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Research Article

ACUTE PHASE PROTEINS AS INDICATORS OF INFLAMMATION IN STREPTOCOCCAL AND STAPHYLOCOCCAL MASTITIS IN BUFFALOES

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ABSTRACT

A study was carried out to evaluate acute phase proteins and other indicators of inflammation in detection of streptococcal and staphylococcal clinical and sub-clinical mastitis in buffaloes. A total of 326 quarter milk samples of 82 murrah buffaloes at an organized farm and 23 quarter milk samples from 21 clinical cases of mastitis in buffaloes received in the College Central Laboratory were subjected to bacteriological examination and somatic cell count. Milk and serum samples from sub-clinically and clinically infected and healthy buffaloes (ten each) were used for estimation of haptoglobin and amyloid A as indicators of inflammation. *Staphylococcus* spp and *Streptococcus* spp. were found to be the major organisms in clinical and sub-clinical cases of mastitic buffaloes. A significant increase in 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.

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 amyloidA 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) and milk amyloid A (MAA) as well as on the concentrations of serum haptoglobin (SHp) and serum amyloid A (SAA). On intensive review of literature it appears that no work has been conducted on acute phase proteins as a marker of mastitis in buffaloes in India and abroad.

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MATERIALS AND METHODS

A total of 326 quarter milk samples of 82 apparently normal murrah buffaloes at an organized farm and 23 quarter milk samples of 21 clinically infected buffaloes received in the College Central Laboratory were subjected to bacteriological examination. The samples were collected by standard milk sampling technique and were examined following standard procedures (Sears *et al*., 1993) for cultural isolation on to 5% sheep blood agar and Mac Conkey's lactose agar plates. The plates were incubated aerobically

at 37^0C 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. Staphylococci 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 1
Frequency distribution of different organisms isolated from milk of buffaloes suffering from clinical and sub-clinical mastitis

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

**E. coli+C. pyogenes* (1), Staphylococci + Streptococci (1)
****Staphylococci+Streptococci (6)

Table 2
Different parameters in sub-clinical and clinical mastitic buffaloes

Group	EC (mS)	SCC ($x10^5$/ml)	Milk Hp (µg/ml)	Serum Hp (µg/ml)	MAA (µg/ml) (µg/ml)	SA (µg/ml)	NA Gase (OD)
Healthy	2.85^{a}±0.28	1.45^{a}±0.48	19.27^{a}±4.16	82.36^{a}±29.58	0.06^{a}±0.03	6.12^{a}±5.44	0.32^{a}±0.12
Sub-clinical	4.18^{b}±0.44	6.60^{b}±0.311	92.00^{b}±16.54	1754.00^{b}±336.24	2.37^{b}±0.81	94.18^{b}±24.15	0.69^{b}±0.10
Clinical	6.35^{b}±1.02	7.65^{b}±0.64	395.00^{c}±170.76	2512.50^{c}±681.82	8.66^{c}±4.52	221.31^{c}±46.69	1.20^{b}±0.34

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

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.

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