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Chloride and lactate as prognostic indicators of calf diarrhea from eighty-nine cases

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ABSTRACT

Importance: Deaths due to neonatal calf diarrhea are still one of the most critical problems of cattle breeding worldwide. Determining the parameters that can predict diarrhea-related deaths in calves is especially important in terms of prognosis and treatment strategies for the disease.
Objective: The primary purpose of this study was to determine mortality rates and durations, survival status, and predictive prognosis parameters based on vital signs, hematology, and blood gas analyses in neonatal diarrheic calves.
Methods: The hospital automation system retrospectively obtained data from 89 neonatal diarrheic calves.
Results: It was found that 42.7% (38/89) of the calves brought with the complaint of diarrhea died during hospitalization or after discharge. Short-term and long-term fatalities were a median of 9.25 hours and a median of 51.50 hours, respectively. When the data obtained from this study is evaluated, body temperature (°C), pH, base excess (mmol/L), and sodium bicarbonate (mmol/L) parameters were found to be lower, and hemoglobin (g/dL), hematocrit (%), lactate (mmol/L), chloride (mmol/L), sodium (mmol/L) and anion gap (mmol/L) parameters were found to be higher in dead calves compared to survivors. Accordingly, hypothermia, metabolic acidosis, and dehydration findings were seen as clinical conditions that should be considered. Logistic regression analysis showed that lactate (odds ratio, 1.429) and Cl^- (odds ratio, 1.232) concentration were significant risk factors associated with death in calves with diarrhea.
Conclusions and Relevance: According to the findings obtained from this study, the determination of lactate and Cl^- levels can be used as an adjunctive supplementary test in distinguishing calves with diarrhea with a good prognosis.

Keywords: Blood gas analysis; lactate; diarrhea; hematology; survival rate

INTRODUCTION

One of the most critical problems of cattle breeding worldwide is neonatal calf mortality [1]. Neonatal calf diarrhea is a disease that occurs predominantly in the first four weeks of life, in which various etiological and predisposing factors play a role [2]. The American National Animal Health Monitoring System (NAHMS) report 2007 reported that 57% of calf deaths

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Conflict of Interest

The authors declare no conflicts of interest.

were due to diarrhea, and most cases occurred in calves less than one-month-old [3]. A similar rate (53.4%) for diarrheal deaths in neonatal calves was reported in Korea [4], and a study conducted in Italy reported the calf mortality rate as 25% [5]. In Türkiye, calf losses due to diarrhea are over 15% in traditional farms, and it is stated that this rate remains above 5% even in modern farms [6,7]. Economic losses concerning diarrhea are due to deaths, costs for treatment and prophylaxis, later growth retardation in calves, and under-selling of animals [7].

Decreased appetite, dehydration, and difficulty standing in calves with diarrhea are usually present. Severe diarrhea, ataxia, acidemia, bacteremia, arrhythmia, and hypovolemia, which can lead to death, can be seen [8,9]. Complications such as sepsis, septic shock, and systemic inflammatory response syndrome (SIRS) seen in neonatal calves after diarrhea cause significant changes in clinical, hematological, biochemical, and blood gas parameters [10].

In calves with diarrhea, hematological and biochemical parameters are affected differently according to the severity of the disease [11]. The hematological findings show a significant increase in the total leukocyte level. It has been reported that neutrophils and monocytes tend to increase, and the lymphocyte ratio tends to decrease [12]. It is well known that the hematocrit (Hct) value is significantly increased in calves with diarrhea [9,10]. Diarrhea increases the loss of electrolytes and water in the feces of calves and reduces milk intake. This process results in dehydration, metabolic acidosis, electrolyte abnormalities (usually decreased sodium and increased or decreased potassium), increased D-lactate concentration, and a negative energy balance (caused by anorexia and nutrient absorption) [9]. A strong ion difference (SID) decrease is solely responsible for strong ion acidosis and academia [5]. Ideally, blood gas analysis with base excess (BE) is used to diagnose and guide treatment. The negative BE of the extracellular fluid defines metabolic acidosis [9,13,14]. Decreased serum glucose, sodium, potassium, and chloride concentrations have also been reported in dehydrated calves with diarrhea [8,15].

Studies investigating new biomarkers that may be effective in determining the prognosis or survival of diarrhea are still up to date [10,16]. However, these biomarkers are still at the scientific research level and have not found enough use in clinical practice. Currently, parameters such as vital parameters, complete blood count, blood gas analysis, and lactate levels are among the complementary tests commonly used by clinicians. These conventional tests can help predict the disease's severity and reveal the metabolic status. By identifying selected parameters with prognostic importance, veterinarians can distinguish between calves with diarrhea with a good prognosis and calves with diarrhea with an elevated risk of death.

The primary purpose of this observational retrospective study is to investigate the hematological, blood gas, vital parameters, environment, etiology, and general conditions that are effective on survival and to determine the threshold values in calves with neonatal diarrhea brought to Erciyes University, Faculty of Veterinary Medicine, Veterinary Teaching Hospital during a calving season. Another aim of the study is to determine the mortality rates and durations of the calves with diarrhea brought to the hospital and to investigate the relationship between hematology and blood gas parameters and mortality rates and durations.

METHODS

Study design

The study was an observational retrospective study. The STROBE checklist guidelines were followed in the preparation of this work. HADYEK, the Local Ethics Committee for the Animal Experiments office of Erciyes University, approved the study (approval No. 23/133*). Animal owners gave consent for their animals' inclusion in the study.

Selection and description of subjects

A single veterinarian examined the diarrheal calves included in the study. Veterinarians with expertise in large animal practice, internal medicine, and surgery decided on inclusion and exclusion from the study.

Inclusion criteria were as follows: Less than one month old, with clinical signs of diarrhea, no medical treatment applied, physical examination findings, complete blood count and blood gas analysis findings, and farm type recorded. Fresh feces taken from a calf in the present study was considered diarrhoeic if it was abnormally frequent, soft, watery, and had an unpleasant odor.

Diarrheic calves with other diseases (pneumonia, omphalitis/omphaloplebitis, arthritis, etc.) and missing complementary tests (hematology, venous blood gas analysis) were not included in the study. The diagnosis of concomitant diseases was made according to the history of the disease, anamnesis information obtained from the animal owner, physical examination findings (abnormal and prominent breathing sounds on auscultation of the lungs, nasal discharge, difficulty in breathing, tenderness in the trachea, pain and swelling on palpation of the joints and umbilical region), blood gas analysis and radiography results. Consultation from a large animal surgeon was requested in cases where it was necessary to confirm concomitant diseases. Decisions were based on consensus.

The calves included in the study came from two different farm types (traditional and modern farms). Traditional farms are defined as businesses that are designed only for animal housing and feeding, without a specific standard, where care, feeding, and management practices are followed by non-technical personnel or primarily by their owners. In these establishments, calves are housed in a corner or adjacent to adult cattle barns, usually built using wooden materials collectively or in the same environment as their mothers. Modern farms are farms where technical personnel (animal keeper, technician, and veterinarian) follow maintenance, feeding, and management practices and have an establishment location and layout project. Animal shelter areas are arranged according to animal welfare criteria in these farms. It defines the establishments where herd management programs (pedigree, vaccination, etc.) are implemented, even if not wholly, where calves are housed in individual huts and manure management systems are available.

To determine the survival status of the calves, the follow-up period was determined as 15 days. The surviving calf was defined as the calf that survived and/or recovered 15 days after discharge from the Veterinary Teaching Hospital (VTH). A dead calf was defined as one that died naturally within 15 days of VTH discharge. The short-term fatality rate (STFR) was defined as the proportion of calves that died in the hospital or "within 24 h" of discharge. The long-term fatality rate (LTFR) was defined as the proportion of calves that died "after 24 h or more" after leaving the hospital. Short-term fatality time (STFT) was defined as the

calves' death time in the hospital and within 24 h after discharge. Long-term fatality time (LTFT) was defined as the death time of the calves that died "after 24 h or more" after leaving the hospital. The calving season refers to the period when the number of calving peaks in the farms where the calves are brought, clustered into the Winter (December, January, February) and Spring (March, April, May) seasons. Stools of calves with diarrhea were scaled between 0–3 according to their consistency [2]. Score 0 was normal (solid but not too harsh); score 1 was semi-formed, pasty (does not hold shape, clumps but spreads slightly); score 2 was loose (spreads easily); score 3; watery.

Physical and blood examination

Calves included in the study were subjected to a complete physical examination, including rectal temperature (°C), pulsation (bpm), respiratory rate, hydration status, sucking reflex, general condition assessment, and stool consistency.

The hydration status of calves was evaluated according to demeanor and skin tent duration (sec) described by Smith [14]. According to this evaluation, the degree of dehydration was normal (< 5%, skin tent duration < 1 sec and normal demeanor), the degree of dehydration mild (6%–8%, skin tent duration 1–2 sec and slightly depressed demeanor), the degree of dehydration moderate (8%–10%, skin tent duration 2–5 sec, depressed demeanor), the degree of dehydration severe (10%–12%, skin tent duration; 5–10 sec, comatose demeanor) and the degree of dehydration comatose (> 12%, skin tent duration > 10 sec, comatose/dead demeanor) were also applied in the present study.

The general status of the calves (mild, moderate, severe, comatose) was determined by clinicians based on clinical findings and supplementary tests. The general clinical conditions of the calves were categorized according to the non-invasive five-point sequential scale clinical evaluation scoring table developed by Dillane et al. [17]. This scoring refers to the classification based on health signs such as demeanor, ear position, mobility, response to environmental warnings, sucking reflex, and degree of dehydration when the calves are brought to the hospital. According to this table, diarrhoeic calves in the present study were categorized as clinically normal, mild, moderate, severe, and comatose.

After the clinical examination of the calves (before starting the treatment), blood samples were collected from the jugular veins of the calves for blood gas and hematological examinations to determine the fluid electrolyte-acid-base balance of the patient and to determine the infection status within the standard practices of the clinic. Blood samples were duly taken into BD Vacutainer K_2 EDTA tubes (Becton Dickinson, USA) for hematology analyses and lithium heparin injectors (Genject, Türkiye) for blood gas analysis. Blood samples were sent to the laboratory (Erciyes University, Faculty of Veterinary Medicine, Animal Hospital, Clinical Hematology, and Biochemistry Laboratory), where the analyses would be conducted immediately (in 5 min). Complete blood count and blood gas analyses of the calves with diarrhea were performed using a complete blood count device (Exigo EosVet; Boule Medical AB, Sweden) and blood gas analyzer (ABL 80 Flex; Radiometer, Denmark) in the laboratory. Blood samples taken into BD Vacutainer K_2 EDTA (Becton Dickinson) tubes were homogenized for 3 min at 40 rpm/min using a roller blood mixer before analysis.

The calculation of SID [17,18] was based on the combined blood serum electrolyte concentrations (natrium [Na^+] + potassium [K^+] − Cl^-).

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Laboratory data

The data collected between 1 December 2021 and 31 May 2022, the peak period of the calving season, were obtained retrospectively from the hospital automation system (Patient Registration System, ERUVetO; V.15042019/2015, Türkiye).

The date the calves were brought to the hospital, the farm type, gender, clinical symptoms, blood gas and complete blood count, treatment practices during hospitalization, survival status during hospitalization, season of arrival, time of death, and diarrhea test results from Erciyes University hospital automation system (Patient Registration System, ERUVetO; V.15042019/2015, Türkiye) were obtained retrospectively. A single researcher performed follow-up examinations of the sick calves by telephone. The calf owners were asked about the survival status of the calves (dead/survived). If the calf died, the time after leaving the hospital was asked. Three attempts were made to reach the owner of the animal. In inaccessibility, the data were defined as missing (unavailable).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 21.0 (SPSS Inc., USA) program. The suitability of the data to normal distribution was evaluated using the Shapiro-Wilk test, Q-Q plot, and histogram. The data sets were expressed as % (number of positive/total cases), median (25th-75th percentile or minimum-maximum) and mean ± standard deviation according to data type. The Independent Samples *t*-test and one-way analysis of variance test were used for normally distributed variables. The Mann-Whitney *U* and Kruskal-Wallis tests were used for non-normally distributed variables. The Bonferroni and Tukey's honestly significant difference tests were used in *post hoc* comparisons.

The relationship between categorical variables was evaluated using Pearson's χ^2) test (and Fisher's exact test). For variables with more than two categories, row (r) × column (c) ($r > 2$ or $c > 2$), the χ^2 test was used.

This analysis included preliminary explorations, including pairwise analyses for the relationship of binary variables using the χ^2 test. This was followed by multivariable modeling using mixed effects logistic regression. The association between the prognostic factors and survived/dead survival status was also analyzed using this method. The prognostic factor with logistic regression analysis was achieved using three steps. Initially, the interrelationships of all variables taken individually with the occurrence of dead were evaluated in a univariate model. Then, any variable with a p value < 0.2 was considered eligible for the next step. In the third step, a final multivariate model was fitted with all the variables that had remained significant during the two previous steps. Backward stepwise logistic regression analysis was used. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the likelihood of association. Receiver operating characteristic (ROC) analysis was performed to determine the variables' prognostic cut-off value, sensitivity, and specificity in dead and survived calves. A p value of < 0.05 was considered statistically significant for all analyses.

RESULTS

The animal material of this study consisted of 89 newborn calves with diarrhea from both sexes (45 males, 44 females) and different breeds (75 Simental, 8 Holstein, 5 Brown Swiss, 1 Crossbreed). The median age of the calves was seven days (range, 2–28). Between 1 December

2021 and 31 May 2022, 203 calves were brought from Kayseri and its surrounding provinces (Sivas, Nevşehir, Yozgat, Niğde, Kırşehir) to Erciyes University, Faculty of Veterinary Medicine, Animal Hospital, Department of Internal Diseases, Ruminant Clinic, and 121 of them (59.6%) were found to have clinical signs of diarrhea (Hospital Automation System, ERUVetO; V.15042019/2015, Türkiye). Thirty-two of these calves (26.5%) were not included in the study due to missing complementary tests (complete blood count, blood gas analysis, physical examination findings) and co-occurring diseases besides diarrhea (respiratory system infection, omphalitis and arthritis accompanying diarrhea). As a result, 89 calves with diarrhea were included in the study. Thirty-eight (42.7%) of these 89 diarrheic calves died during or after treatment, and 49 (55.1%) survived. Two of these 89 calf owners could not be reached because their phones registered in our system were unanswered. 94.4% (84/89) of the calves had come from traditional farms, and 5.6% (5/89) from modern-type farms. It was observed that 40.5% (34/84) of the calves with diarrhea brought from traditional farms and 80.0% (4/5) of the calves from modern farms died.

Enteropathogens taken from stool samples of calves with diarrhea were determined by using lateral flow immunoassay test kits (Anigen Rapid BoviD-5 Ag; BioNote Inc., Korea); as enterotoxigenic *Escherichia coli* (ETEC) K99$^+$ (25.8%, 23/89), *Cryptosporidium* spp. (24.7%, 22/89), bovine rotavirus (BRV) (3.4%, 3/89), BRV + bovine coronavirus (BCoV) (16.9%, 15/89), *Cryptosporidium* spp. + BRV (4.5%, 4/89), BRV + *Cryptosporidium* spp. + ETEC K99$^+$ (1.1%, 1/89), *Cryptosporidium* spp.+ETEC K99$^+$ (1.1%, 1/89). Twenty of these 89 stool samples were evaluated as negative for these five enteropathogens (ETEC K99$^+$, BRV, BCoV, *Cryptosporidium* spp., *Giardia* spp.) and were not evaluated for other enteropathogens (*Salmonella* spp., *Clostridium* spp., etc.).

Physical examination findings

Mean/median body temperatures, respiratory rate, and heart rate of diarrheic calves were 38.1°C (interquartile range [IQR], 36.8–38.6; range, 32.0–39.7), 32/min (IQR, 24–44; range, 16–120) and 120 (IQR, 100.00–128.00; range, 20–240) bpm, respectively. The degrees of dehydration were normal in 5.6% (5/89), mild in 39.3% (35/89), moderate in 25.8% (23/89), and severe in 29.2% (26/89). It was seen that 33.7% (30/89) of their general conditions were mild, 23.6% (21/89) moderate, 36% (32/89) severe, and 6.7% (6/89) comatose.

The parameters that were determined to be statistically significantly low or high in terms of vital (body temperature, heart rate, respiration rate) variables between dead and survived calves are given in **Table 1**. Body temperature (°C) value was significantly lower in those who died than in those who survived (**Table 1**). The differences between vital parameters in calves according to short- and long-term death status are given in **Table 1**.

In the calves included in the study, diarrhea 100% (89/89), weak sucking reflex 57.3% (51/89), no sucking reflex 42.7% (38/89), fatigue 69.7% (62/89), enophthalmos/dehydration 94.4% (84/89), slow to respond when approached 42.7% (38/89), unresponsive when approached 23.6% (21/89), depressed demeanor 59.6% (53/89), comatose situation 6.7% (6/89), lying down 66.3% (59/89), lateral recumbency 6.7% (6/89) were detected.

Short- and long-term fatality rates and times

Out of 38 dead calves, 18 of them (47.4%) died within "first 24 h" and 20 of them (52.6%) died "after 24 h or more." There was a median STFT of 9.25 h (IQR, 5.36–19.38) and a median LTFT of 51.50 h (IQR, 31.50–102.00) in calves that died and a statistically significant difference between the groups ($p = 0.001$).

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Table 1. Comparison of vital (body temperature, heart rate, respiration rate) and hematological parameters according to survival status and death duration of calves with diarrhea

Variables	Survived (n = 49)	Died (n = 38)	*p* value[c]	Died (≤ 24 h) (n = 18)	Died (> 24 h) (n = 20)	*p* value[c]	Reference range [15,28,30]
Body temperature (°C)	38.5 (35.9–39.6)[a,d]	37.0 (32.0–39.7)[b,d]	< **0.001*****	36.17 ± 2.19	36.42 ± 2.05	0.745	38.5–39.5
Respiration rate (min)	36 (28–46)	32 (24–42)	0.254	42.18 ± 19.78	31.76 ± 10.74	0.075	24–36
Heart rate (bpm)	120 (104–140)	116 (96–128)	0.236	117.37 ± 35.66	116.29 ± 22.96	0.918	80–120
WBC (10^9/L)	12.32 ± 5.94	11.67 ± 5.33	0.121	14.15 ± 4.40	13.40 ± 5.93	0.807	4.8–16.3
RBC (10^{12}/L)	7.61 ± 1.69	7.86 ± 2.13	0.063	7.71 ± 1.85	8.45 ± 1.78	0.256	6.2–11.9
Hgb (g/dL)	11.70 ± 2.63[a]	12.02 ± 3.24[b]	**0.030***	11.49 ± 2.81	12.14 ± 2.80	0.509	7.3–14.8
Hct (%)	32.52 ± 7.69[a]	35.48 ± 10.16[b]	**0.008****	33.78 ± 8.27	36.51 ± 9.34	0.385	30.0–45.0
MCV (fL)	42.71 ± 3.37[a]	45.19 ± 5.10[b]	**0.009****	44.03 ± 5.70	43.07 ± 4.17	0.581	33.1–44.2
MCH (pg)	14.90 (14.30–15.60)	15.00 (14.40–15.95)	0.488	14.94 ± 1.57	14.36 ± 1.40	0.267	11–14
MCHC (g/dL)	35.20 (33.00–36.30)	32.40 (31.30–35.85)	0.168	34.39 ± 5.29	33.61 ± 4.39	0.646	30.9–34.6
RDWa (fL)	32.04 ± 3.02	33.92 ± 4.16	0.175	27.54 ± 9.27	29.11 ± 7.46	0.607	31.2–41.6
RDW (%)	17.80 (16.60–19.10)	16.90 (16.50–19.95)	0.594	16.67 ± 2.13[a]	19.32 ± 3.40[b]	**0.026***	15.0–19.4
PLT (10^9/L)	594.00 (431.00–1,019)	461.00 (389.50–897.00)	0.431	408.00 (357.00–463.00)	597.00 (422.50–967.50)	0.246	100.0–800.0
MPV (fL)	4.50 (4.20–4.70)	4.70 (4.20–5.40)	0.108	6.41 ± 3.37	5.87 ± 3.09	0.635	3.5–6.5

The mean and standard deviation are presented for all data which passed normality testing. Variables that failed normality testing are presented as median (25th-75th percentile) and median (min-max).
WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin volume; MCHC, mean corpuscular hemoglobin concentration; RDWa, absolute value of the width of the distribution of red blood cells; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume.
[a,b]Different lowercase letters indicate differences between groups.
[c]Bold styled *p* values indicate differences between groups.
[d]Data were expressed as median (min-max).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2. Rates of dead and survived calves according to the general condition of the calves with diarrhea

General situation	Survived	Died	NR	*p* value[a]	χ^2
Mild (n = 30)	83.3 (25/30)	13.3 (4/30)	3.3 (1/30)	< **0.001***	52.387
Moderate (n = 21)	61.9 (13/21)	33.3 (7/21)	4.8 (1/21)	< **0.001***	16.193
Severe (n = 32)	34.4 (11/32)	65.6 (21/32)	0 (0/32)	< **0.001***	36.104
Comatose (n = 6)	0 (0/6)	100 (6/6)	0 (0/6)	< **0.001***	16.505

Data were expressed as % (number of positive/total cases).
NR, not reachable.
[a]Bold styled *p* values indicate statistical significance.
*$p < 0.001$.

There was a statistically significant correlation between the general status (mild, moderate, severe, comatose) and prognosis (survived, dead) categories of calves with diarrhea ($p < 0.05$). Mortality rates in calves with severe (65.6%) and comatose (100.0%) diarrhea were found to be statistically higher than in calves with mild (13.3%) and with moderate (33.3%) diarrhea (**Table 2**).

Considering the general situation of the calves with diarrhea brought from traditional farms; It was determined that 33.3% were mild, 23.8% moderate, 38.1% severe and 4.8% comatose. The general conditions of the calves with diarrhea brought from modern farms were determined as 40% mild, 20% moderate and 40% comatose (**Supplementary Table 1**).

The median duration of death was determined as 46 h (35.00–66.00) in patients with the mild general condition, median 24.0 h (18.13–115.0) in patients with the moderate condition, median 25.50 h (8.25–75.25) in patients with severe condition, and median 10.25 h (3.54–31.75) in patients with comatose condition. The median death time of the comatose diarrheic calves was statistically significantly lower than the mild diarrheic calves ($p = 0.042$).

Mortality and survival rates in calves with diarrhea according to enteropathogen, age, farm type, sex, season, and farm type categories are given in **Table 3**.

Table 3. Mortality and survival rates according to enteropathogen, age, farm type, sex, and season categories in calves with diarrhea

Variables	Survived	Died	NR	χ^2	*p* value
Enteropathogens				16.871	0.390
ETEC K99$^+$ (n = 23)	56.5 (13/23)	43.5 (10/23)	0.0 (0/23)		
Cryptosporidium spp. (n = 22)	63.6 (14/22)	31.8 (7/22)	4.5 (1/22)		
BRV (n = 3)	66.7 (2/3)	33.3 (1/3)	0.0 (0/3)		
BRV + BCoV (n = 15)	60.0 (9/15)	40.0 (6/15)	0.0 (0/15)		
Cryptosporidium spp. + BRV (n = 4)	50.0 (2/4)	25.0 (1/4)	25.0 (1/4)		
BRV + *Cryptosporidium* spp. + ETEC K99$^+$ (n = 1)	0.0 (0/1)	100.0 (1/1)	0.0 (0/1)		
Cryptosporidium spp. + ETEC K99$^+$ (n = 1)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)		
Undiagnosed (n = 20)	40.0 (8/20)	60.0 (12/20)	0.0 (0/20)		
Age				5.267	0.719
0–7 days (n = 49)	57.1 (28/49)	40.8 (20/49)	2.0 (1/49)		
8–14 days (n = 23)	56.5 (13/23)	43.5 (10/23)	0.0 (0/23)		
15–21 days (n = 16)	50.0 (8/16)	43.8 (7/16)	6.3 (1/16)		
22–30 days (n = 1)	0.0 (0/1)	100.0 (1/1)	0.0 (0/1)		
Sex				3.544	0.123
Male (n = 45)	46.7 (21/45)	48.9 (22/45)	4.4 (2/45)		
Female (n = 44)	63.6 (28/44)	36.4 (16/44)	0.0 (0/44)		
Seasons				5.271	0.236
Winter (n = 43)	58.14 (25/43)	39.53 (17/43)	2.33 (1/43)		
Spring (n = 46)	54.35 (25/46)	43.48 (20/46)	2.17 (1/46)		
Farm type				3.169	0.255
Traditional (n = 84)	57.1 (48/84)	40.5 (34/84)	2.4 (2/84)		
Modern (n = 5)	20.0 (1/5)	80.0 (4/5)	0.0 (0/5)		

Data were expressed as % positive (number of positive/number of total cases), undiagnosed, those are not positive for ETEC K99$^+$, BRV, BCoV, *Cryptosporidium* spp., and *Giardia* spp.
NR, not reachable; ETEC, enterotoxigenic *Escherichia coli*; BRV, bovine rotavirus; BCoV, bovine coronavirus.

Complete blood count findings

The parameters that were determined to be statistically significantly low or high in terms of hematology variables between dead and survived calves are given in **Table 1**. The differences between hematological parameters in calves according to short-term and long-term death status are given in **Table 1**.

It was determined that the ratios of monocytes, hemoglobin (Hgb), Hct, and mean corpuscular volume (MCV) were statistically significantly higher in those who died.

The mean red cell distribution width (%) value of the calves with diarrhea that died in the first 24 h (16.67 ± 2.13) was found to be significantly lower ($p = 0.026$) than the same value of the calves that died in more than 24 h (19.32 ± 3.40).

Blood gas analysis findings

Statistically significantly low or high parameters in terms of blood gas variables between dead and survived calves are given in **Table 4**. The differences in blood gas parameters according to short-term and long-term mortality are given in **Table 4**.

Lactate (6.45 [2.32–11.25] mmol/L), chlorine (Cl^-; 99.55 ± 9.18 mmol/L), Na^+ (140.00 [136.00–142.00] mmol/L), and anion gap (25.35 ± 7.64 mmol/L) parameters were found to be significantly higher in the dead calves compared to survived calves lactate (2.60 [1.60–4.80] mmol/L), Cl^- (92.16 ± 6.85 mmol/L), Na^+ (134.00 [129.00–139.00] mmol/L), and anion gap (20.89 ± 7.52 mmol/L) values.

On the other hand, the pH (7.08 ± 0.27), BE (–12.80 ± 10.30 mmol/L), and HCO_3^- (16.55 ± 8.10 mmol/L) values were found to be significantly lower in the dead calves than the same values obtained from survivor calves (pH, 7.28 ± 0.16, BE, –5.39 ± 9.14; HCO_3^-, 21.10 ± 6.92 mmol/L).

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Table 4. Comparison of blood gas parameters according to survival status and death duration of calves with diarrhea

Variables	Survived (n = 49)	Died (n = 38)	*p* value[f]	Died (≤ 24 h) (n = 18)	Died (> 24 h) (n = 20)	*p* value[f]	Reference range [15,28,30]
pH	7.28 ± 0.16[a]	7.08 ± 0.27[b]	< **0.001****	7.09 ± 0.16	7.10 ± 0.23	0.918	7.35–7.48
pCO_2 (mmHg)	44.46 ± 9.88	48.92 ± 16.63	0.127	56.20 ± 19.11[a]	44.33 ± 13.80[b]	**0.047***	34.0–45.0
pO_2 (mmHg)	30.00 (26.00–34.75)	29.50 (25.00–37.50)	0.443	28.33 ± 10.17	35.50 ± 12.58	0.086	36.0–46.5
Na^+ (mmol/L)	134.00 (129.00–139.00)[a]	140.00 (136.00–142.00)[b]	**0.015***	139.00 ± 5.53	141.72 ± 12.44	0.439	136.5–142.4
K^+ (mmol/L)	5.63 ± 1.21	5.80 ± 1.55	0.581	5.99 ± 1.61	5.74 ± 1.34	0.620	4.1–5.6
iCa^{+2} (mmol/L)	1.20 ± 0.26	1.26 ± 0.22	0.334	1.22 ± 0.20	1.31 ± 0.25	0.265	1.17–1.37
Cl^- (mmol/L)	92.16 ± 6.85	99.55 ± 9.18	< **0.001****	95.07 ± 5.17[a]	101.33 ± 9.58[b]	**0.041***	94.5–105.2
cLactate (mmol/L)	2.60 (1.60–4.80)[a]	6.45 (2.32–11.25)[b]	**0.002****	9.0 (6.20–12.60)[a]	2.50 (2.0–10.35)[b]	**0.005****	0.5–2.0
$cHCO_3^-$(P) (mmol/L)	21.10 ± 6.92[a]	16.55 ± 8.10[b]	**0.007****	16.64 ± 5.81	15.23 ± 9.13	0.610	24.0–33.9
cBE (mmol/L)	−5.39 ± 9.14[a]	−12.80 ± 10.30[b]	**0.001****	−13.12 ± 7.84	−14.52 ± 12.62	0.711	2.6–10.8
	−2.35 (−12.50 to 1.37)	−10.90 (−21.93 to −6.93)		−10.50 (−18.50 to −8.60)	−12.50 (−24.45 to −1.20)		
$ctCO_2$(B) (mmol/L)	20.24 ± 6.36[a]	16.32 ± 7.41[b]	**0.010***	16.77 ± 5.81	14.87 ± 8.14	0.441	NR
cCa_2^+(7.40)	1.18 ± 0.11[a]	1.09 ± 0.11[b]	**0.012***	1.09 ± 0.25	1.08 ± 0.09	0.982	NR
SID[c]	46.00 (44.05–49.90)	48.09 (41.64–51.52)	0.467	49.93 ± 5.61	45.85 ± 6.62	0.048	39.4–48.8
Anion gap (mmol/L)[d]	20.89 ± 7.52[a]	25.35 ± 7.64[b]	**0.009****	27.26 ± 5.65	24.94 ± 8.91	0.372	0.7–20.3
Anion gap,K^+ (mmol/L)[e]	26.06 ± 8.67[a]	31.16 ± 8.15[b]	**0.007****	33.25 ± 6.17	30.69 ± 9.14	0.347	
sO_2 (%)	50.06 ± 15.83[a]	42.36 ± 18.58[b]	**0.043****	33.07 ± 15.08[a]	45.52 ± 18.02[b]	**0.042***	61.28 ± 1.73
ctO_2 (Vol%)	6.99 ± 3.01	6.57 ± 3.15	0.541	5.46 ± 3.72	7.08 ± 2.37	0.139	27.24 ± 1.26

The mean and standard deviation are presented for all data which passed normality testing. Variables that failed normality testing are presented as median (25th-75th percentile).

pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; Na^+, natrium; K^+, Potassium; iCa^{+2}, ionized calcium; Cl^-, chlorine; cLactate, concentration of L-lactate in plasma; $cHCO_3^-$(P), concentration of hydrogen carbonate in plasma (also termed actual bicarbonate); cBE, base excess in blood; $ctCO_2$B, total carbon dioxide concentration in blood; SID, strong ion difference; sO_2, Oxygen saturation; ctO_2, concentration of total oxygen; cCa^{2+}(7.40), concentration of calcium cations at pH: 7.40; NR, reference interval not reported.

[a,b]Different lowercase letters indicate differences between groups.

[c]SID calculated as: $Na^+ + K^+ - Cl^-$.

[d]Anion gap is the quantity difference between cations (positively charged ions) and anions (negatively charged ions).

[e]Anion Gap,K^+ (mmol/L) is difference between the concentration of the cations (sodium and potassium), and the measured anions (chloride and bicarbonate).

[f]Bold styled *p* values indicate differences between groups.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Prognostic factors, according to the univariate logistic regression significantly associated with survival status

The univariate logistic regression model determined the prognostic factors associated with survival status (dead/survived) separately in neonatal calves with diarrhea (**Table 5**). An increase in Na^+, CI^-, lactate, anion gap, Hgb, Hct, and MCV were associated with an increase in the odds of death with an OR of 1.053 (95% CI, 1.000 to 1.109), 1.052 (95% CI, 1.001 to 1.107), 1.145 (95% CI, 1.020 to 1.286), 1.077 (95% CI, 1.018 to 1.140), 1.204 (95% CI, 1.014 to 1.430), 1.073 (95% CI, 1.016 to 1.133) and 1.156 (95% CI, 1.031 to 1.297), respectively.

Prognostic factors, according to the multivariate logistic regression model significantly associated with survival status

The final logistic regression analysis results related to the survival status of calves with diarrhea are given in **Table 6**. An increase in lactate concentration was associated with an increase in the odds of death with an odds ratio of 1.429 (95% CI, 1.110 to 1.839; Wald χ^2 = 7.671; $p = 0.006$). An increase in CI^- was associated with increased odds of death with an odds ratio of 1.232 (95% CI, 1.018 to 1.489; Wald χ^2 = 4.609; $p = 0.032$).

In this study, when the cut-off point for lactate (mmol/L) was taken as 3.75, sensitivity, specificity, and area under the ROC curve were calculated as 88.5%, 61.5%, and 0.722, respectively.

Table 5. Prognostic factors associated with mortality risk in calves with diarrhea in univariate logistic regression model

Variable description	B	SE	Wald	Sig[c].	Exp (B)	95% CI Exp (B)	
						Lower	Upper
Vital signs							
Body temperature (°C)	−1.146	0.280	16.733	< **0.001*****	0.318	0.184	0.551
Respiration rate	−0.013	0.012	1.169	0.280	0.987	0.963	1.011
Hearth rate (bpm)	−0.011	0.008	1.775	0.183	0.989	0.973	1.005
Venous blood gas analysis							
pH	−5.464	1.466	13.894	< **0.001*****	0.004	0.000	0.075
pCO_2 (mmHg)	0.027	0.018	2.151	0.142	1.027	0.991	1.065
pO_2 (mmHg)	0.018	0.023	0.661	0.416	1.019	0.974	1.065
Na^+ (mmol/L)	0.052	0.026	3.826	**0.050***	1.053	1.000	1.109
K^+ (mmol/L)	0.094	0.162	0.334	0.563	1.098	0.800	1.508
iCa^{+2} (mmol/L)	0.953	0.992	0.923	0.337	2.594	0.371	18.144
Cl^- (mmol/L)	0.050	0.026	3.744	**0.050***	1.052	1.001	1.107
cLactate (mmol/L)	0.135	0.059	5.229	**0.022***	1.145	1.020	1.286
$cHCO_3^-$(P) (mmol/L)	−0.082	0.031	6.852	0.009	0.922	0.867	0.982
cBE (mmol/L)	−0.073	0.024	9.356	0.002	0.930	0.888	0.974
$ctCO_2$(B) (mmol/L)	−0.084	0.034	6.143	0.013	0.919	0.860	0.983
cCa_2^+(7.40)	−8.111	3.435	5.577	0.018	0.000	0.000	0.252
Anion gap (mmol/L)[a]	0.080	0.032	6.282	**0.012***	1.083	1.018	1.153
Anion gap K^+ (mmol/L)[b]	0.074	0.029	6.586	**0.010****	1.077	1.018	1.140
sO_2 (%)	−0.027	0.013	3.974	0.046	0.974	0.948	1.000
ctO_2 (Vol%)	−0.045	0.073	0.382	0.537	0.956	0.828	1.103
SID	0.015	0.035	0.181	0.670	1.015	0.948	1.086
Hematology							
WBC (10^9/L)	0.048	0.030	2.506	0.113	1.049	0.989	1.113
RBC (10^{12}/L)	0.240	0.132	3.313	0.069	1.271	0.982	1.644
Hgb (g/dL)	0.186	0.087	4.513	**0.034***	1.204	1.014	1.430
Hct (%)	0.070	0.028	6.380	**0.012***	1.073	1.016	1.133
MCV (fL)	0.145	0.059	6.126	**0.013***	1.156	1.031	1.297
MCH (pg)	0.031	0.116	0.072	0.788	1.032	0.822	1.295
MCHC (g/dL)	−0.058	0.044	1.759	0.185	0.944	0.866	1.028
RDWa (fL)	0.043	0.032	1.849	0.174	1.044	0.981	1.111
RDW (%)	0.045	0.088	0.265	0.607	1.046	0.881	1.243
PLT (10^9/L)	0.000	0.001	0.022	0.882	1.000	0.999	1.001
MPV (fL)	0.025	0.062	0.161	0.688	1.025	0.908	1.158

SE, standard error; Sig., significance; OR, odds ratio; 95% CI Exp (B), 95% confidence interval for the odds ratio; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; Na^+, natrium; K^+, potassium, iCa^{+2}, ionized calcium; Cl^-, chlorine; HCO_3^-, bicarbonate; cLactate, concentration of L-lactate in plasma; $cHCO_3^-$(P), concentration of hydrogen carbonate in plasma (also termed actual bicarbonate); cBE, base excess in blood; sO_2, oxygen saturation; ctO_2, concentration of total oxygen;SID, strong ion difference; WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin volume; MCHC, mean corpuscular hemoglobin concentration; RDWa, absolute value of the width of the distribution of red blood cells; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume.
[a]Anion gap is the quantity difference between cations (positively charged ions) and anions (negatively charged ions).
[b]Anion gap K^+ (mmol/L) is difference between the concentration of the cations (sodium and potassium), and the measured anions (chloride and bicarbonate).
[c]Bold styled *p* values indicate statistical significance.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

DISCUSSION

Neonatal calf diarrhea cases constitute most cases in ruminant clinics [2]. It is imperative to reveal the effects of the investigated parameters on the prognosis and death prediction to minimize the high prevalence of diarrhea cases and the resulting deaths and/or significant economic losses due to treatment costs. When the data obtained from this study is evaluated, body temperature (°C), pH, BE (mmol/L), and sodium bicarbonate (mmol/L) parameters were found to be lower, and Hgb (g/dL), Hct (%), lactate (mmol/L), chlorine (mmol/L), sodium (mmol/L) and anion gap (mmol/L) parameters were found to be higher in dead calves compared to survivors. Accordingly, hypothermia, metabolic acidosis, and dehydration findings are seen as clinical conditions that should be considered. This study determined that

Table 6. Prognostic factors associated with survival status of diarrheic calves in the final multivariate logistic regression model

Variable description	B	SE	Wald	Sig[a]	OR	95% CI for Exp (B)	
						Lower	Upper
pH	−3.075	1.658	3.438	0.064	0.046	0.002	1.192
cNa^{+} (mmol/l)	−0.113	0.082	1.918	0.166	0.893	0.761	1.048
cLactate (mmol/l)	0.357	0.129	7.671	**0.006****	1.429	1.110	1.839
Cl^{-} (mmol/l)	0.208	0.097	4.609	**0.032***	1.232	1.018	1.489
pCO_2 (mmHg)	−0.042	0.028	2.310	0.129	0.959	0.908	1.012
iCa^{+2} (mmol/l)	−2.668	1.446	3.405	0.065	0.069	0.004	1.181
Temperature (°C)	−1.313	0.385	11.632	0.001	0.269	0.126	0.572
Constant	70.204	20.748	11.449	0.001	3.08E+30		

R^2 = 0.470 (Cox & Snell). R^2 = 0.633 (Nagelkerke). Model: $\chi^2(11)$ = 51.464. $p < 0.001$.
SE, standard error; Sig., significance; OR, odds ratio; 95% CI, 95% confidence interval; cNa^{+}, natrium; cLactate, concentration of L-lactate in plasma; Cl^{-}, chlorine; pCO_2, partial pressure of carbon dioxide; iCa^{+2}, ionized calcium.
[a]Bold styled *p* values indicate statistical significance.
*$p < 0.05$, **$p < 0.01$.

the increase in lactate concentrations is one of the prominent parameters in determining the survival status of calves with diarrhea.

Hypothermia is another clinical finding frequently encountered due to dehydration in calves with diarrhea [19]. In the current study, the body temperature value of the dead calves with diarrhea was lower than the survived calves ($p < 0.05$). Boccardo et al. [20] and Keleş et al. [2] reported that a 1-unit increase in body temperature (°C) of calves with diarrhea increased survival rate by 1.2- and 0.7-fold, respectively. In calves with diarrhea with poor general conditions, the prognosis can still be favorable if the body temperature is above 38.0°C [2,19,20].

It was observed that the general conditions of most of the calves with diarrhea (n = 38) who died in the study were in the severe-comatose form (71.1%, 27/38). In addition, 21.1% of the dead calves (n = 38) were moderately (8/38), and 50.0% were severely (19/38) dehydrated. Another possible cause may be poor circulation and decreased tissue perfusion in dead calves. The fact that lactate (mmol/L) levels (median 6.45 [2.48–11.0]) in dead calves were higher than in survived calves (median 2.60 [1.60–4.60]) supports this finding.

The current study determined that 42.7% (38/89) of the calves brought to our hospital with the complaint of diarrhea, whose complementary tests were performed and treated, died during hospitalization or after discharge from hospital. Similarly, Boccardo et al. [21] reported that 69 (43.4%) of 225 diarrheaic calves died. Trefz et al. [8] and Aydoğdu et al. [6] reported the mortality rate in newborn calves with diarrhea as 22.0% and 31.4%, respectively. Diarrhea reduces immune function and causes malabsorption of nutrients, making them more prone to death [13,22]. The high mortality rates in the current study may be related to the high rates of severe and comatose (42.7%, 38/89) calves with diarrhea in the general condition categories of the calves included. This study determined mortality rates in calves with severe and comatose diarrhea as 65.6% (21/32) and 100.0% (6/6), respectively. Another probable reason may be related to the lack of hygienic measures and management practices on farms, most of which are traditional, from which the calves are brought. The present study noted that 94.38% of the calves with diarrhea came from traditional type small-scale (less than 50 animals) farms. However, although the mortality rates are high in calves with diarrhea brought from modern farms, this may be related to the low number (5 calves) and only bringing advanced cases from modern farms.

It was found that 47.4% of the calves with diarrhea died in the "first 24 hours," and 52.6% died "after 24 hours or more." The mean time to death of neonatal calves with diarrhea brought to our hospital is median 25.50 h (range, 2.15–288), STFT is a median of 9.25 h (range, 2.15–24.00), and LTFT was determined as median 51.50 h (range, 25.00–288.00). In the present study, the calves were taken to the farms, where they were returned after treatment, and their general condition improved. Treatment continuations were made by animal owners or by their veterinary surgeons on farms. Especially in diarrhea caused by BRV, BCoV, and *Cryptosporidium* spp., an additional 5–7 days is needed to cease diarrhea. Dehydration and reoccurrence of base deficit due to fluid-electrolyte loss in calves with diarrhea are common complications [13]. Another reason for the high mortality rates in the current study may be the lack of adequate and appropriate treatment in the early or late initiation of treatment and the lack of adequate and timely treatment continuation after being brought to our clinics.

In the current study, mortality rates in diarrheic calves infected with ETEC K99$^+$, BRV + BCoV, BRV, *Cryptosporidium* spp., and *Cryptosporidium* spp. + BRV were determined as 43.5%, 40.0%, 33.3%, 31.8%, and 25.0%, respectively. On the other hand, İder et al. [11] reported mortality rates in diarrheal calves infected with ETEC K99$^+$, BRV + BCoV, and *Cryptosporidium* spp., as 33.3%, 46.7%, and 35.3%, respectively. Indeed, it has been widely described that *E. coli* is responsible for septicemia and neonatal deaths in calves [23,24]. In addition, BRV, BCoV, and *Cryptosporidium* spp. are other enteropathogens that cause significant economic losses and deaths in calves with neonatal diarrhea [11]. These pathogens cause dehydration, metabolic acidosis, electrolyte abnormalities, increased D-lactate concentration, and negative energy balance (caused by anorexia and nutrient absorption) due to septicemia and diarrhea, which can lead to death in neonatal calves [8,9]. The high rate of enteropathogen-related deaths in the calves with diarrhea included in the present study can be explained by the inadequate shelter structure, poor hygiene, and management practices in the farms where the calves are brought [25]. Our study's high number of deaths caused by *E. coli* shows that sanitation and hygienic measures are insufficient.

Dehydration is a common clinical finding in diarrhea and is defined as bodily fluid loss [14]. Hgb (g/dL) and Hct (%) values are important parameters in determining dehydration [12,15]. In the current study, the Hgb (g/dL) and Hct (%) values of the dead calves were higher than the survived calves ($p < 0.05$). It has been reported that as the general condition categories of calves with diarrhea (severe, comatose) worsen, the degree of dehydration increases significantly, related to the severity of diarrhea, the increase in the amount of fluid lost, and the decreased fluid intake [17,26].

Low blood pH value indicates acidemia in calves with diarrhea [8]. Metabolic acidosis in calves with diarrhea was initially attributed to loss of HCO_3^- from the intestines, organic acids in plasma, and a decrease in glomerular filtration rate in response to severe dehydration [27]. In the current study, contrary to the literature [6,15,28] findings, pH, HCO_3^- (mmol/L), and BE (mmol/L) values in dead calves were found to be significantly lower than the same values obtained from survived calves ($p < 0.05$). A possible explanation for our findings may be related to the very poor dehydration degree and general condition of the dead calves, with little or no sucking reflex and excessive loss of fluid and electrolytes, and these adverse conditions persist for a long time. Another likely reason may be related to the delayed arrival of calves to the hospital or delayed recognition of diarrhea/disease.

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In most cases of acute diarrhea, hyponatremia is usually present. The most common cause of hyponatremia is the loss of Na^+ through secretion from the intestines due to diarrhea [29]. In the present study, Na^+ (mmol/L) and Cl^- (mmol/L) levels of calves with diarrhea were found to be lower than the reported reference values [30]. However, the Na^+ concentration (140.00 [136.00–142.00] mmol/L) of dead calves in the current study was significantly higher than that of surviving calves (134.00 [129.00–139.00] mmol/L).

In this study, the SID value in calves that died in more than 24 h was lower than those that died within the first 24 h. A decrease in the SID value indicates strong ion acidosis or acidemia, which is clinically significant and acts as a signal to initiate therapy in the form of fluid administration in diarrheic calves [18,26]. This can be explained by the fact that the HCO_3^- and base deficit values were slightly lower in calves that died in more than 24 h compared to the other group. In addition, the mortality rate in calves that died in more than 24 h (52.6%) and the rate of those whose general condition was severe (57.1%) were found to be higher than those who died within the first 24 h (mortality rate: 47.4% and the rate of those whose general condition was severe: 42.9%). This shows that even minimal changes in the investigated parameters can impact the calves' survival rates.

The Cl^- concentration of both survived and dead calves included in the study was lower than the reported reference value (CI^-, 102 ± 3 mmol/L) for healthy calves [30]. However, the Cl^- concentration (99.55 ± 9.18 mmol/L) of dead calves in the current study was significantly higher than that of surviving calves (92.16 ± 6.85 mmol/L), unlike the results of Lee et al. [15] ($p < 0.05$). The current study determined that a one-unit increase in Cl^- concentration increased the probability of death of diarrheic calves 1.2-fold in the general condition categories (**Table 6**). This may be related to the general condition categories of the dead diarrheic calves, the degree of dehydration, and the worsening of bicarbonate losses compared to the survivors. Therefore, in response to the loss of HCO_3^- in dead calves, the Cl^- concentration in the plasma may have increased due to activating the body's buffer systems just before death. In proportion to the increase in bicarbonate (negatively charged ion) loss, it causes the negatively charged chloride (Cl^-) ion to pass out of the cell. When the bicarbonate concentration in the plasma decreases, the chlorine ion returns to the plasma by a mechanism called "chlorine shift" [31,32]. In this study, although the CI^- values of both survived and dead calves were lower than those of healthy calves, it was observed that this Cl^- decrease somewhat approached the normal value, especially in the dead calves. The rise in Cl^- in the dead compared to the survived can be explained by the "chlorine shift" hypothesis. This finding can be considered a valuable predictive value, especially in predicting prognosis. On the other hand, in another study [15], it was reported that serum levels of Cl^- in diarrheic calves were normal in 48.3%, lower in 18.6%, and higher in 33.1% cases. In addition, cases of metabolic acidosis with an increase in chlorine concentration with an excessive decrease in bicarbonate levels have been reported by many researchers [33,34]. Additionally, despite the incomplete biochemical data in this study, it can be speculated that the higher chloride levels in calves that died compared to those that survived were associated with uremic acidosis. Because, uremic acidosis can cause chloride retention and high anion gap acidosis [31-34]. Detailed data analysis and more comprehensive studies are needed on this issue.

The anaerobic metabolic pathway known as glycolysis is the first step of glucose metabolism and occurs in the cytoplasm of almost all cells. The end product of this pathway, pyruvate, is metabolized to lactate by the enzyme lactate dehydrogenase [35]. It is accepted that the normal blood lactate level in healthy calves is 0.5–2 mmol/L [36,37]. In this study,

concentration of L-lactate in plasma (mmol/L) values of the dead calves with diarrhea were found to be significantly higher than those of the surviving calves ($p < 0.05$), consistent with the literature findings [6]. In addition, lactate (9.0 [6.20–12.60] mmol/L) and partial pressure of carbon dioxide (pCO_2; 56.20 ± 19.11 mmHg) values of calves with diarrhea who died in the first 24 h were found to be statistically significantly higher when compared to calves who died after 24 h (lactate, 2.50 [2.0–10.35] mmol/L, pCO_2, 44.33 ± 13.80 mmHg). Lewis et al. [38] reported that the main change in lactate concentrations during diarrhea occurred 24 h before death. Lactate uptake by the liver is impaired by factors such as acidosis, hypoperfusion, and hypoxia [35,39]. A possible explanation for our findings is that high lactate values in dead calves may be associated with poor general condition, low tissue perfusion, hypoxia, metabolic acidosis, and circulatory dysfunction.

In the current study, the dead calves' blood lactate concentration was significantly higher than the surviving calves ($p < 0.05$). This finding is consistent with the findings of Aydoğdu et al. [6]. Many researchers have reported that blood lactate levels in calves and cattle can be a prognostic indicator and can be used as an indicator of mortality [6,39]. This study determined lactate concentration's cut-off value as 3.75 mmol/L. It was determined that the sensitivity and specificity of calves with lactate concentration of 3.75 mmol/L and above had a mortality risk of 88.5% and 61.5%, respectively. In line with the literature, our findings show that blood lactate concentration can be used as a mortality and prognostic indicator in calves with neonatal diarrhea.

Anion gap can be an essential guide to categorizing causative factors in acid-base imbalances and determining the prognosis in calves with severe diarrhea [15]. In our study, it was observed that the anion gap (mmol/L) value of calves with diarrhea was consistent with the results of Ewaschuk et al. [40] and higher when compared to the reference value ranges [28]. Species, breeds, breeding patterns, and regional differences may cause differences in these parameters. In addition, the mean anion gap (mmol/L) value of the calves with diarrhea that died during hospitalization or after discharge was found to be significantly higher than the surviving calves ($p < 0.05$). Similarly, Lee et al. [15] reported that the dead calves' anion gap value was higher than the survivors.

According to the findings obtained from this study, the determination of lactate (mmol/L) and Cl^- levels can be used as an adjunctive supplementary test in distinguishing calves with diarrhea with a good prognosis and calves with a poor prognosis or risk of treatment failure. The STFT and LTFT are median of 9.25 h and a median of 51.50 h, respectively, which indicates the importance of following neonatal diarrheic calves for at least 72 h and providing replacement fluid-electrolyte and other treatments during this time. In addition, it has been concluded that the high rates of calf deaths can be reduced by treating the disease early since the general condition categories of calves with diarrhea worsen, and the death rates increase. Also, a better understanding of the disease process will reduce calf mortality.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1

Comparison of mortality rates and durations of calves with diarrhea according to their general condition

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