethargy, fever, serous to mucopurulent nasal discharge, conjunctivitis, coughing and recumbency. Among 30 goats in the farm, 23 were affected. Nasal swabs were collected aseptically from four infected goats and dispensed in modified Hayflick's broth. The medium contains PPLO (pleuropneumonia like organisms) infusion broth without crystal violet (17.5 g/L) as basal component and is supplemented with 25 per cent de complemented horse serum,

10 per cent fresh yeast extract, 0.4 per cent glucose, 0.8 per cent sodium pyruvate, 0.4 per cent of phenol red solution, 0.4 mL of five per cent thallium acetate solution and 250 mg ampicillin (Miles and Nicholas, 2010) [7]. The samples were incubated at 37℃ in five per cent CO_2 for about five days. The broth was examined daily for growth, which is indicated by a colour change in the medium. The broth which showed colour change was filtered and transferred to solid medium.

Also, DNA was extracted from the incubated broth after three days of enrichment using Hi Pura multi sample DNA extraction kit (HiMedia, India). The DNA samples were tested using *Mycoplasma* 16S *rRNA* genus specific Polymerase Chain Reaction (PCR). Primer pairs specific to *Mycoplasma* genus (GPO3F 5'-TGGGGAGCAAACAGGATTAGATACC-3' and MGSO 5'-TGCACCATCTGTCACTCTGTTAAC CTC-3') as described by Botes *et al*. (2005) [8] was employed for the study. A 25 µL reaction mixture was set up for the single PCR reaction consisting of

10X PCR master mix	12.5 µL
Forward Primer	2 µL
Reverse Primer	2 µL
Template DNA	6 µL
Nuclease Free Water	2.5 µL

The PCR tubes were placed in a thermal cycler (Eppendorf) and reaction was run as per the following protocol (Cetinkaya *et al*., 2009) [9].

Table 1: Protocol for 16S *rRNA* Genus Specific PCR

Temperature	Time	Steps	
94°C	2 min.	Initial denaturation	
94°C	15 sec.	Denaturation	35 cycles
59.3°C	15 sec.	Annealing	
72°C	15 sec.	Extension	
72°C	5 min.	Final extension	
4°C	10 min.	Hold	

Identification of the amplified products were done in a submerged agarose gel electrophoresis system using one per cent agarose stained with ethidium bromide, and Tris Borate EDTA buffer was used as the matrix at a voltage of 50V. The gel was visualised under a UV transilluminator and results were documented in a Gel Doc system (Biorad).

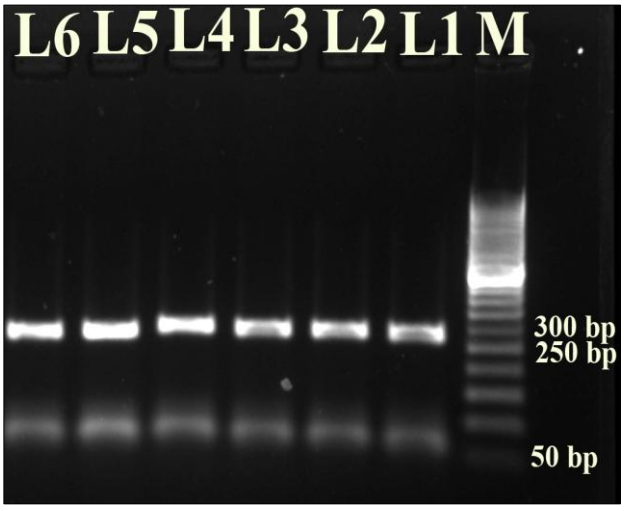
The isolates obtained on solid plate were analysed under the low power objective of microscope and the colonies were subjected to DNA extraction and *16S rRNA* genus specific PCR.

Nasal swabs were also inoculated onto Blood agar (BA) and Sabouraud's Dextrose Agar (SDA) for isolation of bacteria and fungi, respectively. Blood agar plates were incubated at 37°C and the duplicate samples on SDA plates were incubated at 37°C and at room temperature.

3. Results and discussion

In the present study, owner reported respiratory distress, lethargy and reduced production in a herd comprising of 30 goats. On BA, no bacterial organisms of pathogenic significance could be isolated even after 48h. of incubation. No growth could be obtained on SDA even after 7 days of incubation at 37℃ and at room temperature. Diagnosis of caprine mycoplasmosis can be done by various methods, but the gold standard test for confirmatory diagnosis is isolation and identification of the organism. It takes at least two to three weeks for cultural isolation, and the procedure is laborious (Aluotto *et al*., 1970) [10]

Polymerase chain reaction offers a rapid and a sensitive method for the detection and identification of *M. agalactiae* directly from clinical samples (Azevedo *et al*., 2006) [5]. The DNA extracted from the incubated broth was subjected to genus specific PCR for *Mycoplasma* species. A positive PCR result was obtained in case of all the four samples, which was indicated by the presence of a 280 bp fragment in electrophoresed gel under UV Tran illumination (Fig. 1). But, only two samples inoculated with filtered drops of incubated broth showed minute colonies on solid medium. On microscopy, typical fried egg like colonies could be appreciated in these samples (Fig. 2) and is in accordance with the observation of Kibor (1983) [11]. On Diene's staining, dark blue central zone and light blue peripheral zone could be recognised clearly (Fig. 3) as described by Quinn *et al*. (2011) [12]. The isolates were identified as *Mycoplasma* by *16S rRNA* genus specific PCR using the primer pair GPO3F and MGSO, which was found to be accurate in detecting *Mycoplasma* by Botes *et al*. (2005) [8]. The species identification of *Mycoplasma* isolates by biochemical and serological methods is complicated, since the members share many antigenic and genomic features (Thiaucourt and Bolske, 1996) [2]. Hence, in the present study, the amplicons were confirmed as *M. agalactiae* by nucleotide sequencing (Table 2). Even though, all the four samples produced amplicons on16S *rRNA* genus specific PCR, only two samples showed *Mycoplasma* colonies on solid medium. This lower recovery rate of *Mycoplasma* from clinical samples could be attributed to its fastidious nature (Francis *et al*., 2015) [13]. Also, isolation of *Mycoplasma* from nasal swabs was found to be challenging by Litamoi *et al*. (1990) [14] due to contamination with normal flora. This is the first report documenting the detection and isolation of *M. agalactiae* from goats in Kerala.



M – 50 bp DNA ladder
L1 – L4 – Amplicon obtained from broth
L5 – L6- Amplicon obtained from isolates

Fig 1: Amplicons of 16S rRNA gene specific PCR

Fig 2: Colonies obtained on solid medium (10X)

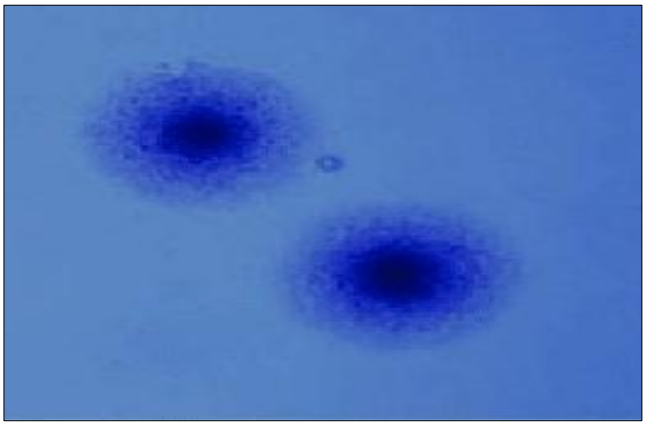


Fig 3: Colonies on Diene's Staining

Table 2: Sequencing Result of *16S rRNA* gene amplicons of *Mycoplasma*

Sample ID	Sequence
F 32	Tggggagcaaacaggattagataccctggtagtccacgccctaaacg atgatcattagttgatggggaactcatcgacgcagctaacgcattaaat gatccgcctgagtagtacgttcgcaagaataaaacttaaaggaattgac ggggatccgcacaagcggtggagcatgtggtttaatttgaagatacgc gtagaaccttacccactcttgacatcttctgcaaagctatggagacatag tggaggttaacagagtgacagatggtgcaa

4. Conclusion

The present study was an investigation in a herd of goats exhibiting respiratory distress, lethargy and significant production loss suggesting the involvement of *Mycoplasma* group of organisms. All the four samples tested showed positive results in PCR while isolates could be obtained from two samples. Even though, conventional isolation and identification is the gold standard technique for the detection of mycoplasmas, because of the laborious and time consuming procedure, it is now replaced by nucleic acid based techniques like PCR. The assay offers a rapid and sensitive alternative for accurate diagnosis of the disease, so that appropriate and timely strategies could be employed to control the disease.

5. Acknowledgement

The authors are grateful to the Dean, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, for providing the facilities to conduct the study.

6. References

1. Nicholas RA. Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. Small Ruminant Research. 2002; 1:45(2):145-9.
2. Thiaucourt F, Bölske G. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses. Rev. Sci. tech. off int. Epiz. 1996; 15(4):1397-414.
3. Kumar P, Roy A, Bhanderi BB, Pal BC. Isolation, identification and molecular characterization of Mycoplasma isolates from goats of Gujarat State, India. Veterinarski Arhiv. 2011; 20:81(4):443-58.
4. Bergonier D, Poumarat F. Agalactie contagieuse des petits ruminants: épidémiologie, diagnostic et contrôle. Revue scientifique et technique-Office international des épizooties. 1996; 15(4):1431-75.
5. Azevedo EO, Alcântara MD, Nascimento ER, Tabosa IM, Barreto ML, Almeida JF *et al*. Contagious agalactia by *Mycoplasma agalactiae* in small ruminants in Brazil: first report. Brazilian Journal of Microbiology. 2006; 37(4):576-81.
6. Kumar A, Rahal A, Chakraborty S, Verma AK, Dhama K. Mycoplasma agalactiae, an etiological agent of contagious agalactia in small ruminants: a review. Veterinary medicine international, 2014.
7. Miles RJ, Nicholas RA. Mycoplasma protocols. Humana press, 2010.
8. Botes A, Peyrot BM, Olivier AJ, Burger WP, Bellstedt DU. Identification of three novel mycoplasma species from ostriches in South Africa. Veterinary microbiology. 2005; 20:111(3-4):159-69.
9. Cetinkaya B, Kalin R, Karachan M, Atil E, Manso-Silvan L, Thiaucourt F. Detection of contagious caprine pleuropneumonia in East Turkey. Revue scientifique et Technique-Office International des Epizooties. 2009; 28(3):1037-44.
10. Aluotto BB, Wittler RG, Williams CO, Faber JE. Standardized bacteriologic techniques for the characterization of Mycoplasma species1, 2. International Journal of Systematic and Evolutionary Microbiology. 1970; 1:20(1):35-58.
11. Kibor AC. Cultural, biochemical and antigenic characteristics of caprine *Mycoplasma* isolated in Kenya. *M.Sc thesis*, University of Nairobi, Kenya, 1983, 111.
12. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitzpatrick EI. Veterinary microbiology and microbial disease. John Wiley & Sons, 2011, 7.
13. Francis MI, Ejeh EF, Raji MA, Egwu GO. Methods of isolation and identification of mycoplasma species of ruminants in Africa-A review. Bulletin of Animal Health and Production in Africa. 2015; 63(4):411-31.
14. Litamoi JK, Wanyangu SW, Simam PK. Isolation of Mycoplasma biotype F38 from sheep in Kenya. Tropical animal health and production. 1990; 1:22(4):260-2.