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## ORIGINAL ARTICLE

# Evaluation of Oxidative Stress in Sheep Affected with *Peste des petits ruminants*

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The aim of the investigation was to evaluate oxidative stress in sheep affected with *peste des petits ruminants* (PPR). Oxidative stress in the affected sheep was evaluated by determining various serum biomarkers viz. vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, glutathione reductase and xanthine oxidase, the mean values of which were  $1.70 \pm 0.07 \mu\text{mol L}^{-1}$ ,  $13.00 \pm 0.10 \mu\text{mol L}^{-1}$ ,  $2.25 \pm 0.07 \mu\text{mol L}^{-1}$ ,  $3.10 \pm 0.06 \mu\text{mol L}^{-1}$ ,  $140.00 \pm 8.00 \text{ kU L}^{-1}$ ,  $294.22 \pm 9.91 \text{ kU L}^{-1}$ ,  $6.99 \pm 0.05 \text{ kU L}^{-1}$  and  $100.10 \pm 3.00 \text{ m U L}^{-1}$ , respectively. The levels of vitamins A, C, E and glutathione decreased significantly ( $p \leq 0.05$ ) and the serum catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased significantly ( $p \leq 0.05$ ) in affected sheep as compared to that in healthy ones. On the basis of the altered levels of serum biomarkers of oxidative stress it was concluded that the animals affected with PPR developed oxidative stress. The findings suggested the relevance of periodic assessment of oxidative status in ruminants for healthier management through supplementation of proper antioxidants as supportive treatment in PPR and in healthy in-contact animals.

*Key words: Biomarkers, oxidative stress, PPR, serum, sheep*

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Oxidative cellular injury can cause cellular dysfunction and, when severe, this form of injury can cause cell death. Steady-state levels of oxidative tissue damage represent a balance between rates of damage caused by pro-oxidant stimuli and rates of antioxidant and tissue repair mechanisms that decrease reactive oxygen species levels and remove oxidatively damaged molecules. Components of the antioxidant defense system

include enzymes and antioxidants that play important roles in determining the extent of free radical-mediated tissue injury (Valyi –Nagy and Dermody, 2005).

Role of reactive oxygen species in the pathogenesis of viral infections has recently gained momentum with the emphasis to find out the efficacy of antioxidants as therapeutic agents in viral diseases of both animal models and patients

(Schwarz, 1996). Pathogenic organism can be considered as pro-oxidant agents because they produce cell death and tissue damage. In addition organism can be eliminated by specific cell defense mechanism which utilize in part, reactive oxygen radicals formed by oxidative stress responses. The cause of the necessarily defense process results in cell damage thereby leading to development of inflammation, a characteristic oxidative stress situation (Romero *et al.*, 1995). *Peste des petits ruminants* (PPR) is an acute and highly contagious disease affecting small ruminants. It is an *office international des epizooties* list A disease caused by morbillivirus and is characterized by high morbidity and high mortality rates resulting into heavy economical losses. Pyrexia, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosae, enteritis and pneumonia are clinical signs followed by death or recovery from the disease (Nisbet *et al.*, 2007). Immunosuppression of the affected animals is the major cause behind the reduced productivity (Kataria *et al.*, 2007).

Involvement of an imbalance in oxidant-antioxidant activity in the pathogenesis of PPR is still not much elucidated but scientific reports, although countable few, are trying to accredit increased oxidative stress as an underlying etiopathogenesis of this disease (Gil *et al.*, 2004). When the fine balance between prooxidative processes and antioxidative system is disturbed oxidative stress occurs. It is acquiring attention of scientific community as a budding problem in the field of veterinary medicine because it has got implications in health and production of animals and is considered to be a major risk factor for the reduction of defense mechanisms and development of diseases.

Research in oxidative stress has been associated in various pathological processes in veterinary medicine (Kataria *et al.*, 2010), however, very little emphasis has been given to infectious disease like PPR in small ruminants. Scientific community is trying to review the infectious pathology in animals with oxidative stress. Looking towards paucity of work associating oxidative stress in pathogenesis of PPR in sheep, the present investigation was carried out with the aim to understand variations in the endogenous serum antioxidants (vitamin A, C and E, and glutathione) and enzymes (catalase, superoxide dismutase, glutathione reductase and xanthine oxidase). Since PPR is an important sheep disease, an appropriate management strategy has to be designed and applied involving periodic monitoring of healthy lot, prevention of contact of former with infected sheep and proper supplementation of antioxidants to in-contact healthy animals and animals under treatment.

## MATERIALS AND METHODS

A series of PPR outbreaks in sheep were investigated during February 2012 – April 2012 in different districts (Bikaner, Nagaur, Churu, Jodhpur) in Rajasthan, India. The affected sheep were of *Marwari* breed belonging to private farmers kept on free range system of feeding and management. The blood samples for serum preparation were drawn from 142 sheep (96 adult and 46 lambs) affected with PPR and 41 (22 adult and 19 lambs) healthy ones.

Blood was collected from jugular vein directly into sterilized tubes without any anticoagulant and serum biomarkers (vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, glutathione reductase and xanthine oxidase) were determined by the methods as given by Kataria *et*

*al.* (2012). The mean values of parameters for PPR affected animals for each biomarker were compared with the respective mean value for healthy animals to find out the levels of significance (Kaps and Lamberson, 2004).

## RESULTS AND DISCUSSION

The mean values of serum biomarkers of oxidative stress are presented in Table 1. The data indicated that vitamin A, vitamin C, vitamin E and glutathione activity decreased significantly ( $p \leq 0.05$ ) and the serum catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased significantly ( $p \leq 0.05$ ) in PPR affected sheep as compared to healthy ones. Among antioxidants maximum changes were observed in the levels of vitamin E, which showed 1.9 times decrease in PPR affected animals. Serum glutathione reductase showed maximum increase of 2.09 times among serum enzymes in PPR affected animals.

Inflammatory responses of the host precipitated by viral infections may involve the generation of reactive oxygen species by infiltrating phagocytic cells and xanthine oxidase mediated humoral responses (Valyi-Nagy and Dermody, 2005). Under certain conditions, antioxidant mechanisms are impaired and free radicals are increased and antioxidant mechanisms may become insufficient to prevent oxidative damage completely. Consequently, oxidative stress develops, which is implicated as a pathogenic factor in a number of viral infections (Beck *et al.*, 2000). Oxidative stress has been suggested to be a mediator of apoptosis induced by a variety of triggers, including virus infections (Buttke and Sandstrom, 1994). An altered status of serum antioxidants in PPR affected animals indicated development of oxidative stress in these animals. Earlier researchers had also

reported alterations in the levels of antioxidant enzymes in PPR affected sheep (Nisbet *et al.*, 2007). They discussed that PPR is a febrile disease that is characterized by respiratory distress, stomatitis and gastroenteritis. There is a balance in the organism between production of free radicals and enzymatic and non-enzymatic anti-oxidant defense mechanisms. Oxidative stress due to an increase in reactive oxygen species or a deficiency in antioxidant defense mechanisms, causes structural and functional modifications of lipid, protein and DNA-containing macromolecules of the cell.

Free radicals can cause protein oxidation, lipid peroxidation and DNA damage (Kocyigit *et al.*, 2005). There has been growing interest in the role of antioxidant function in controlling inflammatory disease states and inflammation is one of the important symptoms of PPR (Kataria *et al.*, 2007). Inflammatory processes are associated with generation of increased number of free radicals. Persistent oxidative stress induced by inflammatory processes is a self-perpetuating process and cause progressive accumulation of DNA damage in target organs (Bartsch and Nair, 2006). Effects of immune suppression on pathogenesis of *peste des petits ruminants* virus infection have been reported (Jagtap *et al.*, 2012). Immunosuppression leads to generation of reactive oxygen species (Kedzierska *et al.*, 2011). Earlier workers have reported that PPR infection causes oxidative stress and consequently lipid peroxidation and the by-products may be used as biomarkers of damage in various tissues (Nisbet *et al.*, 2007). Reactive oxygen and nitrogen metabolites play a complex role in many diseases and in metabolic regulation. Because viruses replicate in living cells, such metabolites influence the growth of viruses in addition to serving as a host defense mechanism (Peterhans, 1997).

Oxidative stress can produce tremendous effect on pathophysiology of virus infections. Scientists are of the opinion to use antioxidant molecules as therapeutic agents in various virus infections (Israel

Gougerot-Pocidalo, 1997). Changes in the levels of antioxidants and enzymes in the affected sheep suggested the presence of oxidative stress in the present study.

**Table1.** Mean  $\pm$  SEM values of serum biomarkers of oxidative stress in healthy and PPR affected sheep

| S. No. | Serum antioxidants                        | Healthy sheep (n=41) | PPR affected sheep (n=142)     |
|--------|---|----------------------|--------------------------------|
| 1      | Vitamin A, $\mu\text{mol L}^{-1}$         | 2.18 $\pm$ 0.08      | 1.70 <sup>b</sup> $\pm$ 0.07   |
| 2      | Vitamin C, $\mu\text{mol L}^{-1}$         | 21.51 $\pm$ 0.10     | 13.00 <sup>b</sup> $\pm$ 0.10  |
| 3      | Vitamin E, $\mu\text{mol L}^{-1}$         | 4.28 $\pm$ 0.08      | 2.25 <sup>b</sup> $\pm$ 0.07   |
| 4      | Glutathione, $\mu\text{mol L}^{-1}$       | 5.87 $\pm$ 0.10      | 3.10 <sup>b</sup> $\pm$ 0.06   |
| 5      | Catalase, kU L <sup>-1</sup>              | 71.00 $\pm$ 9.00     | 140.00 <sup>b</sup> $\pm$ 8.00 |
| 6      | Superoxide dismutase, kU L <sup>-1</sup>  | 167.98 $\pm$ 10.00   | 294.22 <sup>b</sup> $\pm$ 9.91 |
| 7      | Glutathione reductase, kU L <sup>-1</sup> | 3.34 $\pm$ 0.10      | 6.99 <sup>b</sup> $\pm$ 0.05   |
| 8      | Xanthine oxidase, mU L <sup>-1</sup>      | 69.00 $\pm$ 1.00     | 100.10 <sup>b</sup> $\pm$ 3.00 |

Superscript 'b' on the mean shows the significant ( $p \leq 0.05$ ) difference from healthy sheep in a row.

## CONCLUSION

Based upon the decreased antioxidants levels, and increased enzyme activities, it is concluded that PPR affected animals experienced strong oxidative stress. The findings suggested the relevance of periodic assessment of oxidative status in ruminants for healthier management through supplementation of proper antioxidants as supportive treatment in PPR and in healthy in-contact animals. The significance of observations in this study needs to be addressed in future studies related with PPR.

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