Isolation and characterization of mastitis pathogens and milk composition changes in Murrah buffaloes (Bubalus bubalis) during winter season

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ABSTRACT

Present investigation was carried out to study the prevalence of bacterial pathogens in clinical and subclinical mastitis in buffaloes during winter season. A total of 118 Murrah buffaloes were screened using California mastitis Test (CMT). Milk samples were collected and analyzed for fat, protein, lactose, SNF, pH, Electrical conductivity (EC) and Somatic Cell Counts (SCC). Out of 118 milk samples, 60 samples (50.84%) were negative for CMT and 58 samples (49.15%) were detected positive for mastitis incidence. Milk pH and EC was significantly higher (p<0.05) in mastitis affected samples than the normal ones, however, protein, fat, SNF and lactose were lower (p<0.05). SCC ranged between 1.28-1.48 x 10^5 cells/ml (in normal milk samples as compared to 3.85-6.21 x 10^5 cells/ml (p<0.05) in mastitis milk samples. Out of 58 samples only 51 samples exhibited bacterial growth. The culturally examined and characterized samples revealed S. aureus (35.29%) as the predominant bacteria followed by S. agalactiae with an isolation rate of 25.49%. The incidence of coliforms bacteria was not detected in any of the sample cultured and examined. It was concluded that mastitis incidence adversely affect quality of milk by increasing the SCC, pH and EC of milk. Appropriate measures needs to be taken to prevent the incidence of S. aureus bacteria which was the major causative agent.

Key words: Bacteria, EC, Mastitis, pH, Prevalence, SCC.

INTRODUCTION

Mastitis is a very complex and costliest disease causing huge economic losses to the dairy industry globally (Magotra et al., 2015). The economic losses due to mastitis have increased about 115 folds in the last five decades in India, and presently the loss due to mastitis is to the tune of 7165.51 crore/annum (Anonymous, 2012). The first report on losses due mastitis in India was about Rs.52.9 crore annually (Dhanda and Sethi, 1962) which has increased to Rs.6053.21 crore annually in the year 2001 (Dua, 2001). Mastitis have been known to cause a great deal of loss or reduction of productivity, influence the quality and quantity of milk yield, and lead to culling of animals at an unacceptable age (Singh and Singh, 1994). The losses due to mastitis are not only economic, but issues such as animal health and welfare, milk quality, antibiotic usage and the image of the dairy sector are important reasons to focus on mastitis control (Jingar et al., 2014). The disease generally involves interplay between managerial practices and infectious agents that frequently affect the udder like bacteria, viruses, mycoplasma, yeasts and algae (Osumi et al., 2008; Chaneton et al., 2008). Murrah buffalo “the black gold of India” is an important constituent of livelihood in Haryana, Punjab, Uttar Pradesh, Rajasthan, Gujarat and Maharashtra. With 53 % of the world buffalo population, India produces 63 percent of the world buffalo milk, but its quality and production gets deteriorated by clinical and (or) sub-clinical forms of mastitis (Owen et al., 2000). It primarily occurs in response to intramammary bacterial infection (Zhao and Lacasse, 2008). Majority of intramammary infections are of bacterial origin such as Escherichia coli, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae and Streptococcus agalactiae (Varella Coelho et al., 2007; Kuang et al., 2009). Among various causative agents, staphylococci and streptococci are mainly responsible for mastitis infections in lactating animals. Therefore, a bacteriological diagnosis and prevalence study of bacterial pathogens in the herd are critical for rational and effective control of mastitis. Based upon these facts, the present investigation was carried out to study the prevalence of bacterial pathogens responsible for mastitis and the milk composition changes in Murrah buffalos.

MATERIALS AND METHODS

Sample collection: The experiment was conducted on 118 lactating Murrah buffaloes maintained at Livestock Research Centre of the institute farm during the period October 2014-

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March 2015. Data regarding age, parity and lactation stage were collected from the register maintained in record section of the Institute. Milk samples were collected before milking using standard procedures described by the National Mastitis Council (Oliver et al., 2005). Before sample collection, teats were dipped in a pre-milking teat disinfectant, cleaned thoroughly, dried with individual disposable paper towels. The teat ends were sanitized with swabs containing 70% ethyl alcohol. Milk samples were subjected to California mastitis test (CMT) by the standard procedure (Quinn, 1994). Observations were recorded on the basis of gel formation within 30 seconds marked as 0 (negative) and T (trace) were considered negative or normal, while CMT scores of +1 (weak positive), +2 (distinct positive) and +3 (strong positive) were taken as indicators of mastitis. SCC was determined by using EKOMILK SCAN somatic cell analyzer (BULTEH, Europe). Electrical conductivity of fresh milk was measured by using Digital conductivity meter (Century CC 601, cell constant). Milk samples were analyzed for fat, protein and lactose content using Lacto Scan-automatic milk analyzer (Mega netoo, Bulgaria). The samples positive by either of these tests were processed for bacterial isolation.

**Bacteriological culture:** The CMT positive milk samples (n = 51) were subjected to bacteriological culture as per method described by (Quinn et al., 1994). After initial enrichment in brain heart infusion broth overnight, 10 μl of enriched milk samples were streaked onto blood agar, mannitol salt agar and Mackonkey agar media, incubated at 37°C for 24 hours. Each bacterial colony was examined macroscopically for colony morphology, characteristics haemolytic pattern, microscopically by Gram’s stain method and further the identification of all isolates was done using Histaph kit (KB004A HIMEDIA) containing Voges Proskauer’s, Alkaline phosphatase, ONPG, Urease, Arginine utilization, Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose, Maltose and Histrept (KB005A) biochemical kits containing Voges Proskauer’s, Esculin hydrolysis, PYR, ONPG, Arginine utilization, Glucose, Lactose, Sorbitol, Mannitol and Raffinose. The statistical analysis of data was carried out using two-way ANOVA by Sigma Stat 3 programme.

**RESULTS AND DISCUSSION**

Out of 118 lactating buffaloes examined during the study period, 58 samples (49.15%) were found positive for mastitis as indicated by CMT and SCC (Table 2). Protein content in the milk decreased in mastitis affected samples significantly (p<0.05). Mean milk protein concentration was 2.91 in normal quarter and got lower up to 2.91 in quarter affected with mastitis (p<0.05) which is similar to the study reported by Tripaldi et al. (2010), Rawdat and Omaima (2000) and Uallah et al. (2005). The fat and lactose content were significantly (p<0.05) lower in mastitis affected samples as compared to normal milk samples (Table 1) and are streamlined with previous studies (Lindmark-Mansson et al., 2006; Ahmed et al., 2007) showing lower fat, protein and lactose in mastitic milk. However, Dhillon et al. (2000) reported higher lactose content in milk from mastitic animals. The milk pH and electrical conductivity were significantly higher (p<0.05) in mastitic milk samples as compared to normal samples, the respective values were 6.48 & 7.28 and 2.56 mhos and 3.53 mhos (Table 1). The increase in electrical conductivity in mastitic milk could be due to higher concentration of (sodium and chloride) salts as increased permeability of cell membrane during inflammatory process allows exchange of ions between milk and blood. The results obtained in this study are in agreement with the findings reported earlier by Hussain et al. (2012). The mean SCC in the normal milk samples ranged between 1.28-1.48 x 10^5 cells/ml as compared to 3.85-6.21 x 10^5 cells/ml (p<0.05) in mastitis milk samples. Dhalak (2006) reported that mean SCC of California Mastitis Test negative and positive samples were 104 x 10^3 and 1572 x 10^3 /ml of milk, respectively. The increased SCC could be attributed to elevated secretion of epithelial cells and migration of leucocytes from blood into milk during the infection (Dohoo and Meek, 2002) which subsequently influences pH and electrical conductivity of cows and buffaloes milk (Shailza and Singh, 2002). Maximum SCC was found in +3 grade (4.89-6.21 x 10^5 cells/ml) mastitic quarters, while minimum was in +1 grade (1.28-1.48 x 10^5 cells/ml). The percent decreases in mean lactose range in +2 and +3 groups with respect to negative (+) were 3.85-3.70, 5.38-3.98 and 5.89-5.34 respectively (Table 2).

Out of 58 CMT positive samples, 51 samples showed bacteriological colony growth. The major agents involved in bacterial intramammary infection (IMI) isolated from mastitis milk samples were S. aureus, S. epidermidis, S. agalactiae, S. pyogenes, S. dysgalactiae, E. coli, C. perfringens, N. gonorrhoeae, and S. pyogenes.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Normal</th>
<th>Mastitis</th>
<th>P-value</th>
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<tr>
<td>Fat (%)</td>
<td>7.68</td>
<td>3.01</td>
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</tr>
<tr>
<td>Lactose (%)</td>
<td>5.7</td>
<td>3.76</td>
<td>0.05</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.8</td>
<td>2.91</td>
<td>0.05</td>
</tr>
<tr>
<td>SNF (%)</td>
<td>9.7</td>
<td>3.16</td>
<td>0.05</td>
</tr>
<tr>
<td>SCC x 10^5 (cells/ml)</td>
<td>1.26 x 10^5</td>
<td>5.32 x 10^5</td>
<td>0.01</td>
</tr>
<tr>
<td>pH</td>
<td>6.48</td>
<td>7.28</td>
<td>0.05</td>
</tr>
<tr>
<td>EC (mhos)</td>
<td>2.56</td>
<td>3.53</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Score (+1)</th>
<th>Score (+2)</th>
<th>Score (+3)</th>
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<tbody>
<tr>
<td>SCC x 10^5 (cells/ml)</td>
<td>1.28-1.48</td>
<td>3.85-4.16</td>
<td>4.89-6.21</td>
</tr>
<tr>
<td>EC (mhos)</td>
<td>2.18-2.69</td>
<td>3.03-3.38</td>
<td>3.94-4.12</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>5.89-5.34</td>
<td>5.38-3.98</td>
<td>3.85-3.70</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.48-3.29</td>
<td>3.34-2.98</td>
<td>2.91-2.43</td>
</tr>
</tbody>
</table>

Values bearing different superscripts a,b,c, differ (p<0.05) in a row.
S. saprophyticus, S. agalactiae, S. uberis which were present in different combinations in individual animals (Table 3). Out of total 51 cases of mastitis, 18 (35.29%) were found positive for *Staphylococcus aureus* single infection based on growth characteristics on blood agar and mannitol salt agar, while *S. aureus* combination with *S. uberis* was in 8 samples (15.68%) cases. The *Streptococcus agalactiae* incidence was observed in 13 (25.49%) milk samples based on growth characteristics on Christie Atkins and Munch–Petersen reaction (CAMP). It was also observed in combinations with *S. saprophyticus* and *S. uberis* in 1.96% incidences. The percent distribution of *S. epidermidis* single infection was 7 (13.72%) and only 2 (3.92%) animals suffered from *S. saprophyticus* infection. *Staphylococcus* organisms produced large, creamy white, white, grey colored colonies with a complete zone of haemolysis, no zone of haemolysis or partial zone of haemolysis (Fig 1). The *streptococcus* colonies were small grey colored with partial and complete zone of haemolysis (Fig 2). All the isolates were confirmed by Histaph and Histrep biochemical kits (Table 4 & Fig 3 and 4). None of the sample was found positive for coliforms incidence. The relative prevalence rates of various bacterial strains revealed that *S. aureus* (35.29%) was the major causative pathogen of bovine mammary gland followed by *S. agalactiae* (25.49%). Similar results have also been reported by many researchers (Khan and Muhammad, 2005; Ericission et al., 2009; Sentitula et al., 2012; Duguma et al., 2014; Z. Ali et al., 2015; Galvan et al., 2015). Memon et al. (1999) reported *Staphylococcus aureus* as the major pathogen (38%), followed by *Streptococcus uberis* (13%), *E. coli* (11%) and *Klebsiella pneumoniae* (11%). The higher prevalence of *Staphylococcus aureus* may depend on factors such as herd size, milking parlor hygiene, variation in systems of feeding and management (Radostits et al., 2007), while *S. agalactiae* is still a significant cause of chronic mastitis (Keefe, 1997). However, Cheng et al. (2010) reported that *Escherichia coli* was the commonest organism implicated in 82% mastitis cases followed by *Streptococcus uberis* (53%), *Staphylococcus aureus* (41%), *Streptococcus dysgalactiae* (29%) and *Streptococcus agalactiae* (27%). Perez et al. (2015) revealed that the most prevalent pathogens responsible for subclinical mastitis were Gram negative *Proteus vulgaris* (25.0%), *Salmonella spp.* (12%), *Enterobacter aerogenes* (10%), *E. coli* (7.5%), *Proteus mirabilis* (5.0%) and *Klebsiella pneumoniae* followed by only 2.5% *Staphylococcus spp.* The difference in the prevalence rate during different studies could be attributed to sanitary condition of udder, sample size and geographic region (Sadashiva and Kaliwal, 2013). In this study the second most prevalent bacterial species was *Streptococcus agalactiae* with an isolation rate of 25.49%. A low prevalence of mastitis due to other streptococci infection and absence of any coliforms infection suggests the good sanitation and hygienic practices at the farm (Radostits et al., 2007). *S. aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shed through milk serving as source of infection for other healthy cows and transmitted during the milking process (Radostits et al., 2000). It has been stated that
Streptococcus spp. is the most prevalent along with Staphylococcus spp. However, lower prevalence as compared to Staphylococcus spp. is because Streptococcus agalactiae survives poorly outside the udder. Staphylococcus aureus infections do not respond well to antibiotic therapy due to widespread resistance of Staphylococcus aureus to Penicillin –G (Sutra and Poutrel, 1990) leading to a relatively high culling rate. Therefore, reliable and rapid methods for the identification of Staphylococcus aureus from mastitic milk are crucial for the mastitis control and sound udder health management. Hence the present information could be an extra source of information to monitor the udder health and mastitis management.

CONCLUSION

It was concluded that prevalence of mastitis was 49.15% in the studied samples which adversely affected quality of milk by decreasing lactose and increasing the SCC, pH and EC. The isolates also seemed to have well adaption and capability to hide inside udder during the winter season as indicated large number of infected animals. Appropriate measures need to be taken to prevent the incidence of S. aureus bacteria which was the major causative agent followed by S. agalactiae, still a significant cause of chronic mastitis. Thus, the present study reveals the predominance of contagious form of mastitis at the farm that needs to be controlled with appropriate measures to prevent further spread.

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REFERENCES


