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Molecular detection of *Mycoplasma agalactiae* in goats suffering from mastitis

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Abstract

Among various goat diseases, mycoplasmal infections are one of the important infections. Clinical mycoplasmoses often lacks pathognomonic characteristics, so definitive diagnosis of the disease is quite cumbersome. Present study was conducted on mastitic milk samples of 215 goats to detect *Mycoplasma agalactiae* using Polymerase chain reaction (PCR). Results revealed that, out of 215 milk samples, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R in 31 samples. This confirms that 14.42% of mastitic goats were suffering from *Mycoplasma agalactiae* infection.

Keywords: Goats, mastitis, molecular detection, *Mycoplasma agalactiae*

1. Introduction

Goat farming is one of the major sources of revenue for thousands of small scale farmers in countries like India. This species of animal in the country is facing diversified problems including poor management practices, underfeeding, infectious and non-infectious diseases. Among various goat diseases, mycoplasmal infections are one of the important infections which result in significant losses [1]. Clinical mycoplasmoses often lacks pathognomonic characteristics and symptoms can be shared by other clinically significant infections. As a consequence, the diagnosis of an acute caprine mycoplasmal infection can be easily misinterpreted. Earlier, definitive diagnosis required the isolation of the causative mycoplasmas from the affected animals, which were then identified by biochemical, serological tests. Now days, various scientists used polymerase chain reaction for the detection of *Mycoplasma agalactiae* and results suggested that PCR was faster than the traditional tests and it can be used as a confirmatory test in the diagnosis of contagious agalactia.

Samples of preference include milk, nasal, ear swabs and joint fluid. Although the significance of mycoplasmosis is well known but a meager work regarding detection of mycoplasmosis in goats in Madhya Pradesh has been carried out. So in view of the above facts, this study was aimed to detect *Mycoplasma agalactiae* in goat milk using polymerase chain reaction.

2. Materials and Methods

Present study was conducted on 215 mastitic goats belonging to organized as well as unorganized goat farms in and around areas of Jabalpur. Out of 215 goats 137 were suffering from clinical mastitis and 78 were suffering from subclinical mastitis. DNA was extracted from milk samples and Polymerase chain reaction (PCR) was performed to detect the *Mycoplasma agalactiae*. Milk samples were collected under aseptic conditions and 0.1 ml of milk samples were inoculated into mycoplasma broth base containing mycoplasma supplement and incubated in a humid atmosphere containing 5% carbon dioxide at 37°C for 7 days. The broths were checked daily for growth. Positive broth cultures were stored at 4°C till further use [2]. DNA extraction was performed by chelex based extraction of DNA using the Insta Gene Matrix (Bio-Rad laboratories, India Pvt Ltd) [3]. Precisely, for extraction of DNA, 1 ml of mycoplasma broth culture was vortexed for 10 sec and centrifuged for 1 minute at 10,000-12,000 rpm. The supernatant was removed and 200 µl of the Insta Gene matrix was added to the pellet. The suspension was incubated at 56°C for 15-30 min and again vortexed at high speed for 10 sec. The cell suspension was heated in a boiling water bath for 8 minutes and again vortexed at high speed for 10 seconds and spun at 10,000-12,000 rpm for 2-3 minutes.

Then 20 µl of the resulting supernatant was used per 50 µl PCR reaction and the remainder supernatant was stored at -20 °C for further use.

The oligonucleotide primers were synthesized and supplied in lyophilized form by Integrated DNA Technologies, Avantor Performance Materials India Limited, Faridabad. They were reconstituted to 100 µM/µl stocks in sterile nuclease free water (NFW). Primers were used at a working dilution of 50 pmol/µl in sterile NFW. The specific primers were used in the study to identify *Mycoplasma agalactiae* [2].

ma-mp 1 5'-AGCAGCACAAAACCTCGAGA-3' (forward)

ma-mp 1 5'-AACACCTGGATTGTTTGAGT-3' (reverse)

The PCR was carried out in 50µl of reaction mixture which was composed of: PCR master mix 25 µl, Forward and Reverse primers 1µl each, Nuclease free water 3 µl and sample DNA 20 µl. The cycling conditions used for amplifying the ma-mp 81 DNA gene of *Mycoplasma agalactiae* were 30 cycles of denaturation at 94 °C for 1 min, annealing at 54°C for 1 min, extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The amplification of specific PCR product was checked by electrophoresis of the PCR product in 1.5% agarose gel and viewed in UV transilluminator system.

3. Results

The growth of mycoplasmas in broth media was demonstrated by changes in colour and turbidity due to the bacterial biochemical activity and metabolism. A total of 215 milk samples were collected from goats suffering from mastitis and were analyzed with a PCR test for the presence of *Mycoplasma agalactiae*. Out of 215 milk samples, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R were observed in 31 samples. This confirms that 14.42% of mastitic goats were suffering from *Mycoplasma agalactiae* infection i.e contagious agalactia. The results of PCR test is presented in Fig 1.

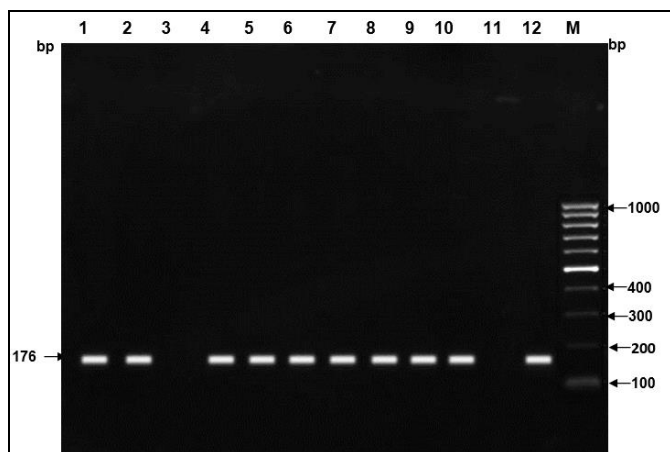


Fig 1: Agarose gel electrophoresis showing amplification of 176 bp fragment specific for *Mycoplasma agalactiae* with M: 100 bp molecular marker; Lane 1-12 shows milk samples; Lane 3 & 11 shows negative samples.

4. Discussion

Use of PCR in confirmatory diagnosis of *Mycoplasma agalactiae* infection is supported by the work of many scientists [4, 5] who stated that PCR is a valuable method for diagnosis of mycoplasma infections. Similarly, Tola *et al.* (1997) [6] extracted DNA from sheep milk to use for polymerase chain reaction (PCR) for diagnosis of

Mycoplasma agalactiae and compared it with traditional culture techniques and the PCR was reported to be much faster than culture, it has also reduced the time required for diagnosis. Other scientists [7,8] also used PCR for identification of *M. agalactiae* from the milk samples collected from suspected goats and reported that PCR can be used as trusty and supersede test in detection of *Mycoplasma agalactiae* from affected goats and among different collecting sites, milk samples are suitable for PCR detection.

The results of present study of detection of *Mycoplasma agalactiae* by using PCR were promising and correlate well with the results of previous studies. In the present investigation, only PCR was adopted for identification of *Mycoplasma agalactiae* in milk samples because it has provided a rapid and early diagnosis when performed in clinical samples. Thus, it enabled to carry out early therapeutic measures when results were positive. Moreover, mycoplasmas are very fastidious pathogens [9] and isolation of mycoplasmas is considered to be one of the most difficult tasks for diagnostic laboratories due to their inability to grow easily in laboratory medium in spite of the great improvement in medium formulations [10]. Additionally, animals with mycoplasma mastitis may shed the pathogen intermittently and shedding of *Mycoplasma* spp. into milk may be below the threshold of detection by standard culture methods [11]. PCR has the advantage of easy use, rapid availability of results and is more suited for processing large number of specimens.

5. Conclusion

In the present study, *Mycoplasma agalactiae* was detected in the mastitic milk samples of 215 goats with the help of Polymerase chain reaction (PCR). Results revealed that, out of 215 milk samples, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R in 31 samples. This confirms that 14.42% of mastitic goats were suffering from *Mycoplasma agalactiae* infection.

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