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RESEARCH ARTICLE

The relationship between hemogram parameters and mortality in neonatal calves with diarrhea

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Neonatal ishalli buzağılarda hemogram parametrelerinin mortalite ile ilişkisi

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Öz

Amaç: Bu çalışmada, farklı etiyolojik etkenlere bağlı gelişen neonatal buzağı ishalinde hemogram parametrelerinin mortalite ile ilişkisinin ortaya konulması amaçlandı.

Gereç ve Yöntem: Çalışmaya 50 ishalli buzağı dahil edildi. Buzağılar hayatta kalma durumlarına göre iyileşen ve ölen buzağılar olarak 2 gruba ayrıldı. Ayrıca dışkı hızlı test kitinde ishale neden olan etiyolojik etken temelinde bakteriyel (*E. coli*), viral (*Rotavirüs* ve *Koronavirüs*) ve paraziter (*Cryptosporidium parvum*) olmak üzere 3 alt gruba ayrıldı. Total lökosit (WBC), lenfosit (Lym), monosit (Mon), granülosit (Gra), eritrosit (RBC), ortalama eritrosit hacmi (MCV), hematokrit (HCT), ortalama eritrosit hemoglobini (MCH), ortalama eritrosit hemoglobin konsantrasyonu (MCHC), eritrosit dağılım genişliği (RDW), hemoglobin (Hb) ve trombosit (PLT) düzeyleri ölçüldü.

Bulgular: Etiyolojiden bağımsız olarak, ölen buzağıların MCHC düzeyleri hayatta kalan buzağılardan daha düşüktü (cut-off: 32.15 g/dL, 68% sensitive, 62% spesifite, p = 0.041). Ayrıca, viral etiyolojiye sahip, ölen buzağıların MCHC düzeyleri, hayatta kalan buzağılardan daha düşüktü (cut-off: 31.75 g/dL, 85% sensitive, 75% spesifite, p = 0.029). *E. coli* etiyolojisine sahip ölen buzağıların RBC düzeyleri, hayatta kalan buzağılardan daha düşüktü (cut-off: 9.27 $\times 10^3$ cells/mL, 83% sensitive, 84% spesifite, p = 0.024). *C. parvum* ile enfekte buzağıların hemogram parametrelerinde istatistiksel olarak anlamlı farklılık yoktu.

Öneri: MCHC ve RBC düzeyleri, yenidoğan buzağı ishalinde mortalite tahminde istatistiksel olarak anlamlıydı. Hemogram parametrelerinin diğer kan parametreleri (kan gazı vb.) ile birlikte değerlendirilmesi mortalite tahmininde daha faydalı olabilir.

Anahtar kelimeler: Buzağı ishali, mortalite, hemogram, etiyoloji

Abstract

Aim: The objective of the present study was to reveal the relationship between hemogram parameters and mortality in neonatal calves with diarrhea from different etiological origins.

Materials and Methods: Fifty calves with diarrhea were enrolled in the study. The calves were divided into 2 groups as surviving and non-surviving calves. In addition, on the basis of the stool quick test and etiological agent, it was divided into 3 subgroups: bacterial (*E. coli*), viral (*rotavirus* and *coronavirus*), and parasitic (*Cryptosporidium parvum*). Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), and thrombocyte (PLT) were measured.

Results: Regardless of etiology, MCHC levels of non-surviving calves were lower than those of surviving calves (cut-off: 32.15 g/dL, 68% sensitivity, 62% specificity, p = 0.041). Also, MCHC levels of non-surviving calves with viral etiology were lower than those of surviving calves (cut-off: 31.75 g/dL, 85% sensitivity, 75% specificity, p = 0.029). RBC levels of non-surviving calves with *E. coli* etiology were lower than those of surviving calves (cut-off: 9.27 $\times 10^3$ cells/mL, 83% sensitivity, 84% specificity, p = 0.024). There was no statistically significant difference in hemogram parameters of calves infected with *C. parvum*.

Conclusion: MCHC and RBC levels were statistically significant in the estimation of mortality in neonatal calf diarrhea. The evaluation of hemogram parameters together with other blood parameters (blood gas, etc.) may be more useful in estimating mortality.

Keywords: Calf diarrhea, mortality, hemogram, etiology

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Introduction

Neonatal calf diarrhea is recognized as one of the major problems for the cattle industry all over the world. Calf diarrhea is the most common cause of morbidity and mortality in calves in the pre-weaning period and accounts for 56.4% of calf mortality in the USA (USDA 2014). In severely infected herds, calf mortality rates can reach to 100%. Other causes of economic losses other than mortality in diarrhea are growth retardation, chronic diseases, and high treatment and care costs (Ok et al 2009, Morris et al 2011, Gibbons et al 2014).

Neonatal calf diarrhea is a multifactorial disease with a complex etiology caused by infectious and non-infectious factors (host, management, nutritional, and environmental factors) (Foster and Smith 2009, Blanchard 2012, Cho and Yoon 2014, Aydoğdu et al 2019). The main etiological agents of primary diarrhea in the neonatal period are enterotoxigenic *E. coli* (ETEC), *rotavirus*, *coronavirus*, *Cryptosporidium parvum* (*C. parvum*), and Salmonella serotypes. Infectious agents cause similar clinical symptoms, but use different inflammatory mechanisms, causing a wide range of severity, prognosis, and mortality rates (Akyüz et al 2017). The K99 strain associated with enterotoxigenic *E. coli* produces a heat-stable enterotoxin (STa) and induces intestinal secretion, resulting in secretory diarrhea. The ability of this bacterial strain to adhere to the intestinal mucosa depends on the age of the calf, and clinical disease is more common in calves less than one week old (Rodostits et al 2007, Smith 2009, Cho and Yoon 2014).

Viral and parasitic pathogens, which play a role in the etiology of calf diarrhea, cause villous atrophy of the intestines, reducing the absorption of water and electrolytes from the intestines and causing osmotic diarrhea (Hodges and Gill 2010). *Rotaviruses* usually affect 2-week-old calves, while *coronaviruses* affect 1-month-old calves. Sugars (glucose and lactose) that cannot be absorbed and digested in the intestinal lumen cause osmosis and thus fluid withdrawal into the intestinal lumen. *Coronaviruses* also destroy crypt cells in the small and large intestines, causing mucous and bleeding (Smith 2009). *Cryptosporidium parvum* is a protozoan parasite that reproduces sexually and asexually in intestinal cells. It causes atrophy of columnar epithelial cells and loss of microvilli, resulting in malabsorptive diarrhea. In this type of infection, prostaglandins induce chlorine (Cl^-) and bicarbonate (HCO_3^-) secretion, while impairing sodium chloride (NaCl) absorption (Foster and Smith 2009, Cho and Yoon 2014).

There are many factors affecting mortality in newborn calf diarrhea, which can be caused by a variety of etiological factors. Biochemical parameters and damage-specific biomarker analyses that take into account various blood gas parameters and organ dysfunctions have been developed to predict the prognosis.

Although hemogram parameters are used separately by researchers in human medicine for mortality, morbidity, and disease severity or differential diagnosis (Senyurt et al 2018, Kaya et al 2021), using 12 hemogram parameters [total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), and thrombocyte (PLT)] for mortality prediction in calf diarrhea has not been found in the literature. In the present study, it was aimed to reveal the relationship between hemogram parameters and mortality in neonatal calves with diarrhea.

Material and Methods

Calves with diarrhea

A convenience sample of 50 calves with diarrhea, 0-20 days-old, and different breeds were enrolled in the study. All calves included in the study were admitted within 24 hours of the onset of diarrhea, and did not receive any veterinary intervention. Calves were determined to be diarrhetic originating from infectious agents based on clinical examinations and laboratory findings. Co-infected calves, different diseases or complications were excluded from the study.

Clinical examination and hospitalization

Breed, age, and gender information of all calves included in the study were recorded. The calves were hospitalized in the intensive care unit for 72 hours, and their daily general health conditions and clinical examinations (heart rate, respiratory rate, and rectal body temperature) were checked. During the hospitalization, treatment applications for the etiological factor and fluid therapy were performed.

Determination of the etiological factor

Stool rapid antigen (BoviD-5 Ag®, BioNote Inc, Gyeonggi-do, Korea) test was used to determine the etiologic agent (*E. coli* K99, *rotavirus*, *coronavirus*, *C. parvum*) in calves with diarrhea. Additionally, the diagnosis of *C. parvum* was confirmed by microscopic examination of stool (Modified Ziehl Neelsen staining technique).

Study groups

The hospitalized calves were divided into 2 groups according to their survival status as surviving calves and non-surviving calves. In addition, on the basis of the etiological agent, it was divided into 3 subgroups: bacterial (*E. coli*, n:18), viral (*rotavirus* and *coronavirus*, n:15), and parasitic (*C. parvum*, n:17).

Collection of blood samples

Blood samples were collected from the calves at the time of admission. Blood samples for complete blood count (CBC) were taken from the jugular vein. Tubes with K_3EDTA were used for CBC analysis. Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), and thrombocyte (PLT) measurements were performed using an automatic cell counter (MS4e, Melet Schlosing Laboratories, France).

Treatment protocol

Daily treatment was performed depending on the etiological agent and fluid/electrolyte imbalance. Fluid therapy was calculated and given according to the clinical examination and hydration status. Ceftiofur (2.2 mg/kg, IM, q 24 h, Excenel flow®, Zoetis) was administered as an antibacterial treatment against *E. coli* and secondary bacterial infection in the viral group. Halofuginone (0.1 mg/kg, PO, q 24 h, Halocur®, MSD) was administered to calves infected with *C. parvum*. In addition, supportive treatment applications of vitamins including a vitamin A, D3, and E combination (1 mL, vitamin A; 500,000 U, vitamin D3; 75,000 U and vitamin E; 50 mg, IM once, Ademin®, DİF) and vitamin C (3 mL, SC q 24 h, Vita-C Vetoquinol®, Novakim). Also, hyperimmune serum (15 mL, SC, once, Septicol®, Vetal) were also performed.

Statistical analysis

SPSS 25 (IBM Corp®, 2017) statistical program was used to evaluate the data. The Kolmogorov-Smirnov test was used to determine the normality of variables and the homogeneity of variances. Since the variables do not have a normal distribution, the study data are presented as median (min/max). Receiver operating characteristic (ROC) analysis was performed to determine the prognostic cut-off value, sensitivity, and specificity of variables in non-survivor and survivor calves with diarrhea. Statistical significance was considered as $p < 0.05$.

Results

A total of 50 neonatal diarrhetic, 28 male and 22 female calves with different breeds (27 Holstein, 15 Simental, 6 Brown Swiss, and 2 Belgian Blue) were enrolled in this study. Moderate or severe dehydration, lack or absence of sucking reflexes, prolongation of capillary filling time, sternal or comatose stance, hypothermia, dryness of the mucous membranes, and depression were observed in all calves with diarrhea. 31 (62%) of the 50 diarrheal calves included in the study survived and 19 (38%) non-survived. The hemogram analysis results of the surviving and non-surviving calves with diarrhea are presented in Table 1. MCHC levels of non-survivor calves were lower than those of surviving calves ($p = 0.041$). No statistically significant

Table 1. The hematological variables in survivor and non-survivor calves with diarrhea, with data being expressed as median and range in parentheses

Variable	Survivors (n=31)	Non-survivors (n=19)	p value
WBC (cells/mL)	18.60 (8.41-38.80)	22.60 (9.36-40.15)	0.549
Lym (cells/mL)	3.95 (1.63-76.88)	7.53 (1.56-37.01)	0.215
Mon (cells/mL)	0.66 (0.26-2.39)	0.56 (0.16-2.28)	0.842
Gra (cells/mL)	12.12 (2.97-21.10)	8.80 (2.98-24.11)	0.976
RBC (×10³ cells/mL)	10.06 (5.63-15.20)	9.28 (7.88-11.14)	0.353
MCV (fl)	38.20 (30.00-45.10)	40.30 (31.60-46.00)	0.165
HCT (%)	37.80 (17.00-57.90)	37.80 (27.30-43.60)	0.624
MCH (pg)	12.40 (10.30-14.10)	12.30 (9.30-15.70)	0.795
MCHC (g/dL)	32.50 (29.00-37.60)	31.10 (23.00-36.70)	0.041
RDW (%)	13.80 (11.20-28.90)	14.00 (11.80-30.80)	0.631
Hb (g/dL)	12.40 (6.40-18.70)	11.70 (7.40-14.20)	0.197
PLT (cells/mL)	376.00 (198.0-1220.0)	370.00 (93.0-1298.0)	0.318

Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), thrombocyte (PLT).

Table 2. The area under the curve (AUC), standard error, confidence interval (95%), optimum cut-off values, respective sensitivity, and specificity of mortality prediction in non-survivor calves with diarrhea

Origin of diarrhea	Variable	AUC	Standard Error	p value	Asymptotic 95% CI		Sensitivity	Specificity	Cut-off Value
					Lower Band	Upper Bound			
Regardless of the etiologic origin	MCHC	0.673	0.083	0.041	0.510	0.836	68	62	32.15
E. coli	RBC	0.826	0.104	0.028	0.623	1.000	83	84	9.27
Viral (Rota-Coronavirus)	MCHC	0.830	0.111	0.032	0.612	1.000	85	75	31.75

Table 3. The hematological variables in survivor and non-survivor calves with *E. coli* origin diarrhea, with data being expressed as median and range in parentheses

Variable	Survivors (n=12)	Non-survivors (n=6)	p value
WBC (cells/mL)	21.36 (17.95-38.80)	14.80 (9.93-36.51)	0.180
Lym (cells/mL)	3.78 (1.63-15.22)	5.68 (2.33-15.37)	0.494
Mon (cells/mL)	0.67 (0.26-1.38)	0.87 (0.35-2.28)	0.291
Gra (cells/mL)	13.52 (6.07-21.10)	9.62 (4.06-21.19)	0.494
RBC (×10³ cells/mL)	10.56 (7.85-13.00)	8.90 (7.92-10.23)	0.024
MCV (fl)	39.30 (33.00-45.10)	40.30 (33.80-44.80)	1.000
HCT (%)	44.60 (30.70-52.40)	37.80 (29.00-39.80)	0.125
MCH (pg)	12.70 (10.80-13.80)	12.90 (9.30-13.80)	0.553
MCHC (g/dL)	32.90 (29.00-34.50)	32.70 (25.50-36.70)	0.892
RDW (%)	12.75 (11.20-27.70)	12.70 (11.80-14.50)	0.494
Hb (g/dL)	13.95 (10.50-16.00)	11.60 (7.40-14.20)	0.083
PLT (cells/mL)	297.50 (198.0-1220.0)	189.50 (93.0-370.0)	0.102

Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), thrombocyte (PLT)

difference was found in the other hemogram parameters ($p > 0.05$). The results of the ROC analysis performed to determine the relationship between hemogram parameters and mortality in calves with diarrhea are presented in Table 2 and Figure 1. As a result of ROC analysis; MCHC cut-off at 32.15 g/dL, area under the curve (AUC) 0.673 (95% confidence interval (CI): 0.510-0.836; $p = 0.041$), with 68% sensitivity and 62% specificity were significant prognostic indicators for mortality in calves with diarrhea.

In this study, *E. coli* in 18 calves, *rotavirus* and *coronavirus* in 15 calves, and *C. parvum* in 17 calves were determined according to stool rapid antigen test. Three subgroups were compared to determine the effect of etiological factors on hemogram parameters and mortality.

Of the 18 *E. coli*-infected calves, 12 (67%) survived, while 6 (33%) died. Hemogram results of surviving and non-surviving diarrhetic calves infected with *E. coli* are

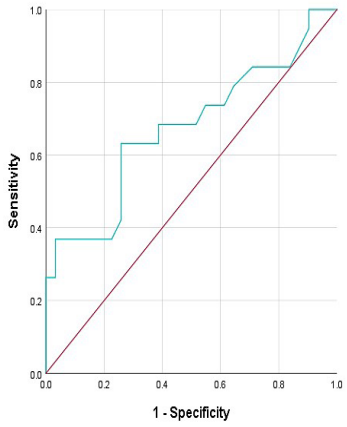

Figure 1. Receiver operating characteristic curve (ROC) analysis for the differentiation between the survivor and non-survivor calves with diarrhea based on the MCHC levels regardless of etiological origin

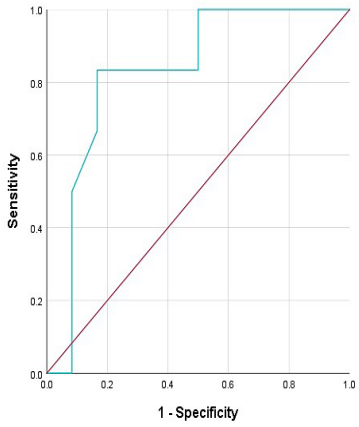

Figure 2. Receiver operating characteristic curve (ROC) analysis for the differentiation between the survivor and non-survivor calves with diarrhea based on the RBC levels in *E. coli* origin diarrhea

presented in Table 3. RBC levels of *E. coli*-infected non-surviving calves were lower than those of surviving calves (p = 0.024). No statistically significant difference was found in other hemogram parameters (p > 0.05). ROC analysis results for determining the relationship between hemogram parameters and mortality in non-surviving *E. coli*-infected diarrhetic calves are presented in Table 2 and Figure 2. As a result of ROC analysis; RBC at cut-off 9.27 $\times 10^3$ cells/mL, AUC 0.826 (95% confidence interval (CI): 0.623-1.000; p = 0.028) with 83% sensitivity and 84% specificity was found to be a significant prognostic indicator in calves with *E. coli* diarrhea.

According to the stool rapid antigen test kit, 8 of 15 calves with viral etiology were infected with *rotavirus* and 7 with *coronavirus*. While 8 (53%) of the calves in this group survived, 7 (47%) non-survived. Of the non-survivor calves, 5 were infected with *coronavirus* and 2 with *rotavirus*. The hemogram results of the surviving and non-surviving diarrhetic calves with viral etiology are presented in Table 4. MCHC levels of non-surviving calves with viral etiology were lower than those of surviving calves (p = 0.029). No statistically significant difference was found in other hemogram parameters (p > 0.05). ROC analysis results for determining the relationship between hemogram parameters and mortality in calves with diarrhea who died with viral etiology are presented in Table 2 and Figure 3. As a result of ROC analysis; MCHC cut-off at 31.75 g/dL, area under the curve (AUC) 0.830 (95% confidence interval (CI): 0.612-1.000; p = 0.032) with 85% sensitivity and 75% specificity was found to be a significant prognostic indicator of mortality in calves with viral diarrhea.

Of the 17 calves infected with *C. parvum*, 11 (65%) survived, while 6 (35%) non-survived. The hemogram results of surviving and non-surviving diarrhetic calves are presented in Table 5. There was no statistically significant difference in hemogram parameters of calves infected with *C. parvum* (p > 0.05).

Table 4. The hematological variables in survivor and non-survivor calves with viral origin diarrhea, with data being expressed as median and range in parentheses

Variable	Survivors (n=8)	Non-survivors (n=7)	p value
WBC (cells/mL)	19.47 (8.41-37.08)	22.66 (10.95-40.15)	0.336
Lym (cells/mL)	5.39 (2.43-76.88)	6.37 (1.56-37.01)	0.694
Mon (cells/mL)	0.81 (0.47-2.03)	0.65 (0.16-2.08)	0.336
Gra (cells/mL)	9.73 (5.51-19.14)	6.41 (2.98-24.11)	1.000
RBC ($\times 10^3$ cells/mL)	9.64 (7.09-15.20)	9.52 (7.88-11.13)	0.955
MCV (fl)	35.15 (30.00-42.70)	41.10 (35.00-46.00)	0.054
HCT (%)	37.35 (23.10-57.90)	39.40 (32.90-43.40)	0.867
MCH (pg)	11.20 (10.30-13.70)	12.20 (9.40-15.70)	0.463
MCHC (g/dL)	32.05 (29.90-34.60)	29.20 (23.00-34.20)	0.029
RDW (%)	18.40 (13.10-28.90)	14.50 (13.40-30.80)	0.613
Hb (g/dL)	11.35 (7.40-18.70)	11.50 (9.50-13.50)	1.000
PLT (cells/mL)	572.00 (217.0-715.0)	411 (134.0-1289.0)	0.867

Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), thrombocyte (PLT)

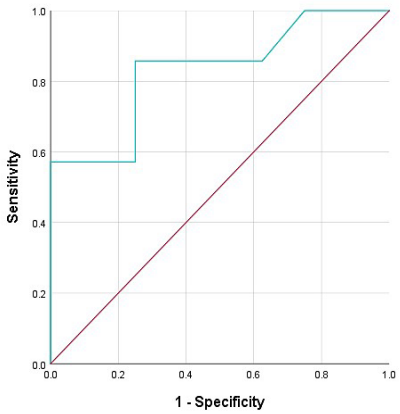

Figure 3. Receiver operating characteristic curve (ROC) analysis for the differentiation between the survivor and non-survivor calves with diarrhea based on the MCHC levels in viral origin diarrhea.

Table 5. The hematological variables in survivor and non-survivor calves with *C. parvum* origin diarrhea, with data being expressed as median and range in parentheses

Variable	Survivors (n=11)	Non-survivors (n=6)	p value
WBC (cells/mL)	16.25 (10.90-24.42)	25.16 (9.36-29.82)	0.149
Lym (cells/mL)	3.93 (2.45-10.71)	9.17 (2.21-10.96)	0.462
Mon (cells/mL)	0.48 (0.29-2.39)	0.50 (0.18-1.25)	0.808
Gra (cells/mL)	11.32 (2.97-18.06)	16.58 (3.36-21.60)	0.301
RBC (×10³ cells/mL)	9.32 (5.63-13.33)	9.76 (8.66-11.14)	0.404
MCV (fl)	38.20 (30.20-40.70)	37.15 (31.60-44.10)	0.808
HCT (%)	33.10 (17.00-53.90)	38.75 (27.30-43.60)	0.525
MCH (pg)	12.40 (11.20-14.10)	12.10 (10.00-12.70)	0.404
MCHC (g/dL)	34.00 (30.10-37.60)	31.20 (28.60-34.60)	0.256
RDW (%)	14.20 (12.50-21.10)	14.20 (12.50-17.30)	0.884
Hb (g/dL)	11.60 (6.40-17.40)	12.20 (9.00-13.50)	0.591
PLT (cells/mL)	468.00 (331.0-1002.0)	468.50 (125.0-550.0)	0.660

Total leukocyte (WBC), lymphocyte (Lym), monocyte (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hemoglobin (Hb), thrombocyte (PLT)

Discussion

In the present study, MCHC levels were found to be lower in non-surviving calves with diarrhea compared to surviving calves with diarrhea, and it was found that the cut-off level of 32.15 g/dL was important in estimating mortality with 68% sensitivity and 62% specificity. RBC at cut-off 9.27 ×10³ cells/mL with 83% sensitivity and 84% specificity in *E. coli* diarrheal calves, MCHC at cut-off 31.75 g/dL with 85% sensitivity and 75% specificity, in calves with diarrhea with viral etiology had significant in mortality prediction. Our finding demonstrated that hemogram parameters were unconvincing in determining mortality in neonatal calf diarrhea.

Diarrhea in newborn calves is considered to be one of the most important diseases because it leads to economic losses caused by mortality, treatment costs and reduced growth rate (Maes et al 2003). Enterotoxigenic *E. coli*, *rotavirus*, *coronavirus*, and *C. parvum* are recognized worldwide as the four main pathogens causing diarrhea in young calves less than 1-month-old. These organisms are responsible for approximately 75-90% of intestinal infections in young calves worldwide (Mayameei et al 2010).

Regardless of the etiological factor, fluid, and electrolyte losses in calves with diarrhea cause dehydration, metabolic acidosis, hyperlactatemia, hypoglycemia, hyperkalemia, hyponatremia, azotemia, hypotension, and sepsis (Trefz et al 2013, Lorenz and Gentilek 2014, Trefz et al 2016, Trefz et al 2017, Ok et al 2020, Naseri and Ider 2021). According to the severity of dehydration and acidosis, clinical findings such as difficulty in standing, loss of sucking reflex, and depression are observed in patients (Fecteau et al 2009, Lorenz and Gentilek 2014, Heller and Chigerwe 2018, Ok et al 2020). In the present study, similar to previous reports, moderate or severe dehydration, loss of sucking reflex, prolonged capillary filling time, difficulty in standing, hypothermia, cold extremities, pallor, and depression were observed in all calves with diarrhea.

Singh et al (2009) reported that the mortality rate in calves varies between 12.5-30%, and 80-85% of fatal cases are observed during the first month of life. Regardless of the underlying pathogens or pathophysiological mechanisms of diarrhea, dehydration, acidemia, hyperkalemia, hypoglycemia, septicemia, endotoxemia, hyperlactatemia, impaired cardiovascular and renal function can lead to increase in mortality rate in diarrhetic calves (Trefz et al 2016, Akyüz et al 2017, Trefz et al 2017, Akyüz et al 2022). In the present study, 31 (62%) of the 50 diarrhetic calves survived, while 19 (38%) non-survived. Of the non-survived calves, 6 (31.5%) were in the *E. coli* group, 7 (37%) were in the viral group, and 6 (31.5%) were in the *C. parvum* group. It was observed that regardless of the etiological factors, the mortality occurred because of complications related to diarrhea.

Evaluation of hemogram parameters in neonatal calf diarrhea provides important data about diagnosis, treatment and prognosis (Jones and Alison 2007, Panousis et al 2018, Atçalı and Yıldız 2020). Etiological factors that lead to diarrhea in newborn calves cause changes in leukocyte and erythrocyte levels by different pathogenesis mechanisms (Fecteau et al 2009, Uzlu et al 2010, Atçalı and Yıldız 2020). In viral and bacterial diarrheas, the plasma volume decreases and leading to an increase in HCT value. However, in cases of parasitic origin, due to anemia, it may remain within the normal range or even decrease (Atçalı and Yıldız 2020, Brar et al 2015). Although Seifi et al (2006) reported a significant difference in the HCT and WBC levels of 24 neonatal calves with diarrhea compared to healthy calves, they could not find a significant difference between the hematological parameters of non-survived and survived calves. Atçalı and Yıldız (2020), in a study conducted on 44 neonatal calves with diarrhea, reported that hemogram is important in

patients, but it does not differ according to etiological factors. It is stated that these changes in hemogram parameters may be caused by stress, inflammation due to enteritis, host defense mechanisms against infectious agents, as well as hemoconcentration due to dehydration (Brar et al 2015). In the present study, WBC and Gra levels were higher than the reference ranges in all non-survived and survivor calves, but there was no significant difference in these parameters according to the etiological factor. In our opinion, independent of the etiological factor, these findings are due to the damage in the intestines, inflammation, and systemic effects of sepsis/endotoxemia in calves with diarrhea.

In the present study, non-surviving calves had lower MCHC levels than surviving calves ($p = 0.041$), and the area under the curve (AUC) 0.673 (95% confidence interval (CI): 0.510-0.836; $p = 0.041$) at the cut-off level of 32.15 g/dL with 68% sensitivity and 62% specificity, were found to be a significant prognostic indicator for estimating mortality in calves with diarrhea. Song et al (2020) found that MCHC levels were lower in calves with diarrhea than in healthy calves. Aydoğdu et al (2019) determined that the MCHC of calves with diarrhea was significantly lower compared to survived calves. In a comprehensive study in 1400 calves with diarrhea, MCHC levels were found to be lower in non-survivor calves than in survivors (Trefz et al 2017). In the present study, we think that the decrease in MCHC levels in non-surviving calves, which indicates hypochromic anemia, may be caused by the deficiency of hematopoietic factors, blood loss and low hemoglobin in erythrocytes (Anwar et al 1999, Eglenti et al 2020). At the same time, although it was not statistically significant, MCV levels were lower than the reference ranges in all calves that non-survived. According to previous reports, it can conclude that when these two indicators were evaluated together, this situation can be associated with microcytic hypochromic iron deficiency anemia in newborn calves with diarrhea. In relation to our opinion, it has been demonstrated that iron deficiency is associated with diarrhea in newborn calves (Prodanović et al 2019).

In addition, when we evaluated the groups according to the etiological agents, the MCHC levels of the non-survivor calves with viral etiology were lower than those of the survivor calves ($p = 0.029$) and the cut-off point of 31.75 g/dL, area under the curve (AUC) 0.830 (95% confidence interval (CI): 0.612-1.000; $p = 0.032$) with 85% sensitivity and 75% specificity, was found to be significant prognostic indicator for the prediction of mortality in diarrhetic calves with viral etiology. Also, 5 of the non-survived calves in the viral group were infected with *coronavirus* and 2 with *rotavirus*. In our opinion, it may be related to the fact that *coronaviruses* cause mucus and bleeding because of losing small intestinal villi and crypt cells of the small and large intestines, and the clinical progression of the disease is more severe than other study groups (Cho and Yoon 2014).

It has been reported that a decrease in the number of erythrocytes and an increase in the number of neutrophils and total leukocytes occur in calf diarrhea with bacterial origin. It has been reported that in calves infected with pathogenic *E. coli* or *Salmonella spp*. neutrophilic leukocytosis and a significant decrease in the number of RBCs and hemoglobin developed (Shehta et al 2022). Also, RBC and MCV levels were found to be lower in calves with sepsis compared to healthy calves (Akyüz et al 2022). In the present study, *E. coli*-infected non-surviving calves had lower RBC levels than surviving calves and 83% sensitivity and 84% specificity was found to be a significant prognostic indicator for estimating mortality in diarrheal calves with *E. coli* origin. The low RBC levels of *E. coli*-infected non-survived calves may be related to the fact that during infection, hemolytic bacterial cytotoxins damage the host cells and cause damage to the endothelium of the small vessels, followed by diffuse intravascular coagulation and finally contribute to the anemia (Anzaldi and Skaar 2010, Aly et al 2016).

Conclusion

In conclusion, changes in hemogram parameters in calf diarrhea caused by different etiological factors showed that different inflammatory mechanisms, the pathogenesis of infectious agents, and severity of clinical findings and these variables affect the prognosis and mortality rates. Although MCHC and RBC levels were statistically significant in the estimation of mortality in neonatal calf diarrhea, convincing results could not be obtained. It has been concluded that the evaluation of hemogram parameters together with other blood parameters (blood gas, etc.) may be more useful in estimating mortality.

Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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Ethical Approval

Ethics approval was obtained from Selçuk University Veterinary Faculty Ethics Committee for the study (number of decisions: 2022/05).