Molecular Prevalence of Mycoplasma capri in Thanjavur region

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Received: 09-08-2020, Accepted: 25-09-2020, Published online: 17-10-2020

Abstract

Goat rearing has emerged as a significant resource in rural areas even under unfavorable environmental conditions. Goat population in India has grown over the past few decades and stands at 148.88 million during 2019 (Census, 2019) owing to their greater socio-economic relevance. Goats, while being generally resistant to diseases, are highly susceptible to respiratory diseases, which account for almost 50\% mortality amongst them. Irrespective of the etiology, the infectious respiratory diseases of sheep and goats contribute to 5.6\% of the total diseases of small ruminants and is responsible for around 28.7 \% mortality. Pneumonia has been noticed as one of the most frequently encountered condition and is responsible for around 28.7\% mortality. Amongst various infections, Mycoplasmosis is one of the most dreaded diseases of goats. The present study was undertaken to detect the Mycoplasma capri infection in cauvery delta region of Tamil Nadu. All the collected tissue materials were subjected to isolation and PCR assay with Mycoplasma group specific primers (GPO- 1 and MGSO) which yielded 715 bp product and Mycoplasma capri specific primers (\textit{P} \textit{4} and \textit{P} \textit{6}) which gave an amplicons of 195 bp products. The findings indicate that the PCR assay is very simple and useful method for detecting the mycoplasma infection directly from the tissue materials in a very short span.

Keywords: Mycoplasma capri, PCR, Infection, detection and tissue materials

doi: https://doi.org/10.51128/jfas.2020.A007 | How to cite this article: Manimaran, K., Balakrishnan, S., Sangeetha, A., Dhanalakshmi, M. K. and Sivakumar, T. 2020. Molecular Prevalence of Mycoplasma capri in Thanjavur region. Journal of Food and Animal Sciences, 01(01): 38-43.

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Introduction

Goat farming is one of the enterprises which have been practiced by a large section of human population specially in developing world and it also plays an immensely important role in employment generation, capital storage and improving house hold nutrition. In spite of the enormous technological and developmental efforts in Livestock improvement sector in the country, the status of goats has largely remained as resource for poor farmers. A number of factors are responsible for economic losses to the goat industry, among them the health of goats is utmost important. The goat population in our country is frequently exposed to ravages of infectious diseases. Pneumonia has been noticed as one of the most frequently encountered condition and is responsible for around 28.7% mortality (Yatoo et al., 2018).

Amongst various infections, Mycoplasmosis is one of the most dreaded disease of goats (Nicholet, 1996; Kumar et al., 2011; Manimaran and Singh, 2017. Mycoplasma causes a number of important diseases viz., CBPP, CCPP, CRD, Arthritis, Contagious agalatiae and Mastitis. Amongst them, Contagious Caprine Pleuro Pneumonia (CCPP) has been a major cause of economic losses to goat industry and still exists at least in 33 countries of Africa and Asia. However in India, the CCPP was first reported by steel (1889) from Bombay and since a number of outbreaks have been observed in different parts of the country. The one of the causative agent Mycoplasma mycoides subsp. capri was first isolated by shirlaw in 1949 in India and later on by Uppal and Kumar (1981) and then Singh et al., (1997) from the kids and goats. To diagnose the CCPP, various serological tests viz., Slide agglutination test (SAT), Latex agglutination test (LAT), Double immunodiffusion (DID), Indirect haemagglutination (IHA), Enzyme linked immunosorbent assay and Complement fixation test (CFT) have been used routinely to diagnose the mycoplasma antibodies in livestock and poultry (Rurangirwa et al., 1981, Hasso and Al-omaran, 1994; Manimaran et al., 2006; Manimaran and Singh, 2016).

However, the conventional method of isolation and identification of the causative agents of Mycoplasma diseases are generally employed in conjunction with the serology which have the limitation of cross reactivity with other Mycoplasma and poor growth of the organisms. To overcome these problems, the PCR assay which is rapid, sensitive and specific was used in the present study to detect the Mycoplasma capri infection directly from the tissue materials in the Cauvery delta region of Tamil Nadu.

Materials and methods

Clinical picture and sample collection

A total of 60 samples were collected from suspected cases of caprine mycoplasmosis from clinically suspected goats of different age groups from kid to adult goats which brought to Veterinary clinical complex for treatment and Department of Veterinary pathology for conducting Post-mortem, Veterinary College and Research Institute, Orathanadu, Thanjavur in and around areas of Cauvery delta region of Tamil Nadu state and all animals were reported with clinical signs like pyrexia (41-43°C), reduced feed intake, water intake, there was nasal discharge with catarrhal in nature at the beginning and becomes mucopurulent in the later stage of disease, respiration was accelerated and painful and accompanied by frequent coughing, closure of one or both eyes, respiratory distress, Mortality 5% and morbidity of 50% was reported. All ages and both sexes were susceptible and prone to infections. Postmortem was carried out from dead animals and the samples include swab from conjunctiva, nasal swab and tracheal swab from live animals as well as heart blood swab, trachea, lungs, liver, spleen and mesenteric lymph nodes tissue samples from dead animals. These samples were collected in Pleuro Pneumonia like Organism (PPLO) transport medium.

Isolation and Identification of Mycoplasma

The collected samples were processed for cultural isolation using PPLO broth and agar. PPLO agar was prepared by mixing two constituents, i.e. Part A and Part B. Part A was prepared by mixing 35 grams of PPLO agar base (Difco) in 700 ml of distilled water. Part B was prepared by mixing 15% heat inactivated horse serum (Invitrogen), 10% yeast extract (HiMedia), 10% glucose (HiMedia), 1% Nicotinamide Adenine Dinucleotide (NAD) (HiMedia), 2.5% Thallium Acetate (HiMedia), Penicillin Benzyl Sodium salt (10000 IU) and the final pH was adjusted to 7.8. Part B was sterilized by filtration. Part A was autoclaved at 121°C for 15 minutes. After cooling, Part A was added to Part B. Similarly, PPLO broth was prepared by making Part A and Part B. Part A was prepared by mixing 14.7 grams of PPLO broth base (Difco) in 700 ml of distilled water. Part B was prepared by mixing 15% heat inactivated Horse Serum (Invitrogen), 10% Yeast Extract (HiMedia), 10% Glucose (HiMedia), 1% NAD (HiMedia), 2.5% Thallium Acetate (HiMedia), Penicillin Benzyl Sodium salt (100000 IU), 0.5% Phenol Red and the final pH was adjusted to 7.8. Part B was sterilized by filtration and Part A was autoclaved at 121°C for 15 minutes. After cooling, Part A was added to Part B. The tissue samples were
homogenized in normal saline using mortar and pestle and 10% tissue were prepared. The processed samples were inoculated into 2 ml of PPLO broth and incubated at 37°C with 5% CO₂ tension for one week. After active growth, it was sub cultured in 5 ml of PPLO broth and again incubated at 37°C with 5% CO₂ tension for one week to 21 days with intermittent shaking until the appearance of sharp yellow colour change indicating the optimum growth. The cultures showing the growth were sub cultured in PPLO agar plate and incubated at 37°C in incubator with 5% CO₂ tension. The plates were viewed under stereomicroscope for presence of fried egg appearance colony.

**Molecular detection**

Cultures suspected for *Mycoplasma* were subjected to PCR. Broth culture (1ml) was centrifuged at 12,000 rpm for 10 min in a microfuge tube to pellet the organism. The pellet was washed twice with phosphate-buffered saline (PBS), Nuclease-free water (NFW) 50 μL was used to dissolve the pellet and the contents were boiled at 95 °C for 10 min followed by immediate cooling at − 20 °C for 5 min. Finally, the tubes were centrifuged at 12,000 rpm for 5 min and the supernatant was used as the template DNA for the PCR. Briefly, the reaction mixture was prepared by adding the following components; 12.5 μl of Red dye master mix (Ampliqon) containing 1.5mM MgCl₂, 1μl each of forward and reverse primers (10 pmol/μl). Appropriate quantity of DNA was added and final volume of 25 μl was made with nuclease free water. Positive and negative controls were included during amplification.

**Mycoplasma group specific PCR**

Initially, the extracted DNA was screened by PCR using *Mycoplasma group specific* primers (GPO- 1 [5'-ACT CCT ACG GGA GGC AGC AGT A-3'] and MGSO [5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC -3']) as per Bashiruddin et al.,1994.

**Mycoplasma capri specific PCR**

*M. capri* specific PCR was performed using primers P4 (5'- ACT GAG CAA TTC CTC TT-T3') and P6 (5'- TTA ATA AGT CTC TAT ATG AAT-3'). The reaction mixture and temperature cycling were similar to the cluster specific PCR except that the annealing temperature of the primer was 46 °C.

**Detection of PCR products**

The PCR products were separated by agarose gel electrophoresis (Broviga, India) using 1.5% agarose gel in 1X tris-acetic acid-EDTA buffer at constant voltage of 80 volts. The ethidium bromide (10 mg/ml) stained gel was visualized under UV and recorded in Gel Documentation system (Universal Hood II, Bio-Rad, USA).

**Results and Discussion**

**Clinical signs**

Animals showed pyrexia with a temperature range of 41-43°C and reduced feed and water intake. There was nasal discharge with catarrhal in nature at the beginning and becomes mucopurulent in the later stage of disease (Fig. 1). Respiration of the animals were accelerated and painful and accompanied by frequent coughing. Other symptoms such as closure of one or both eyes, respiratory distress, Mortality 5% and morbidity of 50% was reported. All ages and both sexes were susceptible and prone to infections. Our findings were in accordance with *Mycoplasma mycoides capri* (*Mmc*) infection along with the classical signs of respiratory system was also observed by (Mondal et al., 2004; Laura et al., 2006; Shah et al.,2017).

**Fig.1:** Bilateral mucopurulent discharge

**Necropsy findings**

Detailed necropsy was conducted from dead animals on 15 adult goats and 5 kids that died in and around Cauvery delta region of Tamil Nadu during the period of study and gross lesions were recorded. Internal examination revealed profuse frothy exudates in tracheal lumen extending up to the bronchi and lungs. Thoracic cavity contained accumulation of serosanguinous fluid, Lung appeared with lung congestion, fibrinous pleuropneumonia (Fig. 2), yellow coloured fluid accumulation in the lung, bilateral lung adhesion. Upon incision, red frothy exudates oozed out from the parenchyma and the consolidated areas were hard to incise with thickening of septa. Similar types of gross lesions were also reported by (Thiaucourt et al., 1996; Awan et al., 2009, Parray et al., 2019) in an outbreak of CCPP caused by *Mycoplasma capricolum subsp. capripneumoniae* (*Mccp*).
Fig. 2. Fibrinous pleuropneumonia

Isolation of Mycoplasma

The processed samples were inoculated into 2 ml of PPLO broth and incubated at 37°C with 5% CO₂ tension for one week. After active growth, it was sub cultured in 5 ml of PPLO broth and again incubated at 37°C with 5% CO₂ tension for one week to 21 days with intermittent shaking until the appearance of sharp yellow colour change indicating the optimum growth. The cultures showing the growth were sub cultured in PPLO agar plate and incubated at 37°C in incubator with 5% CO₂ tension. The plates were viewed under stereomicroscope for presence of fried egg appearance colony (Fig. 3). Similar isolation findings were also reported by Thiaucourt and Bolske, 1996; Nicholas, 2002; Kumar et al., 2011a. Our results were in accordance with Chakraborty et al., 2014 isolated, identified and molecularly characterized mycoplasma isolates from goats of Gujarat State in India and found M. mycoides subsp. capri, M. agalactiae, M. capricolum subsp. capricolum (Mcc) as the main isolates. Similar isolation studies were also done with Yatoo et al., 2019 on Contagious caprine pleuropneumonia.

Molecular detection

The clinical materials viz., lungs, lymphnodes, spleen and liver were subjected to PCR Initially with Mycoplasma group specific primers GPO- 1 and MGSO yielded 715 bp (Fig. 4). Similar findings of 715 bp observed by Mounier et al., (2019) on Isolation and molecular characterization of Mycoplasma spp in sheep and goats in Egypt. Further, it was also characterized by using M. capri specific primers (P4 and P6) which gave an amplicons of 195 bp (Fig. 5) products. Our findings was in accordance with Kumar et al., (2001) stated that the amplified 574 bp fragment from M. mycoides group for differentiation of mycoides group from the capricolum group of the clusters. Similar observation was also made by Amores et al., (2010) found that the PCR for Mycoplasma detection proved to be rapid and sensitive method. Similar findings was also made by Seed and Osman, 2018 on clinical and laboratory diagnosis of Contagious caprine pleuropneumonia in Qassim region of Saudi Arabia and Yatoo et al., 2019 was also observed the similar results on Contagious caprine pleuropneumonia. Earlier reports also recommended molecular diagnostic assays for easy, early and accurate diagnosis of M.capri by PCR assay (Manimaran and Singh, 2017).

Fig. 3. Fried egg appearance (10 X magnification)

Fig. 4. Mycoplasma group specific PCR
Lane-1:100 bp marker, Lane-2 : +ve control, Lane -3-5: clinical samples, Lane -6 : -ve control

Conclusion

It is concluded that PCR, a reliable tool for early detection of the disease condition and molecular diagnosis is more rapid and accurate for early identification of Caprine mycoplasmosis from clinical specimens than conventional culture methods in a very short span.
Fig. 5. Mycoplasma capri specific PCR

PCR. Lane-1: 100 bp marker, Lane-2: +ve control, Lane-3-6: clinical samples, Lane-7: -ve control

Authorship contribution statement
K. Manimaran: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. S. Balakrishnan: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. A. Sangeetha: Writing - original draft, Writing - review & editing. M. Dhanalakshmi, K.: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. and T. Sivakumar: Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Acknowledgment

The authors are thankful to the Dean, Veterinary College and Research Institute, Orathanadu, Thanjavur and Professor and Head, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu, Thanjavur and Professor and Head, Central University laboratory, TANUVAS for providing necessary facilities to carry out this work and the funding by the Director of Research, TANUVAS under TANUVAS sub-project to conduct the research work are duly acknowledged.

Funding
None.

Declaration of Competing Interest
All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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