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Short Communication

# Evaluation of Oxidant and Antioxidant Capacity in Paratuberculosis Positive Cattle*

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**ABSTRACT**

The aim of this study is to determine the oxidant and antioxidant parameters in paratuberculosis positive cattle sera. Paratuberculosis is a zoonotic disease in cattle infected with *Mycobacterium avium*. The analysis did not show any significant difference in serum superoxide dismutase (SOD) and glutathione perioxidase (GSH-Px) activities of paratuberculosis positive group and the control groups. The serum adenosine deaminase (ADA) activity ($P<0.05$) and malondialdehyde (MDA) levels ($P<0.001$) were however observed to be elevated in paratuberculosis positive cattle. In conclusion, it was supposed that elevation in serum ADA activity was due to cell mediated immune system stimulation by *Mycobacterium avium subsp* and the increase in MDA level might be due to the increase in lipid peroxidation because of affected membrane lipids by infection. In spite of that, a nonrelative increase was observed in SOD and GSH-Px activities. This increase suggested that it can be a defense mechanism against increased free radicals together with lipid peroxidation.

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**Authors' Contribution**
**MC conceived and designed the study, collected and analyzed the data and wrote the article. SC helped in data analysis and preparation of manuscript. DD and YK helped in data collection. GFY helped in data analysis and interpretation.**

**Key words**
**Paratuberculosis, SOD, GSH-Px, ADA, MDA**

*Mycobacterium avium* subsp. *Paratuberculosis* (MAP), also named Johne disease, causes paratuberculosis in cattle and is also associated with Crohn disease in humans (Skovgaard, 2005; Pickup *et al*., 2005). MAP is isolated from daily milk, drinking water, various food sources and carcass. Therefore, it is stated that man also may be exposed to this *Mycobacterium avium* subsp. (Beumer *et al*., 2010; Meadus *et al*., 2008). Clinical findings resulting with death and killing in animals infected with this agent are; granulamatositic enteritis, diarrhoea and weight loss. The disease is accepted as the limiting factor in worldwide ruminant production (Harris and Barletta, 2001). Paratuberculosis shortenes the productive lifetime of cattle and also decreases milk production and growth rate (Lombard, 2011). The agent is phagocytosized by the phagocysitic cells at ileum and jejenum and is spreaded to regional lymph nodes and other organs in the following stages of the disease (2-10 years) (Andrews, 1992). Although the disease is present worldwide, knowledge about the pathogenesis is still limited. Incubation period of the disease is considerably long, therefore animals affected can start spreading the bacteria with stool 15-18 months before the clinical symptoms are observed (Bradford, 2001; Mecitoğlu and Demir, 2012). In the subclinical period, variations occur in the antigen response against MAP and in immune response due to the elevation of gamma interferon level (Strickland *et al*., 2005).

Serum adenosine deaminase (ADA) activity has physiologic functions considered to be responsible for cellular immunity (Söğüt *et al*., 2002). ADA plays role in all body tissues and fluids and especially in the formation and differentiation of lymphocytes in lymphoid cells. They show their effect by binding cell surface receptors and by prompting T cells. ADA activity varies due to immune response. It is activated when the immune system is activated and decreases when the immune system is depressed (Söğüt *et al*., 2002; Fischer *et al*., 1976; Gakis *et al*., 1998).

Plasma malondialdehyde (MDA) concentration is the result of nonenzymatic oxidative lipid peroxide destruction and shows toxic effect by binding to nucleic acids, phospholipids and the amino groups of proteins. It is measured as the indicator of lipid peroxidation in oxidative stres (Frei, 1994). Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are the enzymes preventing the accumulation of free radicals and starting of lipid peroxidation. SOD catalyses the conversion of superoxide to hydrogen peroxide, where GSH-Px removes hydrogen peroxide produced by SOD from tissues (Halliwell and Gutteridge, 1989; Dündar and Aslan, 2000). Decrease in these enzyme activities is

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associated with the increase in free radicals destructing the integrity and function of cell membrane (Freeman and Crapo, 1982).

Information about the pathogenesis of this worldwide disease is still limited. The aim of this study was the estimation of ADA which is responsible for cellular immunity, SOD playing role in antioxidant defense system and GSH-Px activities with MDA level which is the indicator of lipid peroxidation in serologically paratuberculosis positive cattle.

*Materıals and methods*

The study was approved by the Veterinary Control and Research Institute Samsun Ethics Committee (Date: 26.01.2015, Number: 31).

Twenty cattle aging 2-3 years were housed in various cattle farms in Samsun region and had not been vaccinated against paratuberculosis previously. The control group comprised 10 healthy cattle.

Blood specimen were obtained aseptically from V. Jugularis and sera were extracted in the laboratory by centrifugation at 3000 rpm for 10 min. Sera were kept at -20ºC until analysed.

Serum specimens were examined for paratuberculosis with commercial antibody ELISA kit (IDEXX) *Mycobacterium paratuberculosis* Antibody Test Kit, Montpellier SAS, France). Sera specimens, positive and negative control sera were diluted 1/20 for a total of 100 µl, using dilution buffer No.12 in a sterile U based microplate. Microplate was placed in ELISA reader (Mindray MR-96A) and was read at 450 nm. Corresponding percent result for each obtained OD value was calculated with the following formula:

$$\text{Results \%} \quad \frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}} \quad x\ 100$$

Results attained according to the above formula were evaluated as; 55% is accepted MAP positive, 45-55% is accepted MAP suspected suspected and less than 45% is accepted as MAP negative.

SOD was determined according to Podczasy and Wei (1988) and GSH-Px according to Paglia and Valentine (1967), both expressed as unit/L protein.

ADA was determined according to Giusti and Galanti (1984). MDA levels were determined according to Yoshioko *et al.* (1979).

Serum protein analyses were performed with commercial kit (Audit, Irland) via autoanalyser (Autolab, The Netherlands).

Student's 't' test was used to determine the statistical differences between groups.

*Results and discussion*

An increase was observed in all analysed parameters in the study group compared to the control group. However, only the increases in MDA and ADA were statistically significant. Results are presented in Table I.

**Table I.- Serum MDA, SOD, GSH-Px and ADA activities in the infected and control groups.**

| | Infected | Control | P value |
|---|---|---|---|
| SOD (IU/ml) | 47.2±5.4 | 34±5.1 | 0.086 |
| MDA (µmol/L) | 1.20±0.22 | 0.21±0.03 | 0.0004 |
| GSH-Px (IU/ml) | 9.48±1.7 | 7±3.9 | 0.58 |
| ADA (IU/L) | 18.7±3.8 | 7.17±2.3 | 0.014 |

In infection with MAP, immune response varies due to the antibody response of T cells and elevation of gamma interferon level (Strickland *et al*., 2005). Cossu *et al*.(2015) reported that T cell mediated immune system is activated in MAP infection. Although ADA activity is present in all cell types, it is extensive in lymphoid tissues, thymus and peripheral lymphocytes and is directly correlated with the differentiation level of lymphoid cells. Therefore, ADA is considered as the nonspesific indicator of cellular immunity and T lymphocyte activation (Baganha *et al*., 1990; Cristalli *et al*., 2001; Boonyagars and Kiertiburanakul, 2010; Suchitra *et al*., 2009). ADA helps in proliferation and differentiation of lymphocytes, and especially T lymphocytes. ADA is particularly sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation. In the present study, increase in ADA activity in MAP infection was observed. This increase in ADA is evaluated as the indicator of cellular immune response, as the result of MAP infection phagocyted by the phagocytic cells in ileum and jejenum and the following transmission to regional lymph nodes.

Free radicals are the most prominant products of antimicrobial activity in the host and their measurements are difficult because of their short life and their high reactivity. Therefore, measurement methods of end products of various reactions are used. The most prevalent of these are, malondialdhyde, which is the indicator of lipid peroxidation and antioxidant activities (SOD, GSH-Px) measurements (Valko *et al*., 2007). Reactive oxygen types (ROS) are required for the defense system against pathogen microorganisms. Neutrophils and macrophages form large amounts of ROS as the result of oxidation (Bayir, 2005). MAP sustains its life in macrophages after passing the intestinal barrier. There, they correspond with reactive nitrogen types and ROS in

cellular defense (Ehrt and Schnappinger, 2009). MAP needs to convert SodA super oxide radicals to hydrogen peroxide to struggle with this local stress and KatG needs MAP proteins to convert this to water and oxygen (Granger *et al*., 2004; Voskuil *et al*., 2011). An oxidant-antioxidant defense mechanism develops between the bacteria and the host. In the present study, with this aim we determined the serum malondialdehyde level to evaluate lipid peroxidation and observed a significant increase ($P<0.001$) when compared with the control group. SOD is one of the most efficient intracellular enzymatic antioxidants. It catalyses the conversion of reactive superoxide anion, which is the first reactive product of oxygen to molecular oxygen and to a less reactive product, hydrogen peroxide (Nelson *et al*., 2006). GSH-Px is an important radical sweeper for hydrogen peroxide. Low concentration hydrogen peroxide is cleaned especially by GSH-Px. This enzyme, prevents the destructive effect of hydrogen peroxide with high spesivity in the medium where reducted glutathion is converted to oxidized glutathion (Munz *et al*., 1997). In the present study, no statistically significant variations were observed in both enzyme activities.

*Conclusion*

To conclude, an oxidant-antioxidant defense system develops between the host and the bacteria after MAP infection. In the present study, MDA, the indicator of oxidation in the host increased, whereas no variations were observed in the antioxidant enzymes SOD and GSH-Px. It is concluded that ADA activity can be beneficial in the diagnosis and therapy of paratuberculosis.

*Statement of conflict of interest*

Authors have declared no conflict of interest.

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