Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Akyüz E, Sezer M, Kuru M, Naseri A (2022).** Changes in Hematology, Some Clinical Biochemical Parameters and Mineral Levels in Neonatal Calves with Sepsis due to Diarrhea. *Van Vet J*, 33(1), 26-30. DOI: https://doi.org/10.36483/vanvetj.1066050

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

# Changes in Hematology, Some Clinical Biochemical Parameters and Mineral Levels in Neonatal Calves with Sepsis due to Diarrhea

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Received: 31.01.2022 Accepted: 06.03.2022

**ABSTRACT** In this study, it was aimed to evaluate hematology, some clinical and biochemical parameters, as well as mineral levels in calves with neonatal sepsis caused by diarrhea. In this study, 30 calves that were 0-10 days old, who did not receive any treatment and who met the criteria for diarrhea and sepsis within 24 hours at the latest, constitute the sepsis group, and 20 healthy calves in the same age group constitute the control group. Venous blood samples were taken from calves with sepsis before treatment and once from healthy calves. The mean heart rate per minute and respiratory rate were determined higher in the group with sepsis than in the control group. In addition, neutrophil counts were found to be higher in the sepsis group compared to the control group. Erythrocyte count and mean erythrocyte volume were found to be low. While the levels of alanine aminotransferase, aspartate aminotransferase, urea and creatinine were statistically significantly higher in the group with sepsis compared to the control group, magnesium concentrations were lower ($p<0.05$). As a result, it was determined that biochemical parameters increased and mineral levels decreased in relation to liver and kidney dysfunction in neonatal calves with diarrhea-induced sepsis. The reason for these data to appear may be the result of dehydration from diarrhea. For this reason, it is important in the treatment to ensure normal tissue perfusion, especially by adding minerals with low levels and performing appropriate fluid therapy. We think that the chance of survival of calves with sepsis will increase by ensuring tissue perfusion and restoring enzyme levels to normal.

***Keywords:*** *Calf, Minerals, Neonatal sepsis.*

**ÖZ**

## İshal Kaynaklı Sepsisli Neonatal Buzağılarda Hematoloji, Bazı Klinik Biyokimyasal Parametreler ve Mineral Düzeylerinin Değişimi

Sunulan çalışmada ishal kaynaklı neonatal sepsisli buzağılarda hematolojik, klinik biyokimyasal parametreler ve bazı mineral düzeylerinin değerlendirilmesi amaçlanmıştır. Çalışmanın hayvan materyalini 0-10 günlük yaşta, herhangi bir tedavi uygulanmamış ve sepsis kriterlerini sağlayan 30 ishalli buzağı sepsisli grubu ve aynı yaş grubunda bulunan 20 sağlıklı buzağı kontrol grubunu oluşturdu. Sepsisli buzağılardan tedavi öncesi ve sağlıklı buzağılardan bir kez venöz kan örnekleri alındı. Sepsisli buzağıların dakikadaki kalp frekansı ve solunum sayıları kontrol grubuna göre yüksek olduğu belirlendi. Hematolojik analizlerde sepsisli grupta nötrofil sayısı kontrole göre yüksek bulunurken, eritrosit sayısı ve ortalama eritrosit hacmi düşük bulundu. Biyokimyasal analizlerde ise sepsisli grupta alanin aminotransferaz, aspartat aminotransferaz, üre ve kreatinin düzeyleri kontrol grubuna göre istatistiksel olarak önemli oranda yüksek, magnezyum konsantrasyonları düşüktü ($p<0.05$). Sonuç olarak ishal kaynaklı sepsisli neonatal buzağılarda karaciğer ve böbrek disfonksiyonu ile ilişkili olarak biyokimyasal parametrelerin yükseldiği, minaral düzeylerinin düştüğü belirlendi. Elde edilen verilerin ortaya çıkmasında ishal kaynaklı dehidrasyonun etkili olabileceği öngörülmüştür. Bu nedenle tedavide özellikle seviyesi düşen minerallerin eklenmesi ve uygun sıvı tedavisi yapılarak normal doku perfüzyonunun sağlanması önemlidir. Doku perfüzyonu sağlanarak, enzim seviyelerinin normale döndürülmesi ile sepsisli buzağıların yaşama şansının artacağını düşünmekteyiz.

***Anahtar Kelimeler:*** *Buzağı, Mineraller, Yenidoğan sepsisi.*

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## INTRODUCTION

Serious fluid and electrolyte losses occur in calves with diarrhea, resulting in death (Fisher and De La Fuente 1972, Aydoğdu et al. 2018). Neonatal sepsis is a serious cause of mortality, especially in calves with insufficient colostrum intake. This situation poses a serious problem for calves in their early life stages (Akyüz et al. 2017; Basoglu et al. 2018; Akyüz et al. 2019; Akyüz and Gökce 2021).

The etiology of sepsis is complex. Mortality usually results from diarrhea, respiratory distress, and multiple organ failure (Chatre et al. 2017). Neonatal calf diseases can be caused by a single factor, or they can occur in a mixed form as a result of the combination of many factors (Erkılıç et al. 2019; Akyüz 2020). Viral, bacterial, and protozoal factors are the most important agents in neonatal calves (Ok et al. 2009; Lorenz et al. 2011; Akyüz and Gökce 2021; Akyüz and Kükürt 2021). Although these agents are orally transmitte, contamination with non-hygienic can also occur in the omphalogen or aerogenous way (Trefz et al. 2013). It activates the live defense mechanisms against the inflammatory situation created by the infectious agents. Systemic inflammatory response syndrome (SIRS) against infectious agents as a defense response and sepsis as a complication may develop. Some symptoms develop after occurring sepsis. Some of these findings can be counted as decreased interest in the environment, anorexia, and diarrhea. Apart from these, the severity of symptoms may vary due to individual differences. Some of these findings can be listed as weakening of the sucking reflex, depression, lethargy, decrease in body temperature, increase in respiratory rate, changes in capillary refill time and dehydration (House et al. 2015; Bonelli et al. 2018; Akyüz 2020).

There are certain criteria when determining sepsis and SIRS. The presence of at least two of these criteria, including hypothermia or hyperthermia, tachycardia, tachypnea, increased arterial partial carbon dioxide pressure, leukopenia or leukocytosis, band neutrophil formation up to 10%, is considered as SIRS. If the presence of infection also accompanies SIRS, it is considered as sepsis (Fecteau et al. 2009; Sen and Constable 2013; Yıldız et al. 2018; Naseri et al. 2019; Beydilli and Gökçe 2019; Akyüz and Gökce 2021). Bacteremia, viremia and endotoxemia can lead to organ failure and death. Bacterial infection (Escherichia coli), viral infections (rotavirus and coronavirus), and parasitic infection (cryptosporidiosis) can cause sepsis and increase the mortality rate in newborn calves (Chatre et al. 2017; Basoglu et al. 2018). Although different treatment options have been developed, the mortality rate in sepsis remains high (Novelli et al. 2010).

In this study, it was aimed to evaluate hematology, some clinical and biochemical parameters, as well as mineral levels in calves with neonatal sepsis caused by diarrhea. In addition, study data may give an idea in terms of determining the major factors causing diarrhea-related sepsis in neonatal calves.

## MATERIAL AND METHODS

### Ethical Declaration

This study was carried out after the approval (KAU-HADYEK 2022-014) of Kafkas University Animal Experiments Local Ethics Committee.

### Animals

#### Septic calves

Thirty calves, 0-10 days old were enrolled in the study. All the calves included in the study met the criteria for diarrhea and sepsis within 24 hours. Calves with congenital abnormalities, asphyxia, received any treatment were excluded from the study.

#### Healthy calves

Twenty healthy calves 0-10 days old enrolled in the study. Calves with congenital abnormalities, asphyxia, diarrhea, and infection suspicion were excluded from the study.

### Procedures

Rapid test kit (BoviD-5 Ag Test Kit®, Bionote Inc., Korea) was used to detect the etiological agent in the feces of calves suspected of neonatal sepsis. SIRS and sepsis criteria were evaluated after initial examination for establishing depression, diarrhea, decreased or no sucking reflex, dehydration, constant urge to lie down. According to this evaluation, neonatal calf SIRS criteria are stated below (Fecteau et al. 2009; Sen and Constable 2013; Yıldız et al. 2018; Akyüz and Gökce 2021); body temperature >39.5 °C or <37 °C, Heart rate (HR) <100 or >160 per minute, respiratory rate (RR) >45 per minute, leukocyte count >12×103/μL or <4×103/μL. The presence of at least 2 of the specified criteria with presence or suspicion of infection was considered as sepsis and included in the study. Blood samples were taken from the calves with sepsis before treatment and in healthy calves once by using Vena jugularis holder and compatible sterile needle tip (Vacuette®, Greiner Bio-One GmbH, Austria). Blood samples were taken into vacuum gel serum tubes (BD Vakutainer®, BD, UK) for serum biochemistry analyses, and vacuum EDTA blood tubes for hematological measurements (BD Vakutainer®, BD, UK). All hematological analyzes were performed within 15 minutes by using a complete blood count device (VG- MS4e®, Melet Schloesing, France). Blood samples taken into vacuum gel serum tubes were kept at room temperature for about 1 hour and then centrifuged at 3000 rpm for 10 minutes (Hettich Rotina 380R®, Hettich, Germany) to extract serum samples. Calcium (mg/dL), magnesium (mg/dL), phosphorus (mg/dL), urea (mg/dL), creatine (mg/dL), lactate dehydrogenase (IU/L), alanine aminotransferase (IU/L), aspartate aminotransferase (IU/L), and alkaline phosphatase (IU/L) concentrations were measured by fully automatic biochemistry device (Mindray BS120®, Mindray Medical Technology, Istanbul, Turkey).

### Statistical Analysis

SPSS 25 (IBM Corp® 2017) statistical program was used to evaluate the data. The Shapiro-Wilk test was used to determine the normality of variables and the homogeneity of variances. All variables had not normal distribution. To compare the data of calves with sepsis and healthy calves Mann-Whitney U test was preferred and data were presented as median (min, max) and mean±standard error of mean (SEM). Statistical significance was considered as $p<0.05$.

## RESULTS

In the present study, according to the rapid test kits results which were applied on stool samples of calves with sepsis; *E. coli* in 12 calves, coronavirus in 7 calves, rotavirus in 3 calves, cryptosporidium in 1 calf, *E. coli* + coronavirus in 2 calves, *E. coli* + rotavirus in 1 calf, coronavirus + rotavirus in 3 calves and *E. coli* + coronavirus + rotavirus mixed in 1

calf were detected. It was determined that all of the calves in the sepsis group had varying degrees of dehydration, mental depression, no sucking reflex, and the calves could not stand up and had a weak interest in the environment. In addition, it was recorded that 12 of the calves in the sepsis group did not receive enough or no colostrum intake.

The clinical findings, hematology, some clinical biochemical parameters, and mineral levels of the calves with sepsis and control group were presented in Table 1. HR and RR were found to be significantly higher in the sepsis group than in the control group (p=0.001, p=0.002, respectively; Table 1). In addition, although there was no statistical significance, the rectal temperature values of the sepsis group were higher than the control group (Table 1).

Among the hematological parameters, the granulocyte count was found to be significantly higher in the sepsis group compared to the control group (p=0.005), while the erythrocyte count and mean erythrocyte volume were found to be significantly lower (p=0.044, p=0.001, respectively; Table 1).

Among biochemical parameters; alanine aminotransferase, aspartate aminotransferase, urea and creatine concentrations were found to be statistically higher in the sepsis group compared to the control group (p=0.001, Table 1). Although calcium, magnesium and phosphorus concentrations were lower in the calves with sepsis compared to the healthy calves, a statistically significant difference was found only for magnesium concentration (p=0.005). No statistically significant difference was found in other parameters in our study.

**Table 1.** Clinical and hematological findings, some clinical biochemical parameters and mineral levels of calves with sepsis and control group.

| Parameters | Sepsis (n=30) Mean ± SEM (Min-Max) | Control (n=20) Mean ± SEM (Min-Max) | p value |
|---|---|---|---|
| **Clinical Findings** | | | |
| Rectal temperature (°C) | 39.40±0.38 (35.20-41.20) | 38.55±0.18 (38.20-39.10) | 0.100 |
| Heart beats/min | 132.00±8.26 (72.00-220.00) | 80.00±4.70 (66.00-120.00) | <0.001 |
| Breaths/min | 51.00±4.05 (24.00-82.00) | 30.00±1.77 (24.00-40.00) | 0.002 |
| **Hematology** | | | |
| Total leukocytes count (×10³/µL) | 11.39±1.16 (5.61-21.16) | 8.03±1.65 (7.04-14.22) | 0.067 |
| Lymphocytes count (x10³/µL) | 1.97±0.25 (1.22-5.70) | 3.34±0.51 (1.24-4.58) | 0.091 |
| Monocytes count (x10³/µL) | 0.29±0.07 (0.20-1.12) | 0.32±0.12 (0.13-1.10) | 0.948 |
| Granulocytes count (x10³/µL) | 9.21±0.87 (3.66-16.11) | 4.78±1.46 (1.90-7.40) | 0.005 |
| Red blood cell count (x10⁶/µL) | 6.06±0.37 (4.17-8.86) | 7.84±0.27 (6.46-9.51) | 0.044 |
| Mean red cell volume (fL) | 41.80±0.38 (37.70-43.80) | 43.90±0.91 (35.90-47.70) | <0.001 |
| Hematocrit (%) | 28.90±2.18 (16.20-39.20) | 37.15±2.04 (23.10-47.10) | 0.074 |
| Hemoglobin (g/dL) | 7.50±0.60 (5.30-12.40) | 9.80±0.42 (6.80-11.70) | 0.076 |
| Platelet count (x10³/µL) | 340±32.46 (68-525) | 401±23.73 (203-677) | 0.109 |
| **Clinical Biochemical Parameters** | | | |
| Alanine aminotransferase (IU/L) | 39.75±5.71 (29.30-139) | 15.25±1.11 (8.10-19.60) | <0.001 |
| Aspartate aminotransferase (IU/L) | 102.10±11.71 (64.10-285.00) | 40.65±5.04 (14.80-66.30) | <0.001 |
| Alkaline phosphatase (IU/L) | 182.20±13.86 (83.60-380.20) | 190.10±29.40 (80.40-333.20) | 0.846 |
| Creatine (mg/dL) | 3.50±0.19 (2.20-5.50) | 1.45±0.08 (1.30-2.00) | <0.001 |
| Urea (mg/dL) | 63.30±7.51 (39.02-139.02) | 27.39±3.91 (18.12-51.36) | <0.001 |
| Lactate dehydrogenase (IU/L) | 758.15±35.06 (428.30-1026.60) | 822.30±82.12 (560.80-1419.20) | 0.214 |
| **Minerals** | | | |
| Calcium (mg/dL) | 10.12±0.23 (9.07-11.45) | 10.38±0.36 (8.98-12.47) | 0.846 |
| Phosphorus (mg/dL) | 5.60±0.50 (4.53-7.41) | 7.01±0.45 (4.06-8.49) | 0.198 |
| Magnesium (mg/dL) | 2.76±0.13 (2.51-4.32) | 3.60±0.18 (2.89-5.29) | 0.005 |

SEM: Standard error of mean. n: Number of calves in the groups. Min: Minimum value within the group. Max: Maximum value within the group. p<0.05: Statistically significant.

## DISCUSSION AND CONCLUSION

Infective agents such as *E. coli*, rotavirus, coronavirus, and cryptosporidium that leading to sepsis isolating more frequently in neonatal calves with diarrhea. They can cause sepsis alone or in a mixed form. It has been reported that in the development of sepsis, in addition to these factors, failure to comply with hygiene rules, insufficient cleaning of equipment contaminated with the infective agent, insufficient colostrum or poor quality may lead to sepsis (Tyler et al. 1999; Ok et al. 2009; Lorenz et al. 2011; Çitil and Gökce 2013; Akyüz and Gökce 2021). In the present study, the determination of *E. coli*, rotavirus, coronavirus, and cryptosporidium agents in the results obtained from the fecal samples of the calves in the sepsis group, and the information that 40% (12/30) did not receive adequate amounts of colostrum were found to be compatible with other studies.

According to the previous studies, clinical findings such as depression, decreased interest in the environment, increase in respiratory and heart rates, increase, or decrease in body temperature, decrease in appetite were reported to occur in sepsis (Aldridge et al. 1993; Fecteau et al. 1997; Çitil and Gökçe 2013; Akyüz et al. 2016; Akyüz 2020; Naseri and Ider 2021; Akyüz and Gökce 2021). Similarly, findings such as the absence of sucking reflex, depression, decreased interest in the environment, and inability to stand were found in calves with sepsis in our study. In addition, respiratory and heart rates were found to be significantly higher, and the rectal temperature was found to be lower in the sepsis group compared to the control group. These findings were consistent with the results of other studies. Host defense and immune mechanisms and trying to compensate for the decreased amount of fluid in the body as a result of sepsis and dehydration (Naseri 2017; Akyüz and Gökce 2021) can explain this situation.

While leukocytosis or leukopenia may occur in calves with sepsis, sometimes there may be no change in the total leukocyte count (Naseri 2017; Akyüz and Gökce 2021). In the present study, the total leukocyte count of the sepsis group was found to be higher than the control group, although there was no statistical difference. The reason for the increase in total leukocytes was thought to be due to the increase in the number of granulocytes because of widespread inflammation.

In neonatal calves, especially in the case of diarrhea, there may be significant changes in hematological and serum biochemistry (Uzlu et al. 2010). Severe fluid loss occurs in sepsis due to loss of appetite and decreased water intake, and diarrhea. Renal dysfunction occurs due to fluid loss and sepsis. As a result of impaired kidney function, serum urea and creatinine values increase (Gökçe and Woldehivet 1999). Fluid loss in calves with diarrhea is the most important reason for increased serum urea and creatinine levels (Dratwa-Chalupnik et al. 2012). In the present study consistent with other studies, urea, and creatinine concentrations of calves in the sepsis group were found to be considerably higher than the control, because of the presence of dehydration, decreased fluid intake due to lack of appetite, and impaired renal function due to the sepsis.

Serum alanine aminotransferase and aspartate aminotransferase concentrations are elevated in neonatal calves with diarrhea (Baser and Civelek 2013; Naseri 2017; Bozukluhan et al. 2017). We thought that the reason for the higher serum alanine aminotransferase and aspartate aminotransferase concentrations in calves with sepsis in our study compared to healthy calves may be due to the deterioration of liver functions due to sepsis. In addition, multi-organ dysfunction caused by sepsis may have caused this increase (Akyüz and Gökce 2021).

One of the first clinical findings in diseased animals is anorexia. Loss of appetite can also occur in serious diseases such as sepsis. Calcium, magnesium, and phosphorus decreases are generally directly related to anorexia (Martin and Lumsden 1987). Magnesium deficiency usually occurs with diarrhea, gastrointestinal disorders, and reduced dietary intake (Elin 1994). In our study, we believe that the low level of these minerals in the sepsis group compared to the control group was related to severe anorexia.

In conclusion, severe liver and kidney dysfunctions caused by SIRS and dehydration in calves with sepsis adversely affect the prognosis. Our findings especially increase in urea, creatinine, alanine, and aspartate aminotransferase concentrations support it. In our acknowledgement it is important to regain impaired kidney and liver functions with appropriate fluid therapy. In addition, we believe that the chance of recovery will be increased by adding calcium, magnesium, and phosphorus to the treatment in the sepsis group.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Idea / Concept: EA
Supervision / Consultancy: EA, MK
Data Collection and / or Processing: EA, MS
Analysis and / or Interpretation: EA, AN
Writing the Article: EA, MS, MK, AN
Critical Review: EA, MS, MK, AN

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