JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2016. Vol. **10**(2), p. 1585-1589

Prevalence and Bacteriological Examination of Clinical and Sub-clinical Cases of Caprine Mastitis in Jammu, India

Anil Taku, Gulzar Ahmad Badroo, Sabahat Gazal, Irfan Ahmad Mir, Mohd Altaf Bhat, Rajinder Kumar Bhardwaj, Rajni Kanta Sharma, Amitoz Kour, Hamid Shah and Faizan Javaid

Division of Veterinary Microbiology and Immunology, FVSc and AH, SKUAST-J, R.S. Pura. 181102, India.

(Received: 09 September 2015; accepted: 14 November 2015)

A total of 109 lactating does were screened for mastitis, both clinical and sub-clinical. The goats with swelling, pain, increased temperature or abnormal milk secretion were considered to have clinical mastitis while California mastitis test was used for detection of sub-clinical mastitis. Milk samples from does detected to be positive for mastitis were collected aseptically for bacterial isolation. The bacteria were identified based on standard microbiological techniques; however, confirmation of the bacterial species was done by PCR using universal primers specific for *16S rDNA* followed by sequencing. Out of 109 lactating does screened, 45 (41.28%) were found positive for mastitis. Among these only seven (6.42%) exhibited clinical manifestations of mastitis while 38 does (34.86%) had subclinical mastitis. *Staphylococci* and *Proteus mirabilis* were found to be the predominant bacteria associated with mastitis. *Bacillus spp.* (including *Bacillus cereus)*, *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii* and *Streptococcus* were also isolated from mastitic milk. Since, enteric pathogens were isolated from 40% of mastitic milk samples, it can be concluded that poor hygienic conditions could be a major factor responsible for mastitis in lactating goats.

Keywords: Prevalence, Clinical Mastitis, Sub-clinical mastitis, bacteria, Does, PCR.

Milk is an ideal food for human being irrespective of ages and undoubtedly the most important one among the foods of animal origin. Goat milk is gaining popularity day by day among the rural households and serves an important source of milk to small marginal farmers. The goat milk is highly nutritious and has a similar nutritional profile to those of human's breast milk. But milk quality may be affected by bacterial contamination of mammary gland, which causes clinical and subclinical mastitis (Boscos *et al*., 1996). The disease causes serious economic losses due to the loss of milk production (Ameh and Tari, 1999). Losses are related to a reduction in milk production itself, as well as associated losses including the cost of treatment and the discarding of contaminated milk. Various factors such as poor managemental conditions, inadequate hygiene, increased relative humidity, and heavy rainfall and teat injuries predispose the goats to mastitis (Abu-Samra *et al.* 1988).

Mastitis in goats can be of clinical or sub-clinical nature. Clinical mastitis is characterized by signs of inflammation: swelling, pain, increased temperature and abnormal milk secretion. However, mastitis in goat is mainly of subclinical type (MacDougall *et al*., 2001) in which the milk appears normal with no visible abnormalities in udder tissue except an elevated Somatic Cell Count (SCC) in milk which is commonly used as a milk quality standard at the individual animal and herd level (McDougall *et al*., 2001). The annual incidence of clinical mastitis in small ruminants is generally lower than 5%, but this incidence can increase

* To whom all correspondence should be addressed.
Mob.: +91-9622028848;
E-mail: mirirfan441@gmail.com

sporadically. The prevalence of subclinical mastitis has been estimated at 5–30% or even higher (Contreras *et al*., 2003).

Early diagnosis of subclinical mastitis is of vital significance because changes in udder tissue take place much earlier than they become apparent. Various approaches, based on physical and chemical changes of milk and cultural isolation of organisms are practiced for diagnosis of mastitis in goats (Contreras *et al*., 2007). California mastitis test (CMT) and sodium lauryl sulphate test (SLST) are considered to be reliable screening tests for sub-clinical mastitis. These are simple, rapid screening tests based upon the amount of cellular nuclear protein present in the milk sample. However, cultural isolation of the organism is the gold standard test for mastitis. A number of surveys have been conducted on mastitis in dairy cows but only a few studies have been carried out to determine the etiology of caprine mastitis (Mir *et al*., 2013). The study was conducted to determine the prevalence of clinical and subclinical caprine mastitis in Jammu, and to determine the bacterial etiological agents associated with mastitis in lactating does.

MATERIALS AND METHODS

Sampling

Milk samples were collected from lactating does at goat farms located in and around Jammu. A total of 109 does were evaluated for clinical evidence of mastitis. The udder was subjected to a thorough physical examination which consisted of visual observation and manual palpation of the individual half of the udder and the teats. Each milk sample was also subjected to California mastitis test for determination of subclinical mastitis and pH of each sample was also recorded.

California Mastitis Test (CMT)

The California mastitis test was conducted to diagnose the presence of subclinical mastitis. This screening test was performed according to the procedure given for mastitis by Quinn *et al*. (2002). The result was scored as 0, +1, +2 or +3 depending on the intensity of reaction where was for Negative results, +1 for Subclinical Mastitis while +2 and +3 for serious mastitis infection.

Bacteriological examination of milk

Milk samples were collected from does with clinical and sub-clinical mastitis. The udder was thoroughly disinfected with 70% ethanol before collection of milk sample. First 3-4 streams of milk were discarded and samples were collected in sterilized bottles. The samples were immediately brought to the laboratory on ice for further processing. All the samples were subjected to bacterial culture.

The milk samples were initially inoculated on Blood agar containing 5% sheep blood and MacConkey's agar plates. Both the plates were incubated aerobically at 37°C for 24-48 hrs. Isolated colonies were selected and sub cultured on Nutrient agar (Himedia, Mumbai) or other suitable media and incubated aerobically at 37 °C for 24 - 48 h for further biochemical identification. Various biochemical tests viz. Catalase, Oxidase, Coagulase, Urease, Indole, Methyl red, Voges-Proskauer and Citrate tests were carried out. Sugar fermentation tests viz. fermentation of Glucose, Lactose, Mannitol, Adonitol, Arabinose, Sorbitol, Mannitol, Rhamnose and sucrose were conducted. The bacteria were identified based on their colony characters, Gram's staining and biochemical profile (Quinn *et al*., 1994).

Polymerase Chain Reaction and Sequencing

For confirmation of species, the bacterial isolates were subjected to PCR using 16S rDNA-specific primers (16S 1525R and 16S 27F) followed by sequencing. DNA was extracted from the isolates by suspending a loop full of confluent bacterial growth in 1 ml of sterile distilled water followed by boiling for 10 min. The DNA was used as a template for PCR. PCR amplifications were performed in 0.2 ml thin-walled PCR tubes. The PCR mixture contained a final concentration of 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 3.5 mM $MgCl_2$, 1.0 ìM concentration of each primer, 0.2 mM concentrations of each 2'-deoxynucleoside 5'-triphosphate and 1.0 U of Taq DNA polymerase. The amplification cycle consisted of initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C 1min, 50°C for 1 min; 72°C for 3min. Final extension was carried out at 72°C for 20 min. The sequence of 16S27F and 16S1525r primer used in the study is 5'AGAGTTTGATCMTGGCTCAG 3' and 5'AAGGAGGTGWTCCARCC 3' respectively.

RESULTS AND DISCUSSION

Out of 109 lactating does screened, only seven exhibited clinical manifestations of mastitis while the milk samples from 38 does were found positive by California mastitis test and were thus confirmed as cases of subclinical mastitis. Thus, the prevalence of clinical mastitis in does was 6.42% while that of subclinical mastitis was 34.86%. Several authors have reported the prevalence of subclinical mastitis in a dairy goat to range between 19.4 and 47% (Contreras *et al*., 2003), from 20 to 50% (Bergonier and Berthelot, 2003) and from 5 to 30% (Ndegwa *et al*., 2002). This large variability of prevalence may be caused by different host and management risk factors that influence intra-mammary infection of goats.

During the clinical examination, it was observed that in most of the goats right quarter was affected more frequently. The only right quarter was affected in twelve of 38 goats with subclinical mastitis whereas in 26 goats both right and left quarters were affected. The probable reason for this could be the sitting posture of goats due to which right quarter comes in contact with the ground more often.

Table 1. The details of bacteria isolated from subclinical cases of caprine mastitis

Bacteria	No. of samples positive (Clinical mastitis)	No. of samples positive (Subclinical mastitis)
P. mirabilis	4	9
Staphylococci	3	9
(Coagulase positive Staphylococci)	(2)	(5)
(Coagulase negative Staphylococci)	(1)	(4)
Bacillus spp. (including B. cereus)	-	8 (5)
E. coli	-	3
Pseudomonas aeruginosa	-	3
Citrobacter freundii	-	2
Streptococcus spp.	-	1
No growth	-	3
Total	7	38

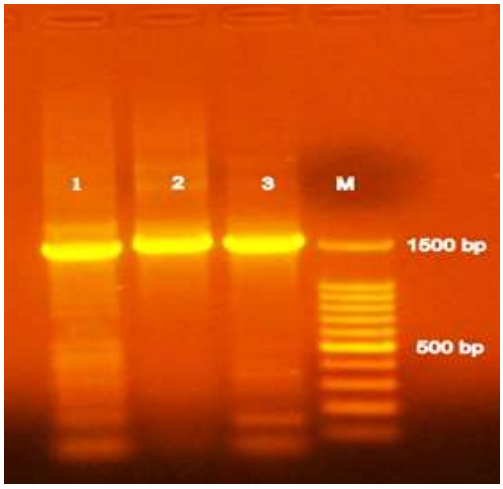

Fig. 1. PCR amplified *16S rDNA* gene Product (1500 bp) of the isolates using Universal *16S rDNA* Primers

The bacterial culture of the milk samples from does with mastitis (clinical and subclinical) revealed bacterial colonies in 42 samples whereas no growth was observed in three samples. Bacteria were isolated from all the seven cases of clinical mastitis while bacteria could be isolated from 35 out of 38 cases of subclinical mastitis. The bacterial isolates were identified based on standard tests followed by PCR (Fig. 1) and further confirmation was done based on sequence comparison with already known sequences available in Genbank using BLAST (Basic Local Alignment Search tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) at the server of the National Centre for Biotechnology information (NCBI).

The results of BLAST showed predominant bacteria isolated was *Proteus mirabilis* followed by *Staphylococcus*, *Bacillus*

spp., *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii* and *Streptococcus spp*. The details of bacteria isolated from mastitic goats are provided in table1. All the cultures were deposited in the Veterinary Type Culture Collection centre, Hisar.

In the present study, *Proteus mirabilis* was found to be the predominant bacteria responsible for clinical mastitis while *Proteus* and *Staphylococci*, both, were the predominant bacteria isolated from cases of subclinical mastitis. This is in contrast to the earlier reports where *Staphylococcus* was found to be the most common causal bacteria responsible for causing intra-mammary infection in sheep and goats (Najeeb *et al*., 2013; Bergonier and Berthelot, 2003). *Proteus spp*., members of family *Enterobacteriaceae* are commonly found in the gastrointestinal tract of mammals. The members of *Enterobacteriaceae* viz. *Proteus spp*., *E.coli* and *Citrobacter freundii* accounted for 18 of total 45 isolates (40%) obtained from mastitic milk samples. The presence of such a high percentage of enteric pathogens in the milk samples suggest that poor hygienic conditions could be a major factor contributing to mastitis in goats. *Streptococcus* sp. was isolated from only a single case of sub-clinical mastitis. Although, *Streptococci* are the second group of microorganisms in importance, after *Staphylococcus*, responsible for mastitis in ruminants (Bergonier *et al*. 1999). Also, Mir *et al*. (2013) found the cases of clinical mastitis in does of Jammu region due to *Streptococcus zooepidemicus. But, the incidence of Streptococcus in our study can be less due to poor sanitary conditions of farms screened which mostly resulted in environmental mastitis having the predominance of enteric pathogens*.

Apart from enteric bacteria, Staphylococci and Bacilli were isolated frequently from mastitic milk samples of goats. Even though Staphylococci have been well recognised as etiological agents of mastitis in goats (Contreras *et al*., 2003), Bacilli particularly *Bacillus cereus* is rarely associated with caprine mastitis (Quinn *et al*., 1994). *Bacillus cereus* has been associated with cases of food poisoning in humans and therefore, consumption of the milk infected with this bacterium can cause human health problems.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support provided by ICAR under VTCC project for carrying out the research work.

REFERENCES

1. Abu Samra M.T., El Sanoqs, S.M., Abdalla, H.A., GameJi, A. A., Aziz, A. A, Abbas, B., Ibrahim, K. E. E. and Idrlsa, S.O. Gangrcnous mastitis in goats. *Cornell Veterinary.* 1988; **78**: 281-300.
2. Ameh, J.A. and Tari, I.S. Observations on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. *Small Ruminant Res*. 1999; **35**: 1–5.
3. Bergonier, D. and Berthelot, X. New advances in epizootiology and control of ewe mastitis. *Livest Prod. Sci*. 2003; **79**: 1-16.
4. Bergonier, D., Berthelot, X. Romeo, M. Contreras, A. Coni, V. Santis, E. De, Roselu, S. Barillet, F. Lagriffoul, G. and Marco. J. Fréquence des différents germes responsables de mammites cliniques et subcliniques chez les petits ruminants laitiers, *In* F. Barillet and P. Zervas (ed.), Milking and milk production of dairy sheep and goats. Wageningen Pers, Wageningen, The Netherlands. 1999; 130–136.
5. Boscos, C., Stefanakis, A., Alexopoulos, C. and Samartzi, F. Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats. *Small Ruminant Research*. 1996; **21**:139–147.
6. Contreras, A., Sierra, D., Sanchez, A., Corrales, J.C., Marcoc, J.C., Paape, M.J. and Gonzalo, C. Mastitis in small ruminants. *Small Rum. Res*. 2007; **68**:145-153.
7. Contreras, A.C., Luengo, A. Sanchez and Corrales, J.C. The role of intramammary pathogens in dairy goats. *Livest Prod. Sci*. 2003; **79**: 273-283.
8. MacDougall, S., Murdough, P. Pankey, W. Delaney, C. Barlow, J. and Scruton, D. Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. *Small Rum. Res*. 2001; **40**: 245-254.

9. Mir, I.A., Taku, A., Iqbal, A., Khan, M.A. and Wani, N. Clinical mastitis by Streptococcus equi subsp. zooepidemicus in lactating goats of Jammu, India. *The Indian Journal of Small Ruminant Research.* 2013; **19** (1). 110-111.
10. Najeeb, M. F. Anjum, A. A. Ahmad, M. U. D. Khan, H. M. Ali, M. A and Sattar, M. M. K.
11. Bacterial etiology of Subclinical Mastitis in Dairy Goats and Multiple Drug Resistance of the isolates. *The Journal of Animal & Plant Sciences*. 2013. **23**(6): 1541-1544.
12. Ndegwa, E. N, Mulei, C. M., Munyua, J. C. and Moti, S. J. Prevalence of microorganism associated with udder infections in dairy goats on small-scale farms in Kenya. *Journal of Science African Veterinary Association*. 2001; **72**: 97–98.
13. Quinn P.J, Carter ME, Markey BK, Carter GR (1994). Clinical Veterinary Microbiology, Published by Wolfe Publishing, an imprint of Mosby-Year Book Europe Limited Printed in Spain by Grafos, S.A. Arte Sobre Papel ISBN 0723417113; pp 21-26, 209-236.
14. Quinn, P.J., M.E. Carter, B.K. Markey and Carter, G.R. Clinical Veterinary microbiology. Harcourt publishers,Virginia, 2002; 331-344.