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Evaluation of oxidative stress in pigs affected with classical swine fever

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Abstract. The aim of the present investigation was to evaluate oxidative stress in pigs affected with classical swine fever. Oxidative stress in the affected pigs was evaluated by determining various serum biomarkers, namely: vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, glutathione reductase and xanthine oxidase, the mean values of which were 1.50 ± 0.06 $\mu molL^{-1}$, 11.00 ± 0.07 $\mu molL^{-1}$, 2.12 ± 0.06$\mu molL^{-1}$, 3.00 ± 0.07$\mu molL^{-1}$, 159.00 ± 7.00 kUL^{-1}, 325.32 ± 10.00 kUL^{-1}, 10.00 ± 0.06 kUL^{-1} and 120.00 ± 6.00 mUL^{-1}, respectively. The levels of vitamins A, C, E and glutathione decreased significantly ($p \leq 0.01$) and those of catalase, superoxide dismutase, glutathione reductase and xanthine oxidase activities increased significantly ($p \leq 0.01$) in affected pigs as compared to those in healthy ones. Among antioxidants, maximum changes were observed in the levels of vitamin C, which showed 2.36 times decrease in diseased animals. Serum glutathione reductase showed a maximum increase of 2.5 times among serum enzymes in diseased animals. Based on the altered status of serum oxidative stress biomarkers it was concluded that the animals affected by swine fever developed oxidative stress. The findings suggest the relevance of periodic assessment of oxidative status in pigs, for healthier management through supplementation of proper antioxidants as supportive treatment in swine fever and in healthy in-contact animals.
Key Words: biomarkers, pig, oxidative stress, serum, swine fever.

Introduction. Classical swine fever virus (CSFV) causes a severe disease in pigs that is characterized by hemorrhage, disseminated intravascular coagulation, and leucopoenia. It is a serious contagious viral disease with a widespread worldwide distribution with economical importance. This infectious disease of pigs is caused by an RNA virus belonging to family Flaviviridae, genus *Pestivirus*. The severity of CSF infection is believed to be determined by different factors, including the virulence of the strain as well as factors related to the host (Nielsen et al 2010) and the virulence of CSF virus strains ranges from avirulent to highly virulent (Mayer et al 2003). The disease has been reported from different parts of India from time to time (Bhattacharya 2001; Kumar et al 2007; Kataria et al 2010a).

Looking towards the unpleasant fact that CSF outbreaks can cause enormous losses in pig populations, the scientific community is pointing strongly towards the minimization of the economical damage by developing a baseline CSF control strategy. This may include periodic screening of general the health status of animals. Researchers have investigated the function of CSFV non-structural protein 5A (NS5A) by examining its role in the induction of oxidative stress and related intracellular events (He et al 2012). Therefore, detection of oxidative stress can be one aspect of the control strategy. 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 oxidative damaged molecules.

The ability of pigs to neutralize the reactive oxygen species plays a key role in their welfare and scientists have recommended the application of such methods for the assessment of farmed animals' welfare (Brambilla et al 2001). The 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 (Schwarz 1996). The pathogenic organisms can be considered as pro-oxidant agents because they produce cell death and tissue damage. In addition, the organism can be eliminated by a specific cell defence mechanism that utilizes in part, reactive oxygen radicals formed by oxidative stress responses. The cause of the necessarily defence process results in cell damage thereby leading to development of inflammation, a characteristic oxidative stress situation (Romero et al 1995). When the fine balance between the pro-oxidative processes and the antioxidative system is disturbed oxidative stress occurs.

The scientific community has started looking towards oxidative stress as a serious problem, due to its implications in animal health and breeding. It is considered to be a major risk factor in the reduction of the defence mechanisms, which is associated with various pathological processes known in veterinary medicine (Kataria et al 2010b); however, very little emphasis has been put on viral diseases, like swine fever. It is important to review the infectious pathology of pigs, since swine fever is an important pig disease. To develop an appropriate management strategy, a periodic monitoring of the healthy lot is recommended along with proper supplementation of antioxidants to in-contact healthy animals and animals under treatment. Looking towards the scarcity of work associating oxidative stress in swine fever, the present investigation was carried out with the aim to understand variations in the levels of biomarkers of oxidative stress, which are endogenous antioxidants (vitamin A, C and E, and glutathione) and enzymes (catalase, superoxide dismutase, glutathione reductase and xanthine oxidase).

Materials and Methods. An outbreak of swine fever was recorded in the summer season in a pig population comprised of exotic crossbred pigs in Rajasthan state, India. The disease was diagnosed based on clinical signs, post-mortem observations and laboratory confirmation by c-ELISA. Blood samples were collected from tail vein directly into sterile tubes without any anticoagulant from affected and healthy adult animals during the outbreak (10 each). Serum biomarkers (vitamin A, vitamin C, vitamin E, glutathione, catalase, superoxide dismutase, glutathione reductase and xanthine oxidase) were determined, following the methods described by Kataria et al (2012). The mean value of each biomarker of the animals affected by swine fever was compared to the corresponding mean value of the healthy animals to find out the levels of significance (Kaps & Lamberson 2004).

Results and Discussion. The mean values of serum biomarkers of oxidative stress are presented in Table 1.

Table1

Serum biomarkers (Mean ± SEM) of oxidative stress in healthy pigs and pigs affected by swine fever (n=10)

Serum biomarkers	Healthy pigs	Affected pigs	Differences (± d)
Vitamin A, µmol L^{-1}	2.20 ± 0.09	1.50 ± 0.06	0.70**
Vitamin C, µmol L^{-1}	26.00 ± 0.08	11.00 ± 0.07	15.00**
Vitamin E , µmol L^{-1}	4.45 ± 0.07	2.12 ± 0.06	2.33**
Glutathione, µmol L^{-1}	6.90 ± 0.08	3.00 ± 0.07	3.90**
Catalase, kU L^{-1}	79.00 ± 8.00	159.00 ± 7.00	80.00**
Superoxide dismutase, kU L^{-1}	155.10 ± 11.00	325.32 ± 10.00	170.22**
Glutathione reductase, kU L^{-1}	4.00 ± 0.08	10.00 ± 0.06	6.00**
Xanthine oxidase, mU L^{-1}	72.00 ± 7.00	120.00 ± 6.00	48.00**

The data indicates that the activity of vitamin A, vitamin C, vitamin E and glutathione decreased significantly ($p \leq 0.01$) and the serum catalase, superoxide dismutase,

glutathione reductase and xanthine oxidase activities increased significantly ($p \leq 0.01$) in pigs affected by swine fever when compared to healthy ones. Among antioxidants maximum changes were observed in the levels of vitamin C, which showed a 2.36 fold decrease in animals affected by swine fever. Serum glutathione reductase showed a 2.5 fold maximum increase of serum enzymes, in animals affected by swine fever.

The role of various antioxidants in controlling diseases (Kataria et al 2007) explains the depletion of antioxidants during the disease state (Kataria et al 2012). Under certain conditions, antioxidant mechanisms are impaired and free radicals are increased and antioxidant mechanisms may become insufficient to completely prevent oxidative damage. Consequently, oxidative stress develops, which is implicated as a pathogenic factor in a number of viral infections (Beck et al 2000). An altered status of serum antioxidants in pigs affected by swine fever indicated the development of oxidative stress. Stress signalling from mitochondria and the endoplasmic reticulum leads to the induction of the proapoptotic transcription factor. Many viruses including African swine fever virus use the endoplasmic reticulum as a site of replication and/or envelopment, and this activity can lead to the activation of endoplasmic reticulum stress and apoptosis (Netherton et al 2004). There is a balance in the organism between production of free radicals and enzymatic and non-enzymatic anti-oxidant defence mechanisms. When free radicals are generated in excess, protein oxidation, lipid peroxidation and DNA damage may occur (Kocyigit et al 2005). Persistent oxidative stress induced by inflammatory processes is a self-perpetuating process and causes progressive accumulation of DNA damage in target organs (Bartsch & Nair 2006). Immunosuppression leads to generation of reactive oxygen species (Kedzierska et al 2011). 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 defence mechanism (Peterhans 1997). Oxidative stress can produce tremendous effect on pathophysiology of virus infections. The scientific opinion encourages the use of antioxidant molecules as therapeutic agents in various virus infections (Israel & Gougerot-Pocidalo 1997). Recent studies have shown that pigs supplemented with antioxidants developed the potential to prevent free radical induced damage (Zhu et al 2012). Therefore depletion in the levels of antioxidants along with altered levels of enzymes in the affected pigs suggested the presence of oxidative stress in the present study.

Conclusions. Based upon the decreased antioxidants levels, and increased enzyme activities, it is concluded that pigs affected by classical swine fever experienced potent oxidative stress. The findings suggested the relevance of periodic assessment of oxidative status in pigs for healthier management through supplementation of proper antioxidants as supportive treatment in swine fever and in healthy in-contact animals. These findings provide novel information on the development of oxidative stress which is poorly researched in swine fever cases. It will also help in providing an insight into the mechanism by which alteration in the antioxidants are associated with the viral infection. Knowledge of the alterations can help in assessing significance of sensitivity of diagnostic tests for infectious diseases under field conditions which can be used to design a surveillance program that increases the effectiveness of the control policy.

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