

The Antioxidant Status and Biochemical Parameters in Kid Goats Naturally Infected with Peste Des Petits Ruminants Virus

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ABSTRACT

Background: Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants. The disease is high economical importance because of the high mortality rate. Oxidative stress is an active field of research in small ruminant medicine and has been implicated in numerous disease processes including sepsis, mastitis, acidosis, enteritis, pneumonia, respiratory, and joint diseases. Compared to human medicine, only a limited number of conditions have been investigated in regard to the effects of oxidative stress in small ruminants. The aim of this study was to determined and compared the oxidative status and some biochemical parameters in kid goats with PPR.

Materials, Methods & Results: The study was performed on 15 healthy hair of kid goats (control group) and 15 kids naturally infected with Peste des Petits Ruminants (PPR). Competitive enzyme linked immunosorbent assay (C-ELISA) was used for serological detection of PPRV specific antibodies, and a reverse transcription polymerase chain reaction (RT-PCR) was performed for the detection of PPR virus. Concentrations of plasma biochemical parameters were analysed by a clinical chemistry analyser, and blood biochemical indices determined, including total protein, albumin, alkaline phosphatase (ALP), aspartate amino transferase (AST), γ - glutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL). The plasma CAT activity, plasma GSHPx activity and plasma lipid peroxidation level was measured according to the specific methods. Besides, vitamin C values were colorimetrically determined using a phosphotungstic method acid method and vitamin E values were determined spectrophotometrically method. Plasma MDA concentrations were markedly increased in the group of kid goats with PPR ($P < 0.001$) whereas GSHPx ($P < 0.01$), and CAT ($P < 0.01$) activities were significantly depressed as well as concentrations of vitamins E ($P < 0.05$) and vitamin C ($P < 0.001$). Significant differences between groups were showed relative to plasma total protein ($P < 0.05$), albumin ($P < 0.05$), ALP ($P < 0.05$), AST ($P < 0.01$), GGT ($P < 0.05$), LDH ($P < 0.05$), glucose ($P < 0.001$), VLDL ($P < 0.05$), LDL ($P < 0.01$), and HDL ($P < 0.05$).

Discussion: The clinical and postmortem findings of PPRV infection may be sufficient for the diagnosis in the endemic areas, yet laboratory confirmation is essential for definitive diagnosis because of the clinical similarity of PPR to rinderpest. In this study used both C-ELISA and RT-PCR in the diagnosis of suspected disease. The decrease level of VLDL, LDL, and HDL in the kids with PPR were consistent findings with liver damage, and the cause of decrease could be inadequate synthesis of cholesterol that main structure of lipoproteins due to liver dysfunction. Plasma MDA concentrations were found to be increased in the kid goats with PPR compared to the control group, while decreases of GSHPx and CAT activities were observed. Because of GSHPx and CAT are involved in the conversion of radicals into less effective metabolites, these changes coupled to the increase of MDA concentrations, suggest that an excessive ROS production occurred during PPR infection. This study has highlighted the occurrence of an oxidative stress with important differences in antioxidant status as reflected by assessment of some enzymatic and non-enzymatic antioxidants in kids infected by PPRV. In conclusion, this study has highlighted the occurrence of an oxidative stress with important differences in antioxidant status as reflected by assessment of some enzymatic and non-enzymatic antioxidants in kids infected by PPRV. Furthermore, the liver was effected by PPRV infection.

Keywords: antioxidant, biochemistry, kids, goats, liver, PPRV.

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INTRODUCTION

PPRV is a morbillivirus and is related to rinderpest virus, canine distemper virus, and the human measles virus [13,41]. Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants [12,32]. The PPRV spreads close contact by aerosol route between infected and susceptible animals [3,41]. The infection is characterized by fever, erosive, necrotic stomatitis, diarrhea, anorexia, prulent ocular and nasal discharges, and respiratory distress [41,46]. Morbidity and mortality could be very high especially in non-vaccinated young animals than in adults [13,15,26]. The PPRV infection more severe in goats as compared to sheep [12,33,43]. The disease is high economical importance because of the high mortality rate and restriction on livestock trading [1,33].

A free radical can be described as any atom or a group of atoms or molecules in which there is at least one unpaired electron in the outermost shell [38]. Usually, organism has sufficient antioxidant reserves to cope with the production of free radicals [7,41] but the imbalance between the production of ROS (Radical Oxygen Species) and the availability of antioxidant molecules may result in oxidative stress [6,21,40]. The components of the antioxidant system have been classified as preventive and chain breaking antioxidants. Glutathione peroxidase (GSHPx) and catalase (CAT) are anti-oxidant enzymes [14,29,40]. The chain-breaking antioxidants act after initiation of a chain reaction. This class of antioxidants includes reduced lipid soluble vitamin E [41], and water soluble ascorbate [38]. Whenever equilibrium between ROS and antioxidants is broken, progressive oxidation of other biological substrates (such as lipids, DNA and proteins) occurs, establishing an oxidative stress status that may impair health both directly and indirectly whenever equilibrium between ROS and antioxidants is broken, progressive oxidation of other biological substrates (such as lipids, DNA and proteins) occurs, establishing an oxidative stress status that may impair health both directly and indirectly [14,40]. Direct effects include peroxidation damage to important lipids and macromolecules. Indirect changes include effects on cellular membranes and components, modifying metabolic pathways and resulting in altered physiology [7].

The aim of this study was to determined and compared the oxidative status and some biochemical

parameters between kid goats with PPR and healthy controls on the basis of plasma lipid peroxidation intensity (MDA), antioxidant enzyme activities, chain breaking antioxidants, and some biochemical parameters.

MATERIALS AND METHODS

Animals and Samples

The study was performed on 15 healthy hair obtained from kid goats (control group) and 15 from kids naturally infected with Peste des Petits Ruminants (PPR) in the province of Elazığ, Eastern of Anatolia, Turkey. Ages of the affected kid goats ranged from 2 and 4 month olds. The kids had a history of six days of disease. All the animals were kept under the same care and feeding conditions.

Blood samples were taken by veni puncture from the jugular vein into heparinized and nonheparinized vacutainer tubes. The nonheparinized blood samples were kept at room temperature for 2 h in order to obtain serum. Plasma and serum was separated by centrifugation (700 g, at +4°C) and stored at -20°C until analysis.

Detection of PPR antibodies

Competitive enzyme linked immunosorbent assay (C-ELISA) was used for serological detection of PPRV specific antibodies. Serum was collected from 15 clinically ill kid goats analysed for the detection of antibodies against to PPR by a commercial C-ELISA kit (PPR Competition, Competitive ELISA kit)¹. The ELISA was performed according to the manufacturer's instruction as described elsewhere [2].

Detection of PPR virus

A reverse transcription polymerase chain reaction (RT-PCR) was performed for the detection of PPR virus. The tissue samples such as lymph node, spleen, lung, and oro-nasal swaps were used as RT-PCR material. The reaction was carried out with a PPRV-specific primer set (PPRVF1b: AGTACAAA-GATTGCTGATCACAGT and PPRVF2d: GGGTCTCGAAGG CTAGGCCCGAATA) originally designed by Forsyth and Barrett [17] in order to amplify a 448-bp cDNA product from the F gene. A lyophilized live PPR vaccine, produced by the Etlik Veterinary Control and Research Institute Ankara, Turkey, was used as the positive control. RNA was extracted from the positive

control or tissue homogenate from the field samples using RNeasy Mini Kit, (Qiagen, RNeasy Mini Kit)² according to manufacturer's protocol. The RT-PCR was performed with Qiagen One- Step RT-PCR kit (Qiagen, RT-PCR kit)². The 20 µL reaction mixture contained 7 µL Molecular Grade Water, 0.8 µL 10 pmol of forward and reverse primers, 4.0 µL buffer, 0.8 µL dNTP mix, 0.8 µL enzyme mix, 4.0 µL 5x Q-Solution, 2.6 µL template RNA. The thermocycling profile was as follows: reverse transcription at 50°C for 30 min, initial denaturation and activation of polymerase at 94°C for 15 min, followed by 35 cycles of denaturation, annealing and extension at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, respectively, and final elongation at 72°C for 7 min (Thermal Cycler)³. The RT-PCR products were analysed by electrophoresis at 80V for 2 h on 1.5% agarose gel stained with ethidium bromide. PCR products with a molecular size of 448 bp were considered indicative for PPRV [6].

Serum Biochemistry

Concentrations of plasma biochemical parameters were analysed by a clinical chemistry analyser (Advia 1200)⁵, and blood biochemical indices determined, including total protein, albumin, alkaline phosphatase (ALP), aspartate amino transferase (AST), γ - glutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL).

Oxidative stress parameters

The plasma CAT activity was measured as previously described by GOTH (20). Briefly, 0.2 mL of plasma samples was incubated in 1.0 mL substrate (65 µmol hydrogen peroxide per mL of a 50 mM phosphate buffer, pH 7.0) at 37°C for 60 s. The enzymatic reaction was terminated with 1.0 mL of a 32.4 mM ammonium molybdate solution. Hydrogen peroxide was measured at 405 nm against blank containing all the components except the enzyme on a spectrophotometer (Schimadzu UV-1208)⁵. The catalase activity was expressed as kU/L.

The plasma GSHPx activity was determined according to the method of Lawrance [31]. The reaction mixture contained of 50 mM of a potassium phosphate buffer (pH 7.0), 1 mM of EDTA, 1 mM of sodium azide (NaN₃), 0.2 mM of reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 U/ml of

oxidized glutathione (GSSG)-reductase, 1 mM of GSH, and 0.25 mM of H₂O₂. Enzyme source (0.1 mL) was added to 0.8 mL of the above mixture and incubated for 5 min at 25°C before the initiation of the reaction by the addition of 0.1 mL of peroxide solution. The absorbance at 340 nm was recorded for 5 min on a spectrophotometer. The activity was calculated from the slope of the lines as micromoles of NADPH oxidized per minute. The blank value (the enzyme was replaced with distilled water) was subtracted from each value. The protein concentration was also measured by the method of Lowry [37]. The results were expressed as U/g of proteins.

The plasma lipid peroxidation level was measured according to the concentration of thiobarbituric acid reactive species [42]. The amount of produced MDA was used as an index of lipid peroxidation. Briefly, one volume of the test sample and two volume of stock reagent (15%, w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was heated for 15 min in boiling water. After cooling, the precipitate was removed by centrifugation at 500 g 10 min and then absorbance of the supernatant was measured at 532 nm against a blank containing all reagents except test sample on a spectrophotometer. The lipid peroxidation level was expressed as µmol/L.

Antioxidant vitamins analysis

Vitamin C values were colorimetrically determined using a phosphotungstic acid method described by Kyaw [30] and vitamin E values were determined spectrophotometrically according to Martinek's method [39]. All vitamin analyses were performed in the serum.

Statistical analysis

The statistical analysis was performed using SPSS Ms package program (Windows Release 10.0)⁶. T tests for independent samples were used for evaluating data between groups. Results were expressed as means \pm standard deviations, and $P < 0.05$ was taken as the level of significance.

RESULTS

PPRV antibodies were detected by C-ELISA in all serum samples of clinically sick kid goats. PPRV was isolated and identified in RT-PCR from the tissue, such as blood samples and oro-nasal swabs collected

from all the sick goats. Moreover we can observe the specific 448 bp band obtained from the DNA amplification of F protein-coding gene using the primers PPRVF1b: AGTACAAAAGATTGCTGATCACAG T and PPRVF2d:GGGTCTCGAAGG CTAGGCCC-GAATA (Figure 1).

As shown in Table 1, plasma MDA concentrations were markedly increased in the group of kids with PPR ($P < 0.001$) whereas GSHPx ($P < 0.01$), and

CAT ($P < 0.01$) activities were significantly depressed as well as concentrations of vitamins E ($P < 0.05$) and vitamin C ($P < 0.001$).

Significant differences between groups were showed relative to plasma total protein ($P < 0.05$), albumin ($P < 0.05$), ALP ($P < 0.05$), AST ($P < 0.01$), GGT ($P < 0.05$), LDH ($P < 0.05$), glucose ($P < 0.001$), VLDL ($P < 0.05$), LDL ($P < 0.01$), and HDL ($P < 0.05$) [Table 2].

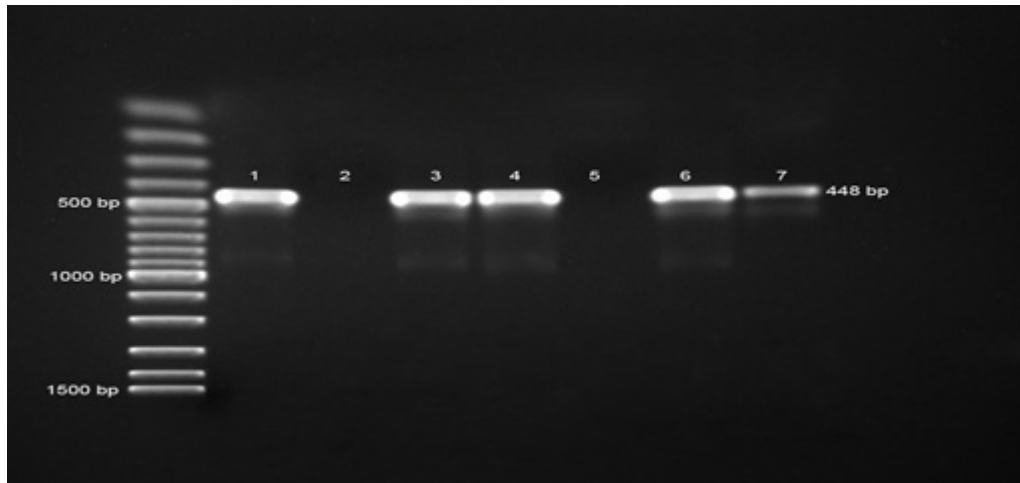


Figure 1. Agarose Gel Electrophoresis of PPRV. PCR Positive and Negative specimens and controls. Line 1- Positive control; Line 2-Negative Control; Line 3- Positive lymph node sample; Line 4- Positive lymph node sample; Line 5- Negative sample; Line 6- Positive blood sample; Line 7- Positive oro-nasal swap sample.

Table 1. Plasma oxidative stress parameters and antioxidant vitamin concentrations in kid goats naturally infected by PPR (n = 15) and in healthy controls (n = 15). Results are expressed as mean \pm standard deviation.

Parameter	Groups		P
	PPR	Healthy	
MDA (mmol/L)	2.00 \pm 0.39	1.33 \pm 0.38	***
GSHPx (U/g protein)	0.80 \pm 0.19	1.06 \pm 0.11	**
CAT (kU/L)	22.2 \pm 6.6	31.9 \pm 11.8	**
Vitamin E (mg/L)	0.86 \pm 0.11	1.11 \pm 0.15	*
Vitamin C (mg/L)	2.87 \pm 0.5	3.92 \pm 0.4	***

Data are given as means \pm standard deviation; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Plasma biochemical parameters and total NEFA concentrations in kid goats naturally infected by PPR (n = 15) and in healthy controls (n = 15). Results are expressed as mean \pm standard deviation.

Parameter	Groups		P
	PPR	Healthy	
T.protein (g/dL)	6.76 \pm 0.26	7.00 \pm 0.21	*
Albumin (g/dL)	2.98 \pm 0.17	3.19 \pm 0.23	*
ALP (U/L)	63.6 \pm 9.6	56.4 \pm 7.5	*
AST (U/L)	124.5 \pm 13.5	95.5 \pm 9.2	**
GGT (U/L)	64.6 \pm 10.1	53.1 \pm 3.73	*
LDH (U/L)	241.1 \pm 46.5	204.6 \pm 33.5	*
Glucose (mg/dL)	45.2 \pm 7.3	66.2 \pm 6.9	***
VLDL	2.13 \pm 0.5	3.26 \pm 1.7	*
LDL	27.4 \pm 8.8	39.0 \pm 10.2	**
HDL	43.2 \pm 8.0	63.5 \pm 23.4	*

Data are given as means \pm standard deviation; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

In the Turkey, Eastern of Anatolia is one of the regions made intensive of livestock. The presence of PPRV infection in Turkey has been reported before different studies [19,41,44,52]. Although the clinical and postmortem findings may be sufficient for the diagnosis of PPR in the endemic areas, yet laboratory confirmation is essential for definitive diagnosis because of the clinical similarity of PPR to rinderpest [34,36]. Immunocapture [35] and reverse transcription polymerase chain reaction (RT-PCR) followed by nucleotide sequencing [11,17,48] are the current diagnostic methods for all morbillivirus infections. Furthermore, due to simplicity, high sensitivity, and economy, several competitive enzyme-linked immunosorbent assays (c-ELISA) have been recognised as suitable systems for use for diagnosis and seroepidemiological surveillance [8,9,23,24,45]. We used both C-ELISA and RT-PCR in the diagnosis of suspected disease. We determined that the diagnostic value of necropsy materials such as lymph node, oro-nasal swap and blood were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR.

The liver is not a primary target for the PPR virus and may be fatty degenerated [26,50]. Toplu *et al.* [50] and Yarim *et al.* [51] reported multifocal areas of coagulative necrosis and vacuolation of hepatocytes and abnormal liver function test in sheep naturally infected with PPR and significant increases in mean serum concentrations of AST and ALT. In this study, small multifocal necrotic foci and haemorrhagic areas were seen as macroscopically in the liver. Capsular fibrosis, severe hidropic degeneration in the hepatocytes, multifocal necrosis foci, disorganisation in the hepatic cord, and some haemorrhagic areas were seen in the microscopic examination. Furthermore, proliferation of the bile ducts, and mononuclear cell infiltration were seen in the periportal perivascular areas. These histopathological findings were confirmed that the liver damage was formed during PPRV infection. Significant increases especially ALP, AST, and GGT, and significant decreases in total protein and albumin in the kids with PPR was consistent to the liver damage.

The liver plays a central role in the regulation of lipoprotein synthesis and degradation as well as in the storage of lipids in various lipids. Therefore, liver dysfunction can be associated with dys- or paralipo-

proteinaemia [47]. In this study, the decrease level of VLDL, LDL, and HDL in the kid goats with PPR were consistent findings with liver damage, and the cause of decrease could be inadequate synthesis of cholesterol that main structure of lipoproteins due to liver dysfunction.

The glucose level was lower in the PPR group than control. The cause of the low concentration in the infected kid goats could lose of their appetite.

The determination of lipid peroxidation status (MDA concentrations) is among the most widely used methods for determination of the oxidative stress. Increased malondialdehyde (MDA) concentration in plasma is a marker of lipid peroxidation [25,39]. Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids. It leads to the creation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes, and one of the most common of these intermediates is MDA [10,28]. In this study, plasma MDA concentrations were found to be increased in the kids with PPR compared to the control group, while decreases of GSHPx, and CAT activities were observed. Because of GSHPx and CAT are involved in the conversion of radicals into less effective metabolites, these changes coupled to the increase of MDA concentrations, suggest that an excessive ROS production occurred during PPR infection.

Vitamin E (in the form of α -tocopherol) is the major lipid-soluble antioxidant of lipoproteins and biomembranes [27,31,49]. Vitamin C (ascorbic acid) functions as a potent water soluble chain-breaking antioxidant in the biological fluids, but it can not scavenge the radicals within the lipid region of the membranes [16]. The synergism between ascorbic acid and α -tocopherol in the inhibition of lipid peroxidation is well known. Vitamin C enhances the antioxidant activity of vitamin E by recycling the α -tocopheroxyl radical back to α -tocopherol and the depletion of the α -tocopherol is markedly reduced [5,20,29,37].

In this study, the concentrations of serum vitamin E, and vitamin C, which are responsible for protecting the cells from damage caused by lipid peroxidation, were found significantly lower in the PPR group than in the control group. The decrease of plasma antioxidant vitamins could results to their over utilisation due to the disease related oxidative stress.

CONCLUSION

In conclusion, this study has highlighted the occurrence of an oxidative stress with important differences in antioxidant status as reflected by assessment of some enzymatic and non-enzymatic antioxidants in kids infected by PPRV. There is no effective therapy for PPRV infection especially young goats, and due to oxidative stress might be an aggravating factor of the disease, it can be useful to add the antioxidant vitamins such as vitamin E and C, to the classical treatment procedures for get ride of the disease.

MANUFACTURERS

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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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