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Abiramy A
Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, KVAFSU, Karnataka, India

Ramesh PT
Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, KVAFSU, Karnataka, India

Basava Reddy K
Ph.D. Scholar, Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, KVAFSU, Karnataka, India

Corresponding Author
Basava Reddy K
Ph.D. Scholar, Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, KVAFSU, Karnataka, India

Incidence and molecular identification of pathogens in mastitis milk of goats

Abiramy A, Ramesh PT and Basava Reddy K

Abstract
Goat milk is considered to have better digestibility, alkalinity, buffering capacity and certain therapeutic values in human nutrition. Mastitis is considered as an important economic trait that causes great reduction in milk production and increases morbidity and mortality. A total of 200 milk samples were collected from goats of different breeds. Pure cultures of various Bacterial pathogens like Staphylococcus, *E. coli*, *Mycoplasma* spp were isolated. The determination of Bacteriological Status (BS) of milk samples is regarded as a "Gold Standard" for the determination of the udder health status. All the bacterial pathogens isolated from milk samples were confirmed by PCR. A total of 157 Staphylococci spp. isolates, 7 *Enterobacter* spp. and 8 *Mycoplasma* spp. isolates were confirmed as positive with PCR.

Keywords: goat, mastitis, pathogenic bacteria, PCR

Introduction
In India, goat farming is well established but unorganized and mostly reared by landless farmers (Prasad *et al*., 2005) [19]. According to an economic survey, India ranks first in the world with the total milk production of about 198.4 million tonnes in 2019-20. However, goat milk contributes 3.2 per cent of the national milk production and most of it is auto-consumed by the rural population (70%). Goat milk differs from cow or human milk in having better digestibility, alkalinity, buffering capacity and certain therapeutic values in human nutrition. Therefore, awareness about advantage of consumption of goat milk should be popularized so that production and utilization of goat milk could be enhanced.

The small ruminant population in our country is frequently exposed to ravages by various infectious diseases *viz*. bacterial, viral etc. Among them, mastitis plays a major role for high economical losses in the form of decreased milk production and increased treatment costs (Watson and Buswell, 1984 and Bor *et al*., 1989) [22, 5]. Mastitis is usually classified into two forms *viz*., subclinical and clinical. In clinical form, it could be diagnosed visually or by palpation of mammary gland, whereas subclinical form is diagnosed *via* enumeration of somatic cells and /or isolation of bacterial agents (Keisler *et al*., 1992 and Radostits *et al*., 2000). Sub-clinical form of goat mastitis is considered as a great threat as it causes great reduction in milk production up to 21% and also causes kid mortality due to feeding of mastitis milk (Mhase *et al*., 2007) [17]. Hence, assessment of udder health in goats should be prioritised. Studies on various diagnostic techniques for detection of mastitis in goats have been in limelight in research. Electrical conductivity (EC), Somatic cell count (SCC), bacteriological examination, enzyme estimation and physical properties of milk (pH and specific gravity) gives indirect information on the composition of milk in normal and diseased conditions. The determination of Bacteriological Status (BS) of milk samples is regarded as a "Gold Standard" for the determination of the udder health status. Identification of bacteria in most clinical laboratories is currently based on biochemical tests, serotyping, and enzymatic profiles and molecular techniques (PCR). PCR is considered as a fast, highly specific and an excellent technique for the rapid detection of pathogens, including those that are difficult to culture. Pathogens such as *E. coli*, *Staphylococcus aureus*, *Corynebacterium spp.* Coagulase negative *Staphylococci*, *Streptococccus dysagalactiae*, *Streptococcus uberis* are much obvious for causing mastitis and frequently isolated bacterial isolates in goat milk (Harmon, 1994 and Bergonier *et al*., 2003) [12, 3]. Monitoring of milk samples throughout the lactation period is recommended to check for mycoplasma infections in dairy goat herds as they lead to mastitis and causes mortality (Contreras *et al*., 2008 and Fox *et al*., 2003) [6, 3]. Hence the present study was conducted to screen the presence of pathogens that cause subclinical and clinical mastitis

in goats using molecular diagnostic tools.

Materials and Methods

A total of 200 milk samples were collected from goats of different breeds from Veterinary hospital, Veterinary College, Hebbal, organized farms and unorganized household farmers in and around Bangalore. Samples were immediately transported to laboratory under refrigeration condition. Bacteria were isolated and pure cultures were obtained. Mycoplasma was isolated using PPLO broth and stored at 4°C until further study. All the bacterial pathogens isolated from milk samples were confirmed by biochemical profiles subjected to PCR.

DNA extraction from pure bacterial culture

QIAamp MiniSpin Columns kit was used for extraction of bacterial DNA from pure culture. Eluted DNA was checked for its quality and quantity using Nanodrop sphectrophotometer and stored at -20 ºC until further use

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed as per the method described by Yokoigawa *et al.* (1999) [23] for *E.Coli* and McAuliffe *et al.* (2005) [15] and was also carried out for staphylococcus and mycoplasma. The genes included for the PCR are 'Tu' elongation factor (tuf) gene for *Staphylococci* spp, *alr* gene for *E. coli and Uni* gene for *Mycoplasma.* The primers designed for the present study are presented in table 1.

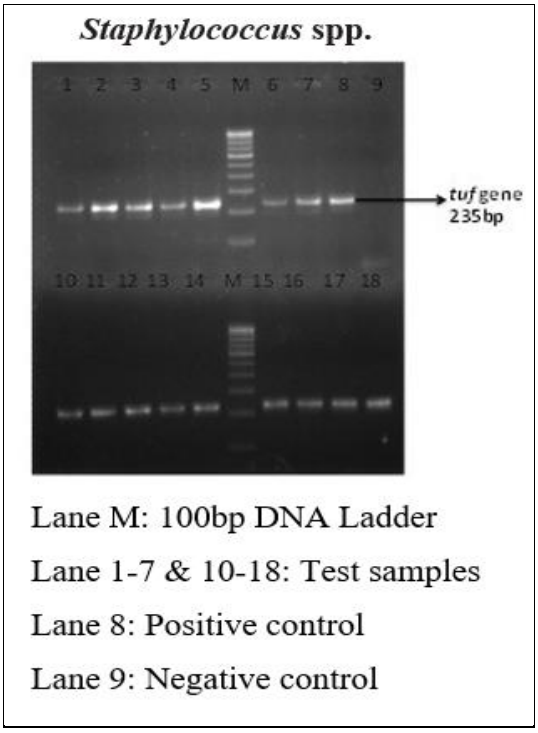
Table 1: List of primers used for *Staphylococcus, E.Coli & Mycoplasma* spp.

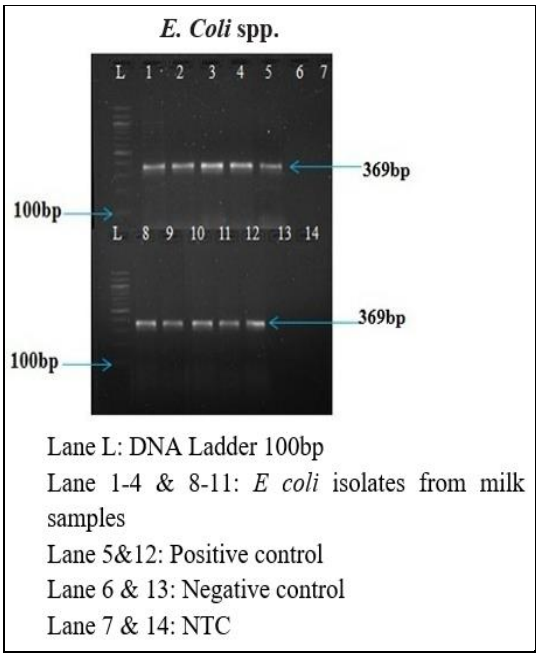
Gene	Primer sequence	Product length
Staph tuf-F	5'- GAA GAA TTA TTA GAA TTA GT -3'	235
Staph tuf-R	5' GTG ATT GAG AAT ACG TCC TCA AC -3'	
EC-*alr*-F	5'-CTG GAA GAG GCT AGC CTG GAC GAG-3'	369
EC-*alr*-R	5'-AAA ATC GCC ACC GGT GGA GCG ATC-3'	
Mycoplasma Uni-F	5'- CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3'	250
Mycoplasma Uni-R	5'- ACC TAT GTA TTA CCG CG -3'	

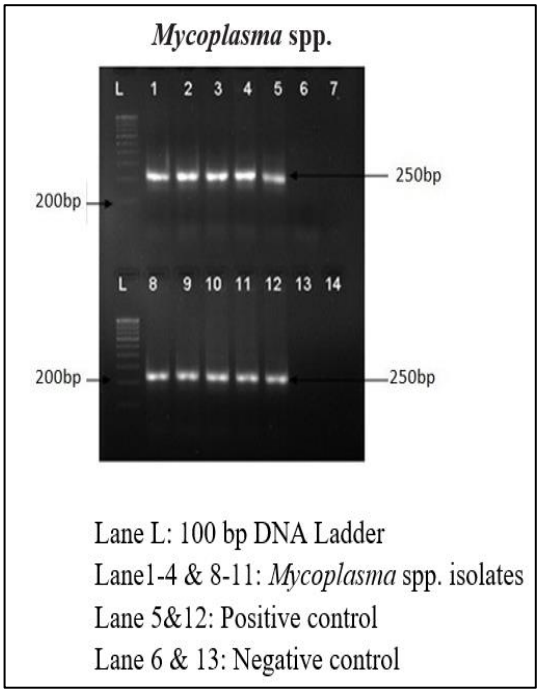
Genomic DNA from *Staphylococcus*, *E.coli* and *Mycoplasma* culture from bacteriological research laboratory, department of Microbiology, Veterinary College, Hebbal, Bangalore was used as positive control to PCR and negative control in PCR consisted of reaction mixer inoculated with ultrapure water in substitution of DNA. Reaction mixture was prepared in 0.2 ml thin walled pcr tubes that contained 3μl of DNA template, 1 μlof Forward and reverse primers, 12.5 μl of master mix that make up to 25 μl with molecular grade water. PCR was performed at the followed conditions viz., initial denaturation for 5 min at 94 ^{0}C, denaturation for 30 sec at 94 ^{0}C, annealing (50 ^{0}C for 30 sec in staphylococcus, 57 ^{0}C for 30sec in *E. Coli* and 56 ^{0}C for 45 sec in Mycoplasma spp.), extension at 72 ^{0}C for 30sec and final extension at 72 ^{0}C for 10min for 30 cycles.

Results and Discussion

The PCR products were checked on agarose gel electrophoresis to screen the causative pathogens and scored as positive based on the product size (235, 369 and 250 bp in *Staphylococcus*, *E.coli* and *Mycoplasma*). The images were documented using gel documentation system (Bio Rad, USA).







The overall incidence of subclinical mastitis was 72.5 per cent, clinical mastitis was 8.5 per cent and total mastitis cases recorded was 81 per cent. Gupta *et al.* (1999) [11] recorded subclinical mastitis in goats on a yearly basis and found that the incidence was highest (14.76%) in 1993 and lowest was (4.68%) in 1991. In the present study total of 157 Staphylococci spp. isolates, 7 *E. coli*. and 8 *Mycoplasma* spp. isolates showed positive with biochemical tests and were confirmed as positive with PCR.

The results, obtained in this isolation studies were very much in accordance with the earlier studies of Marogna *et al.* (2012) who recorded *Staphylococcus* spp. (236 samples, 73.5%), with *S. epidermidis* (53 samples, 22.5%), *S. aureus* (44 samples, 18.7%), and *S. caprae* (41 samples, 17.4%) as being the most frequently identified species. Menzies and Ramanoon (2001) [16] observed that *E. coli*, *Staphlyococcus aureus*, *Streptococci* spp. and *Pseudomonas* spp. were commonly isolated from cases of clinical caprine mastitis

In the present study, total of 8 (4%) isolates of *E.coli* obtained from subclinical, clinical mastitis. Similar findings were also reported by El- Bassiony *et al.* (2008) [8], Ajuwape *et al.* (2005) [1], Mhase *et al.* (2007) [17], Bezerra *et al.* (2003) [4], Fernandez *et al.* (2005) [9] and Mohan *et al.* (2004) [18]. Smith and Sherman (1994) opined that *E. coli* is an environmental pathogen causing infection. The variation in the number of *E. coli* isolates in the present study were attributed to geographical area, environmental pathogen, difference in year and type of inflammatory reaction

In this study 8 (4%) *Mycoplasma* spp. were isolated which is in concordance with Egwu *et al.* (2001) [7] who reported a 40 per cent prevalence of *Mycoplasma* spp. in udder-halves with mastitis and 29 per cent prevalence in udder-halves without mastitis. The lesser prevalence in the present study might be due to large sample size (n=200). Similarly, Al- Momani *et al*. (2006) who isolated *Mycoplasma* spp. of 8 (13%) and 7 (2.3%) from milk sample and nasal swabs respectively.

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