

productivity, metritis and mastitis problems. Similar higher prevalence of *Salmonella* antibodies has been reported earlier in female than in male in goats (Chandra *et al.*, 2006, 2007) and horses (Singh and Sharma, 2000).

*Salmonella* has often been reported to be associated with abortion in mares either as primary cause or as secondary opportunist when mares are under stress from other viral and bacterial infections or physical stress due to work or environment (Kilborne, 1983). High prevalence of *Salmonella* antibodies in breeding mares (with no history of vaccination in last three years) in India are in concurrence to bacteriological findings reported earlier (Singh and Sharma, 2000, Singh, 2007, Singh *et al.*, 2007).

The study concludes that anti-*Salmonella* IgGs were more commonly present in serum samples of mares and dogs than in cattle and buffaloes. Significantly, female animals had high sero-positive *Salmonella* infection than male animals. Similarly, sick and aborted cows were significantly more sero-positive for *Salmonella* infection than the healthy stock indicating that salmonellosis might be an important infection in animals in Northern India.

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## SUBCLINICAL MASTITIS IN BUFFALOES: INVESTIGATIONS ON OXIDATIVE STRESS

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### ABSTRACT

Investigations were conducted on oxidative stress in 25 cases of sub-clinical mastitis in buffaloes. Ten apparently healthy animals were kept as control. The oxidative stress in sub-clinical mastitis was assessed by estimation of serum inorganic phosphorus (Pi) along with blood and milk malondialdehyde-levels. Serum Pi level was found to be significantly lower ( $P=0.01$ ) in diseased buffaloes as compared to healthy controls which indicated a depleted body antioxidant system. Further, a significant increase ( $P=0.01$ ) of milk and blood malondialdehyde levels was observed in buffaloes suffering from sub-clinical mastitis suggesting a manifold increase of lipid peroxidation. The findings reflected that oxidative stress is one of the important factors related to pathogenesis of sub-clinical mastitis in buffaloes.

**Key words:** Oxidative stress, sub-clinical mastitis, buffaloes, malondialdehyde

Mastitis is characterized by physical, chemical and bacteriological changes in milk and pathological alterations of glandular tissue. It occurs in both clinical as well as sub-clinical forms. Sub-clinical mastitis (SCM) has 15-40 times more prevalence than clinical mastitis. The SCM is a herd problem and it usually remains unnoticed because no gross clinical signs of inflammation or alterations in milk composition are evident. In the recent past, attention is given to the role of oxidative stress in relation to pathogenesis of mastitis (Ranjan *et al.*, 2005, Kumar *et al.*, 2007). Serum inorganic phosphorus (Pi) and blood malondialdehyde (MDA) levels are considered to be good indicators of oxidative stress in animals (Mata and Bhardwaj, 1985, Gahlawat *et al.* 2007).

### MATERIALS AND METHODS

The study was conducted on buffaloes from Animal Farm, CCS HAU, Hisar and Hisar city as well as its surrounding areas. A total of 1080 quarters of 280 lactating buffaloes were screened for the prevalence of SCM of which 40 quarters in 25 animals were found positive. Diagnosis of SCM was based on somatic cell count, California mastitis test and milk conductivity test. The cases selected for present study were positive

for at least two of these three tests. Sub-clinically infected mastitis cases were investigated for oxidative stress status by estimating malondialdehyde (MDA) in whole blood and milk as well as serum inorganic phosphorus levels. Ten healthy buffaloes from both Animal Farm, CCS HAU, Hisar as well as Hisar city were kept as controls. Approximately, 10 ml of milk sample from each quarter was collected aseptically in sterilized test tubes. For the estimation of serum inorganic phosphorus (Pi) levels, about 15-20 ml blood was collected in test tubes through jugular puncture and serum was separated. The serum samples were stored at  $-20^{\circ}\text{C}$ . The serum Pi level was measured by UV Endpoint Method using AUTOPAK commercial kit of Bayer Diagnostics India Ltd. For estimation of MDA, blood was collected in test tubes containing heparin as anticoagulant. The MDA levels in the fresh blood and milk were estimated using thiobarbituric acid reaction as described by Okhawa *et al.* (1979). Statistical analysis was done as per standard procedure described by Snedecor and Cochran (1994).

### RESULTS AND DISCUSSION

Blood and milk biochemical alterations depicting oxidative stress in SCM cases in buffaloes are given in Table. Serum Pi concentration in sub-clinically

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Table  
Blood and milk biochemical alterations depicting oxidative stress in sub-clinical mastitis in buffaloes

Parameter	Healthy control (n=10)	Sub-clinical mastitis (n=25)
Serum Pi mg %	5.56±0.29 <sup>a</sup>	2.71±0.18 <sup>b</sup>
Blood MDA nmol/ml	28.26±0.83 <sup>a</sup>	38.61±0.65 <sup>b</sup>
Milk MDA nmol/ml	17.96±0.61 <sup>a</sup>	47.46±0.86 <sup>b</sup>

Means with different superscripts in a row vary significantly (P=0.01), Pi: Inorganic phosphorus, MDA: Malondialdehyde

infected buffaloes were found to be significantly lower (P=0.01) as compared to healthy controls. This is in agreement with earlier reports of Bozhkova and Tsvetkov (1976) and El Zubeir *et al.* (2005) who found lower levels of Pi in milk and blood samples of cows suffering from SCM. However, Dwivedi *et al.* (2004) did not record any significant alteration in Pi levels in mastitic cows. Serum Pi is considered to be a major player in energy metabolism in body as it is a component of ATP, which is major energy source for all the metabolic pathways. Earlier, Mata and Bhardwaj (1985) suggested the role of phosphorus in causing oxidative stress in post-parturient hemoglobinuria in buffaloes. In recent past, attention has been paid to the relationship of oxidative stress to the pathogenesis of mastitis. Ranjan *et al.* (2005) reported enhanced erythrocytic peroxide levels and reduced blood zinc as well as plasma ascorbic acid concentrations suggesting decline of blood antioxidant status in mastitic dairy cows. Therefore, the findings of this study are comparable with the earlier reports.

In present investigations, a significant increase (P=0.01) of serum and milk MDA levels was observed in SCM in buffaloes as compared to healthy controls. The MDA is a by-product of lipid peroxidation reactions in body and is normally produced in healthy animals, however, during the mastitis it is excessively produced. Relationship of oxidative stress in mastitis has been studied by Kumar *et al.* (2007) who have reported a significant increase of blood and milk MDA levels in both sub-clinical and clinical forms of mastitis in buffaloes. Further, elevated milk MDA levels have also been reported in SCM in cows (Shaheen *et al.*, 2008). A significant increase of MDA levels in the present investigation provides good support to oxidative

stress theory in relation to pathogenesis of this disease. Earlier workers have reported significant decrease of serum copper, zinc, selenium, ascorbic acid and vitamin E in mastitis in cows and buffaloes which further support the role of oxidative stress in pathogenesis of mastitis (Tuteja, 1999, Ranjan *et al.*, 2005, Shaheen, 2005, Rezamand *et al.*, 2007). All these earlier reports along with the present findings provide a platform for further detailed studies involving a larger number of cases on this aspect of disease.

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