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# HAEMATOLOGICAL ALTERATIONS IN CALVES WITH ACUTE RESPIRATORY DISTRESS SYNDROME DUE TO ASPIRATION PNEUMONIA: A PROSPECTIVE STUDY

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## Abstract

The aim of this prospective study was to investigate the arterial blood gas (ABG) analysis, which is considered the gold standard, and complete blood count (CBC) as a complementary test in neonatal calves with acute respiratory distress syndrome (ARDS) due to aspiration pneumonia. Ten healthy and 20 calves with ARDS due to aspiration of milk/colostrum were enrolled in the study. Clinical examinations were performed at admission. ABG analyses were performed to determine the presence of ARDS and investigate the extent of lung ventilation/damage. CBC analysis was performed from venous blood samples. Heart and respiratory rates and body temperature values were higher in diseased calves than healthy ones ($p <$ 0.000). Arterial pH, partial pressure of oxygen in arterial blood ($PaO_2$) and saturation of oxygen in arterial blood ($SaO_2$) were lower ($p < 0.001$) in the diseased calves, while partial pressure of carbon dioxide in arterial blood ($PaCO_2$) and lactate levels were higher ($p < 0.000$) than those in healthy ones. Also, leukocyte (WBC), lymphocyte, monocyte, granulocyte, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) levels were higher in the diseased calves than the healthy calves ($p < 0.032$). As a result of the correlation analysis, the only correlation was determined to be between $PaO_2$ and WBC, which was moderately negative. As a result, it was observed that leukocytosis developing in respiratory diseases that cause severe inflammatory processes such as aspiration pneumonia was negatively correlated with $PaO_2$ and could potentially exacerbate hypoxia,

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and ABG evaluation with CBC could provide useful clinical data in calves with ARDS due to aspiration pneumonia.

**Key words:** arterial blood gas, complete blood count, aspiration pneumonia, diagnosis, calf

# HEMATOLOŠKE PROMENE KOD TELADA SA AKUTNIM RESPIRATORNIM DISTERS SINDROMOM ZBOG ASPIRACIONE PNEUMONIJE: PROSPEKTIVNA ISTRAŽIVANJA

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## Kratak sadržaj

Cilj ove prospektivne studije bio je istraživanje analize arterijskih gasova krvi (AGK) koja se smatra zlatnim standardom, kao i kompletne krvne slike (CBC) kao dopunskog testa kod teladi u neonatalnom periodu sa akutnim respiratornim distresnim sindromom (ARDS) usled aspiracione pneumonije. Ispitivanje je obuhvatilo 10 zdrave i 20 teladi sa ARDS izazvane aspiracijom mleka/kolostruma. Klinički pregledi su izvršeni prilikom prijema životinja. AGK analize su izvršene da bi se odredilo prisustvo ARDS i ispitao kapacitet plućne ventilacije odnosno stepen njenog oštećenja. Analiza CBC vršena je iz uzoraka venske krvi. Vrednosti srčane i respiratorne frekvencija bile su više kod obolele teladi nego kod zdrave ($p <$ 0.000). Arterijski pH, parcijalni pritisak kiseonika u arterijskoj krvi ($PaO_2$) i saturacija kiseonika u arterijskoj krvi ($SaO_2$) bile su niže ($p < 0.001$) kod obolele teladi, dok su vrednosti parcijalnog pritiska ugljen dioksida u arterijskoj krvi ($PaCO_2$) kao i nivoi laktata bile više ($p < 0.000$) kod obolelih jedinki u odnosu na zdrave. Pored toga, nivoi leukocita (WBC), limfocita, monocita, granulocita, srednjeg korpuskularnog volumena (MCV) i srednjeg korpuskularnog hemoglobina (MCH) bili su viši kod obolele nego kod zdrave teladi ($p < 0.032$). Na osnovu rezultata korelacione analize jedina korelacija je utvrđena između $PaO_2$ i WBC, koja je procenjena kao umereno negativna. Shodno tome, ustanovljeno je da je leukocitoza, koja se razvija u okviru respiratornog oboljenja i izaziva teške inflamatorne procese kao što je aspiraciona pneumonija, u negativnoj korelaciji sa $PaO_2$ i potencijalno može da pogorša hipoksiju. Analiza arterijskih gasova krvi zajedno sa CBC mogu pružiti korisne kliničke podatke za telad sa ARDS izazvane aspiracionom pneumonijom.

**Ključne reči:** arterijski gasovi krvi, kompletna krvna slika, aspiraciona pneumonija, dijagnoza, tele

## INTRODUCTION

One of the most frequent causes of economic loss in livestock is respiratory disease, which has a multifactorial origin and leads to clinical and pathological abnormalities in the respiratory system. In addition to infectious causes, aspiration pneumonia (AP) is caused by the intake of foreign substances such as milk, colostrum, liquid supplements, or medications that damage the lung tissue as a result of inappropriate drenching methods/bottles or forced feeding (Hattab et al., 2022). Due to the irritating properties of the aspirated material, gangrenous bronchopneumonia may develop in cases where substantial amounts of fluid have been aspirated, albeit death is nearly always immediate (Akyüz et al., 2022). The initiation of clinical symptoms is frequently subtle since clinical and pathological characteristics of aspiration pneumonia are highly variable (Lopez and Martinson, 2017; Hattab et al., 2022).

Lung endothelial and epithelial damage develops as a result of aspiration/inhalation of foreign matter. After aspiration of liquid substances, acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) may develop in cases where gas exchange is impaired (Ider et al., 2022). The pulmonary alveolar and bronchial epithelium are also disrupted in cases of ARDS (Osaka et al., 2011) which is characterised by hypoxia ($PaO_2 < 60$ mmHg), respiratory acidosis, hypercapnia ($PaCO_2 > 45$ mmHg), tachypnea (respiratory rate>45/min), and abdominal respiration with wheezing (Bleul, 2009; Yıldız and Ok, 2017).

Complete blood count (CBC) and arterial and/or venous blood gas measurements are primary clinical diagnostic tools. Although the findings have been reported to be non-specific, CBC analysis is recommended in cases of aspiration pneumonia (Kogan et al., 2008). The use of arterial blood samples has been suggested in the evaluation of lung ventilation (Bleul et al., 2007). However, the technical difficulty of obtaining arterial blood samples under farm conditions in veterinary medicine causes the preference for venous blood samples (Nagy et al., 2002). Therefore, the aim of this prospective study is to investigate arterial blood gas (ABG) analysis, which is considered the gold standard in assessing lung oxygenation and ventilation-perfusion, and venous CBC findings as a complementary test and their correlation with each other in calves with ARDS due to aspiration pneumonia, and to acquire clinical information about the extent of the damage from venous blood samples in cases where arterial blood samples cannot be obtained.

## MATERIAL AND METHODS

This study was approved by the decision of the Ethics Committee of the Faculty of Veterinary Medicine of Harran University, with session number 2021/004, dated 07.05.2021 and numbered 01-08. All institutional and national guidelines for the care and use of study animals were followed. All calf breeders gave their consent before the commencement of the study.

### *Animal Selection*

Twenty neonatal calves of Holstein breed, whose developed non-specific respiratory disease symptoms such as cough, nasal and/or ocular discharge, tachypnea, and respiratory distress after being fed on milk/colostrum with inappropriate methods/bottles constituted the Diseased Group. Ten healthy neonatal calves of Holstein breed which were determined to be healthy in clinical and laboratory examinations (Dillane et al., 2018), constituted the Healthy Group. Calves from dystocia, prematurity, congenital abnormalities, and those with diarrhoea and infection suspicion were excluded from the study. In addition, the formation of aspiration pneumonia due to inhalation of different liquid materials such as digestive stimulants or vitamin-mineral complex solutions was not included in the study as the alteration and severity of the investigated parameters may be affected by the character and acidity of the aspirated material. All calves included in the study were treated with appropriate treatment protocols (intravenous fluid therapy, antimicrobial, anti-inflammatory, mucolytic and intranasal oxygen administrations) following clinical examination and sampling. However, the follow-ups of the diseased calves could not be performed due to communication and/or economic issues.

### *Clinical examinations*

Clinical examinations were performed both to detect the presence of pneumonia and to determine the present medical condition and included evaluation of hydration status, palpable lymph nodes and mucous membranes, measurements of heart and respiratory rate, and heart and lung auscultation. In addition, thoracic ultrasonography was performed to detect the presence of pneumonia in all the diseased calves. Using a 5 MHz micro-convex probe (Mindray Z60, China), the area of interest was wetted with 70% isopropyl alcohol without clipping the hair and scanned from the $10^{th}$ intercostal space towards the cranial. Clinical and ultrasonographic examinations were performed by the same veterinary staff applying the same examination protocols.

### *Blood Sampling*

Venous blood samples (5 mL) were taken once from vena jugularis at the time of admission from all the calves included in the study. The time of blood sampling, and thus the first admission to the hospital and the onset of the symptoms, was a median of 5 (1 – 12) hours after inappropriate colostrum/milk drenching. Arterial blood samples (1 mL) were taken by a puncture of arteria auricularis caudalis, as previously reported (Nagy et al., 2002). After the sampling, it was ensured that there were no air bubbles and inadequate or excessive anticoagulant. After the sampling, the specimen was analysed immediately. For CBC analysis, venous blood samples (using tubes with $K_3EDTA$), and for ABG analysis, arterial blood samples were used to determine the presence of ARDS and investigate the lung ventilation/damage.

### *Arterial Blood Gas Analysis*

Arterial blood pH, partial pressure of carbon dioxide in arterial blood ($PaCO_2$), partial pressure of oxygen in arterial blood ($PaO_2$), saturation of oxygen in arterial blood ($SaO_2$), and lactate measurements were performed using an automatic blood gas analyzer (epoc® Blood Analysis System, Siemens, Germany).

### *Complete Blood Count Analysis*

Total leukocyte (WBC), lymphocyte (Lym), monocytes (Mon), granulocyte (Gra), erythrocyte (RBC), mean corpuscular volume (MCV), hematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and haemoglobin (Hb) measurements were performed using an automatic cell counter (pocH-100i, Sysmex®, Japan).

### *Criteria for definition of Acute Respiratory Distress Syndrome*

The criteria for the presence of ARDS were hypoxia ($PaO_2 < 60$ mmHg), respiratory acidosis, hypercapnia ($PaCO_2 > 45$ mmHg), tachypnea (respiratory rate > 45/min), and abdominal respiration with wheezing (Bleul, 2009). Among these parameters, the presence of at least two criteria along with $PaO_2 < 60$ mmHg was taken into consideration.

### *Statistical Analysis*

Statistical software (SPSS 25.00, IBM®, USA) was used to evaluate the data. We used a one-sample Kolmogorov-Smirnov test to determine whether the variables had normal distributions. Non-parametric data were evaluated by Mann-Whitney U test, and presented as median (min, max). A Spearman's correlation was performed to determine the relationship between ABG and venous CBC results. Sizes of correlation were considered 0.5 – 0.7 (-0.5 - -0.7) moderately positive (negative), 0.7 – 0.9 (-0.7 - -0.9) highly positive (negative), and 0.9 – 1 (-0.9 - -1) very highly positive (negative). Statistical significance was considered as $p < 0.05$ and $p < 0.01$.

## RESULTS

### *Anamnestic Data*

Anamnestic data revealed that all the calves included in the Diseased Group developed respiratory disease-related symptoms after forced feeding on milk/colostrum with inappropriate methods/bottles. The samples were taken during the calving season, between June and August. Also, all the calves of the present study, which were housed in individual pens, were naturally born (> 280 days gestation) on the farm and were able to stand within 1 hour of life. The body weights of the calves in the groups were similar (Table 1). All the calves were given 2 litres of milk/colostrum within the first 6 hours of their life. The time of admission for all calves to the hospital was the day they were born. Considering the first 6 hours of colostrum administration, the mean time to hospital admission was 11 (7 - 20) hours after delivery. The complete history of each diseased calf is visualized (Figure 1.) as the symptom onset is sudden in cases of aspiration pneumonia.

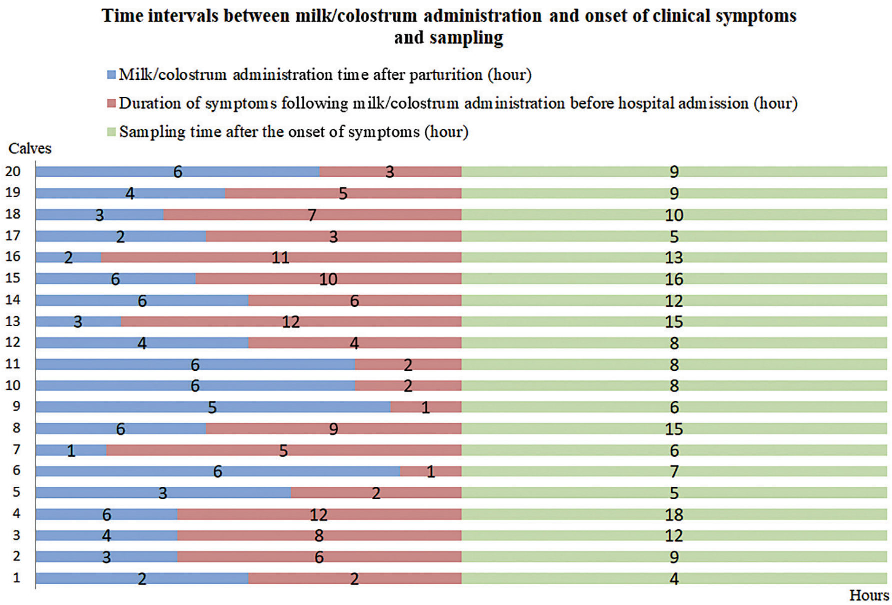


Figure 1. The complete history of time intervals of each diseased calf

## *Clinical Examination Findings*

Clinical examinations revealed findings to suspect aspiration pneumonia, such as extended neck (14 out of 20), bilateral mucoid/purulent nasal discharge (8 out of 20), a painful expression (13 out of 20), and widespread crackles over the affected lung during auscultation (20 out of 20). In addition, as a result of the thoracic ultrasonographic examination, poorly defined lung surface dorsally (12 out of 20), B-lines origination from pleura > 3 lines (20 out of 20), and loss of A-lines (14 out of 20) were detected (Figure 2). The results of clinical examination findings of the healthy animals and the calves with aspiration pneumonia are presented in Table 1. The heart and respiratory rates and body temperature of the calves with aspiration pneumonia were significantly higher than the healthy calves ($p < 0.000$).

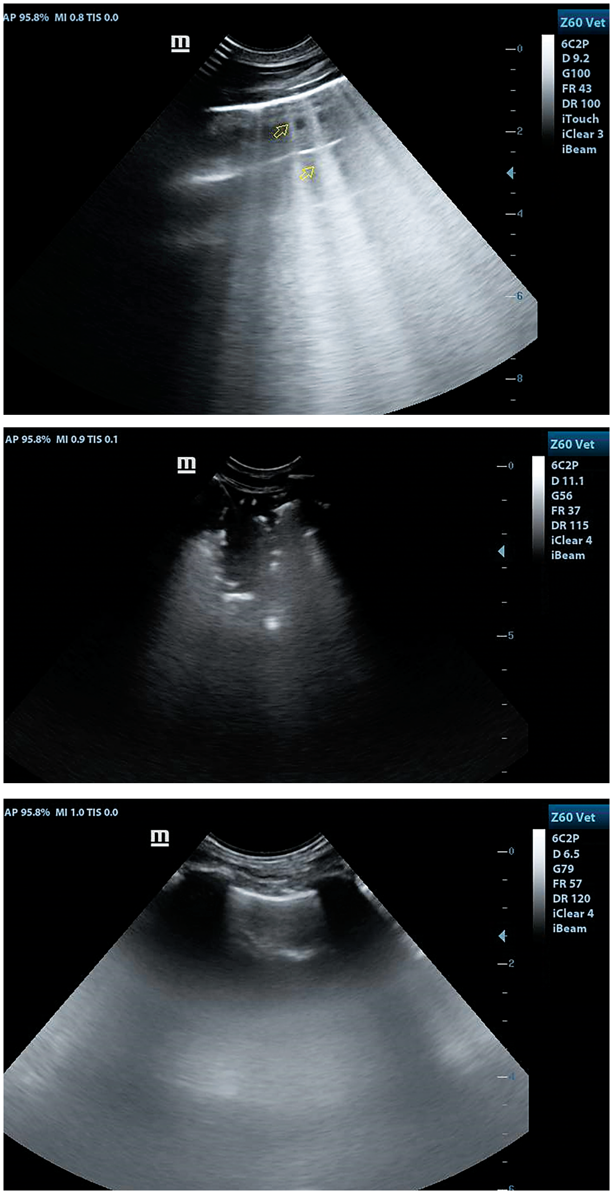


Figure 2. Thoracic ultrasonography examination findings (A. More than 3 B-lines in number, in confluent view, indicating loss of peripheral lung aeration due to interstitial disease involvement.; B. The ill-defined lung surface and anechoic area showing possibly aspirated fluid material.; C. The loss of the A-lines between the acoustic shadows formed by the ribs)

Table 1. Clinical examination findings

| Parameters | Healthy Group (n=10) | | | | | Diseased Group (n=20) | | | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | Q1 | median | Q3 | max | min | Q1 | median | Q3 | max | |
| Body weight (kg) | 42 | 43.75 | 48 | 51.75 | 55 | 34 | 39.75 | 47 | 50.25 | 54 | 0.154 |
| Heart rate (beats/min) | 86 | 88 | 99 | 110 | 120 | 78 | 140 | 148 | 160 | 178 | 0.000 |
| Body temperature (°C) | 37.6 | 38.07 | 38.5 | 39.02 | 39.4 | 38.8 | 39.8 | 40.05 | 40.6 | 41.2 | 0.000 |
| Respiratory rate (breaths/min) | 36 | 38 | 42 | 50.5 | 55 | 52 | 64 | 74.5 | 77 | 90 | 0.000 |

## *Arterial Blood Gas Analysis*

The results of ABG analysis findings of the healthy calves and the calves with aspiration pneumonia are presented in Table 2. Statistically significant differences were observed in pH, $PaCO_2$, $PaO_2$, $SaO_2$, and lactate levels. While the pH, $PaO_2$, and $SaO_2$ levels of the calves with aspiration pneumonia were lower than those in the healthy calves ($p$ < 0.001), $PaCO_2$ and lactate levels were significantly higher ($p$ < 0.000).

Table 2. Arterial blood gas analysis findings of the healthy and the diseased calves

| Parameters | Healthy Group (n=10) | | | | | Diseased Group (n=20) | | | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | Q1 | me-dian | Q3 | max | min | Q1 | me-dian | Q3 | max | |
| pH | 7.35 | 7.36 | 7.38 | 7.41 | 7.45 | 6.81 | 7.12 | 7.26 | 7.37 | 7.44 | 0.001 |
| $PaCO_2$ (mmHg) | 20.6 | 20.95 | 26.8 | 32.65 | 35.8 | 37.4 | 44.07 | 54.1 | 60.97 | 87.9 | 0.000 |
| $PaO_2$ (mmHg) | 79.7 | 82.8 | 85.25 | 89.6 | 93.8 | 8.6 | 22.4 | 25.65 | 31.15 | 41.6 | 0.000 |
| $SaO_2$ (%) | 86.5 | 90.92 | 95.5 | 90.55 | 99.6 | 5.8 | 26.75 | 39.2 | 43.67 | 53.9 | 0.000 |
| Lactate (mmol/L) | 0.2 | 0.3 | 0.62 | 0.9 | 0.92 | 1.13 | 2.65 | 5.01 | 7.49 | 13.33 | 0.000 |

pH: Power of hydrogen, $PaCO_2$: Partial pressure of carbon dioxide in arterial blood, $PaO_2$: Partial pressure of oxygen in arterial blood, $SaO_2$: Saturation of oxygen in arterial blood. Min: Minimum, Q1: First quartile, Q3: Third quartile, Max: Maximum.

### *Complete Blood Count Analysis*

The results of the CBC analysis of the healthy calves and the calves with aspiration pneumonia are presented in Table 3. As a result of CBC analysis, it was determined that WBC, lymphocyte, monocyte, granulocyte, MCV, and MCH levels of the calves with aspiration pneumonia were higher than that of the healthy ones ($p < 0.000$, $p < 0.012$, $p < 0.028$, $p < 0.000$, $p < 0.032$ and $p < 0.025$, respectively).

Table 3. CBC findings of the healthy and the diseased calves

| Parameters | Healthy Group (n=10) | | | | | Diseased Group (n=20) | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | Q1 | me-dian | Q3 | max | min | Q1 | me-dian | Q3 | max | |
| WBC ($x10^9/L$) | 5.4 | 7.43 | 9.4 | 9.4 | 12.5 | 6.38 | 14.57 | 19.59 | 25.45 | 47.67 | 0.000 |
| Lym-phocyte ($x10^9/L$) | 1.6 | 2.05 | 3.0 | 4.02 | 4.59 | 0.89 | 2.20 | 4.97 | 11.34 | 31.62 | 0.012 |
| Monocyte ($x10^9/L$) | 0.33 | 0.37 | 0.49 | 0.55 | 1.02 | 0.08 | 0.42 | 0.65 | 1.15 | 3.34 | 0.028 |

| Parameters | Healthy Group (n=10) | | | | | Diseased Group (n=20) | | | | | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | Q1 | median | Q3 | max | min | Q1 | median | Q3 | max | |
| Granulocyte ($x10^9/L$) | 2.9 | 4.17 | 5.26 | 7.08 | 9.37 | 2.14 | 8.98 | 12.86 | 15.81 | 17.83 | 0.000 |
| RBC ($m/mm^3$) | 5.62 | 6.41 | 7.96 | 10.12 | 10.62 | 4.05 | 6.19 | 8.19 | 10.54 | 12.83 | 0.782 |
| MCV (fl) | 30.5 | 34.52 | 44.85 | 49.62 | 52.5 | 27.8 | 42.7 | 48.65 | 70.77 | 76.7 | 0.032 |
| Haematocrit (%) | 23.2 | 25.02 | 37.55 | 45.92 | 52.1 | 21.6 | 33.92 | 40.85 | 48.28 | 64.2 | 0.242 |
| MCH (pg) | 7.1 | 9.8 | 11.6 | 12.6 | 15.3 | 4.6 | 11.12 | 13.5 | 20.75 | 28.2 | 0.025 |
| MCHC (g/dL) | 16.7 | 19.15 | 26 | 31.97 | 34 | 14.8 | 24.65 | 27.9 | 30.30 | 58.2 | 0.351 |
| Hb (g/dL) | 5.9 | 7.6 | 8.4 | 11.97 | 15.4 | 5 | 8.82 | 11.3 | 13.62 | 17.4 | 0.198 |

WBC: Total leukocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Hb: Haemoglobin. Min: Minimum, Q1: First quartile, Q3: Third quartile, Max: Maximum.

## *Correlation Between ABG and CBC Findings*

The results of Spearman's correlation test are presented in Table 4. The only correlation detected between ABG and CBC variables was between WBC and $PaO_2$, which was moderately negative (r = -0.567).

Table 4. Spearman's correlation analysis results

| Parameters | pH | $PaCO_2$ | $PaO_2$ | $SaO_2$ | Lactate | WBC | Lym | Mon | Gran | RBC | MCV | HCT | MCH | MCHC | Hb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | 1.000 | **-.568**** | .319 | **.604**** | **-.639**** | -.091 | -.072 | -.062 | .024 | -.319 | .214 | -.079 | .077 | -.060 | -.101 |
| **$PaCO_2$** | | 1.000 | -.700** | **-.784**** | **.723**** | .427* | .240 | .114 | .283 | .301 | .025 | .185 | .115 | .006 | .247 |
| **$PaO_2$** | | | 1.000 | **.852**** | **-.677**** | **-.567**** | -.498** | -.250 | -.438* | -.026 | -.225 | -.128 | -.260 | .127 | -.060 |
| **$SaO_2$** | | | | 1.000 | **-.778**** | -.463** | -.287 | -.158 | -.368* | -.063 | -.086 | -.008 | -.161 | -.018 | -.046 |
| **Lactate** | | | | | 1.000 | .399* | .177 | .170 | .366* | .062 | .367* | .409* | .247 | -.198 | .134 |
| **WBC** | | | | | | 1.000 | **.563**** | **.632**** | **.834**** | -.146 | .257 | .097 | .409* | .317 | .268 |
| **Lym** | | | | | | | 1.000 | .401* | .103 | .038 | -.107 | -.138 | -.132 | -.008 | -.171 |

| Parameters | pH | $PaCO_2$ | $PaO_2$ | $SaO_2$ | Lactate | WBC | Lym | Mon | Gran | RBC | MCV | HCT | MCH | MCHC | Hb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mon** | | | | | | | | 1.000 | .479** | -.095 | .213 | .176 | .384* | .310 | .322 |
| **Gran** | | | | | | | | | 1.000 | -.330 | **.514**** | .199 | **.650**** | .352 | .360 |
| **RBC** | | | | | | | | | | 1.000 | **-.547**** | .315 | -.451* | -.092 | .290 |
| **MCV** | | | | | | | | | | | 1.000 | .500** | .723** | -.163 | .197 |
| **HCT** | | | | | | | | | | | | 1.000 | .406* | -.210 | **.630**** |
| **MCH** | | | | | | | | | | | | | 1.000 | .399* | **.556**** |
| **MCHC** | | | | | | | | | | | | | | 1.000 | .487** |
| **Hb** | | | | | | | | | | | | | | | 1.000 |

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

pH: Power of hydrogen, $PaCO_2$: Partial pressure of carbon dioxide in arterial blood, $PaO_2$: Partial pressure of oxygen in arterial blood, $SaO_2$: Saturation of oxygen in arterial blood, WBC: Total leukocyte, Lym: Lymphocyte, Mon: Monocyte, Gran: Granulocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Hb: Haemoglobin.

## DISCUSSION

Respiratory diseases, which are considered as one of the most critical health problems of the livestock industry, are very significant due to their economic impact, and the foremost cause is pneumonia. Cattle can acquire haematogenous pneumonias; however, most cases are bronchogenic or of inhalation/aspiration origin (Gülersoy and Şen, 2017). The most common respiratory diseases in neonatal calves are reported as postnatal hypoxia-hypercapnia, pulmonary hypertension, aspiration pneumonia, and bacterial and viral pneumonias (Vallés, 2013).

It has been reported that ARDS and ALI may develop due to impaired alveolar gas exchange and hypoxia that develops following incidental aspiration in cases of aspiration pneumonia (Son et al., 2017). Clinically, calves with ARDS due to aspiration pneumonia are characterized by cough, fever, tachypnea, cyanosis, and abnormal lung sounds (Ider et al., 2022). In the present study, the respiratory rate, body temperature, and the heart rate of the calves with ARDS due to aspiration pneumonia were significantly higher than the healthy calves ($p < 0.000$) (Table 1). In addition, tachypnea, weakness, lethargy, mild to moderate cyanosis, tachycardia, and fever were present in all the calves with ARDS due to aspiration pneumonia. The clinical findings of the diseased calves in this study were due to the combination of factors and septic nature of aspiration pneumonia (McGuirk and Simon, 2008), and the development of respiratory distress (Ider et al., 2022).

Abnormal blood gas alterations such as hypoxia, hypercapnia, and respiratory acidosis are common findings in calves with respiratory distress (Bleul, 2009). In the present study, pH, $PaO_2$, and $SaO_2$ levels of the calves with ARDS due to aspiration pneumonia were significantly lower ($p < 0.001$), while $PaCO_2$ and lactate levels were higher ($p < 0.000$) than that of the healthy calves (Table 2). However, $PaCO_2$ levels of some of the diseased calves (6 out of 20) were determined to be less than 45 mmHg, and this finding was thought to be related to the volume of aspirated milk/colostrum (Marik, 2001). These findings indicate that significant changes in ABG balance and elevated lactate levels in calves with ARDS were due to aspiration pneumonia associated with hypoxia, lung endothelial and epithelial damage, and impaired gas exchange (Yıldız and Ok, 2017; Ider et al., 2022).

The primary clinical diagnostic tools are CBC and arterial and/or venous blood gas measurements (Kogan et al., 2008). It was reported that respiratory diseases lead to direct lung function disturbances with alterations in gas exchange and changes in the haematological profile (Šoltésová et al., 2015). Due to the septic and acute characteristics of aspiration pneumonia, it has been reported that severe infection may develop (Dhillon et al., 2020). In the present study, WBC, lymphocyte, monocyte, granulocyte, MCV, and MCH levels of the calves with ARDS due to aspiration pneumonia were significantly higher than that of the healthy calves ($p < 0.032$) (Table 3). Elevated WBC, lymphocyte, monocyte, and granulocyte levels are indicators of an inflammatory process (Šoltésová et al., 2015). In the previous reports, differences in RBC count, haemoglobin and haematocrit levels were reported. These findings could be a consequence of the adaptation and compensatory processes reflecting an existing pulmonary disease, hypoxaemia, and the stimulation of erythropoiesis (Hanzlicek et al., 2010; Fraser et al., 2014). In a study in rats, hypoxia was determined by five indicators: WBC, granulocyte and RBC counts, reticulocyte count/percent, and MCH levels (Kondashevskaya et al., 2021). However, in the present study, RBC, haematocrit, MCHC and Hb levels of the diseased calves were not statistically different from the healthy ones. These findings may be related to the fact that these compensation mechanisms have not been activated yet. An elevation in MCV level may result from reticulocytosis, which is quite uncommon in chronic respiratory diseases, but in acute ones (Tsantes et al., 2004). Therefore, higher MCV and MCH levels of the diseased calves in this study may be associated with hypoxia tolerance and acuteness of the cases (Dzhalilova and Makarova, 2020).

Since blood serves as the primary channel for transferring $CO_2$ in the opposite direction and $O_2$ from the lungs to tissues, studies aimed at identifying the effects and/or reflections of hypoxia mostly on blood (Kondashevskaya et al., 2021). That is why the use of arterial blood samples has been suggested in evaluating lung ventilation/damage (Bleul et al., 2007). During infectious or non-infectious conditions causing hypoxia, erythroid cells continue performing their specific functions, changing their numbers, size, or $O_2$ content. Thus, under hypoxic conditions, WBC counts in small laboratory rodents, mainly neutrophils, are reported to be elevated (Kondashevskaya et al., 2021). As a result of the correlation analysis, which is one of the main topics of the present study, a moderate negative correlation was determined between $PaO_2$ and WBC count (Table 4). This finding may demonstrate that leukocytosis developing due to aspiration pneumonia may have a possible negative contribution to the exacerbation of hypoxia and could worsen the clinical manifestation as a consequence of rapid oxygen consumption by the leukocytes (Chillar et al., 1980). Therefore, since aspiration pneumonia in calves is an often-fatal emergency (Hattab et al., 2022), it may be considered to evaluate CBC as a complementary test along with ABG, at least for the initial assessment, and the prediction of the clinical outcome (McKeever et al., 2016).

The major limitation of the present study is the limited number of animals that may influence the significance and correlations of some investigated blood parameters. The authors recommend evaluating the present results with a larger number of animals. In addition, the lack of histopathological examination indicating lung damage and loss to follow-ups due to social or structural reasons can be considered limitations. Although the results of the present study are promising, evaluation of ABG and CBC findings along with serum biochemistry parameters may allow more accurate and faster assessment of emergency aspiration pneumonia cases based on a routine analysis.

## CONCLUSION

Aspiration pneumonia, which is often caused by inappropriate drenching and/or forced feeding, is a frequent condition that causes hypoxia, lung endothelial and epithelial damage, ARDS, and even sudden death depending on the amount of aspirated liquid. It emerged that significant changes occur in ABG and venous CBC findings of neonatal calves with ARDS due to aspiration pneumonia. It was observed that leukocytosis that develops in respiratory diseases such as aspiration pneumonia which causes a severe inflammatory process, could potentially exacerbate hypoxia. As a result, it was concluded that CBC analysis with ABG measurement findings could provide valuable clinical data in calves with ARDS due to aspiration pneumonia.

## Author's Contribution

EG and CB made contributions to conception, methodology, involved in data collection, formal analysis and drafting the manuscript. İG and AŞ carried out the data collection and drafting of the manuscript. All authors revised the manuscript critically and EG together with CB prepared the final draft of the manuscript etc. All authors read and approved the final manuscript.

## Competing interest

The authors declare that they have no competing interest.

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