Volume 12, Issue 4, Page 123-128, 2023 https://doi.org/10.46810/tdfd.1343189 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

A Study On The Relationship Between Severity Of Clinical Findings And Serum IL-6 Level In Neonatal Calves With Diarrhoea

Gürsel AYDIN[1], Mustafa Sinan AKTAŞ[2*]

[1] Atatürk University, Graduate School of Health Sciences, Veterinary Internal Medicine Department, Erzurum, Türkiye

[2] Atatürk University, Veterinary Faculty, Internal Medicine Department, Erzurum, Türkiye

Gürsel AYDIN ORCID No: 0000-0002-0912-3352

Mustafa Sinan AKTAŞ ORCID No: 0000-0002-7206-5757

**Corresponding author: sinanaktas@atauni.edu.tr*

(Received: 15.08.2023, Accepted: 06.12.2023, Online Publication: 28.12.2023)

Keywords
Diarrhea, IL-6, Neonatal calves

Abstract: The aim of the study is to investigate the relationship between clinical course and serum IL-6 level in neonatal calves with diarrhea. The study material consisted of 40 calves in the neonatal period. The calves were divided into 4 groups: group I (healthy, control, n=10), group II (with mild diarrhea, n=10), group III (with moderate diarrhea, n=10) and group IV (with severe diarrhea, n=10). The breed, age, sex and clinical findings of the calves were recorded. Etiological analysis was performed on stool samples taken from calves. Haematological analyzes were performed on the blood samples taken and serum IL-6 levels were determined using the ELISA test kit. WBC and NEU numbers of calves with diarrhea in group II, group III and group IV were found to be numerically higher than healthy calves in group I (P>0.05). Calves in group II (239.76±11.05), group III (293±48.7) and group IV (300±25.06) had higher serum IL-6 levels than calves in group I (211.58±10.07) (P< 0.01). While IL-6 levels of group IV were higher than group III (P>0.05), IL-6 levels of group III and IV were higher than group II (P<0.01). According to the data obtained from this study, it was concluded that serum IL-6 is an significant biomarker indicating the degree of clinical severity.

Neonatal Dönem İshalli Buzağılarda Klinik Bulguların Şiddeti İle Serum IL-6 Düzeyi Arasındaki İlişki Üzerine Bir Araştırma

Anahtar Kelimeler
IL-6, İshal, Neonatal buzağı

Öz: Bu çalışmanın amacı, neonatal dönem ishalli buzağılarda klinik seyir ile serum IL-6 düzeyi arasındaki ilişkiyi araştırmaktır. Çalışma materyalini neonatal dönemdeki 40 buzağı oluşturdu. Buzağılar grup I (sağlıklı, kontrol, n=10), grup II (hafif ishalli, n=10), grup III (orta şiddette ishalli, n=10) ve grup IV (şiddetli ishalli, n=10) olmak üzere 4 gruba ayrıldı. Buzağıların ırk, yaş ve cinsiyet bilgileri ile klinik bulguları kaydedildi. Buzağılardan alınan dışkı örneklerinde etiyolojik analiz yapıldı. Alınan kan örneklerinde hematolojik analizler yapıldı ve serumda IL-6 düzeyleri, ELISA test kiti kullanılarak belirlendi. Grup II, grup III ve grup IV'deki ishalli buzağılarda WBC ve NEU sayıları grup I'deki sağlıklı buzağılara göre rakamsal olarak yüksek olduğu belirlendi (P>0.05). Grup II (239.76±11.05 ng/L), grup III (293±48.7 ng/L) ve grup IV'de (300±25.06 ng/L) bulunan buzağıların serum IL-6 düzeyleri grup I'deki (211.58±10.07 ng/L) buzağılara göre daha yüksek olduğu belirlendi (P<0.01). Grup IV'ün IL-6 düzeyi grup III'e göre rakamsal olarak yüksekken (P>0.05), grup III ve IV'ün IL-6 düzeyleri, grup II'ye göre yüksek olduğu belirlendi (P<0.01). Bu çalışmadan elde edilen verilere göre, neonatal dönem ishalli buzağılarda serum IL-6'nın hastalığın klinik şiddetinin derecesini gösteren önemli bir belirteç olduğu sonucuna varılmıştır.

1. INTRODUCTION

Diarrhea, pneumonia (especially aspiration pneumonia), joint diseases, umbilical cord diseases, vitamin, mineral substance and trace element deficiencies, trauma, congenital anomalies, and nutritional disorders are common neonatal period diseases in calves, and among these diseases, neonatal calf diarrhea is the most common disease throughout the world [1,2,3].

Coronavirus, Rotavirus, Cryptosporidium, Clostridium perfiringens (Cl. perfiringens) and *Escherichia coli (E. coli)* are the most common causes of diarrhea in neonatal calves, and they can cause inflammatory and functional damage in the intestine [4,5,6]. The clinical signs observed in diarrhea formed in calves during the neonatal period are very variable and may show a course ranging from mild watery diarrhea to coma from a lying position according as the severity of diarrhea and inflammation [6]. In addition, neonatal calf diarrhea can cause significant changes in haematological parameters such as total leukocytes (WBC), lymphocytes (LYM), neutrophils counts (NEU) and hematocrit concentration (HCT) [7,8].

Interleukins, especially Interleukin-6 (IL-6), are used as an important marker in determining early inflammation and prognosis in various diseases [9]. IL-6 is a cytokine produced in response to tissue damage and infections [10]. Multiple cell types are associated with the production of this cytokine, including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, T and B cells [11]. The biological consequences of IL-6 production have been associated with both pro- and anti-inflammatory effects [12].

There are few studies evaluating the diagnostic and prognostic potential of IL-6 in cattle [13,14,15]. In neonatal diarrheal calves, on the other hand, IL-6 levels were investigated in methodically different studies such as the comparison of those with diarrhea and healthy ones, and those who recovered and those who did not recover in calves with diarrhea and septicemia [16,17]. Based on the hypothesis of the relationship between the severity of the disease and inflammation, in the presented study, it was aimed to investigate the relationship between clinical course and serum IL-6 level in neonatal calves with diarrhea.

2. MATERIAL AND METHOD

2.1. Animals and Groups

In the study different ages, breeds and sexes 10 healthy and 30 untreated diarrheic neonatal calves were used (Ethics Committee Decision No: 2020-13/191). Clinical findings [body temperature (T, C°), respiration rates (R, num/min), and pulse rates (P, beats/min)], dehydration status, sucking reflexes, and whether they could stand or not of the calves were recorded. Neonatal calves had a problem other than diarrhea (pneumonia, arthritis, omphalitis, etc.) were not included in the study.

The study population was comprised of 40 neonatal calves, 10 of which had healthy (Group-I) and 30 of which were diarrheic calves, additionally diarrheic calves were divided into three groups as mild (Group-II), moderate (Group-III) and severe (Group-IV) according to the criteria for clinical dehydration and depression [18].

Group-I (Control, n=10): This group consisted of different breeds and sexes, 8.3±4.3 days old, who were healthy calves according to clinical and haematological findings.
Group-II (n=10): This group consisted of different breeds and sexes, 7.9±5.6 days old, and mild diarrhea calves with a clinical score of 1 according to the criteria were given in Table-1.

Group-III (n=10): This group consisted of different breeds and sexes, 7±5 days old, and moderate diarrhea calves with a clinical score of 4 according to the criteria were given in Table-1.

Group-IV (n=10): This group consisted of different breeds and sexes, 7.3±4 days old, and severe diarrhea calves with a clinical score of 7 according to the criteria were given in Table-1.

Table-1: Clinical scoring in neonatal calves with diarrhea [18].

Eyeball retraction	0: Not crashed
	1: Mild crashed
	2: Severe crashed
Skin elasticity	0: Back to normal immediately
	1: Back to normal after 1-3 seconds
	2: Back to normal after ≥4 seconds
Sucking reflex	0: Strong regular suction
	1: Poor ineffective suction
	2: No sucking reflex
Stool consistency score	1: Watery
	2: Pasteus
	3: Solid

2.2. Collections of Samples

Blood samples from all calves were taken from V. Jugularis and transferred into 4 mL tubes with EDTA (Becton Dickinson Co, USA) and 10 mL tubes with gel (BD Vacutainer System, Plymouth, UK). Thereafter serum was obtained by centrifugation at 3000 rpm for 10 minutes. The serum samples were stored at -80 °C until analysis (maximum 3 months) Haematological analysis were performed immediately. Additionally fecal samples were taken into sterile fecal containers by rectal stimulation.

2.3. Diagnosis of Etiologic Agents

Diagnosis of etiologic agents (*Rotavirus, Coronavirus, Cryptosporidium, Cl. perfringens* and *E. coli*/F5-K99) in fecal samples were made using rapid diagnosis test kit (Rainbow Calf Scours-BIO K 306 Ag Test Kit, Biox Diagnostics, Belgium). The use of this kit was carried out in accordance with the manufacturer's instructions.

2.4. Haematologic Analysis

The count of white blood cell (WBC), lymphocyte (LYM), neutrophil (NEU), erythrocyte (RBC), hemoglobin (HGB) and hematocrit (HCT) of the calves were determined by hematology analyzer (Abacus Junior Vet 5® Hungary).

2.5. Determination of IL-6

Determination of IL-6 in serum samples was by a sandwich enzyme-linked immunosorbent assay (ELISA) using bovine IL-6-specific antibody (Sun Red Biotechnology Company, Cat No: 201-04-0008). The analysis was carried out in accordance with the recommendations of the manufacturer.

2.6. Statistical Analyses

The analyzes of the clinical, haematological and IL-6 values of the calves in the groups were performed using the General Linear Model procedure and the SPSS 20.0 package program. Significance controls of means found significant between groups were determined by Duncan Multiple Comparison Test.

3. RESULTS

3.1 Clinical Findings

The findings of the clinical examination [T (C°), P (Beats/min), R (Num/min)] of calves in the groups are presented in Table 2. Body temperatures of the calves in groups III and IV were found to be lower than those of the calves in groups I and II (P<0.01).

Table-2: Clinical findings [T (C°), P (Beats/min), R (Num/min)] of the calves in the groups (Mean ±SE).

Parameters/Groups	Group I (Mean ±SE)	Grup II (Mean± SE)	Grup III (Mean ±SE)	Grup IV (Mean ±SE)	P
T (C°)	38.5± 0.3^{a}	38.6± 0.5^{a}	36.9± 1.5^{b}	36.07± 1.5^{b}	**
P (Beats/min)	113.1± 14.3	119.2± 33.3	111± 25.2	105.8± 29.5	NS.
R (Num/min)	30.9± 5.4	34.6± 10.5	40.7± 12.8	35.8± 8.0	NS.

a, b: The difference between the means with different letters on the same line is statistically significant. **: P<0.01, NS: Not Significant.

3.2. Etiologic Agent Findings

Enteropathogens diagnosed in the feces of diarrhoeic neonatal calves are given in Table 3. Such as *E. coli*, *Rotavirus*, *Coronavirus*, *Cryptosporidium* and *Cl. perfiringens* enteropathogens or their combinations were determined.

Table-3. Etiological findings of calves in all groups

Etiology/Groups	Group I (n=10)	Group II (n=10)	Grup III (n=10)	Group IV (n=10)
E. coli	-	2	5	4
Rotavirus	-	2	2	1
Coronavirus	-	1	-	-
Cryptosporidium	-	2	-	-
Cl. perfiringens	-	-	-	-
E.coli + Rotavirus	-	1	1	2
E.coli + Coronavirus	-	-	1	-
Rotavirus + Coronavirus	-	-	-	1
Rotavirus + Cl. perfiringens	-	1	1	1
E.coli + Rotavirus + Coronavirus	-	-	-	1
E.coli + Rotavirus + Cryptosporidium	-	1	-	-

3.3. Haematological Findings

The haematological parameters findings of calves in all groups are given in Table-4. It was determined that the WBC and NEU numbers in the group II, III and IV were numerically higher than the group I (P>0.05), and there was no difference in the LYM numbers between the groups (P>0.05). RBC numbers in the group II, and IV were higher than the group I (P<0.01). Additionally, it was detected that the HGB numbers and HCT percentages in the group II, III and IV were higher than the group I (P<0.01).

Table-4: Haematological parameters findings of calves in all groups (Mean ±SE).

Parameters Groups	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)	P
WBC (10^3/µl)	8.8±1.4	13.3±8.4	12.6±5.3	11.7±5.1	NS.
LYM (10^3/µl)	4.9±1.1	4.8±2.4	5.7±3.2	3.4±1.4	NS.
NEU (10^3/µl)	3.6±1.0	8.2±7.5	6.7±5.9	7.8±3.9	NS.
HCT (%)	22.4±3.7^{c}	36.1±10.8^{b}	35±9.6^{b}	47.2±11.6^{a}	**

a, b, c: The difference between the means with different letters on the same line is statistically significant. **: P<0.01, NS: Not Significant.

3.4. IL-6 Findings

Serum IL-6 levels of calves in all groups are given in Table-5. It was determined that the serum IL-6 levels in the group II, III and IV were higher than the group I (P<0.01).

Table-5: Serum IL-6 levels of calves in all groups (Mean ±SE).

Parameter /Groups	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)	P
IL-6 (ng/L)	211.58± 10.07^{c}	239.76± 11.05^{b}	293± 48.7^{a}	300± 25.06^{a}	**

a, b, c: The difference between the means with different letters on the same line is statistically significant. **: P<0.01.

4. DISCUSSION AND CONCLUSION

Despite the advances in herd management, animal care, stables, animal feeding, cattle industry and timely use of biopharmaceuticals, neonatal calf diarrhea remains a major cause of economic loss in the cattle industry worldwide [19]. IL-6 is defined as an early and reliable prognostic marker in various diseases [20]. In the present study, it was aimed to determine serum IL-6 levels according to the clinical course of the disease in calves with neonatal diarrhea and determine whether a relationship between the clinical course or not IL-6.

Among the many infectious agents that cause diarrhea in neonatal calves, *E. coli*, *Salmonella* and Clostridium species are the most common bacterial agents [21], *Rotavirus* and *Coronavirus* are the most common viral agents, and *Cryptosporidium parvum* spp is the most common protozoal agent [22]. In the study presented in accordance with these reports, *Rotavirus*, *Coronavirus*, *E. coli*, *Cryptosporidium*, *Cl. perfiringens* or a combination of these agents have been identified. In the neonatal period, *Coronavirus*, *Rotavirus*, *Cryptosporidium* and enterotoxic *E. coli* are the most common agents that can cause inflammatory and functional damage, especially in the intestine [4,5].

In calves with diarrhea, body temperature is mostly within the normal range, and in a few cases it may be elevated or decreased due to dehydration [23]. Similar to body temperature, respiration and pulse rates may be normal in diarrhoeic calves [24], and hypothermia and tachypnea may occur [25]. In the present study, although there were no difference between respiratory and pulse rates of calves in the groups II, III and IV, it was determined that body temperatures of calves in the groups III and IV were lower than those in the groups I and II.

White Blood Cell, LYM and NEU numbers may increase in diarrhoeic neonatal calves, and leukocytosis may occur. It is stated that the primary reason for this situation may be related to the reactions of the body's immune system against infectious factors, inflammatory reactions, severity of diarrhea, stress and hemoconcentration [26,27,28,29,30]. Atcali and Yildiz [31], determined that there was no significant difference in LYM levels in neonatal diarrheal calves compared to healthy ones in their study, and they interpreted this situation as etiological differences, duration of diarrhea, severity of inflammation, low number of animals, and the fact that some of the animals were at the initial stage of diarrhea. When the same researchers took into account the haematological values that could not be determined statistically significant in their studies, they observed that the lowest and highest levels of these values were in a very wide range and therefore there was no difference. In the presented study, although the WBC and NEU numbers in the groups II, III and IV were numerically higher than the group I, the possible reason why they were not statistically significant was the high standard deviations of the groups, similar to the reasons stated in the above notifications. Barua et al [32] state that the increase in HCT percentages in calves with diarrhea is due to hemoconcentration associated with dehydration and hypovolemia. In the presented study, HCT percentages in the groups II, III and IV were higher than the in group I. The possible reason for this situation may be hemoconcentration, as stated by Barua et al [32].

Cytokines function in many basic processes for life and disease. Among these different functions, their role in inflammation has attracted attention in relation to disease development and treatment [33]. Interleukins, and especially IL-6, are used as a marker of early inflammation and prognosis in various diseases [9]. An extremely important advantage of IL-6 is that it can stay in the circulation longer than other proinflammatory cytokines [34].

There are many studies in the literature that have been conducted in different animal species and in which IL-6 has been evaluated. It has been used as a reliable parameter to monitor dogs in intensive care units, the average concentration of IL-6 of these dogs was determined to be significantly higher in the non-survivor group compared to the survivors [35]. In addition, it was determined that serum IL-6 levels were increased in dogs with septic peritonitis [36], babesiosis [37], and different liver diseases [38]; in cats with sepsis and septic shock [39], idiopathic cystitis [40], and feline infectious peritonitis [41]; in horses with metabolic syndrome pain [42]; endurance training [43], and acute abdominal disease [44]; in pigs with vesicular stomatitis [45]; in lambs with experimental endotoxemia [46]; in cattle with subclinical mastitis [15]; subclinical endometritis [47], and subclinical parasitemia [48], compared to healthy ones.

Methodologically, different studies have been conducted to evaluate IL-6 in diarrhoeic neonatal calves. Fischer et al [17], in their study to investigate the value of IL-6 as a prognostic marker in neonatal diarrheal calves, divided the calves into two groups as those who recovered and those who did not, according to their clinical findings on the 7th and 10th days. They determined that IL-6 levels were very high at the onset of diarrhea in non-healing calves. In the results of the study; They concluded that IL-6 is a useful complementary parameter for disease prognosis in neonatal calf diarrhea, and high IL-6 values may prompt the Veterinarian to monitor calves more closely and adapt the therapeutic strategy accordingly, thus avoiding animal suffering and economic losses. Albayrak and Kabu [16] determined that IL-6 levels were significantly higher in calves with diarrhea compared to healthy ones. As a result of their studies, they evaluated that routine measurement of serum IL-6 levels is a valuable parameter in evaluating the course and prognosis of diseases. Carroll et al. [49], determined that serum IL-6 levels were very high compared to the control group in calves to which they administered endotoxin intravenously. Kırbaş et al. [50] determined that calves with septicemia had significantly higher IL-6 levels on day 0 compared to the control group, and determined that the levels were significantly lower than the control group after treatment. Similar to the results obtained in the above reports, in the presented study, it was determined that serum IL-6 levels were parallel to the clinical course in neonatal diarrheal calves. Cytokines often exert stimulant (pro-inflammatory) or depressive (anti-inflammatory) effects in inflammatory conditions [51]. As can be understood from the literature reports given above, IL-6, which is one of the proinflammatory cytokines, increases in all conditions that cause inflammation, including diarrhea. The data obtained from this study also support this information.

It was concluded that serum IL-6 level increased in neonatal diarrheic calves as the disease became clinically

more severe, is an significant biomarker indicating the degree of clinical severity in neonatal diarrheic calves.

Acknowledgement
This study is summarized from the master's thesis of Gürsel Aydın

REFERENCES

[1] Aslan V. Calf diarrhea and its treatments, neonatal calf losses symposium. Selçuk University, Faculty of Veterinary Medicine, Konya, Turkey, 59-69, 1986.
[2] Collery P, Bradley J, Fagan J, Jones P, Redehan E, Weavers E. Causes of perinatal calf mortality in the Republic of Ireland. Irish Veterinary Journal. 1996; 49: 491-496.
[3] Poulsen KP, McGuirk SM. Respiratory disease of the bovine neonate. Veterinary Clinics of North America: Food Animal Practice. 2009; 25: 121-137.
[4] Al Mawly J, Grinberg A, Prattley D, Moffat J, French N. Prevalence of endemic enteropathogens of calves in New Zealand dairy farms. New Zealand Veterinary Journal. 2015; 63: 147-152.
[5] Kaske M. Physiological function of the gastrointestinal tract and pathophysiological changes in neonatal diarrhea of calves. Deutsche tierärztliche Wochenschrift. 1993; 100: 434-439.
[6] Mercmanual [Internet]. Diarrhea in neonatal ruminants; 2022 [Cited: January, 2022]. Available from: https://www.merckvetmanual.com/digestive-system/intestinal-diseases-in ruminants/diarrhea-in-neonatal-ruminants
[7] Taylor JA. Leukocyte responsees in ruminants. In: Bernart FF, Joseph GZ, Nemi CJ. Editors. Schalm's Veterinary Hematology. Lippincott Wiliams and Wilkins, Philadelphia; 2000. P. 391-400.
[8] Uzlu E, Karapehlivan M, Çitil M, Gökçe E, Erdoğan HM. Investigation of serum sialic acid and some biochemical parameters in calf with diarrhea symptoms. YYÜ Veteriner Fakültesi Dergisi. 2010; 21: 83-86.
[9] Rincon M. Interleukin-6: From an inflammatory marker to a target for inflammatory diseases. Trends Immunology. 2012; 33: 571-577.
[10] Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspectives in Biology. 2014; 2: 1-17.
[11] Mauer J, Denson JL, Bruning JC. Versatile functions for IL-6 in metabolism and cancer. Trends Immunology. 2015; 36: 92-101.
[12] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta. 2011; 1813: 878-888.
[13] Ishıkawa Y, Nakada K, Hagıwara K, Kırısawa R, Iwaı H, Morıyoshı M, Sawamukaı Y. Changes in interleukin-6 concentration in peripheral blood of pre- and post-partum dairy cattle and its relationship to postpartum reproductive diseases. The Journal of Veterinary Medical Science. 2004; 66: 1403-1408.
[14] Nakajima Y, Mikami O, Yoshioka M, Motoi Y, Ito T, Ishikawa Y, Fuse M, Nakano K, Yasukawa K. Elevated levels of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) activities in the sera and milk of cows with naturally occurring coliform mastitis. Research in Veterinary Science. 1997; 62: 297-298.
[15] Sakemi Y, Tamura Y, Hagiwara K. Interleukin-6 in quarter milk as a further prediction marker for bovine subclinical mastitis. Journal of Dairy Research. 2011; 78: 118-121.
[16] Albayrak H, Kabu M. Determining serum haptoglobin and cytokine concentrations in diarrheic calves. FÜ Sağlık Bilimleri Veteriner Dergisi. 2016; 30: 113-117.
[17] Fischer S, Bauerfeind R, Czerny CP, Neumann S. Serum interleukin-6 as a prognostic marker in neonatal calf diarrhea. Journal of Dairy Science. 2016; 99: 6563-6571.
[18] Walker PG, Constable PD, Morin DE, Drackley JK, Foreman JH, Thurmon JC. A reliable, practical, and economical protocol for inducing diarrhea and severe dehydration in the neonatal calf. Canadian Veterinary Research. 1998; 62: 205-213.
[19] Picco NY, Alustiza FE, Bellingeri RV, Grosso MC, Motta CE, Larriestra AJ, Vissio C, Tiranti KI, Terzolo HR, Moreira AR, Vivas AB. Molecular screening of pathogenic Escherichia coli strains isolated from dairy neonatal calves in Cordoba province, Argentina. Revista Argentina de Microbiología. 2015; 47: 95-102.
[20] Fisher DT, Michelle M, Appenheimer S, Evans S. The two faces of IL-6 in the tumor microenvironment. Seminars in Immunology. 2014; 26: 38-47.
[21] Kasa A, Tulu D, Negera C. Review of common bacterial cause and management of neonatal calf diarrhea in cattle. International Journal of Microbiology Research. 2020; 11: 98-104.
[22] Kongsted H, Jonach B, Haugegaard S, Angen O, Jorsal SE, Kokotovic B, Larsen L, Jensen TK, Nielsen JP. Microbiological, pathological and histological findings in four Danish pig herds affected by a new neonatal diarrhoea syndrome. BMC Veterinary Research. 2013; 9: 206.
[23] Sahal M, Terzi OS, Ceylan E, Kara E. Calf Diarrhea and Prevention Methods. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi. 2018; 58: 41-49.
[24] Fecteau G, Pare J, Van Metre DC, Smith BP, Holmberg CA, Guterbock W, Jang S. Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. Canadian Veterinary Journal. 1997; 38: 101-104.
[25] Lofstedt J, Dohoo IR, Duizer G. Model to predict septicemia in diarrheic calves. Journal of Veterinary Internal Medicine. 1999; 13: 81-88.
[26] Brar A, Ahuja CS, Sood NK, Sandhu BS, Gupta K. Haematological changes in neonatal diarrheic calves of different age groups. Indian Journal of Veterinary Pathology. 2015; 39: 73-77.
[27] Brar TK, Singh KD, Kumar A. Effect of different phases of menstrual cycle on heart rate variability (HRV). Journal of Clinical Diagnostic Research. 2015; 9: 1-4.

[28] Günes V, Unver A, Citil M, Erdoğan HM. The prevalance of Escherichia coli O157 serotype and Clostridium perfringens type A α-toxin in neonatal diarrhoeic calves in Kars disrict. Kafkas Universitesi Veteriner Fakültesi Dergisi. 2004; 10: 41-45.
[29] Seifi HA, Mohri M, Shoorei E, Farzaneh N. Using haematological and serum biochemical findings as prognostic indicators in calf diarrhoea. Comparative Clinical Pathology. 2006; 15: 143-147.
[30] Sentürk S. Fluid therapy in diarrheic calves. Journal of Faculty Veterinary Medicine. 2001; 20: 161-167.
[31] Atcalı T, Yıldız R. The effects of different factors on hemogram parameters on neonatal calves with diarrhea. Mehmet Akif Ersoy University Journal of Health Sciences Institute. 2020; 8: 119-127.
[32] Barua SR, Rakib TM, Das S, Masuduszaman M, Hossain MA. Haematological and serological changes in neonatal diarrheic calves infected with bovine rotavirus. Multidisciplinary Advances in Veterinary Science. 2018; 2: 356-366.
[33] Hirano T. IL-6 in inflammation, autoimmunity and cancer. International Immunology. 2021; 33: 127-148.
[34] Song M, Kellum JA. Interleukin-6. Critical Care Medicine. 2005; 33: 463-465.
[35] Schüttler J, Neumann S. Interleukin-6 as a prognostic marker in dogs in an intensive care unit. Veterinary Clinical Pathology. 2015; 44: 223-228.
[36] Martiny P, Goggs R. Biomarker guided diagnosis of septic peritonitis in dogs. Frontiers in Veterinary Science. 2019; 6: 208.
[37] Goddard A, Leisewitz AL, Kjelgaard-Hansen M, Kristensen AT, Schoeman JP. Excessive pro-inflammatory serum cytokine concentrations in virulent canine babesiosis. PLoS ONE. 2016; 11(3): e0150113.
[38] Neumann S, Kaup FJ, Scheulen S. Interleukin-6 (IL-6) serum concentrations in dogs with hepatitis and hepatic tumours compared with those with extra-hepatic inflammation and tumours. Comparative Clinical Pathology. 2012; 21: 539-544.
[39] Troia R, Mascalzoni G, Agnoli C, Lalonde-Paul D, Giunti M, Goggs R. Cytokine and chemokine profiling in cats with sepsis and septic shock. Frontiers in Veterinary Science. 2020; 7: 305.
[40] Parys M, Yuzbasiyan-Gurkan V, Kruger JM. Serum cytokine profiling in cats with acute idiopathic cystitis. Veterinary Internal Medicine. 2018; 32: 274-279.
[41] Goitsuka R, Ohashi T, Ono K, Yasukawa K, Koishibara Y, Fukui H, Ohsugi Y, Hasegawa A. IL-6 activity in feline infectious peritonitis. Journal of Immunology. 1990; 144: 2599-25603.
[42] Krzysztof M, Katarzyna B, Nezir Yaşar T, Agnieszka S, Jakub N. The activity of IL-6 and TNF-α in adipose tissue and peripheral blood in horses suffering from equine metabolic syndrome (EMS). Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2014; 20: 493-499.
[43] Witkowska-Pilaszewic O, Baska P, Czopowicz M, Zmigrodzka M, Szarska E, Szczepaniak J, Nowak Z, Winnicka A, Cywinska A. Anti-inflammatory state in Arabian Horses introduced to the endurance training. Animals. 2019; 9: 616.
[44] Barton MH, Chrysann C. Tumor necrosis factor and Interleukin-6 activity and endotoxin concentration in peritoneal fluid and blood of horses with acute abdominal disease. Journal of Veterinary Internal Medicine. 1999; 13: 457-464.
[45] Velazquez-Salinas L, Pauszek SJ, Stenfeldt C, O'Hearn ES, Pacheco JM, Borca MV, Verdugo Rodriguez A, Arzt J, Rodriguez JJ. Increased virulence of an epidemic strain of vesicular stomatitis virus is associated with interference of the innate response in pigs. Frontiers Microbiology. 2018; 9: 1891.
[46] Kirbas A, Karakus E, Ozkanlar S, Gedikli S, Hanedan B, Topcu A, Bayraktutan Z. Comparative efficacy of the steroids administered by inhalation and parenteral route in lambs with experimentally induced endotoxemia. International Journal of Veterinary Science. 2015; 4: 199-205.
[47] Brodzki P, Kostro K, Krakowski L, Marczuk J. Inflammatory cytokine and acute phase protein concentrations in the peripheral blood and uterine washings of cows with subclinical endometritis in the late postpartum period. Veterinary Research Communications. 2015; 39: 143-149.
[48] Sevimli K, Kuş FS. Acute-phase proteins and Interleukin-6 (IL-6) in cattle infected with subclinical parasitic infections. Acta Scientiae Veterinariae. 2015; 43: 1271.
[49] Carroll JA, Arthington JD, Chase CC. Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves. Journal of Animal Science. 2009; 87: 4167-4172.
[50] Kırbas A, Kandemir FM, Celebi D, Hanedan B, Timurkan MO. The use of inflammatory markers as a diagnostic and prognostic approach in neonatal calves with septicaemia. Acta Veterinaria Hungarica. 2019; 67: 360-376.
[51] Moreland LW, Scott SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, Ettlinger RE, Cohen S, Kopman WJ, Mohler K, Widmer MB, Blosch CM. Treatment of rheumatoid arthritis with a recombinant human TNF receptor (p75)-Fc fusion protein. The New England Journal of Medicine. 1997; 337: 141-147.