Bovine mastitis is an inflammatory reaction of one or more quarters of the mammary gland to bacterial, chemical, thermal or mechanical injury (Haghkhah et al., 2010) and costs around Rs. 72 billion per year (Bansal and Gupta, 2009). The inflammatory response stimulates acute phase response in the body. The acute phase response refers to a group of non-specific host responses to a wide variety of stimuli and is characterized by changes in concentrations of a number of hepatically synthesized plasma proteins - the acute phase proteins. Serum amyloid A and haptoglobin are the two major acute phase proteins in cattle. They are potentially useful as disease markers owing to their low concentration in normal animals, the rapid increase in their concentration during the acute phase of inflammation and their rapid decrease with the resolution of the disease (Eckersall et al., 2001). Clinical mastitis is easy to detect, however, sub-clinical mastitis is a major problem for the dairy industry as there are no visible changes in the udder or milk. Sub-clinical mastitis is frequently diagnosed by cultural examination, California mastitis test (CMT), electrical conductivity test or by laboratory analysis of somatic cell count (SCC). These procedures have their own limitations. Therefore, it is of great importance to investigate biomarkers that could be used for rapid detection of sub-clinical mastitis. The aim of this work was to evaluate the influence of streptococcal and staphylococcal clinical and sub-clinical mastitis on the concentrations of milk haptoglobin (MHp), serum haptoglobin (SHp), milk amyloid A (MAA) and serum amyloid A (SAA) was observed in clinically and subclinically infected buffaloes as compared to healthy buffaloes. Concentration of milk and serum haptoglobin and amyloid A accurately reflected the degree of inflammation and could be used as a marker for detection of clinical and sub-clinical mastitis.

**Key words:** Sub-clinical mastitis, clinical mastitis, acute phase proteins, haptoglobin, amyloid A.
at 37°C for 24-48 h. The animals which had calved recently (less than two weeks) or those in late lactation (more than nine months) were not included in the study. Animals having milk samples apparently normal but culturally positive and showing somatic cell count > 5 lacs/ml were identified as sub-clinically infected.

Milk samples were also subjected to estimation of SCC (Schalm et al., 1971), NAG-ase test (Kitchen et al., 1978) and electrical conductivity (EC) with the help of a portable mastitis detector manufactured by AHI, Plastic Moulding Co. New Zealand

Out of these animals, blood and milk samples from 10 healthy, 10 sub-clinically and 10 clinically infected animals were subjected to estimation of acute phase proteins i.e., MAA, SAA, MHp and SHp with kits (PhaseTM Range, Tridelta, Development Ltd. Ireland) following the manufacturer’s protocol. To identify milk samples infected with *Staphylococcus* spp. and *Streptococcus* spp., the Gram-positive cocci were subjected to catalase test to differentiate between staphylococci and streptococci. On the basis of oxidation and fermentation, oxidase and catalase tests, Gram-positive cocci were classified into micrococci and staphylococci. *Staphylococcus*ococi were further characterized into coagulase-positive and coagulase-negative (CNS) types on the basis of coagulase production. The bacterial cultures were stocked in semi-solid agar and blood-agar slants for further use. The organisms which on preliminary examination were found to be staphylococci, were further characterized for different species on the basis of coagulase test, DNAase, phosphates, different sugar fermentation, unease, clumping factor and latex agglutination. No significant difference between the values of different parameters between coagulase negative and coagulase positive staphylococci infected milk samples, was found, hence for further analysis, both were combined.

The organisms which on preliminary examination were found to be streptococci, were further characterized for different species on the basis of CAMP test (Christie et al., 1944), latex agglutination, hippurate test and sugar fermentation (arabinose, trehalose, raffinose, salicin and sorbitol). Basic statistics was determined by using the SPSS programme, version 11.0.

**RESULTS AND DISCUSSION**

Animals having milk samples apparently normal but culturally positive and showing somatic cell count >5 lacs/ml were identified as sub-clinically infected. Out of 38 culturally positive sub-clinical infected quarters, a total of 44 isolates were recovered (Table 1). Of the 38 culturally positive sub-clinical infected quarters, 22 quarters were positive only for *Staphylococcus* spp., 10 only for *Streptococcus* spp. and the remaining six quarters revealed the mixed infection of *Staphylococcus* spp. and *Streptococcus* spp. Of the 44 isolates, 63.64% were *Staphylococcus* spp. and 36.36% were *Streptococcus* spp.

Of 23 samples from 21 clinical cases of mastitis, 22 (95.65 %) samples were found culturally positive and 24 isolates were obtained. Of these 24 isolates, frequency of isolation of different organisms was *Staphylococcus* (62.50%), *Streptococcus* (29.16%) and *E. coli* and *Corynebacterium pyogenes* (4.16% each).

A significant increase in the concentrations of MHp, SHp, MAA and SAA was observed in sub-clinically and clinically infected animals (Table 2). Values of NAGase and SCC were also significantly higher in mastitic animals (clinical and sub-clinical) as compared to healthy animals. A significant difference was also observed in concentration of acute phase proteins between clinically and sub-clinically infected animals. Both sub-clinically and clinically infected animals showed a significant increase in EC as compared to healthy animals. Though clinically infected buffaloes had higher SCC, EC and NAGase activity than sub-clinically infected buffaloes; the difference was not statistically significant.

In the present study *Staphylococcus* spp. and *Streptococcus* spp. were the major organisms isolated

<table>
<thead>
<tr>
<th>Nature of cases</th>
<th>Clinical cases</th>
<th>Sub-clinical cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of buffaloes</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>Quarters examined</td>
<td>23</td>
<td>326</td>
</tr>
<tr>
<td>Quarters culturally positive</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td>Quarters sterile</td>
<td>1</td>
<td>288</td>
</tr>
<tr>
<td>Total isolates</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td><em>Staphylococcus</em> alone</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td><em>Streptococcus</em> alone</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>C. pyogenes</em></td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mixed infections</td>
<td>2*</td>
<td>6**</td>
</tr>
</tbody>
</table>

*E. coli+C. pyogenes (1), Staphylococci + Streptococci (1)  
*Staphylococci+Streptococci (6)
from clinical and sub-clinical cases of mastitis in buffaloes. Etiological agents isolated in our study are similar to previous reports (Sharma and Sindhu, 2007; Chavan et al., 2007; Sindhu et al., 2009; Roychoudhury and Dutta, 2009; Charaya et al., 2013). The only difference was that in the present investigation, *E. coli* and *C. pyogenes* were also isolated. Our findings are supported by Hiss et al. (2004) who also detected *C. bovis*, *Staphylococcus* spp, *Streptococcus* spp. and *E. coli* from clinical mastitis in cows. These pathogens have been reported from India and abroad from both cows and buffaloes. In contrary, *E. coli* (Eckersall et al., 2001) and *Streptococcus uberis*, *S. dysgalactiae* (Nielsen et al., 2004) were the main organisms responsible for clinical mastitis in dairy cows in Denmark.

Reports are not available on the concentrations of acute phase proteins in healthy and mastitic buffaloes. However, studies conducted by different workers (Eckersall et al., 2001; Nielsen et al., 2004; Eckersall et al., 2006; O’Mahony et al., 2006; Kovac et al., 2007; Kumar et al., 2012; Thulasiraman et al., 2013) have demonstrated higher serum concentrations of SAA and haptoglobin in cows suffering from sub-clinical and clinical mastitis and suggested the diagnostic value of the acute phase proteins in differentiating healthy and mastitic animals. We also observed that the concentrations of milk and serum haptoglobin and amyloid A accurately reflected the degree of inflammation and could be used as markers for detection of clinical and sub-clinical mastitis. Reports on milk and serum concentrations of Hp and AA in relation to bacterial isolates are few and limited to cows only.

In cows, *S. aureus* and *S. uberis* have been shown to increase acute phase protein concentration in sub-clinical and clinical mastitis (Gronlund et al., 2003; Pedersen et al., 2003; Gronlund et al., 2005; Pyorala et al., 2011). In cows, Gronlund et al. (2005) analysed acute phase proteins in chronic subclinical mastitis and Hp concentrations ranged from <0.3 µg/ml to 358 µg/ml in samples being positive for udder pathogens (*S. aureus* and *S. agalactiae*, *E. coli* and coagulase negative Staphylococci) which also support our results. Safi et al. (2009) concluded that measuring haptoglobin and amyloid A in milk was more accurate than serum analysis for the diagnosis of sub-clinical mastitis in Holstein Friesian cows. Although parameters such as EC and NGase may help differentiating healthy animals from infected ones but their limitation is that they are influenced by physiological factors whereas haptoglobin and amyloid A are specific indicators of bovine mastitis.

In conclusion, our study provides strong evidence for production of significant amount of acute phase proteins in milk during SCM. These can be rapid and sensitive markers of inflammation as compared to SCC. The advantage of haptoglobin and serum amyloid A over other markers of mastitis is attributable to the fact that they are not present in the milk of healthy dairy buffaloes and are not influenced by factors other than mastitis. Therefore, estimation of acute phase proteins in milk is a useful diagnostic tool to detect streptococcal and staphylococcal clinical and sub-clinical mastitis and to monitor herd health.

**ACKNOWLEDGEMENTS**

Thanks are due to Sh. Randhir Singh and Sh. Bhupendra Singh of Veterinary College Central Lab. for their help and cooperation.

**REFERENCES**


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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>EC (mS)</th>
<th>SCC (x10⁵/ml)</th>
<th>Milk Hp (µg/ml)</th>
<th>Serum Hp (µg/ml)</th>
<th>MAA (µg/ml)</th>
<th>SA (µg/ml)</th>
<th>NA Gase (µg/ml)</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>2.85±0.28</td>
<td>1.45±0.48</td>
<td>19.27±4.16</td>
<td>82.36±29.58</td>
<td>0.06±0.03</td>
<td>6.12±5.44</td>
<td>0.32±0.12</td>
<td></td>
</tr>
<tr>
<td>Sub-clinical</td>
<td>4.18±0.44</td>
<td>6.60±0.31</td>
<td>92.00±16.54</td>
<td>1754.00±336.24</td>
<td>2.37±0.81</td>
<td>94.18±24.15</td>
<td>0.69±0.10</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>6.35±1.02</td>
<td>7.65±0.64</td>
<td>395.00±170.76</td>
<td>2512.50±681.82</td>
<td>8.66±4.52</td>
<td>221.31±46.69</td>
<td>1.20±0.34</td>
<td></td>
</tr>
</tbody>
</table>

Within the column, the values with different superscripts differ significantly (<0.05)


