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Lung Inflammatory Response in Neonatal Diarrheic Bovine Calves with Respiratory Disease Syndrome (RDS)

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Abstract

To investigate the lung inflammatory response during diarrheic episodes in neonatal calves with respiratory disease syndrome, 27 newly born bovine Friesian calves of both sexes (up to 30 days old) were studied, of which 17 diarrheic calves with RDS admitted to the Hospital of the Faculty of Veterinary Medicine Zagazig University and ten apparently healthy calves with no history of any previous illness kept as a control group in the same age range. The examined calves were admitted with a history of diarrhea with respiratory disease syndrome and presented with a variety of clinical signs, including anorexia, diarrhea, cough, dyspnea, and variable degrees of nasal discharge dehydration, weight loss, dullness and pale mucous membranes. The laboratory findings reveal significant changes in diarrheic calf blood parameters, with the RDS group showing significantly lower pH, PaO_2, HCO_3, BE, and significantly higher $PaCO_2$ and blood lactate values compared to healthy group. Serum glucose, Na, and Cl levels were also significantly lower, whereas serum K levels were significantly higher compared to healthy group. There was a positive correlation between pH and PaO2, HCO_3, and BE concentrations, but a negative correlation between pH and $PaCO_2$ and lactate concentrations. The lung-specific epithelial and endothelial biomarkers in healthy and diarrheic neonatal calves with ARD included in the present study revealed that, serum ADMA and SP-D levels were significantly lower, while ET-1 concentrations increased significantly in diarrheic calves with RDS compared to control group measurements. The relationship between ADMA, ET-1 and SP-D concentrations, showed a very strong negative correlation between ADMA and SP-D concentrations and the concentration of ET-1. Whereas a strong positive correlation between ADMA and SP-D concentrations.

KEYWORDS
Bovine calves, diarrhea, asymmetric dimethylarginine, endothelin-1, lung biomarkers.

INTRODUCTION

Neonatal calf diarrhea is a commonly reported disease and a major cause of economic loss to cattle producers. Calf mortality due to diarrhea is reported to be high in the 3rd week of life, and mostly attributed to infectious causes (Cho and Yoon, 2014). Immunodeficiency, season effects (Berber *et al.*, 2021), difficult parturition, and poor management conditions are all important causes of calf mortality. Diarrhea and respiratory disease are the two most common medical conditions that negatively impact the health of dairy calves (Medrano-Galarza *et al.*, 2018). Both are clinical signs that are most common in calves under one month old. Both conditions are complicated and require different treatment approaches.

Diarrhea in young calves is a syndrome with a mixed etiology that is exacerbated by a combination of environmental, dietary, physiological, and management factors. While, pneumonia is a fairly predominant condition that affects the claves and causes major economic losses as well as death (Zhang *et al.*, 2015). Recent advances in human medicine have led to the use of biomarkers specific to pulmonary epithelium and endothelial damage as a noninvasive method of assessing lung inflammatory response in diarrheic patients (Galie *et al.*, 2004; Garcia-Laorden *et al.*, 2017).

Asymmetric dimethylarginine (ADMA), endothelin-1 (ET-1), and surfactant protein-D (SP-D) concentrations are lung-specific epithelial and endothelial biomarkers used to assess endothelial cell proliferation and mortality in bronchopneumonia (Gutierrez *et al.*, 2001; Nickel *et al.*, 2011; Zhang *et al.*, 2015; Ider *et al.*, 2021).

Asymmetric dimethylarginine is an oxidative stress end product and a naturally occurring amino acid that prevents the production of nitric oxide. Increased ADMA concentrations reduce nitric oxide concentrations, resulting in an increase in vascular tone. As a result, it has been proposed that ADMA can be used as a biomarker in bronchopneumonia (Zhang *et al.*, 2015).

Endothelin-1 is a peptide that is abundant in the lung and is important in the development of bronchopneumonia due to the presence of endothelin receptors on the vascular smooth muscle cells (Galie *et al.*, 2004). Surfactant protein-D is secreted by type II pneumocytes and is important in ensuring and maintaining alveolar surface integrity. SP-D concentrations in patients with acute respiratory disease syndrome (ARDS) decreased with destruction

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of type II pneumocytes in the lungs, depending on the severity of lung damage (Greene *et al*., 1999). Changes in SP-D concentrations in ARDS patients could provide useful information about the disease's prognosis (Ware *et al*., 2010; Calfee *et al*., 2011). In humans and other species, elevated surfactant protein D levels in bronchoalveolar lavage fluid and blood have been linked to early alveolar inflammation and basal membrane damage.

Therefore, we aimed to determine the concentration of lung-specific epithelial and endothelial biomarkers (ADAM, ET-1 and SP-D) and to determine the prognostic value of these markers in predicting mortality in neonatal calves with diarrhea complicated with respiratory disease.

MATERIALS AND METHODS

Animals

The present study was performed using 27 newly born bovine Frisian calves (with an age of up to 30 days old) of both sexes. Of which, 17 diarrheic calves with respiratory disease syndrome (RDS) were collected from calves admitted for clinical examination at the Hospital of the Faculty of Veterinary Medicine, Zagazig University, Egypt, were enrolled in the study. Ten apparently healthy calves with no history of any previous illness were kept as a control group in the same age range. Because this study did not include any experimental work, approval from the Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC) was not required.

Clinical examination

Diseased calves were admitted with a history of diarrhea combined with respiratory disease syndrome and presented with a variety of clinical signs, including anorexia, diarrhea, cough, dyspnea, and variable degrees of nasal discharge dehydration, weight loss, dullness, and pale mucous membranes. A thorough clinical examination was performed (Constable *et al*., 2016) which included body temperature, respiration, and heart rate. Ultrasonographic examination was performed for both diseased and control calves on both sides of the thorax according to standardized examination techniques (Tharwat and Oikawa, 2011).

Collection of blood samples

At the time of admission, blood samples were collected from healthy and control calves for hematology, arterial blood gas analysis and lung-specific biomarker measurements. Two blood samples were collected from the jugular vein, the first placed in an EDTA tube for whole blood collection and the second in a plain tube for serum collection.

Hematological analysis

Whole blood sample was used for hematological analysis, including hematocrit (%), hemoglobin (%), erythrocyte count (106/ml) and total leucocytic count were performed using an automated cell counter (HA-Vet Hematology Analyzer®, Clindiag Systems B.V.B.A, Belgium).

Blood gas analysis

An arterial blood sample was collected using heparinized syringes for measuring blood gases (pH, $PaCO_2$, PaO_2, HCO_3, base excess (BE) and lactate concentration) using a blood gas analyzer (ST-200 CC, Blood Gas Analyzer®, Sensacore, India).

Lung inflammation biomarker analyses

The second blood samples were centrifuged for 10 minutes at 3000 rpm, serum samples were collected and then kept frozen at -20 °C until further analysis. Serum ADMA, ET-1 and SP-D concentration of all calves were measured using commercial bovine-specific ELISA test kits according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai, China): bovine asymmetrical dimethylarginine ELISA kit (Bioassay Technology Laboratory), bovine endothelin-1 ELISA kit (Bioassay Technology Laboratory) and bovine SP-D ELISA kit (Bioassay Technology Laboratory).

Thoracic ultrasonography

The ultrasonographic examination was done according to previously described studies. The area of ultrasonographic examination extends from the 3rd to 11th intercostal spaces (ICS) and was prepared by clipping, shaving, and cleaning with alcohol, and then ultrasonographic gel was used. Lungs were examined from both sides according to a standard examination technique. Ultrasonography was performed with a 3.5 MHz convex transducer (WED, WELLD®, Shenzhen Well D Medical Electronics Co., Ltd., China). Lungs were examined from a dorso-ventral plane by holding the transducer parallel to the ribs (Tharwat and Oikawa, 2011; El-Zahar *et al*., 2021).

Statistical analysis

All data were statistically analyzed using SPSS Statistics® 22.0 (Version 22.0, Armonk, NY: IBM Corp). The data were tested for normal distribution using Shapiro Wilks W Test and were found normally distributed. The obtained results were analyzed using analysis of variance ANOVA test, all data are listed as mean±SE. Differences between parameters were tested for significance at probability level of $P<0.05$. Pearson correlation was used for estimating the relationship between the concentration of blood gas parameters and ADMA, ET-1, and SP-D concentrations in diarrheic and healthy calves.

RESULTS

Clinical findings

Based on clinical examination, the most common clinical signs in calves with diarrhea and respiratory disease syndrome at the time of hospitalization are summarized in Table 1. Severe clinical signs (depression, inappetence, loss of body weight, hyperventilation, dyspnea, nasal and ocular discharge, and coughing) were present in the majority of cases. Mean rectal temperature was 39.1±0.6, mean heart rate was 127±2.37 bpm, mean respiratory rate was 37.17±1.19 rpm. Sixty five percent (n = 11) of the calves were considered to have mild dehydration, while 35% (n = 6) calves were classified as having moderate dehydration. On lung percussion, all diseased animals had a reduced resonance sound, while lung auscultation revealed a 24% exaggerated vesicular sound, 70% wheezes, 6% pleuritic frictional sounds on lung auscultation. There were 24% of diarrheic calves who coughed up on stimulation and 76% who coughed spontaneously. The clinical evaluation revealed that the other ten calves (controls) showed no symptoms as well.

Hematological analysis

The mean values of hematological indices in healthy and diarrheic calves with RDS are summarized in Table 2. Erythrocyte count (7.19±0.12) and hemoglobin (8.1±0.17) were significantly lower in the diseased calves compared to the control group measurements (9.11±0.12 and 11.92±0.24, respectively). While the total white blood cell count (17.4±0.42), neutrophils (73.62±0.63) and HCT (38.48±0.25) increased significantly when compared to the control group measurements (9.43±0.22, 59.1±2.9 and 31.97±0.44, respectively).

Blood gas analysis and glucose

The mean values of arterial blood gas analysis in healthy and diarrheic calves with RDS are summarized in Table 2. The results revealed that diarrheic calves with respiratory disease syndrome had significantly lower values for pH (7.12±0.01), PaO_2 (30.26±1.8), HCO_3 (18.17±0.33), BE (-6.22±0.4) while significantly higher values for $PaCO_2$ (60.73±1.3) and blood lactate (52.69±4.5). In addition, serum glucose (76.03±1.15), Na (124.5±1.02) and Cl (73.84±1.6) levels were significantly ($p < 0.05$) lower in diarrheic calves with RDS compared to the control group measurements, whereas serum K (5.39±0.11) levels were significantly ($p < 0.05$) higher.

Inflammation biomarker analysis

The mean values of lung inflammation biomarkers in healthy and diarrheic calves with RDS are summarized in Table 3. Serum

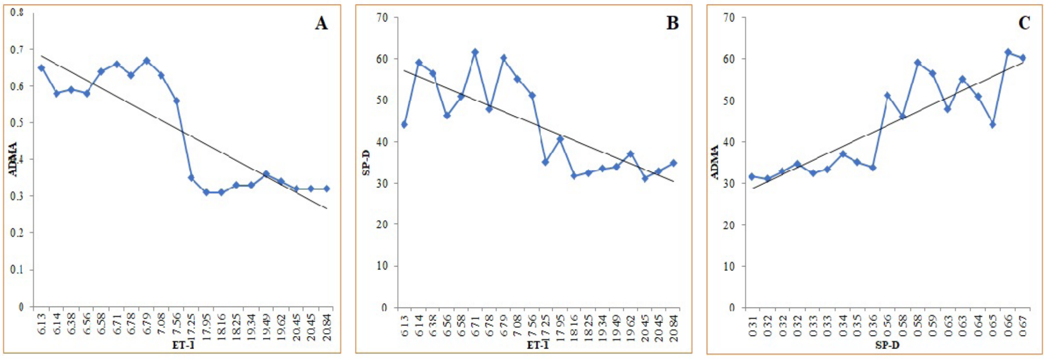

Figure 1. Correlation analysis graph between serum lung-specific epithelial and endothelial biomarkers (ADMA, ET-1 and SP-D). (A) strong negative correlation between ET-1 and ADMA (r = -0.975). (B) strong negative correlation between ET-1 and SP-D (r = -0.901). (C) strong positive correlation between SP-D and ADMA (r = 0.900).

Table 1. Frequencies of clinical signs and physical examination findings in healthy and diarrheic calves with RDS included in the present study.

Clinical signs and physical examination findings	Healthy calves (n = 10) (Mean±SE)	Diarrheic calves with RDS (n = 17) (Mean±SE)
Body weight (Kg)	42.4±2.8[a]	27.32±2.1[b]
General systemic statement		
- Body temperature °C	38.5±0.3[a]	39.1±0.6[b]
- Heart rate (beats/min)	96.9±2.7[b]	127±2.37[a]
- Respiratory Rate/ minute	24.34±1.2[b]	37.17±1.19[a]
Coughing		
- Coughing up on stimulation, n (%)	n = 0	n = 4 (24%)
- Spontaneous coughing, n (%)	n = 0	n = 13 (76%)
Percussion of lung		
- Reduced resonance, n (%)	n = 0	n = 17 (100%)
- Increased resonance, n (%)	n = 0	n = 0 (00)
Auscultation of the lungs		
- Exaggerated vesicular sound, n (%)	n = 0	n = 4 (24%)
- Wheezing sound, n (%)	n = 0	n = 12 (70%)
- Absence of lung sounds, n (%)	n = 0	n = 0
- Pleuritic frictional sounds, n (%)	n = 0	n = 1 (6%)
Ocular discharge		
Moderate amount, n (%)	None	n = 10 (59%)
Heavy amount, n (%)	None	n = 7 (41%)
Sunken eyes, n (%)	None	n = 14 (82%)
Dehydration		
Mild, n (%)	None	n = 11 (65%)
Moderate, n (%)	None	n = 6 (35%)

The data are presented as Mean±S.E and n = absolute number.; a,b Means carrying different superscripts differ significantly

ADMA (0.33±0.05) and SP-D (34.32±0.89) levels were significantly ($p<0.05$) lower, while ET-1 (19.18±0.38) concentration was increased significantly ($p< 0.05$) in diarrheic calves with RDS compared to control group measurements.

The relationship between the serum lung-specific epithelial and endothelial biomarkers (ADMA, ET-1 and SP-D) concentrations were measured using Pearson correlation by plotting the results of ADMA and SP-D concentrations against the measurements of the ET-1 concentrations in the same samples of 17 diarrheic calves with RDS; the results showed a very strong negative correlation ($r = -0.975$, $r = -0.901$, respectively) (Figures 1.A and 1.B). Whereas a strong positive correlation between ADMA and SP-D concentrations ($r = 0.900$) (Figures 1.C).

Correlations between the arterial blood gas parameters and the lung-specific biomarkers (ADMA, ET-1 and SP-D) concentrations in diarrheic calves with RDS and control group are presented in Table (4). Positive correlations between blood pH, PaO_2, HCO_3, BE and ADMA whereas a negative correlation was found among PaCO2, lactate and ADMA (Figure 2). A positive correlation between $PaCO_2$, lactate and ET-1, whereas a negative correlation was found among blood pH, PaO_2, HCO_3, BE and ET-1 (Figure 3). Positive correlations between blood pH, PaO_2, HCO_3, BE and SP-D whereas a negative correlation was found among $PaCO_2$, lactate and SP-D (Figure 4).

Ultrasonographic findings

The ultrasonographic examination of lungs in healthy control

Table 2. The hematological findings and mean values of glucose, acid-base balance parameters and electrolyte levels in healthy and diarrheic neonatal calves with RDS included in the present study.

Parameters	Healthy calves (n=10)	Diarrheic calves (n=17)
Hematological analysis		
Hemoglobin (g/l)	11.92±0.24[a]	8.1±0.17[b]
HCT (%)	31.97±0.44[b]	38.48±0.25[a]
RBCs ($x10^6$/ul)	9.11±0.12[a]	7.19±0.12[b]
WBCs ($x10^3$/ul)	9.43±0.22[b]	17.4±0.42[a]
Neutrophils %	59.1±2.9[b]	73.62±0.63[a]
($x10^3$/ul)	5.57±0.06[b]	6.94±0.01[a]
Blood gas analysis		
pH	7.46±0.03[a]	7.12±0.01[b]
$PaCO_2$ (mmHg)	37.11±0.61[b]	60.73±1.3[a]
PaO_2 (mmHg)	66.25±0.35[a]	30.26±1.8[b]
HCO_3 (mmol/l)	30.72±0.15[a]	18.17±0.33[b]
BE (mmol/l)	1.3±1.2[a]	-6.22±0.4[b]
Lactate (mg/dl)	19.14±1.2[b]	52.69±4.5[a]
Glucose (mg/dl)	114.31±1.38[a]	76.03±1.15[b]
Na^+ (mmol/l)	136.68±0.46[a]	124.5±1.02[b]
K^+ (mmol/l)	4.43±0.04[b]	5.39±0.11[a]
Cl^- (mmol/l)	98.9±1.1[a]	73.84±1.6[b]

The data are presented as Mean±S.E.; [a,b] Means carrying different superscripts differ significantly

Figure 2. Correlation analysis graph between ADMA and blood gas parameters (pH, $PaCO_2$, PaO_2, HCO_3, BE and lactate. (A) Positive correlation between ADMA and pH ($r = 0.973$). (B) strong negative correlation between ADMA and PaCO2 ($r = -0.946$). (C) Positive correlation between ADMA and PaO2 ($r = 0.957$). (D) Positive correlation between ADMA and HCO3 ($r = 0.971$). (E) Positive correlation between ADMA and BE ($r = 0.813$). (F) strong negative correlation between ADMA and lactate ($r = -0.828$).

group showed the pleural layers as long, smooth, white linar echo lines that move synchronously with respiration. The normal lung parenchyma was imaged as reverberation artifacts that appear regularly and parallel to the pleura owing to the presence of air (Figure 5).

Diseased calves suffered from bronchopneumonia showed small hypoechoic circular zones on the lung surface with a comet-tail artifact on ultrasonographic examination. In severe pneumonia with lung consolidation, reverberation artifacts were ill defined and unclear (Figure 6).

In calves with pleurisy, the ultrasonographic examination showed echogenic fluid in the pleural sac with the presence of fibrin shreds and the pleura appeared thick and corrugated, pleural effusion revealed hypoechoic fluid (Figure 7).

Table 3. The lung-specific epithelial and endothelial biomarkers in healthy and diarrheic neonatal calves with ARD included in the present study.

Parameters	Healthy calves (n = 10)	Diarrheic calves (n = 17)
ADMA (nmol/ml)	0.62±0.12[a]	0.33±0.05[b]
ET-1 (ng/l)	6.67±0.14[b]	19.18±0.38[a]
SP-D (ng/ml)	53.34±1.9[a]	34.32±0.89[b]

The data are presented as Mean±S.E.; [a,b] Means carrying different superscripts differ significantly

Figure 3. Correlation analysis graph between ET-1 and blood gas parameters (pH, $PaCO_2$, PaO_2, HCO_3, BE and lactate. (A) Negative correlation between ET-1 and pH (r = -0.972). (B) Positive correlation between ET-1 and $PaCO_2$ (r = 0.950). (C) Negative correlations between ET-1 and PaO_2 (r = -0.967). (D) Negative correlation between ET-1 and HCO3 (r = -0.978). (E) Negative correlation between ET-1 and BE (r = -0.784). (F) Positive correlation between ET-1 and lactate (r = 0.869).

Table 4. Correlation results between arterial blood gas parameters and ADMA, ET-1, and SP-D concentrations in diarrheic and healthy calves (Pearson correlation analysis).

Parameters	pH	$PaCO_2$	PaO_2	HCO_3	BE	Lactate	ADMA	ET-1	SP-D
pH	1	-0.965**	0.969**	0.985**	0.771**	-0.854**	0.973**	-0.972**	0.878**
$PaCO_2$		1	-0.985**	-0.967**	-0.768**	0.864**	-0.946**	0.950**	-0.848**
PaO_2			1	0.979**	0.739**	-0.860**	0.957**	-0.967**	0.853**
HCO_3				1	0.787**	-0.853**	0.971**	-0.978**	0.885**
BE					1	-0.664**	0.813**	-0.784**	0.681**
Lactate						1	-0.828**	0.869**	-0.730**
ADMA							1	-0.975**	0.900**
ET-1								1	-0.901**
SP-D									1

ADMA, asymmetric dimethylarginine; BE, base deficit; ET-1, endothelin-1; PaCO2, arterial partial pressure of carbon dioxide; PaO2, arterial partial pressure of oxygen; SP-D, surfactant protein. **P < 0.01.

DISCUSSION

Calf diarrhea and pneumonia are the two primary diseases affecting dairy calves' welfare, causing massive economic and productivity losses to the global bovine industry (Cho and Yoon, 2014; Medrano-Galarza *et al.*, 2018). Both are the leading causes of neonatal morbidity and mortality in dairy and beef calves under a month old (Altuğ *et al.*, 2013; Uetake, 2013; Lorenz *et al.*, 2011; Boussena and Sfaksi, 2009). Both are multifactorial diseases with distinct management approaches (Windeyer *et al.*, 2014). The most common clinical observations in bovine diarrheic neonatal calves (up to 30 days old) with signs of respiratory disease syndrome were depression, inappetence, loss of body weight, hyperventilation, dyspnea, exaggerated vesicular sound, wheezes, nasal and ocular discharge, and coughing were present in the majority of cases, and this was in accordance with a previous study by Constable *et al.* (2016). The obtained results were also in agreement with previous findings reported by Ider *et al.* (2021), who observed that the most clinical signs appeared are mainly the presence of apnea or tachypnea, abdominal or wheezing respirations, cyanotic or pale mucous membranes, prolonged capillary refill time, and hypothermia were present in premature calves with RDS.

The mean body temperature, respiration, and heart rate all increased significantly, while calves' body weight decreased significantly. This observation agrees with previous reports (Walker *et al.*, 1998; Leal *et al.*, 2008). In fact, it appears that compensatory polypnea occurs in response to acidosis to eliminate excess CO_2 in order to achieve normal pH values, and tachycardia compensates for hypovolemia caused by diarrheal episodes. The rectal temperature could be influenced by the duration of the diarrheal episode and the severity of dehydration (Leal *et al.*, 2008; Berchtold, 2009; Torche *et al.*, 2020).

In the current study, the mean values of arterial blood gas analysis, serum electrolytes concentrations and hematological indices in diarrheic calves with RDS revealed that erythrocyte count and hemoglobin concentration were significantly lower, while the total white blood cell count, neutrophils and HCT increased significantly when compared to the control group measurements. In fact, leukocytosis and neutrophillia in calves suffered from diarrhea than apparently healthy group is due to the pathogenic infection. Thus, our results coincided with those of previous reports (Leal *et al.*, 2008; Freitas, 2009; Malik *et al.*, 2013). Hematocrit provides important information about the overall erythrocytes volume in addition; it tended to be higher at birth and then decreased with age. Furthermore, hemoglobin is related to the rate of oxygen transported in the bloodstream. Hematocrit and hemoglobin reflect accurately the hydration status and used to assess it (Malheu, 2007).

Diarrheic claves with respiratory disease syndrome elicit significant changes in blood results where it showed significantly lower values of pH, PaO_2, HCO_3, BE, and significantly higher values for PaCO2 and blood lactate. In addition, serum glucose, Na and Cl levels were significantly lower, whereas serum K levels were significantly higher. Thus, our results coincided with those of previous reports (Klein *et al.*, 2008; Sayers *et al.*, 2016). The low blood pH value indicates acidemia in calves with diarrhea. In general, metabolic acidosis may be a compensatory decrease in PCO_2 due to hyperventilation. However, since calves with severe acidosis have already lost much respiratory function, hypoventilation does not serve a compensatory function (Constable *et al.*, 2016).

Previous research on calves with RDS indicated that changes in blood gases and acid-base balance varied in severity (Yildiz *et al.*, 2017; Ok *et al.*, 2020). In neonatal calves with RDS, mixed

Figure 4. Correlation analysis graph between SP-D and blood gas parameters (pH, $PaCO_2$, PaO_2, HCO_3, BE and lactate. (A) Positive correlation between ET-1 and pH (r = 0.878). (B) Negative correlation between ET-1 and PaCO2 (r = -0.848). (C) Positive correlations between ET-1 and PaO2 (r = 0.853). (D) Positive correlation between ET-1 and HCO3 (r = 0.885). (E) Positive correlation between ET-1 and BE (r = 0.681). (F) Negative correlation between ET-1 and lactate (r = -0.730).

acidosis (respiratory-metabolic acidosis) is prevalent in addition to hypercapnia and hypoxia. In our investigation, a positive association was seen between pH and PaO_2, HCO_3, and BE concentrations, while a negative correlation was seen between pH and $PaCO_2$ and lactate concentrations. Blood pH and BE are frequently regarded as useful indicators for detecting the respiratory and metabolic acidosis in newborn calves. The decrease in blood pH is caused by insufficient CO_2 removal from the lungs. A high lactate concentration, in addition to PCO_2, contributes to the development of acidosis. Lactate is an indirect marker of tissue hypoxia produced when there is hypoxia and poor tissue perfusion. Lactate is responsible for metabolic acidosis in newborns with asphyxia and plays an important role in the development of acidosis (Bleul and Gotz, 2013; Ok *et al*., 2020).

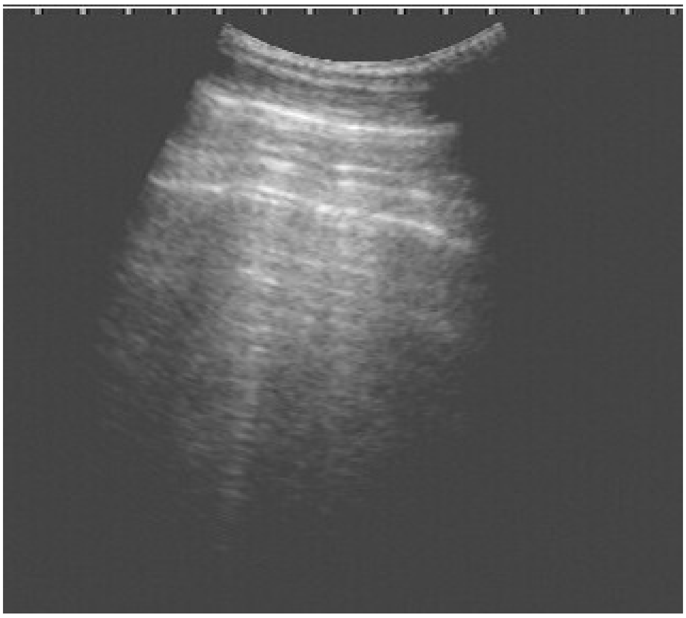

Figure 5. Ultrasonography of the normal lung in healthy calves using a 3.5 MHz transducer. The pleural layers appear as white linear echogenic lines. Reverberation artifacts appear as echogenic lines parallel to the pulmonary surface.

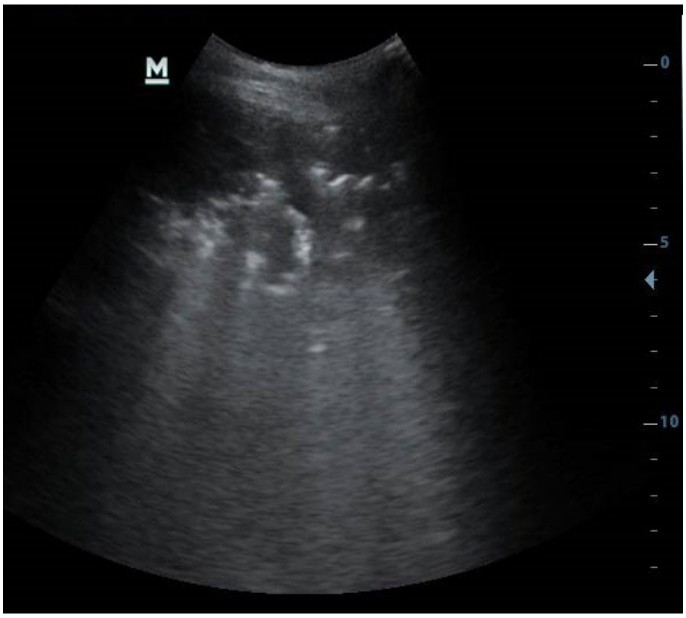

Figure 6. Ultrasonography of the lung of calves suffered from bronchopneumonia using a 3.5 MHz transducer. Showed small hypoechoic circular zones on the lung surface with a comet-tail artifact on ultrasonographic examination.

In the neonatal diarrheic calves with RDS in this investigation, elevated $PaCO_2$ and lactate concentrations and decreased PaO_2, HCO_3, and BE indicate extensive tissue hypoxia as a result of compromised lung function, and these cases indicate severe respiratory acidosis. Metabolic acidosis in these calves was originally attributed to intestinal HCO_3- loss, as well as the presence of organic acids in plasma and a decrease in the glomerular filtration rate in response to severe dehydration (Klein *et al*., 2008).

The concentrations of serum Na and Cl levels were significantly decreased while serum K level was significantly increased in the diarrheic calves with RDS compared to healthy calves. The observed changes in the levels of serum Na, Cl and K could be due to excessive water loss with feces which leads to dehydration and impaired cell membrane permeability (Sayers *et al*., 2016; Constable *et al*., 2016).

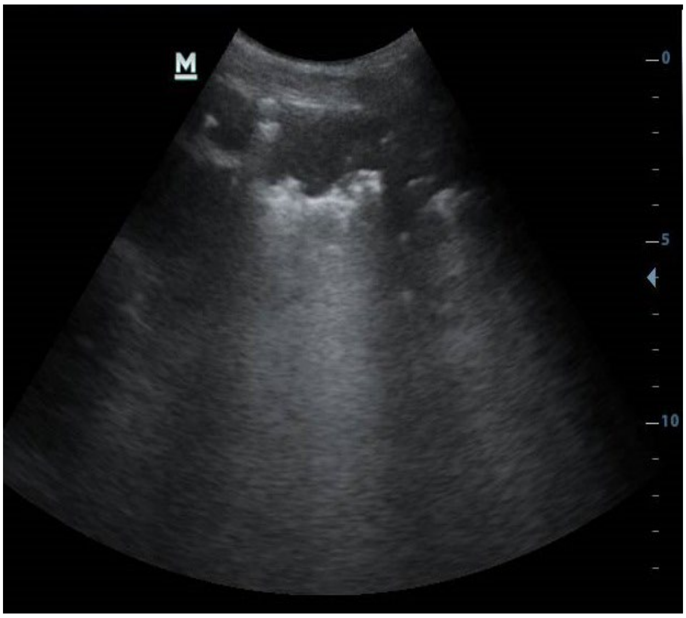

Figure 7. Ultrasonography of the lung of calves with pleurisy using a 3.5 MHz transducer. The ultrasonographic examination showed echogenic fluid in the pleural sac with the presence of fibrin shreds and the pleura appeared thick and corrugated.

The lung-specific epithelial and endothelial biomarkers in healthy and diarrheic neonatal calves with ARD included in the present study revealed that, serum ADMA and SP-D levels were significantly lower, while ET-1 concentration increased significantly in diarrheic calves with RDS compared to control group measurements. By investigating the relationship between the ADMA, ET-1 and SP-D concentrations, Pearson correlation was performed, and the results showed a very strong negative correlation between ADMA, SP-D concentrations against the concentration of ET-1. Whereas a strong positive correlation between ADMA and SP-D concentration. The obtained results were also in agreement with previous findings reported by Ider *et al*. (2021), who observed that the ADMA and SP-D concentrations of premature calves with RDS were lower and serum ET-1 concentrations higher than those of non-RDS premature and healthy calves.

Additionally, by investigating the relationship between the alterations of these lung-specific markers and the blood gas concentrations in calves with RDS compared to control group measurements, the results showed positive correlations between blood pH, PaO_2, HCO_3, BE and ADMA whereas a negative correlation was found among PaCO2, lactate and ADMA. A positive correlation between $PaCO_2$, lactate and ET-1, whereas a negative correlation was found among blood pH, PaO_2, HCO_3, BE and ET-1. Positive correlations between blood pH, PaO_2, HCO3, BE and SP-D whereas a negative correlation was found among $PaCO_2$, lactate and SP-D. These results were in agreement with those obtained in previous reports (Rubens *et al*., 2001; Galie *et al*., 2004; Ok *et al*., 2020; Ider *et al*., 2021).

ADMA is an endogenous inhibitor of nitric oxide synthase, increased ADMA levels result in a decrease in nitric oxide, which raises vascular tone. As a result, in patients with congestive bronchopulmonary dysplasia, heart failure, and pulmonary hypertension, an increase in the concentration of ADMA is thought to be an indication of poor prognosis when it coincides with endothelial damage (Trittmann *et al*., 2015; Klok *et al*., 2011; Zhang *et al*., 2015; Kavurt *et al*., 2017).

Endothelin-1 is a polypeptide with significant vasoconstriction activity, and it has been established that it has a variety of fibrinogenic actions (Rubens *et al*., 2001; Ok *et al*., 2020). In conjunction with the development of bronchopneumonia, hypoxia and acidosis in neonatal diarrheic calves induced RDS, plasma ET-1 concentrations and $PaCO_2$ increased and pH and PaO_2 decreased in response to RDS (Galie *et al*., 2004). However, increases in plasma and pulmonary ET-1 concentrations were found to be positively correlated with the severity of hypoxia in rats, and ET-1 may play a role in hypoxia-related pulmonary arterial narrowing or pulmonary hypertension (Kamiyama *et al*., 2014).

Type II pneumocytes release surfactant protein D, which is essential for preserving the surface integrity of alveoli and offers crucial information regarding the prognosis of the illness. It was discovered that SP-D concentrations reduced following the death of type II pneumocytes in the lungs and were closely correlated with the degree of lung injury (Ware *et al*. 2010; Calfee *et al*., 2011; Yildiz *et al*., 2019). Surfactant protein D (SP-D) is a type of collagenous glycoprotein that serves several functions in the lung. SP-D appears to be primarily a host defense molecule, binding a diverse range of pathogens such as viruses, bacteria, fungi, and pneumocystis. Furthermore, immunologic methods have been used to measure it in the systemic circulation, and it may be useful biomarkers of disease (Ider *et al*., 2021). In humans and other species, elevated surfactant protein D levels in bronchoalveolar lavage fluid and blood have been linked to early alveolar inflammation and basal membrane damage. If elevated levels of SP-D can be reliably found in the blood of cattle with bronchopneumonia, it may be possible to replace the existing diagnostic test as a more accurate one.

CONCLUSION

It is concluded that, serum asymmetric dimethylarginine, endothelin-1 and surfactant protein D concentrations are useful in determining lung inflammatory response and damage during diarrheal episodes in newly born bovine calves, particularly during the first month of life.

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CONFLICT OF INTEREST

There is no conflict of interest to disclose regarding the content of this article.

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