Endothelin-1, pentraxin-3, acute phase proteins and oxidative stress before and after treatment in neonatal calves with sepsis caused by Escherichia coli K99

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Abstract

This study aimed to detect changes in acute-phase proteins endothelin-1 (ET-1), pentraxin-3 (P-3), ceruloplasmin (Cp), haptoglobin (Hp), and albumin, in calves with diarrhea caused by *Escherichia coli K99* neonatal sepsis before and after treatment. Diagnostic and prognostic clinical, biochemical, and hematological parameters were evaluated. A total of 30 calves, divided into a sepsis group and a healthy control group, were assessed. The sepsis group was composed of 20 newborn calves (0–10 days old) which met neonatal sepsis criteria, did not receive any treatment, and were referred to the clinics of the Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, within 24 hours after the detection of clinical findings. The control group consisted of 10 healthy calves (0–10 days old) for comparison. Clinical examinations (respiratory rate, rectal temperature, heart rate, capillary refill time, sucking reflex) were performed before (0th h) and after (7th d) treatment. Blood samples were drawn via the jugular vein from sick calves before and after treatment and once from control animals. The P-3, ET-1, Cp, Hp, and malondialdehyde (MDA) concentrations in the sepsis group before treatment were statistically higher than post-treatment and control group values ($P < 0.001$); while the glutathione (GSH) concentration was statistically lower ($P = 0.002$). In conclusion, the high pre-treatment concentrations of P-3, ET-1, Cp, Hp, and MDA in the sepsis group were of diagnostic importance. The approximation of these parameters after treatment to the mean values of the control group was also of prognostic value.

Introduction

Diarrhea caused by *Escherichia coli*, enterotoxins cause a decrease in absorption and an increase in secretion. As a result, fluid loss occurs, and the electrolyte balance is disturbed. Absorption problems are seen as severe damage progresses in the intestinal villi. Dehydration, metabolic acidosis, and sepsis develop due to diarrhea, and related deaths occur (Mulcahy et al. 2010; Şen et al. 2013; Akyüz et al. 2017). Neonatal sepsis is a serious cause of mortality in calves (Radostits et al. 2006; Basoglu et al. 2018; Akyüz 2020; Akyüz and Gökce 2021), and *E. coli* is responsible for the majority of economic loss related to neonatal calves in the first 5 days after birth (Ok et al. 2009; Ok et al. 2020). The etiology of sepsis is very complex. Death usually occurs due to diarrhea, respiratory system problems, and multiple organ failure (Asadi et al. 2015; Merhan et al. 2016; Akyüz et al. 2016; Chatre et al. 2017). Neonatal calf diseases can be caused by a single factor or due to a combination of many factors (Ok et al. 2009; Erkılıç et al. 2019; Akyüz 2020), including viral, bacterial, and protozoal components (Ok et al. 2009; Lorenz et al. 2011; Akyüz and Gökce 2021; Akyüz and Kükürt 2021). Although the transmission route of these agents is usually via the oral route from contaminated equipment that does not meet hygienic conditions, spread can also occur by omphalogen or aerogenous routes (Trefz et al. 2013). Inflammatory situations created by infectious agents lead to activation of the body's defense mechanisms. As a result, systemic inflammatory response syndrome (SIRS) against general infectious agents and sepsis may develop. Affected animals may experience decreased interest in the environment, anorexia, diarrhea, weakening of the sucking reflex, depression, lethargy, drop in body temperature, increase in respiratory rate, prolongation of capillary refill time (CRT), and dehydration (House et al. 2015; Bonelli et al. 2018; Akyüz

2020). Deaths due to sepsis usually occur due to bacteremia, viremia, and endotoxemia. Bacterial infections such as *E. coli*, viral infections such as rotavirus and coronavirus, and parasitic diseases such as cryptosporidiosis that cause sepsis are responsible for many newborn calf deaths (Chatre et al. 2017; Basoglu et al. 2018; Akyüz and Gökce 2021). Although different treatment options have been tried, sepsis severity and mortality rates remain high (Novelli et al. 2010).

The importance of using biomarkers to diagnose sepsis is increasing in both human and veterinary medicine (Moore et al. 2007; Köse and Maden 2013). Acute-phase proteins are blood proteins used to evaluate the body's response to infection, inflammation, and trauma (Murata et al. 2004; Petersen et al. 2004; Coşkun and Şen 2011). These proteins play a significant role as biomarkers in the evaluation and diagnosis of sepsis and in monitoring the response to treatment (Meisner, 2005; Dupuy et al. 2013; Sönmezer and Tülek 2015). Haptoglobin (Hp) and ceruloplasmin (Cp) are positive acute-phase proteins (Murata et al. 2004; Coşkun and Şen 2011; Bozukluhan et al. 2021; Ceron et al. 2005; Erkılıç 2019). The role of Hp is to prevent iron loss after forming complex structures by binding hemoglobin (Cray 2012; Tuna and Ulutaş 2015; Erkılıç 2019). Cp, synthesized from the liver (Murata et al. 2004; Erkılıç 2019), is a plasma antioxidant that binds free copper in the serum and provides protection against the damage that can be caused by iron during inflammatory events (Cray 2012; Tuna and Ulutaş 2015; Erkılıç 2019).

Pentraxin-3 (P-3) is an acute-phase protein released from macrophages, dendritic cells, leukocytes, and endothelial cells during the inflammatory response (Libby et al. 2009). Studies have found a positive correlation between disease severity and plasma P-3 levels (Kao et al. 2013; Sönmezer and Tülek 2015). Endothelin-1 (ET-1) is a very potent vasoconstrictor released by endothelial cells. In endotoxemias, prepro-ET-1 is released from the heart and lungs and increases in direct proportion to disease mortality rates (Shah 2007; Tschaikowsky et al. 2000; Sönmezer and Tülek 2015). Glutathione (GSH), a substance made from the amino acids glycine, cysteine, and glutamic acid, is intended to protect against oxidative tissue damage. The intracellular concentrations of these enzymes are very high (Parlakpınar et al. 2013; Akyüz et al. 2021). The organic compound Malondialdehyde (MDA) is widely used to measure oxidative stress and determine cellular damage (Akyüz et al. 2021; Bozukluhan et al. 2021).

This study aimed to evaluate the diagnostic and prognostic value of acute-phase proteins, oxidative stress, as well as clinical, biochemical, and hematological parameters before and after treatment in calves with diarrhea and neonatal sepsis caused by *E. coli K99*.

Material And Methods

Animals

The animals evaluated in this study were 30 calves divided into two groups. The sepsis group was composed of 20 newborn calves (0-10 days old) which met neonatal sepsis criteria but did not receive any treatment, referred to the clinics of the Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University within 24 hours after clinical findings were detected. A control group

consisting of 10 healthy calves (0-10 days old) housed in the closed barn system in the University of Veterinary Medicine Education, Research, and Application Farm were used for comparison.

Procedures

Agent Detection from Feces

A rapid test kit (BoviD-5 Ag Test Kit®, Bionote Inc., Korea) was used to detect etiological factors in the feces of calves with neonatal sepsis. Only samples with positive *E. coli* confirmed by feces rapid test kit results were included in the study. Mixed samples were not included. Positive feces samples were sent to Kafkas University Veterinary Faculty Microbiology Laboratory for confirmation.

Obtaining and Measuring Blood Samples

Blood was collected in gel-containing vacuum tubes (BD Vacutainer®, BD, UK) for biochemical measurements and K_2EDTA-containing tubes for hematological measurements (BD Vacutainer®, BD, UK). Total leukocyte count (WBC, $x10^3/\mu L$) and other hematological parameters were determined using a complete blood count device (VG-MS4e®, MELET SCHLOESING Labs., Osny, France) within 30 minutes of sample collection. For analysis requiring sera, after approximately 1 hour at room temperature, blood samples were centrifuged for 10 min at 3000 rpm (Hettich Rotina 380R®, Hettich, Germany) and stored at -20°C until measured. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein (TP), cholesterol, triglycerides, glucose, creatinine, urea, and creatine kinase (CK) were assessed using a fully automated biochemistry device (Mindray BS120®, Mindray Medical Technology Istanbul, Turkey). Albumin was measured colorimetrically (Epoch®, Biotek, USA) with the methods reported for Hp by Skinner et al. (1991) and Cp by Colombo and Richterich (1964). Cattle P-3, long (PTX3®, Cat: ELK8840, ELK Biotechnology, Wuhan, China) and cattle ET-1 (EDN-1®, Cat: ELK8839, ELK Biotechnology, Wuhan, China) were measured colorimetrically with ELISA kits. Serum measurements of MDA were performed according to Yoshioka et al. (1979) and GSH according to Beutler et al. (1963). The obtained data were processed using a spectrophotometric microplate reader (Spectramax Plus®, Marshall Scientific, Product Code: MD-SMP, NH, USA).

Polymerase Chain Reaction Procedure

Polymerase chain reaction (PCR) was performed to confirm the *E. coli K99* strain detected in the feces samples using the rapid test kit. For PCR, 1 g of feces was diluted with 3 ml of phosphate-buffered saline (PBS), from which 1 mL was taken and added to 9 mL of buffered peptone water and incubated at 37°C for 18 hours. At the end of the incubation, 1 mL of the culture was centrifuged at 8000 rpm for 10 minutes. The supernatant was removed, and the pellet was washed 3 times with PBS. At the end of washing, DNA extraction was performed by adding 100 µL of nuclease-free water and boiling the pellet at 99ºC for 10 minutes (Franck et al. 1998). The primer pair in Table 1 was used to determine the K99 gene

region of the obtained DNA. For the 50 µL PCR mix, 5 µL of 10x PCR buffer (Thermo Fisher EP0402®, Thermo Fisher Scientific, USA), 1.5mM MgCl2 (Thermo Fisher EP0402®, Thermo Fisher Scientific, USA), 1mM dNTP (Ampliqon A502004®, 40mM mix, Ampliqon A/S, Denmark), 0.5 µM from each primer sequence (Sentebiolab®, Turkey), 1.25 U Taq polymerase (Thermo Fisher EP0402®, 5U/µl, Thermo Fisher Scientific, USA), and 5 µL of the obtained bacterial DNA were added to nuclease-free water. Amplification of the prepared mixture consisted of 25 cycles of denaturation at 94°C for 30 seconds, bonding at 50°C for 45 seconds, elongation at 70°C for 90 seconds, with 3 seconds per cycle added. The final bonding was conducted at 70°C for 10 minutes (Franck et al. 1998). The K99 strain found in the collection of the laboratory of the Microbiology Department of the Faculty of Veterinary Medicine of Kafkas University was used as a positive control. The primer pair used was per Roosendaal et al. (1984) (Table 1).

Table 1 Study primer pair

Virulence factor	Primary Sequence 5'- 3'	Reference	Product size
K99 (F)	TATTATCTTAGGTGGTATGG	(Roosendaal et al. 1984)	314
K99 (R)	GGTATCCTTTAGCAGCAGTATTTC		

SIRS and Sepsis Evaluation

There are specific criteria when determining sepsis and SIRS. The presence of at least two of the following criteria is considered SIRS: Hypothermia or hyperthermia, tachycardia, tachypnea, increased arterial partial carbon dioxide pressure, leukopenia or leukocytosis, and band neutrophil formation with a 10% history. If infection accompanies SIRS, it is considered sepsis (Fecteau et al. 2009; Sen and Constable 2013; Yıldız et al. 2018; Beydilli and Gökçe 2019; Akyüz and Gökçe 2021). Calves with symptoms of depression, diarrhea, low/lack of sucking reflex, dehydration, and constant urge to lie down were examined and evaluated per sepsis criteria. The SIRS criteria for neonatal calves were: body temperature >39.5°C or <37°C, pulse rate per minute <100 or >160, respiratory rate per minute >45, leukocyte count >12×10^3/µL or <4×10^3/µL (Fecteau et al. 1997, 2009; Yıldız et al. 2018; Akyüz and Gökce 2021). Calves having at least two of the specified criteria assessed as SIRS and with the presence of infection evaluated to have sepsis were included in the study.

Treatment

Calves with sepsis were kept under observation during treatment in separate compartments. Fluid therapy was provided to these animals according to the severity of dehydration. For this purpose, the calves were intravenously administered 0.9% NaCl (PVC®, 1000 mL Eczacıbaşı Baxter, Istanbul, Turkey), 1.3% $NaHCO_3$ (Bikarvil®, Vilsan, Ankara, Turkey), and 5% dextrose (Polifleks®, Polifarma, Tekirdağ, Turkey). For infection control, they were intramuscularly administered enrofloxacin (Baytril %10®, Bayer, Germany) 2.5-5 mg/kg for 7 days. They were orally administered antipyretic neomycin sulfate and bismuth subcarbonate-containing powder (Cesamolin®, Topkim, Turkey) 10-20 mg/kg for 3 days. For vitamin supplementation,

the calves were parenterally administered vitamin B complex (Berovit B12®, Ceva, Australia) in a practical dose of 8-10 mL/calf for 7 days, vitamin C (Maxivit-C®, Bavette, Turkey) 4-6 mg/kg for 7 days, and vitamin ADE in a single subcutaneous dose of 1 mL/50 kg (Ademin®, Ceva, Australia). For mineral supplementation, they were subcutaneously administered a solution of Ca, P, and Mg (Kalsimin®, Vilsan, Germany) at a single dose of 10 mL/50 kg. The calves were also given a single 4-6 mg/kg dose of nonsteroidal anti-inflammatory solution (Bavet Meloxicam®, Bavet, Turkey).

Statistical analysis

The normal distribution of the data of the sepsis group before and after treatment and the control group were evaluated using visual methods (histogram graph and Q-Q graph) and the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to compare normally distributed groups. After evaluating the homogeneity of variances with Levene's test, a Tukey HSD test was applied for post hoc comparison. Pearson correlation coefficients were calculated to define the correlation between variables. The data obtained in the study were reported as mean ± standard error (SEM). All analyses were performed with the SPSS® software program (SPSS Statistics 26.0, Chicago, IL, USA). Differences obtained in group comparisons were considered significant at $P<0.05$.

Results

Clinical examinations of the calves that met the sepsis criteria revealed different degrees of diarrhea, no or reduced sucking reflex, depression, little interest in the environment, and inability to stand. The K99 gene region was detected by PCR in all stool samples of the 20 calves with sepsis (Fig. 1). Physical examination of the calves with sepsis found the mean values of CRT and heart and respiratory rates per minute before treatment to be statistically significantly higher than post-treatment and control calf values ($P < 0.001$, Table 2).

Table 2
Clinical, hematological, and biochemical parameters of calves

Parameters	Sepsis Group		Control Group	P-value
	Before Treatment	After Treatment		
	Mean ± SEM			
Rectal temperature (°C)	38.52 ± 0.43	38.42 ± 0.07	38.38 ± 0.06	0.948
Breaths/min	59.05 ± 3.05^{b}	35.16 ± 0.67^{a}	28.60 ± 0.89^{a}	< 0.001
Heartbeats/min	158.20 ± 8.66^{b}	93.90 ± 3.99^{a}	80.40 ± 1.80^{a}	< 0.001
Capillary refill time/sec	3.00 ± 0.14^{b}	1.40 ± 0.06^{a}	1.50 ± 0.04^{a}	< 0.001
Total leukocytes count (×10^3/μL)	15.85 ± 1.60^{b}	9.15 ± 0.92^{a}	7.66 ± 0.28^{a}	< 0.001
Lymphocytes count (x10^3/μL)	5.10 ± 0.73^{b}	3.15 ± 0.35^{ab}	3.04 ± 0.43^{a}	0.019
Monocytes count (x10^3/μL)	0.34 ± 0.05	0.42 ± 0.03	0.56 ± 0.13	0.120
Granulocytes count (x10^3/μL)	9.43 ± 0.74^{b}	5.58 ± 0.77^{a}	3.85 ± 0.65^{a}	< 0.001
Red blood cell count (x10^6/μL)	7.08 ± 0.37^{a}	7.84 ± 0.27^{ab}	8.63 ± 0.23^{b}	0.015
Mean red cell volume (fL)	43.54 ± 0.67	42.43 ± 0.56	41.50 ± 0.94	0.164
Hematocrit (%)	36.82 ± 2.07	30.85 ± 1.86	35.99 ± 2.28	0.077
Hemoglobin (g/dL)	8.78 ± 0.60	9.22 ± 0.55	10.51 ± 0.42	0.190
Platelet count (x10^3/μL)	453.52 ± 73.12	497.05 ± 41.58	288.91 ± 25.46	0.083
Alanine aminotransferase (IU/L)	35.23 ± 2.89^{b}	26.14 ± 2.41^{b}	15.04 ± 0.68^{a}	< 0.001
Aspartate aminotransferase (IU/L)	84.04 ± 6.70^{b}	64.36 ± 4.22^{b}	42.23 ± 3.62^{a}	< 0.001
Alkaline phosphatase (IU/L)	275.66 ± 22.52^{b}	193.72 ± 16.26^{a}	162.43 ± 11.06^{a}	< 0.001
Gamma-glutamyl transferase (IU/L)	383.82 ± 72.21	250.26 ± 86.17	302.98 ± 52.97	0.435
Creatine (mg/dL)	3.17 ± 0.20^{b}	1.87 ± 0.10^{a}	1.46 ± 0.10^{a}	< 0.001
Urea (mg/dL)	66.59 ± 7.94^{b}	42.82 ± 3.65^{a}	23.81 ± 2.65^{a}	< 0.001

$^{a, b}$: The mean values with different letters in the same line represent the difference between sepsis and control groups. (P < 0.05). SEM: standard error of the mean

Parameters	Sepsis Group		Control Group	P-value
	Before Treatment	After Treatment		
	Mean ± SEM			
Lactate dehydrogenase (IU/L)	742.30 ± 34.72	760.48 ± 56.31	771.71 ± 60.70	0.925
Glucose (mg/dL)	50.70 ± 5.37[a]	82.80 ± 4.12[b]	97.15 ± 2.31[b]	< 0.001
Total protein (g/dL)	6.99 ± 0.41	6.72 ± 0.28	6.47 ± 0.27	0.652
Albumin (g/dL)	2.69 ± 0.09[a]	2.92 ± 0.13[b]	2.96 ± 0.07[b]	0.019
Cholesterol (mg/dL)	37.75 ± 2.77[ab]	28.89 ± 1.96[a]	39.79 ± 2.65[b]	0.010
Creatine kinase (IU/L)	416.49 ± 84.52	498.64 ± 95.83	360.49 ± 74.70	0.697
Triglyceride (mg/dL)	23.80 ± 4.19[a]	16.15 ± 1.71[a]	42.60 ± 7.64[b]	0.001

[a, b]: The mean values with different letters in the same line represent the difference between sepsis and control groups. (P < 0.05). SEM: standard error of the mean

Before treatment, WBC, lymphocyte, and granulocyte counts were higher in calves with sepsis than after treatment and controls, while erythrocyte counts were lower (P < 0.05, Table 2).

In calves with sepsis, pre-treatment ALT, AST, ALP, urea, and creatinine concentrations were statistically significantly higher than after treatment and control concentrations (P < 0.001). On the other hand, serum glucose was at its lowest level before treatment in the sepsis group but increased after treatment, approaching control animal values. Albumin, cholesterol, and triglyceride concentrations were statistically significantly lower in the sepsis group before treatment compared to the control group (P < 0.05, Table 2).

Haptoglobin and Cp acute-phase protein concentrations were considerably higher in the sepsis group compared to the controls; however, after treatment, concentrations reached values close to the controls (P < 0.001, Fig. 2). While the GSH concentration was lower in the sepsis group compared to the control group before treatment (P = 0.002, Fig. 2), the MDA concentration was higher (P < 0.001, Fig. 2). ET-1 and P-3 concentrations before treatment were higher in the group with sepsis compared to post-treatment and controls (P < 0.001, Fig. 2).

Haptoglobin, Cp, GSH, MDA, P-3, and ET-1 were compared with all other study data (Table 3). A positive correlation was determined with Hp, Cp, P-3, ET-1, and total leukocytes. Hp, P-3, and ET-1 negatively correlated with erythrocyte count. Hp, Cp, and P-3 positively correlated with ALT and AST. Hp, Cp, GSH, MDA, P-3, and ET-1 parameters correlated differently with physical findings, hematology, clinical, and biochemical parameters (Table 3). While a significant positive correlation was determined between Hp and Cp, MDA, P-3, and ET-1, a negative correlation was determined with GSH. Cp showed similar

correlations to Hp in ET-1, P-3, and MDA comparisons among themselves (Table 3). GSH, on the other hand, showed a significant negative correlation with Hp, MDA, P-3, and ET-1 (Table 3).

Table 3
Pearson correlation of measured parameters

Parameters	Hp	Cp	GSH	MDA	P-3	ET-1
	Pearson Correlation Coefficient (R)					
Total leukocytes count (×10^3/μL)	0.481**	0.445**	-0.176	0.126	0.556**	0.579**
Lymphocytes count (x10^3/μL)	0.369**	0.136	-0.170	0.048	0.383**	0.440**
Monocytes count (x10^3/μL)	-0.189	-0.341*	0.144	-0.135	-0.267	-0.272
Granulocytes count (x10^3/μL)	0.554**	0.453**	-0.280*	0.282*	0.493**	0.531**
Red blood cell count (x10^6/μL)	-0.342*	-0.276	0.182	-0.195	-0.429**	-0.341*
Mean red cell volume (fL)	0.206	0.305*	-0.166	-0.095	0.288*	0.167
Hematocrit (%)	0.200	0.293*	-0.073	-0.031	0.108	0.109
Hemoglobin (g/dL)	-0.184	-0.206	0.264	-0.184	-0.236	-0.145
Platelet count (x10^3/μL)	0.065	0.019	-0.166	0.095	0.128	0.127
Alanine aminotransferase (IU/L)	0.475**	0.446**	-0.056	0.214	0.618**	0.277
Aspartate aminotransferase (IU/L)	0.555**	0.281*	-0.381**	0.223	0.512**	0.294*
Gamma-glutamyl transferase(IU/L)	0.161	0.190	-0.110	0.098	0.064	0.050
Alkaline phosphatase (IU/L)	0.005	-0.127	-0.091	-0.008	-0.137	-0.125
Glucose (mg/dL)	-0.636**	-0.328*	0.344*	-0.440**	-0.574**	-0.342*
Cholesterol (mg/dL)	0.192	0.036	-0.170	0.171	0.070	0.144
Creatine (mg/dL)	0.734**	0.422**	-0.364**	0.253	0.755**	0.541**
Urea (mg/dL)	0.522**	0.244	-0.251	0.354*	0.578**	0.428**
Total protein (g/dL)	0.170	0.074	0.015	0.108	0.033	0.011
Lactate dehydrogenase (IU/L)	-0.050	-0.090	0.011	-0.038	0.003	-0.124
Creatine kinase (IU/L)	-0.032	-0.018	-0.092	-0.019	0.072	-0.070
Triglyceride (mg/dL)	-0.037	-0.247	0.100	-0.073	-0.210	-0.248
Hp (g/L)	-	0.609**	-0.481**	0.577**	0.891**	0.647**

*: Correlation is significant at the 0.05 level (2-tailed), **: Correlation is significant at the 0.01 level (2-tailed). Hp: Haptoglobin, Cp: Ceruloplasmin, GSH: Glutathione, MDA: Malondialdehyde, P-3: Pentraxin-3, ET-1: Endothelin-1

Parameters	Hp	Cp	GSH	MDA	P-3	ET-1
	Pearson Correlation Coefficient (R)					
Cp (mg/dL)	0.609**	-	-0.254	0.269	0.616**	0.542**
Albumin (g/dL)	-0.383**	-0.309*	0.176	-0.186	-0.387**	-0.427**
GSH (umol/mL)	-0.481**	-0.254	-	-0.399**	-0.441**	-0.434**
MDA (nmol/mL)	0.577**	0.269	-0.399**	-	0.467**	0.286*
P-3 (ng/mL)	0.891**	0.616**	-0.441**	0.467**	-	0.712**
ET-1 (pg/mL)	0.647**	0.542**	-0.434**	0.286*	0.712**	-
Rectal temperature (°C)	0.083	-0.122	-0.130	0.217	0.059	0.050
Heartbeats/min	0.681**	0.523**	-0.421**	0.278	0.675**	0.560**
Breaths/min	0.841**	0.570**	-0.503**	0.501**	0.743**	0.677**
Capillary refill time/sec	0.761**	0.541**	-0.320*	0.459**	0.758**	0.539**

*: Correlation is significant at the 0.05 level (2-tailed), **: Correlation is significant at the 0.01 level (2-tailed). Hp: Haptoglobin, Cp: Ceruloplasmin, GSH: Glutathione, MDA: Malondialdehyde, P-3: Pentraxin-3, ET-1: Endothelin-1

Discussion

Sepsis is an in vivo response to infection associated with a high mortality rate. Early diagnosis and initiation of appropriate treatment are essential in the fight against sepsis (Dupuy et al. 2013; Bachmann et al. 2005; Sönmezer and Tülek 2015). In light of this information, in our study, a rapid test kit for feces examination was used for early diagnosis in calves, and treatment was started within 24 hours at the latest after sepsis symptoms appeared.

Studies have reported that clinical findings such as depression, decreased interest in the environment, increase in respiratory and pulse rates, rise or fall in body temperature, and diminished appetite occurs with sepsis (Aldridge et al. 1993; Fecteau et al. 1997; Çitil and Gökçe 2013; Yıldız et al. 2018; Beydilli and Gökce 2019; Akyüz et al. 2016; Akyüz 2020; Akyüz and Gökce 2021). In the present study, the physical examination findings before treatment in the sepsis group were similar to the results of other studies. These effects are most likely due to the deterioration of hemostasis due to SIRS and the inability of organs and systems to perform their functions properly. In animals with sepsis, tachycardia develops due to fluid loss and SIRS (Fecteau et al. 2009; Naseri 2017; Yıldız et al. 2018; Akyüz and Gökce 2021). In this study, tachycardia detected before treatment in calves with sepsis may have been due to dehydration and SIRS. Tachypnea occurs as a result of compensation mechanisms associated with sepsis and infectious

conditions (Fecteau et al. 1997; Fecteau et al. 2009; Naseri 2017). Similarly, in our study, it was determined that tachypnea occurred due to sepsis. CRT prolongation in sepsis results from multiple organ dysfunction, adversely affecting the cardiovascular system (Fecteau et al. 2009; Akyüz and Gökce 2021). CRT was prolonged in our sepsis group compared to the control group due to cardiovascular system deterioration prior to treatment.

It has been reported that leukocytosis may occur in calves with sepsis (Dellinger et al. 2013; Naseri 2017; Yıldız et al. 2018; Beydilli and Gökce 2019). In our study, leukocytosis associated with the increase in lymphocytes and granulocytes before treatment in the sepsis group was similar to the results of other studies. The possible cause of this leukocytosis may be due to SIRS-induced inflammation. In addition, the high number of total leukocytes in the group with sepsis may have triggered the body's defense mechanisms against existing infection. Depending on disease severity, a decrease in the number of erythrocytes can be observed in calves with sepsis (Pardon and Depres 2018; Beydilli and Gökçe 2019). Consistent with the literature, the low number of erythrocytes found in our study may be due to the absence of or low level of sucking reflex, the disruption of erythrocyte production, and the deterioration of organ functions due to sepsis.

Increases in serum ALT, AST, and ALP have been reported in neonatal calves with diarrhea (Baser and Civelek 2013; Naseri 2017; Bozukluhan et al. 2017). We found serum ALT, AST, and ALP to be higher in calves with sepsis before treatment than after treatment and the control group, which may be a result of a deterioration in liver function due to sepsis. Dehydration is the most important reason for increased serum urea and creatinine concentrations in calves with diarrhea (Dratwa-Chalupnik et al. 2012). It has been reported that serum creatinine and urea concentrations are higher in calves with sepsis than in healthy calves (Ercan et al. 2016; Akyüz and Gökce 2021). In our study, the high pre-treatment serum urea and creatinine concentrations in calves with sepsis compared to healthy calves may be the result of dehydration. This opinion is supported by the improvement of renal function as a result of fluid therapy in the sepsis group and the fact that serum urea and creatinine values reached the levels of the control group after treatment. In calves with diarrhea, liquid electrolyte losses impair hemostasis, and carbohydrate stores are depleted, resulting in hypoglycemia (Dratwa-Chalupnik et al. 2012). Depletion and loss of carbohydrates, and the cessation of food intake, are known to cause hypoglycemia (Seifı et al. 2006). Serum glucose concentrations in calves with sepsis were reported to be lower than in healthy calves (Ercan et al. 2016). In our study, the blood glucose concentration before treatment was also lower in the sepsis group than in the control group. This result is likely due to lack of food intake, glucose loss through diarrhea, and depletion of carbohydrate stores due to sepsis. There may be a decrease in the concentration of albumin, a negative acute-phase protein associated with inflammation and diarrhea (Uzlu et al. 2010; Marcato et al. 2018; Akyüz and Gökce 2021). The results of our study are consistent with the literature in this regard. Low albumin levels in the sepsis group before treatment compared to the control group may be due to sepsis-induced inflammation. In many acute cases, plasma triglyceride levels were normal or low (Akgün et al. 1998). In our study, triglyceride levels were lower in calves with sepsis compared to controls. While cholesterol levels increase in nephrotic syndrome or protein-losing nephropathy, hypothyroidism, acute pancreatitis, and cholestasis, the levels decrease in protein-losing

enteropathy, hypoadrenocorticism, and acute inflammatory response (Carpienter and Scruel 2002). In our study, the low cholesterol concentration in the sepsis group was most likely due to an inflammatory reaction and enteritis.

Acute-phase proteins, blood proteins generally synthesized in the liver, are used to evaluate the body's response in cases of inflammation or infection (Pradeep 2014). One of the acute-phase proteins, Hp, which is important for ruminants, is typically at a low level in healthy animals but increases in cases of inflammation, trauma, and infection (Petersen et al. 2004; Mcgrotty et al. 2003; Erkılıç 2019). Thus, Hp is a clinically useful parameter to determine the severity of the inflammatory response in ruminants (Murata et al. 2004; Çitil 2003; Heegaard et al. 2000; Coşkun and Şen 2011). Merhan et al. (2016) reported that Hp and Cp levels increased in a study of calves with diarrhea. Pourjafar et al. (2011) also reported an increase in Hp concentration. This increase in Hp was probably due to tissue destruction. In addition, the Hp acute-phase protein increase may be the result of activation of the body's defense mechanisms against infection and the severe inflammation that develops in sepsis. After treatment, Hp concentration decreased due to improvement in animal health. Cp is also a positive acute-phase protein (Ceron et al. 2005; Erkılıç 2019) synthesized in the liver (Murata et al. 2004; Erkılıç 2019). Cp, which is used less commonly in cattle than Hp, is reported as an indicator of infection (Guys et al. 2005; Petersen et al. 2004). Cp acts on defense system cells and causes an increase in the antimicrobial power of these cells (Cerone et al. 2000). In a study conducted on calves, the level of Cp increased in cases of enteritis (Murata et al. 2004; Coşkun and Şen 2011). In this study, the increase in Cp may be due to its effect on the defense system. In addition, the reason for this increase is thought to be due to the acute-phase response, infection, and plasma antioxidant properties.

Inflammation and infection activate many cells with phagocytic activity such as monocytes and macrophages (Tokoyuni 1999) and cause the formation of free radicals due to excessive oxygen consumption (such as hydrogen peroxide, superoxide anion). Studies have reported that the oxidant-antioxidant balance is disrupted, and oxidative stress occurs in bacterial, viral, and parasitic diseases such as traumatic reticuloperitonitis (Atakişi et al. 2010), sheeppox in sheep (Bozukluhan et al. 2018), and hypodermosis in cattle (Merhan et al. 2017). In this study, the oxidant-antioxidant balance was disturbed, the MDA concentration increased, and the GSH concentration decreased, which may be due to free radicals formed by phagocytes, which play an important role in host defense. As a result of treatment, the MDA concentration decreased, while the GSH concentration increased.

Pentraxin-3 is an acute phase protein released from macrophages, dendritic cells, leukocytes, and endothelial cells during the inflammatory response (Libby et al. 2009). Studies have found a positive correlation between disease severity and the plasma P-3 level (Kao et al. 2013; Sönmezer and Tülek 2015). Consistent with the literature, pre-treatment P-3 was higher in the sepsis group than in the controls in our study. This result may be due to the presence of leukocytosis in the sepsis group, the widespread inflammation caused by SIRS, and the increase in acute-phase proteins associated with infection. ET-1 is secreted by endothelial cells (Shah 2007; Tschaikowsky et al. 2000; Sönmezer and Tülek 2015). The higher ET-1 values in the sepsis group in our study compared to the control group may be the result of

endothelial damage caused by sepsis. In light of the data presented, it was determined that the oxidative stress load increased, the antioxidant capacity decreased, concentrations of Hp, Cp, and P-3 acute phase proteins increased, and the negative acute phase protein albumin decreased in calves with sepsis. In addition, it was determined that endothelial damage occurred. Accordingly, there were changes in the related enzymes due to an increase in Cp and some organ tissue dysfunction.

Conclusion

It is of diagnostic importance that Hp, Cp, MDA, ET-1, and P-3 levels were significantly higher before treatment in calves with sepsis than after treatment and in controls. After treatment, these parameters decreased and reached close to control levels, which is important for prognosis evaluation. In addition, Hp, Cp, GSH, MDA, ET-1, and P-3 had significant correlations; therefore, evaluation of these parameters together is essential to determine a sepsis diagnosis swiftly and accurately. It is vital to start treating sick calves as soon as possible after clinical findings appear and a diagnosis of sepsis is made. The survival of all calves with sepsis in this study indicates that early diagnosis and treatment are critical. In conclusion, Hp, Cp, GSH, MDA, ET-1, and P-3 are important diagnostically and prognostically in neonatal calves with sepsis due to *E. coli K99.*

Declarations

Author contributions EA conceived and supervised the study. EA, OM, MS, and YUB collected and analyzed data. OM analyzed biochemical measurements. MRC and MŞ performed the PCR procedure. EA, GG, EEE, MK, and KB contributed to writing the article. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

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Data availability The datasets generated and analyzed during the current study are not publicly available due to all the results in the form of means and statistics are presented in this paper, but are available from the corresponding author on reasonable request.

Ethical Approval This study was conducted with approval number "KAU-HADYEK/2020-078" from the local ethics committee of Kafkas University.

Competing interest The authors declare no conflicts of interest associated with this study or its results.

Consent to participate All authors participated and helped voluntarily in the research.

Consent for publication All authors read and approved the final manuscript.

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Figures

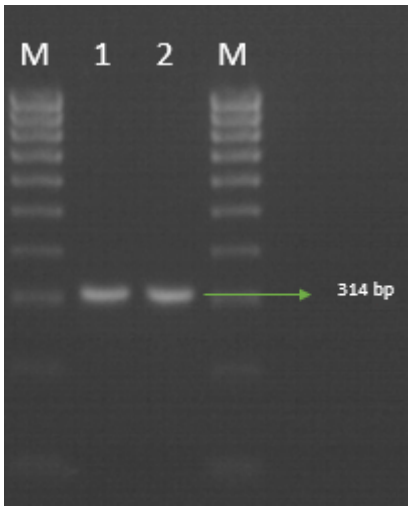

Figure 1

E. coli K99 PCR image obtained from a stool sample of a calf with sepsis. M: 100bp Marker 1: Positive control, 2: *E. coli K99* positive sample.

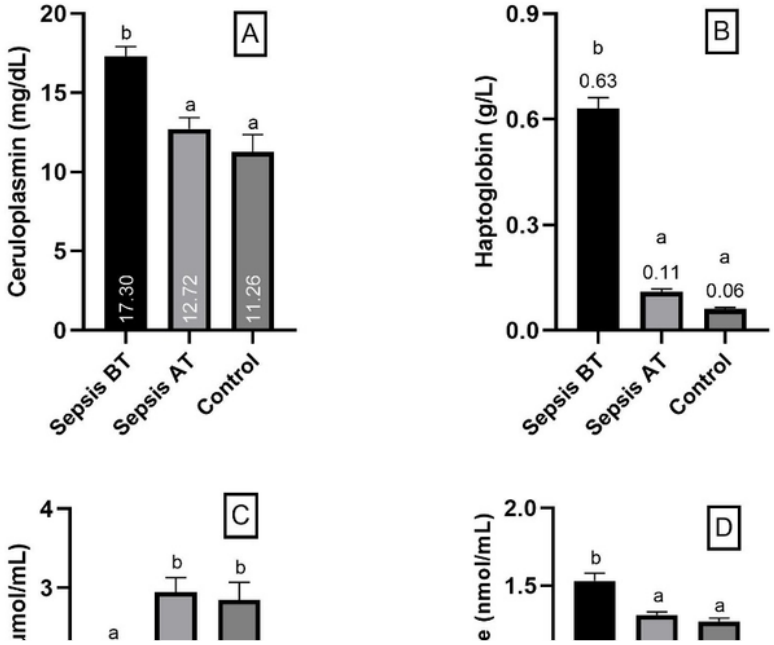

Figure 2

A. Ceruloplasmin levels of calves with sepsis and healthy (P<0.001). **B.** Haptoglobin levels of sepsis and healthy calves (P<0.001). **C.** Glutathione levels of sepsis and healthy calves (P=0.002). **D.** Malondialdehyde levels of calves with sepsis and healthy (P<0.001). **E.** Endothelin-1 levels of calves with sepsis and healthy (P<0.001). **F.** Pentraxin-3 levels of calves with sepsis and healthy (P<0.001). **BT:**

Before treatment. **AT:** After treatment. $^{a-c}$**:** Different letters indicate significant statistical difference between sepsis and control groups.