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Bovine Viral Diarrhea Virus Infection in Cattle - Antioxidant Status and Some Biochemical Parameters

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ABSTRACT

***Background*:** Bovine viral diarrhea virus (BVDV) infections in cattle result in significant economic losses due to reproductive performance deficiencies caused by gastrointestinal, respiratory system infections, and transplacental infections. BVDV is one of the most important and widespread pathogens in cattle worldwide, including Turkey. Methods such as virus neutralization, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase and polymerase chain reaction (RT-PCR) are used for the detection of the disease. The diagnosis of the disease in its subclinical form is challenging due to the lengthy and costly procedures involved. Investigating oxidative stress parameters in ruminants with various diseases contributes significantly to diagnosis and prognosis. This study aimed to investigate some oxidative stress and biochemical parameters in cattle infected with BVDV.

***Materials, Methods & Results*:** In the study, blood samples were collected from 80 Simmental breed cows aged between approximately 4 and 8 years to determine the presence of BVDV antibodies using the ELISA method. Based on the results obtained, study groups were organized. The study included a group of 10 animals with positive antibody levels as the infected group, and a group of 10 animals with negative antibody levels as the healthy group. Blood samples were taken from the animals, and serum separation was ensured. In the obtained serum samples, levels of vitamin E, vitamin A, β-Carotene, catalase, GSH-Px, and MDA were determined using spectrophotometric methods. In addition, serum total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca), and phosphorus (P) were measured using commercial test kits and an autoanalyzer. In the study, it was observed that the differences in serum MDA, vitamin E, vitamin A, β-carotene, and catalase levels were statistically significant between the healthy and BVDV-infected groups ($P < 0.001$). The activity of GSH-Px was also found to be statistically different between the groups ($P < 0.01$). Among the biochemical parameters, HDL, LDL, and AST levels were found to be statistically significant between the healthy and BVDV-infected groups ($P < 0.001$). Additionally, ALP and glucose levels were found to be statistically significant ($P < 0.01$). However, although there were differences in the levels of total protein, albumin, Ca, and P between the groups, these results were not statistically significant.

***Discussion*:** Although the diagnosis of the disease was partially made based on clinical observations in BVDV infections, the ELISA method was used for accurate diagnosis. Furthermore, it was found that there was a significant difference in MDA concentration between the healthy and infected groups, indicating oxidative damage caused by the virus. Similarly, significant differences in vitamin E, vitamin A, β-carotene, GSH-Px, and catalase levels were observed between the groups, indicating a decrease in antioxidant values due to the infection. In addition, differences in ALP, AST, glucose, LDL, and HDL levels were found between the groups. This difference is thought to be related to the effects of the disease agent on the liver and systemically. This study demonstrates that, in addition to the viral pathogen, antioxidant and biochemical values are important criteria in the detection of the disease.

Keywords: antioxidant, bovine, BVDV, MDA, serum biochemistry.

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INTRODUCTION

Bovine Viral Diarrhea Virus (BVDV) infection is a globally prevalent pathogenic disease [38,45]. In Turkey, this infection is caused by a virus belonging to the Pestivirus genus in the Flaviviridae family [16]. Countries that do not implement comprehensive control programs against BVDV infection have a persistently infected (PI) animal rate of approximately 1-2% [13]. In Turkey, the prevalence of this disease is reported to be between 46-86%, and the presence of persistently infected animals can vary between 0.07-4.9% [5]. The virus exhibits different levels of virulence and can manifest clinically or subclinically [19,39]. Symptoms observed in affected animals include embryonic deaths, abortion, infertility, mummification, congenital malformations, fever, diarrhea, leukopenia, and thrombocytopenia [12]. Transmission occurs through direct contact with animals experiencing acute infection. The disease is transmitted after contact with infected animals' tears, nasal discharge, saliva, urine, semen, feces, sweat, and milk. Additionally, persistent infected (PI) animals serve as primary sources of infection, and direct contact with them is a significant factor in viral spread [4,37].

Oxidative damage occurs due to the infection [18]. Antioxidants play an important role in preventing oxidative damage in living organisms [2,32]. Antioxidants act by inhibiting the reactions that produce free radicals [14]. This study aims to determine serum lipid peroxidation (MDA), certain antioxidant, and biochemical parameters in healthy cattle and those infected with BVDV in the Bingöl region.

MATERIALS AND METHODS

Animals

The study population consisted of 80 Simental breed cows between the ages of 4-8 years that were brought to the Bingöl University Faculty of Veterinary Medicine clinics for different purposes. The study was conducted after obtaining ethical approval from the local ethics committee.

Working Groups

In the study, blood samples were taken from the vena jugularis of animals in sterile vacuum tubes for BVDV antibody detection. (IDEXX BVDV Ag/Serum Plus Test)[1] was used to determine the presence of BVDV antibodies in serum samples obtained from blood samples. The test protocol was performed as recommended by the manufacturer, and the results were evaluated by reading at a wavelength of 450 nm on the ELISA[2] reader. Ten animals with positive antibody levels constituted the patient group of the study. In contrast, the other 10 animals with negative levels included the healthy group.

Preparation of blood samples

Blood samples taken from the vena jugularis of the study group animals in vacuum tubes were centrifuged at 1500 *g* for 10 min in the laboratory, and serum samples were placed in separate Eppendorf tubes and stored in a deep freezer at -30°C until the analysis was performed.

Oxidative stress parameters

Serum vitamin E was measured by a spectrophotometer according to the method of Kayden et al. [28] (Shimadzu UV-120-01)[3]. Plasma vitamin A and β-Carotene levels were determined by the spectrophotometric method described by Suzuki & Katoh [42]. Serum GSH-Px activity level was measured as described by Lawrence and Burk [30]. Serum Catalase (CAT) enzyme was determined as described by Aebi [1]. Malondialdehyde (MDA), the end product of serum lipid peroxidation, was measured by a spectrophotometer according to the method of Placer *et al*. [34]. Lowry's method was used to determine protein concentration, and the results were determined as U/g protein [31].

Biochemical parameters

Biochemical analyses were performed using an autoanalyser (Olympus AU 600)[4] via commercial test kits for total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca) and phosphorus (P).

Statistical analysis

The statistical evaluation of the study was calculated using the (SPSS 20.0)[5] package program, and an independent *t*-test was used to determine the difference between the groups. The data were shown as ± average standard error, and significance was evaluated based on $P < 0.05$.

RESULTS

Serum MDA, GSH-Px, CAT, Vitamin E, Vitamin A and β-Carotene values were given in Table 1 and Figure 1 of this study. T Protein, Albumin, ALP, AST, glucose, LDL, HDL, Ca and P levels were shown in Table 2 and Figure 2.

The difference in serum MDA, E vitamin, A vitamin, β-Carotene and CAT levels was statistically significant among the healthy group with BVDV ($P < 0.001$). The difference in GSH-Px activity among the groups was found to be statistically significant ($P < 0.01$).

Serum biochemical parameters HDL, LDL and AST levels were found to be statistically significant among healthy groups with BVDV ($P < 0.001$). In addition, ALP and Glucose levels were found to be statistically significant ($P < 0.01$). On the other hand, it was found that there was a difference in T Protein, Albumin, Ca and P levels among the groups, but it was not statistically significant.

Table 1. Serum oxidative stress parameters and antioxidant vitamin concentrations in cattles naturally infected by BVDV (n = 10) and in healthy controls (n = 10). Results are expressed as mean ± standard deviation.

Parameters	Groups		*P*
	Healthy	BVDV	
MDA (mmol/L)	1.63 ± 0.09	2.10 ± 0.18	***
GSH-Px (U/gr protein)	1.27 ± 0.12	1.01 ± 0.16	**
CAT (kU/L)	29.84 ± 1.68	24.86 ± 1.07	***
Vitamin E (mg/L)	5.96 ± 0.31	4.61 ± 0.46	***
Vitamin A (µmol/L)	0.960.17	0.70 ± 0.05	***
B-carotene (µmol/L)	1.42 ± 0.05	1.02 ± 0.13	***

Data are given as means ± standard deviation; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2. Serum biochemical parameters in cattles naturally infected by BVDV (n = 10) and in healthy controls (n = 10). Results are expressed as mean ± standard deviation.

Parameters	Groups		*P*
	Healthy	BVDV	
T. protein (g/dL)	6.82 ± 1.54	6.36 ± 0.85	-
Albumin (g/dL)	2.75 ± 0.58	2.88 ± 0.27	-
ALP (U/L)	55.24 ± 4.01	62.95 ± 3.45	**
AST (U/L)	94.60 ± 6.43	119.57 ± 3.94	***
Glucose (mg/dL)	63.16 ± 3.76	55.86 ± 4.32	**
LDL (mg/dL)	36.67 ± 1.29	29.81 ± 1.98	***
HDL (mg/dL)	59.48 ± 2.62	38.97 ± 1.96	***
Ca (mg/dL)	9.20 ± 2.31	9.44 ± 0.78	-
P (mg/dL)	5.64 ± 1.93	5.86 ± 1.61	-

Data are given as means ± standard deviation; $^{**}P < 0.01$; $^{***}P < 0.001$.

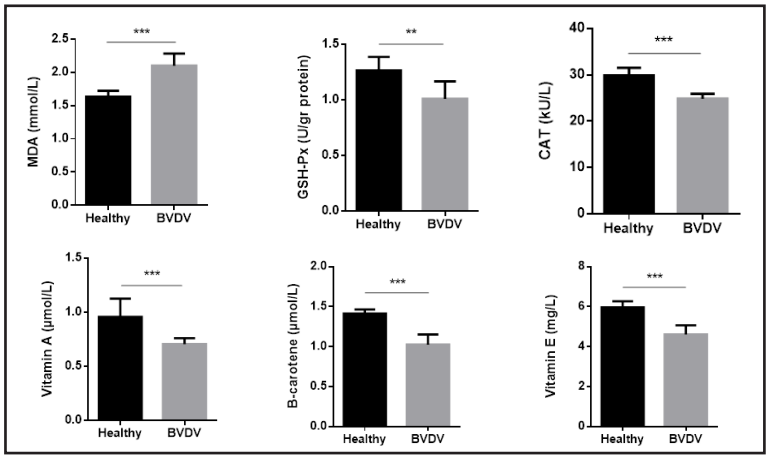

Figure 1. Serum oxidative stress parameters and antioxidant vitamin concentrations in cattles naturally infected by BVDV and in healthy controls. [$^{**}P < 0.01$; $^{***}P < 0.001$].

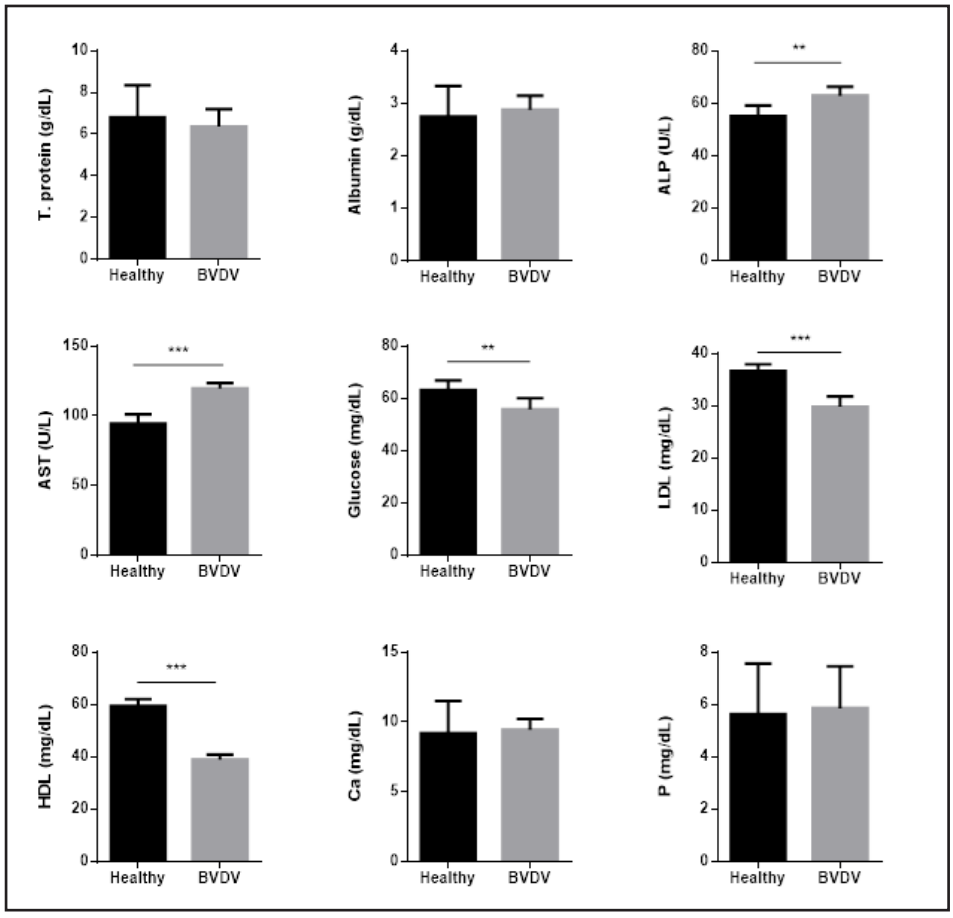

Figure 2. Serum biochemical parameters in cattles naturally infected by BVDV and in healthy controls. [$^{**}P < 0.01$; $^{***}P < 0.001$].

DISCUSSION

BVDV infection is a disease that is common all over the world and causes especially significant economic losses [36,41]. Numerous studies have recently been conducted in Turkey indicating the presence of this infection in cattle [9,20-22,27,46]. BVDV is a respiratory, digestive and especially reproductive system disease and is an important disease seen in cattle at all periods and ages. Its treatment is costly and causes death and economic losses as well. It is stated that besides in the aetiology of the disease, stress factors such as hunger, extreme heat and cold, transportation and fatigue have an important effect on the occurrence of the disease [15,17,23,44].

Numerous studies in young animals show an association between vitamin deficiency and the prevalence of diseases [6,33]. It is stated that vitamin E has a significant effect on the reproductive system [17]. In another study, it is stated that it protects from free radicals depending on the antioxidant concentration in the tissues and serum of mammals [10]. In another study, it is stated that it has an important effect in protection against mastitis, reproductive disorders and other diseases in dairy cows in ruminants [7,35]. In this study, it was observed that the difference in vitamin E levels between healthy and sick cattle was statistically significant, and it is thought that the decrease in vitamin E level is due to its use against oxidative damage due to infection. Vitamin A, which is a fat-soluble vitamin, is an essential vitamin for functions such as immunity and cellular change, protection of respiratory and gastrointestinal epithelial surfaces, growth, reproduction and vision [40]. Vitamin A is not abundant, but its precursor, β-Carotene, is widely found in plants. With adequate intake of β-carotene, the need for vitamin A is provided. Vitamin A is an important antioxidant found mostly in the liver in animal tissues [25,43]. In this study, the difference in vitamin A and β-Carotene levels was found to be lower in the group with BVDV

compared to the healthy group, and this is estimated to be due to its use against oxidative damage due to infection.

The determination of the oxidative stress state is commonly determined by the determination of the lipid peroxidation concentration. An increase in malondialdehyde (MDA) is an indicator of lipid peroxidation [2,26]. Lipid peroxidation is a non-enzymatic chain reaction. It directly damages the membrane structure and indirectly produces reactive aldehydes and damages other cell components [2,11]. In this study, MDA concentration was found to be higher in the group with BVDV compared to the healthy group. Likewise, a decrease was found in GSH-Px and CAT activity. Here, it is thought that the decrease in enzymes is used in the conversion of radicals formed due to the increase in MDA. BVDV virus affects the lymphoreticular system by causing direct damage to the lymphoreticular system and causing the release of immunosuppressive substances in the organs they infect, resulting in immunosuppression [8,24,29]. In a study, it was determined that 100% of the BVDV virus was found in the liver, spleen and various lymph nodes [3]. Thus, depending on the localization of the virus in these organs, it causes some abnormal values, especially in liver function tests. In this study, parameters such as T. Protein, Albumin, ALP, AST, Glucose, LDL, HDL, Ca and P were investigated. In the statistical analysis, it was observed that the difference in ALP, AST, Glucose, LDL and HDL levels between the group with BVDV and the healthy group was statistically significant, while the change in other parameters was not significant. It is thought that the change in these parameters may have resulted from the negative effects of the virus on the organ and general system.

CONCLUSION

As a result, in this study, it was determined that there was a significant difference between some biochemical and antioxidant values due to oxidative stress in BVDV-infected cattle compared to healthy ones. There is no direct treatment for BVDV-infected animals. Therefore, it is thought that the application of antioxidant vitamins such as vitamin A and vitamin E may be significantly beneficial in order to reduce oxidative stress and possible damage in the disease.

MANUFACTURERS

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Ethical approval. The experimental protocols were approved by the local Animal Use Committees of Bingol University (Bingol, Turkiye).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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