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

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A total of 109 lactating does were screened for mastitis, both clinical and subclinical. 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.
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 MgCl₂, 1.0 iM 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 1 min, 50°C for 1 min; 72°C for 3 min. 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% (Ndewga et al., 2002). This large variability of prevalence may be caused by different host and management risk factors that influence intramammary 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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No. of samples positive (Clinical mastitis)</th>
<th>No. of samples positive (Subclinical mastitis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. mirabilis</em></td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>(Coagulase positive</td>
<td>(2)</td>
<td>(5)</td>
</tr>
<tr>
<td>Staphylococci</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Coagulase negative</td>
<td>(1)</td>
<td>(4)</td>
</tr>
<tr>
<td>Bacillus spp. (including B. cereus)</td>
<td></td>
<td>8 (5)</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No growth</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>38</td>
</tr>
</tbody>
</table>

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 table 1. 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.

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**REFERENCES**


