Clinio-Bacteriological Investigation of Sub-Clinical and Clinical Mastitis in Dairy Goats

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Abstract
Mastitis is common managerial problem of dairy goats. In the present study, prevalence of mastitis in Dera Din Pannah, Beetal, Teddy, Desi and non-descriptive dairy goat breeds, investigation of pathogenic bacteria of mastitis and their susceptibility to various antibiotics were tested. Milk samples were collected from district Muzaffargarh, Punjab and subjected to Surf Field Mastitis Test for screening. The positive samples were cultured on blood agar; tryptose agar and MacConkey’s agar for bacterial isolation *Staphylococcus aureus, Streptococcusagalactiae, Streptococcusysagalactiae and Bacillus cereus* were investigated. 235 milk samples from lactating goats of different breeds were tested. Out of which 51 (21.70%) were found positive for subclinical (18.29%) and clinical mastitis (3.4%). Enrofloxacin, Ciprofloxacin, Amoxicillin, Gentamycin, Oxytetracycline, Penicillin, Amoxicillin, Norfloxacin, Chloramphenicol and Streptomycin were used for anti-bio-gram profiling against bacterial isolates. Chloramphenical, norfloxacin, gentamicin, penicillin and ciprofloxacin were found effective in vitro.

Key words: Mastitis, Enrofloxacin, Investigation, Antibiotics

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Introduction
Mastitis is one of the multifactorial and expensive diseases of the dairy animals as it not only reduces the milk production but also leads to detrimental changes in the milk composition [1]. There is dire need to curtail this disease to produce the milk according to World trade organization standard (WTO). It damages the teats which results partial or complete loss of milk of lactating animals. Mastitis occurs in major three forms: subclinical, clinical and chronic. In clinical mastitis, changes in color, presence of clots and massive increase in leukocytes in milk occurs. There will be inflammatory signs in the glandular tissues of teats which are detected by visual observation. In subclinical form there will be no visual signs but increase in the somatic cell count (SCC) and it can only be detected through screening tests. An inflammatory signs continued from several months reveals the chronic mastitis [2]. Subclinical form also has a negative impact on hygienic milk quality and yield [3]. Maintenance of dietary values of milk is necessary for human and animal health but due to mammary gland infection quality of milk reduces by deteriorating physiochemical properties of milk [4]. Diagnosis of mastitis is done through various test mainly surf field mastitis (SFM), [5] California mastitis tests (CMT) under field conditions. This study was conducted to determine the prevalence, bacteriological investigation and antibiogram profiling of mastitis causing bacteria in lactating dairy goats.

Materials and methods
The study was conducted in district Muzaffargarh of Pakistan, which is located about 35 km from Multan of Punjab province. A total of 470 milk samples were collected from 235 lactating dairy goats at 10 different villages. The dairy goats were of different ages and various breeds namely Dera Din Pannah, Beetal, Teddy, Desi and non-descriptive.

Clinical udder examination
Udders of lactating goats were observed visually and through palpation for presence of lesions and inflammatory signs i.e., heat, pain, redness and swelling and loss of function.

Physical examination of milk
Milk from each teat was taken and examined for alteration in consistency and color.

Milk samples collection
Milk samples were collected from each teat following the procedure as described by National Mastitis Council [6]. The teat ends were thoroughly washed with water and dried with clean towel. Then teats were disinfected using swabs dipped into KMnO4 (1:1000) Solution. Approximately 3-4ml of milk from each quarter will
be drawn onto paddle cups for the Surf Field Mastitis Test. Then 10ml of positive milk sample will be collected from each teat directly into clean and sterile plastic tubes.

**Surf Field Mastitis Test**
In this test method was followed as described by Rehman and Muhammad by using reagent (3% surf solution) [7]. About 1 ml milk was drawn into the black paddle cup and 5 ml surf was properly mixed in circular fashion. After 30 seconds, there was formation of gel like flakes for the positive milk samples. Obtained scores were presented from 01-05, with 1 for negative, 2 for trace, 3 for weak positive, 4 for a distinct positive and 5 for a strong positive.

**Microbiological Investigation**
All positive milk samples for mastitis were transported to Microbiology laboratory department of Microbiology, University of Veterinary and Animal Sciences, Lahore. Each milk sample primarily was cultured on blood agar by using spread out technique. Individual colonies were purified by multiple streaking. Pure bacterial isolates were identified on the basis of culture characters, microscopic morphology and biochemical profile following identification flow charts of Bergey’s Manual of Systematic Microbiology (2010).

**Anti-biogram Profiling**
All the bacteriological investigated positive isolates were subjected for anti-biogram by disc diffusion (Bhatt et al. 2011). Antibacterial discs of Enroflooxin, Ciproflooxin, Ampicillin, Gentamycin, Oxytetracycline, Penicillin, Amoxicillin, Norfloxacin, Chloramphenicol and Streptomycin has been used.

**Statistical analysis**
The results were analyzed statistically by Pearson chi-square test using SPSS version 20.0.

**Results**
Out of 235 samples, 52 (21.70%) were positive. Prevalence of subclinical, clinical and chronic mastitis was 26.8, 19.67, 19.23 and 18.60% respectively as mentioned in the table 1-4. Incidence of mastitis increases along with the age (Table 5). Bacteria isolated were *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae* and *Bacillus cereus* with percentage 80.8, 19.6, 15.68 and 3.9 respectively (Table 6). Chloramphenical, Norfloxacin, Gentamicin, Penicillin and Ciproflooxin were found effective in vitro among Enroflooxin, Ciproflooxin, Ampicillin Gentamycin, Oxytetracycline, Penicillin, Amoxicillin Norfloxacin, Chloramphenicol and Streptomycin under antibiogram profiling.

**Table 1: Positive cases showing total prevalence**

<table>
<thead>
<tr>
<th>Subclinical-mastitis</th>
<th>Clinical-mastitis</th>
<th>Chronic-mastitis</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>07</td>
<td>03</td>
<td>21.7</td>
</tr>
</tbody>
</table>

**Table 2: Prevalence of subclinical mastitis (SCM), clinical mastitis (CM) and chronic mastitis (ChM)**

<table>
<thead>
<tr>
<th>Prevalence of SCM (%)</th>
<th>Prevalence of CM (%)</th>
<th>Prevalence of ChM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80.4</td>
<td>13.7</td>
<td>5.88</td>
</tr>
</tbody>
</table>

**Table 3: Comparative prevalence of Mastitis in different dairy goats breeds**

<table>
<thead>
<tr>
<th>Beetle</th>
<th>Teddy</th>
<th>Dera Din Pannah</th>
<th>Desi</th>
<th>Non-descriptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.8</td>
<td>19.7</td>
<td>26.3</td>
<td>19.2</td>
<td>18.6</td>
</tr>
</tbody>
</table>

**Table 4: Prevalence of Mastitis in dairy goats of various ages**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>2-3</th>
<th>3.4</th>
<th>4.5</th>
<th>5.6</th>
<th>6.7</th>
<th>7.8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (%)</td>
<td>30</td>
<td>37</td>
<td>38</td>
<td>44</td>
<td>44</td>
<td>47</td>
<td>58</td>
</tr>
</tbody>
</table>

**Table 5: Bacteriological investigation**

<table>
<thead>
<tr>
<th>Bacteria Isolated</th>
<th>Staph. aureus</th>
<th>Strep. agalactiae</th>
<th>Strep. dysagalactiae</th>
<th>Bacillus cereus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage (%)</td>
<td>60.8</td>
<td>19.6</td>
<td>15.7</td>
<td>3.9</td>
</tr>
</tbody>
</table>

**Discussion**
Mastitis is the most costly and prevalent disease of lactating cattle, buffalo, sheep and Goat causing major economic losses [8] including veterinary services, treatment and labor costs, reduced milk production, increased incidence of subsequent mastitis, culling of cow and poor milk quality. Mastitic milk contains threshold level of bacteria and their toxin which are detrimental to human health [9, 10]. There has been no diagnostic reported case of Sub-Clinical mastitis in lactating dairy goats in district Muzaffargarh which is most sheep and goat populated area. For detection of intramammary infections (IMI’s) in lactating dairy goats, surf field mastitis test (SFMT) was used that is simplest, cheapest test and can be easily used in the field.
conditions [7]. In the present study, the highest incidence of mastitis was due to the reason that in these species the milk samples were collected from the goats having udder complaints. In Peshawar, mastitis incidence rate in lactating goats has been reported 80.0, 26.7, 33.3, 57.1 and 42.0%, respectively five successive years [11]. In the present study, more incidence rate of mastitis is recorded in the village where goats are fed through grazing in the field. The prevalence of subclinical and clinical mastitis in Connecticut and Rhode Island and New York was 36.4 and 38.2% respectively [12] which does not correlate with the present study. There has been no recorded prevalence report of mastitis in various breeds of lactating goats in Pakistan. In regard to this, comparative prevalence of sub clinical Mastitis in five different breeds of lactating Goats has been determined. Highest prevalence was found in Beetle breed then in Dera Din Pannah as compared to other breeds of lactating goats due to more milk yield as compared to other breeds. Moreover, the Beetle breed has long pendulous teats and there is more exposure of teats to the soil which results more chances of infections during grazing. Gram positive bacteria were the main pathogens. Previous research also reveals that these pathogens are the major mastitis causing microbes in dairy goats. Staphylococcus aureus was the main isolated bacterium in the sub-clinical mastitis which gives justification that this has wide spread natural distribution in environment causing many diseases. During the present study as the Streptococcus agalactiae and Streptococcus dysgalactiae were the common mastitis causing microbes in the dairy goats. These results are in agreement with the results of Scott McDougall [13, 14]. There are growing concerns regarding the increased prevalence of antibiotics resistance worldwide. The use of large amount of antibiotics for disease control in food animal production is suspected to play a role in the spread and persistence of antimicrobial resistant zoonotic bacteria. In the present study, all isolates were found to be sensitive to commonly available antibiotics but most sensitive were Chloramphenical, Norfloxacain, Gentamicin, Penicillin and Ciprofloxacin. Mohammad Rafiqul Islam has found oxytetracycline, ampicillin, amoxcillin most sensitive [2] against above mentioned bacteria. This study indicates high percentage of sub-clinical mastitis in various breeds of dairy goats due to unhygienic measures in dairy environment. Good management practices with proper sanitation are required to prevent the incidence of intra-mammary infection in dairy goats. It is suggested that strict hygienic measures should be adopted during the production and handling of milk in field condition to lessen the public health hazards. Awareness of the small ruminant’s handlers about the significance of hygienic milking practices would minimize the adverse effects of mastitis on the yield and quality of goat milk. It would be beneficial if the lactating goats in the herd are screened regularly to diagnose sub-clinical mastitis and to achieve optimum milk production.

References