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ORIGINAL ARTICLE

Serum Antioxidant Status to Assess Oxidative Stress in Brucella Infected Buffaloes

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The investigation was carried out to assess oxidative stress on the basis of serum antioxidant status in the buffaloes of *Murrah* breed having brucellosis. For this serum antioxidants like vitamin A, vitamin C, vitamin E and glutathione and various enzymes like catalase, superoxide dismutase, glutathione reductase and xanthine oxidase were determined. Results indicated that vitamin A, vitamin C, vitamin E and glutathione levels decreased significantly ($p \leq 0.05$) in affected buffaloes as compared to healthy buffaloes. Serum catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased significantly($p \leq 0.05$) in affected buffaloes as compared to healthy buffaloes. Results indicated that the antioxidant status was altered in the brucella infected buffaloes. Depletion of levels of antioxidants in the serum reflected towards the development of oxidative stress in buffaloes having brucellosis.

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Oxidative stress is acquiring attention of scientific community as a budding problem in the field of veterinary medicine. The activity of antioxidative enzymes and the capacity of non enzymatic antioxidants are known as the antioxidative status of the organism. If the balance between prooxidative processes and antioxidative system is disturbed oxidative stress occurs. Oxidative stress is considered to be a major risk factor for the reduction of defense mechanisms and development of diseases. Farm animals undergo several periods of severe challenge of the antioxidative system.

Research in oxidative stress has been implicated in numerous disease processes including sepsis, mastitis, acidosis, ketosis, enteritis, pneumonia, respiratory, and joint diseases. However, compared to human medicine, only a limited number of conditions have been investigated in regard to the effects of oxidative stress in animals and very little

emphasis has been given to brucellosis related oxidative stress. Brucellosis is a significant public health problem in areas of the world where brucella infections are endemic in herbivorous animals.

Brucella is a Gram-negative, facultative, intracellular pathogen that produces cell toxicity by altering plasma membrane and inducing cell apoptosis. It is stated that the oxidative killing pathways of host macrophages represent a primary mechanism utilized by these host phagocytes to control the intracellular replication of the brucellae. Therefore, it is possible that brucellosis may be related to increased free radical production and antioxidant depletion, and oxidative stress may be implicated in the pathogenesis of brucellosis. The oxidative killing by polymorphonuclear leukocytes and macrophages plays a primary role in the elimination of intracellular brucellae. The organism has ability to resist the oxidative killing by several incompletely understood mechanisms and thereby can survive and multiply in the these phagocytic cells of the host (Young, 2005). Brucella pathogen can stimulate cerebral lipid peroxidation in the infection without causing significant inflammation (Kataria *et al*., 2010a). Countable few reports are there in milch animals showing oxidative stress against brucella infection. Oxidative stress can be monitored with several biomarkers including antioxidants and enzymes in the laboratory. Measures of oxidative stress allow the assessment of real status of physiological defenses and prevention of the appearance of correlated pathologies. The *Murrah* is the most important Indian breed of buffalo and is the premier milking buffalo. These animals contribute greatly in enhancing economical status of farmers. Looking towards the higher occurrence of brucella infection in buffaloes with paucity of research on oxidative stress, the present investigation was planned to assess antioxidant status in brucella infected *Murrah* buffaloes of India.

MATERIALS AND METHODS

During a brucellosis surveillance study in an organised private dairy farm sera were collected from 200 animals. From the lot, 60 sera each from healthy and brucella infected buffaloes were used in the study to evaluate oxidative stress by determining antioxidants.

Serum antioxidants included vitamin A, vitamin C, vitamin E, and glutathione and enzymes included catalase, superoxide dismutase, glutathione reductase and xanthine oxidase. Vitamins A and C were determined by the methods of Varley (1988) with little modification (Anonymous, 2010); vitamin E by Nair and Magar (1955) with modification (Kataria *et al*., 2010b); and glutathione by Owens and Belcher (1965) with modifications for serum samples (Kataria *et al.* 2010b). Serum catalase was determined by the method of Goldblith and Proctor (1950) with little modifications (Kataria *et al*., 2010b); superoxide dismutase by Winterbourn *et al.* (1975) with modification (Kataria *et al.,* 2010b); glutathione reductase by King (1965) with little modification (Kataria *et al*., 2010b); and xanthine oxidase by Litwack *et al.* (1953) with little modification (Kataria *et al.,* 2010b).

RESULTS AND DISCUSSION

The mean values of serum antioxidants *viz*. vitamin A, vitamin C, vitamin E and glutathione and serum enzymes *viz.* catalase, superoxide dismutase, glutathione reductase and xanthine oxidase are presented in table 1.

Table 1: Mean ± SEM values of serum antioxidants in healthy and brucella infected buffaloes

S.No.	Serum antioxidants	Healthy Buffaloes	Brucella infected buffaloes
1	Vitamin A, µmol L^{-1}	2.00±0.03	1.74^{b}±0.02
2	Vitamin C, µmol L^{-1}	20.12± 0.10	14.24^{b}± 0.12
3	Vitamin E , µmol L^{-1}	4.20 ±0.09	2.20^{b} ±0.02
4	Glutathione, µmol L^{-1}	5.45±0.10	3.08^{b} ±0.05
5	Catalase, kU L^{-1}	68.55 ±10.00	132.65^{b} ±8.56
6	Superoxide dismutase, kU L^{-1}	160.22 ±12.90	284.34^{b} ±9.21
7	Glutathione reductase, kU L^{-1}	3.10± 0.11	5.93^{b} ± 0.05
8	Xanthine oxidase, mU L^{-1}	81.12 ±1.80	110.12^{b} ±3.11

Superscript 'b' on the means shows the significant ($p \leq 0.05$) difference from healthy buffaloes

Results indicated that vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly in affected buffaloes as compared to healthy buffaloes. Serum catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased significantly in affected buffaloes as compared to healthy buffaloes. In brucella affected buffaloes, the status of serum antioxidants was changed. Decrease in the levels of antioxidants and enzymes indicated towards development of oxidative stress in brucella infected buffaloes.

These results corroborated the earlier findings in brucella affected cows by Kataria *et al.* (2010a). Erdogan *et al.* (2008) also observed that brucella infection suppressed antioxidant enzyme activities. Oxidative events against brucella infection are not well elucidated in animals. The oxidative burst represents the pathogenesis of brucellosis. Bucella are internalized into phagocytic cells and are killed by reactive oxygen and nitrogen species. Therefore brucellosis is related to increased free radical production and antioxidant depletion (Serefhanoglu *et al*., 2009).In brucellosis, stimulation of macrophages is a significant stage in the progress of the disease in which cells serve as an effector system of cytokines, chemokines and free radicals, to produce highly damaging reactive nitrogen species and reactive oxygen species (Klebanoff *et al*., 1980). Organisms have several enzymatic and non-enzymatic antioxidant systems that overwhelm harmful effects of these free radicals. Under certain conditions, antioxidants mechanisms are impaired and/or free radicals are increased and antioxidant mechanisms may become insufficient to prevent oxidative damage completely. Consequently, oxidative stress develops (Aruoma,1996). Reactive oxygen species can cause protein oxidation, lipid peroxidation and DNA damage (Kocyigit *et al*., 2005). Karsen *et al.* (2011) concluded that patients with brucellosis were exposed to potent oxidative stress.

In conclusion, according to the data obtained from the present study, decreased antioxidants levels, and increased enzyme activities showed that the buffaloes with brucellosis infection were exposed to potent oxidative stress. The study also pointed out towards the relevance of periodic assessment of antioxidant status in farm animals for proper supplementation of antioxidants. Various antioxidant indices can be used to monitor the treatment of brucellosis.

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