Neopterin, procalcitonin, total sialic acid, paraoxonase-1 and selected haematological indices in calves with aspiration pneumonia

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Abstract

This study aims to determine serum neopterin, procalcitonin, total sialic acid (TSA), paraoxonase-1 (PON1), and some haematological indices in calves with aspiration pneumonia (ASP). The patient group consisted of 30 calves of the Simmental breed, 0–28 days old, diagnosed with ASP upon clinical examination, anamnesis, auscultation examination, and radiographic results. The control group consisted of 10 healthy calves, otherwise with the same characteristics. A significant difference was found between calves with ASP and the control group of calves in serum iron (Fe, $P < 0.001$), total iron-binding capacity (TIBC, $P < 0.05$), PON1, TSA, procalcitonin, neopterin, and total leukocyte count (WBC) indices as a result of the analyses ($P < 0.001$). Serum procalcitonin concentration was found to be 285.71 ng/ml in the ASP group and 30.34 ng/ml in the control group. Serum neopterin concentration was found to be 37.68 nmol/l in the ASP group and 15.14 nmol/l in the control group. Serum procalcitonin, neopterin, and TSA concentrations were found to be significantly higher in the ASP group compared to the control group. It was concluded that biomarkers such as serum procalcitonin, neopterin, and TSA are high in calves with ASP, and as a result, these markers provide diagnostically and prognostically important information.

Biomarkers, calves, foreign body pneumonia, haematology, neopterin

The function of the respiratory system is to provide oxygen and carbon dioxide exchange in the alveoli, to provide acid-base balance in the organism, to metabolize corticosteroids, serotonin, and prostaglandin, and to activate angiotensin (Shahriar et al. 2002; Bulut 2019). Respiratory system diseases lead to a very high loss of productivity, an increase in the labour force, and even deaths in severe cases, causing serious economic damages (Friton et al. 2005). In cattle, respiratory system diseases are the most common. The main reason for this is that the lungs of cattle are very small compared to their bodies, due to their anatomical structure (Kurtdede and Kalınbacak 2016).

Pneumonia is called inflammation of the lung tissue. It is mostly accompanied by pleuritis. Loss of appetite, fever, dyspnoea, serous or mucopurulent discharge in the nasolacrimal duct, tachypnoea, and tachycardia are among the clinical symptoms of pneumonia (Bulut 2019). Pneumonia may have a different character depending on the agent's route of entry (aerogenic, haematogenous, lymphogenic), animal species, age, and body resistance (Lopez and Martinson 2017). The disease is very important in

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terms of causing loss of productivity and especially death in young animals (Dörtkardeş 2018). Pneumonia is categorized as bronchopneumonia (fibrinous, suppurating, aspiration pneumonia), interstitial, embolic, or granulomatous pneumonia (Çiftçi and Erer 2020). Bronchopneumonia is the inflammation of the bronchi, bronchioli, and alveoli. It is common and mostly occurs in the cranial lobes of the lungs (Adamu et al. 2013; Çiftçi and Erer 2020). Aspiration pneumonia (ASP) is one of the most common respiratory problems in neonatal calves (Güneş et al. 2013). Liquid substances such as milk and medication often go down the lungs as a result of malnutrition of the calves. Aspiration pneumonia frequently develops as a result (Kurtdede and Kalınbacak 2016). It is caused by the inhalation of large amounts of foreign material fluid into the lungs. This disease is also called gangrenous, foreign body, drug delivery or lipid pneumonia. It mostly occurs by trying to drink anything by mouth in cases where the swallowing reflex is impaired (Kurtdede and Kalınbacak 2012).

Neopterin is a compound in the structure of pteridine released as a result of the stimulation of tissue macrophages by γ-interferon (Çaycı et al. 2009; Akyüz 2020). There is an increase in neopterin concentrations in all kinds of viral and bacterial inflammation. Studies have found that patients with pneumonia have high neopterin concentrations (Murr et al. 2002; Çaycı et al. 2009). Neopterin finds use in respiratory system diseases, inflammatory conditions, different cancer types, evaluation of the severity and prognosis of diseases, sepsis and urinary system diseases (Lhee et al. 2006, Ünüvar and Aslanhan 2019). Plasma neopterin concentration is actually an indicator of active monocyte and macrophage activation in the circulation. A change in neopterin plasma concentration can be considered a direct reflection of the activation of monocytes and macrophages against disease (Brunkhorst et al. 1999; Ruokonen et al. 2002).

Procalcitonin (PCT) is a prehormone of the calcitonin hormone (Ercan et al. 2016; Hacımustafaoğlu 2017; Kırbaş et al. 2019; Akyüz 2020). It increases in conditions such as sepsis and pneumonia (Hacımustafaoğlu 2017; Akyüz 2020). Serum PCT concentrations are quite specific in demonstrating the inflammatory response in pneumonia (Galstian et al. 2003; Taşcı et al. 2007). Serum procalcitonin concentrations have higher sensitivity and specificity in distinguishing bacterial pneumonia from viral pneumonia (Taşcı et al. 2007).

Sialic acid is the acetylated derivative of neuraminic acid that binds to the unreduced carbohydrate chains of glycoproteins and glycolipids (Ülfer et al. 2017). Sialic acid increases as a result of the increase in these acute-phase proteins in cases of inflammation (Alturfan et al. 2007; Joshi and Patil 2010; Kuru et al. 2020). The diagnosis, differential diagnosis, clinical course of many diseases are monitored and their prognoses are evaluated by utilizing these changes (Karapehlivan and Maraşlı 2004). Diseases in which sialic acid increases include cardiovascular system diseases, bacterial and parasitic infections, cancer, diabetes, chronic liver diseases, pneumonia (Karapehlivan and Maraşlı 2004; Chrostek et al. 2011; Merhan et al. 2020). Total sialic acid levels have been reported to increase in calves with pneumonia (Karapehlivan et al. 2007).

Paraoxonase-1 (PON1) is an endogenous antioxidant produced by liver cells. It is protective against lipid peroxidation caused by free radicals on cell surfaces (Aguiar et al. 2012; Çevik et al. 2012; Giera et al. 2012). Serum activity of PON1 decreases during inflammation or sepsis (Torrente et al. 2019; Kuru et al. 2020; Ruggerone et al. 2020). During the acute phase response, changes in high density lipoproteins (HDL) inactivate the PON1 enzyme and hepatic gene expression of PON1 is inhibited. Therefore, PON1 is considered a negative acute phase protein (Feingold et al. 1998; Novak et al. 2010).

An acute phase response occurs in the organism against inflammation, tissue injury, infection, neoplastic growth or immunological disorders. Local reactions in acute phase response infiltration of leukocytes and capillaries includes increased permeability. As a result, defense cells become active (Yılmaz and Gökçe 2017). Total leukocyte count (WBC) increases in respiratory system diseases in cattle. There may be changes in haemoglobin, red blood cell count, and platelet count (Šoltésová et al. 2015; Yılmaz and Gökçe 2017).

This study aims to determine how serum neopterin, PCT, and total sialic acid concentrations and PON1 activity change as a result of inflammation in calves diagnosed with aspiration pneumonia. We believe that evaluating the specified biomarkers together in calves with ASP will increase the specificity of the study.

Materials and Methods

Ethical Statement

This study was conducted at the Department of Internal Medicine of the Faculty of Veterinary Medicine upon the approval of the Local Ethics Committee of Animal Experiments of Kafkas University, Kars, Turkey (KAÜ-HADYEK/2020-109). All institutional and national guidelines for the care and use of study animals were followed. All calf breeders gave their consent before commencement of the study.

Animals

Thirty calves of the Simmental breed, 0–28 days old, diagnosed with ASP upon clinical examination, anamnesis, auscultation examination, and radiographic results constituted the patient group. Glutaraldehyde test-positive calves with one or more results of the loss of appetite, fever, dyspnoea, serous, or mucopurulent discharge in the nasolacrimal duct, tachypnea, and tachycardia were included. Ten healthy calves with the same characteristics constituted the control group. In addition, control blood samples were obtained from calves housed in a closed barn system at Kafkas University Veterinary Education, Research and Application Farm.

Blood sampling

Blood samples were taken once into serum tubes (8.5 mL, BD Vakutainer®, BD, UK) from vena jugularis using a compatible sterile needle tip (Vacuette®, Greiner Bio-One GmbH, Austria) with the help of a holder. Blood samples were kept at room temperature for about 1 h and centrifuged at 1,500 *g* for 10 min (Hettich Rotina 380R®, Hettich, Germany) to obtain serum samples. Two mL of blood samples were taken into K_2EDTA tubes (BD Vakutainer®) for total leukocyte count. Haematological indices were measured daily. The serum samples to be used for biochemical measurements were stored at −20 °C until analysis.

Biochemical and haematological analyses

Serum PCT and neopterin concentrations were determined using commercial cattle specific ELISA kits (Bovine Neopterin ELISA Kit®, Bovine Procalcitonin ELISA Kit®, Sunred, Sunred Biotechnology Company, China). ELISA tests were performed as recommended by the manufacturer and optical densities were determined in the ELISA microplate reader at 450 nm wavelength (Epoch®, Biotek, USA). Regression analysis was performed and neopterin and procalcitonin values were read. Total leukocyte count (WBC $\times 10^3/\mu l$) was determined using a complete blood count device (VG-MS4e®, Melet Schloesing, France). Total iron-binding capacity (TIBC) was obtained by summing serum iron (Fe) and unsaturated iron-binding capacity (UIBC) levels. Transferrin saturation (TS) of the serum was obtained by calculating serum Fe and TIBC levels using the formula TS (%) = Fe/TIBC × 100 (Merhan and Özcan 2010). PON1 (Rel Assay Diagnostic, Turkey), Fe and UIBC (Biolabo®, France) were measured with a commercial test kit. Total sialic acid (TSA) was colorimetrically measured according to the method reported by Sydow (1985).

Glutaraldehyde test procedure

The test was performed by mixing 2 ml of blood and 2 mL of 1.4 % glutaraldehyde stock solution. The mixture was put into a 10 ml glass tube. It was turned upside down at 30-s intervals. Coagulation within 15 min was considered positive. The test results were considered as follows: 0–5 min, severe inflammation; 5–10 min, moderate; 10–15 min, mild inflammation. Coagulations longer than 15 min were considered normal (Turgut 2000).

Radiological imaging and evaluation

Radiographic images were taken in the right and left latero-lateral, ventro-dorsal position, with 35 × 43 cm size cassettes, 70 kV and 10 mAs doses, and using the radiographic imaging device (Fujifilm FCR Prima T2®, Mindray Medical Technology, Turkey). Care was taken to ensure that the radiographs were taken when the animals were standing and during the inspiration phase.

Histopathological examinations

Necropsies of 4 calves that died in the study were performed at the Department of Pathology, Faculty of Veterinary Medicine, Kafkas University. Tissue samples (lung, intestine, liver, etc.) taken following the systemic necropsy of animals were placed in 10% buffered formaldehyde (Merck®, Sigma-Aldrich, Germany) solution. Paraffin blocks were prepared after routine tissue follow-up procedures were applied. Five µm thick sections were taken from the paraffin blocks. The sections prepared were stained with haematoxylin-eosin (Merck®, Sigma-Aldrich) to reveal histopathological changes. Sections were examined and photographed under a light microscope (Olympus Bx53®, Olympus Global, Japan).

Statistical analyses

Statistical analysis of the data was performed using SPSS® (SPSS 18.0, IL, USA) software. The statistical differences between the groups with normal distribution according to Shapiro-Wilk test were compared by independent sample *t*-test. Pearson correlation coefficients were calculated to define the correlation between the variables. The obtained results were given as mean ± standard error of the mean (SEM). $P < 0.05$ was considered significant in the evaluation of the results.

Results

Physical findings

Clinical observations in calves with ASP included loss of appetite, dyspnoea, and serous or mucopurulent discharge in the nasolacrimal duct. Physical examination results of the rectal temperature, pulse, and respiratory rate per minute were found to be higher in the ASP group compared to the control group ($P < 0.001$, Table 1).

Table 1. Comparison of aspiration pneumonia and control group calves.

Indicator	Group	n	Mean ± SEM	*P* value*
Rectal temperature (°C)	Control	10	38.55 ± 0.07	< 0.001
	ASP	30	39.05 ± 0.09	
Respiration rate per min	Control	10	28.60 ±0.89	< 0.001
	ASP	30	46.53 ± 2.52	
Pulse rate per min	Control	10	85.20 ± 2.53	< 0.001
	ASP	30	129.73 ± 5.94	
Iron (g/dl)	Control	10	97.35 ± 2.37	< 0.001
	ASP	30	85.58 ± 1.59	
Total iron-binding capacity (g/dl)	Control	10	234.14 ± 1.56	< 0.05
	ASP	30	222.86 ± 2.26	
Transferrin saturation (%)	Control	10	41.38 ± 1.18	> 0.05
	ASP	30	38.61 ± 0.96	
Total leukocyte count ($\times10^3/\mu l$)	Control	10	7.42 ± 0.60	< 0.001
	ASP	30	17.01 ± 1.30	
Lymphocyte count ($\times10^3/\mu l$)	Control	10	4.12 ± 0.35	< 0.05
	ASP	30	7.79 ± 0.97	
Monocyte count ($\times10^3/\mu l$)	Control	10	0.58 ± 0.11	> 0.05
	ASP	30	0.78 ± 0.08	
Granulocyte count ($\times10^3/\mu l$)	Control	10	2.93 ± 0.48	< 0.001
	ASP	30	8.44 ± 0.78	
Red blood cell count ($\times10^6/\mu l$)	Control	10	7.38 ± 0.25	< 0.001
	ASP	30	9.27 ± 0.39	
Haemoglobin count (g/dl)	Control	10	9.45 ± 0.47	< 0.05
	ASP	30	10.74 ± 0.40	
Platelet count ($\times10^3/\mu l$)	Control	10	450.90 ± 65.37	> 0.05
	ASP	30	615.83 ± 75.82	

ASP - calves with aspiration pneumonia; Control - healthy calves; n - number of calves in groups; SEM - standard error of mean; *$P < 0.05$ is considered significantly different

Glutaraldehyde test findings

All calves in ASP group were positive for glutaraldehyde test. Calves in the ASP group in the study; 18 severe, 9 moderate and 3 mild inflammations were determined. In addition, coagulation over 15 min was detected in all calves in the control group and evaluated as normal.

Radiological examination findings

In the findings of radiological examination of 22 calves with ASP; there was an increase in opacity in the cranial lobes of the lung due to consolidation (replacing the air in the bronchi by a liquid or solid structure). The consolidation rate in the bronchi decreased caudally, and the severe opacity was replaced by radiolucent parts containing air (Plate I, Fig. 1A). In addition to the increased opacity observed in the cranio-ventral lobes of the lung, it was observed in eight calves that the opacity towards the caudal lobes continued with a decrease (Fig. 1B).

Biochemical and haematological findings

The changes and statistical differences of PON1, TSA, PCT and neopterin in both the control and ASP groups are given in Fig. 2. Iron concentration ($P < 0.001$), TIBC ($P < 0.05$), and PON1 activity ($P < 0.001$) were found to be lower in the ASP group compared to the control group (Fig. 2A). There was no significance even though TS was low in the ASP group ($P > 0.05$). Significant increases in TSA (Fig. 2B) and neopterin (Fig. 2D) levels, especially PCT, were noted in the ASP group ($P < 0.001$). Serum PCT concentration was found to be approximately 10 × higher in the ASP group compared to the control group (Fig. 2C). Serum PCT, neopterin and TSA levels in ASP and control were 285.71 ng/ml and 30.34 ng/ml, 37.68 nmol/l and 15.14 nmol/l, 76.04 mg/dl and 65.91 mg/dl, respectively. The total leukocyte count was found to be higher in the ASP group compared to the control group ($P < 0.001$). Total leukocyte count was found to be 14.71×10^3/µl in the ASP group and 38.6×10^3/µl in the control group. The values of Fe, TIBC, TS, and PON1 were found to be low whereas those of TSA, PCT, neopterin, WBC, lymphocytes, monocytes, granulocytes, red blood cell, haemoglobin, and platelet count were found to be high in the ASP group (Table 1). Pearson correlation among parameters is given in Table 2.

Pathological findings

In macroscopic examinations, aspirated foreign material was found in the trachea, bronchi and bronchioles of the calves (Plate I, Fig. 3). Oedema and pneumonia were observed in the lungs of the calves. The lungs were larger than normal and the intestines of the calves

Table 2. Pearson correlation between indicators.

Indicator	Fe (g/dl)	TIBC (g/dl)	TS (%)	PON1 (U/l)	TSA (mg/dl)	Procalcitonin (ng/ml)	Neopterin (nmol/l)
TIBC (g/dl)	-0.153						
TS (%)	0.893**	-0.502**					
PON1 (U/l)	0.261	0.488**	0.02				
TSA (mg/dl)	-0.267	-0.073	-0.201	-0.084			
Procalcitonin (ng/ml)	-0.497**	-0.415**	-0.216	-0.518**	0.379*		
Neopterin (nmol/l)	-0.477**	-0.08	-0.352*	-0.373*	0.406**	0.631**	
WBC ($\times 10^3$/µl)	-0.188	-0.12	-0.095	-0.596**	0.227	0.443**	0.452*

**Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed); Fe - iron; TIBC - total iron-binding capacity; TS - transferrin saturation; PON1 - paraoxonase-1; TSA - total sialic acid; WBC - total leukocyte count.

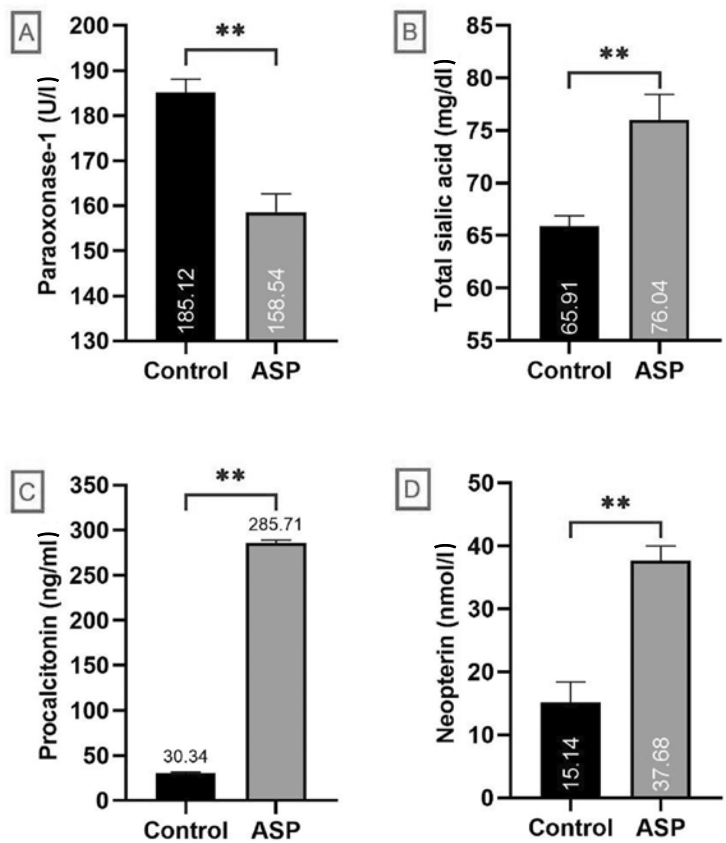

Fig. 2. A - Comparison of paroxanase-1 in control and ASP group. B - Comparison of total sialic acid in control and ASP group. C - Comparison of procalcitonin in control and ASP group. D - Comparison of neopterin in control and ASP group.
** Significance at $P < 0.001$; ASP - aspiration pneumonia

were also quite haemorrhagic. Histopathological examinations revealed the presence of foreign material aspirated in the lung alveoli (Plate II, Fig. 4A,B,C). In addition, severe hyperaemia in the lungs and thickening of the interalveolar septa were observed. In 2 of the calves, shedding of villus epithelium in the intestines and enteritis was observed. Hepatitis was observed in the liver in a single case. It was noted that other organs preserved their normal histological structure and did not exhibit a remarkable lesion.

Discussion

Loss of appetite, fever, dyspnoea, serous or mucopurulent discharge in the nasolacrimal duct, tachypnoea and tachycardia are among the clinical symptoms of pneumonia (Bulut 2019). Aspiration pneumonia is classified as a type of bronchopneumonia (Çiftçi et al. 2020). In the present study, the respiratory rate, pulse rate, and rectal temperature were found to be significantly higher in the ASP group compared to the control group ($P < 0.001$). The reason for these increases is the defence reflex as a result of compensation mechanisms.

There is an increase in neopterin concentrations in all kinds of viral and bacterial inflammations (Murr et al. 2002; Çaycı et al. 2009). Serum neopterin concentrations measured in different types of bovine pneumonia were found to be higher compared to healthy blood sera (Tuzcu et al. 2020). Other studies have found that patients with pneumonia have high neopterin levels (Murr et al. 2002; Çaycı et al. 2009). It was confirmed in the present study by the glutaraldehyde test that calves with ASP developed severe inflammation. Serum neopterin concentration in the study was found to be high in calves with ASP as a result of severe inflammation compared to the control group. We think that the reason for this increase is the stimulation of alveolar macrophages of the lung, which are tissue macrophages, by γ-interferon.

Procalcitonin increases in conditions such as bacterial sepsis and pneumonia (Hacımustafaoğlu 2017). Procalcitonin provides important information in demonstrating the inflammatory response (Galstian et al. 2003; Taşcı et al. 2007; Akyüz 2020). Serum PCT concentrations were higher in bovine pneumonia caused by different factors (Tuzcu et al. 2020). In our study, serum PCT concentration was high in calves with ASP as an indicator of inflammation similar to neopterin. We also think that the increase in total leukocytes may be the result of bacterial inflammation. The information in literature and the reason for the increase in neopterin and PCT concentrations in the present study were found to be in agreement.

Total sialic acid is an important biomarker in the prognosis and diagnosis of inflammatory diseases (Uzlu et al. 2010; Deveci et al. 2018). In the case of inflammation, TSA concentration increases in parallel with the acute phase proteins (Alturfan et al. 2007; Joshi and Patil 2010). Total sialic acid levels increase in calves with pneumonia (Karapehlivan et al. 2007). Similarly, in our study, TSA concentration was high in calves with ASP, in accordance with the literature. We believe that TSA increases due to increased levels of fibrinogen, immunoglobulin, and acute-phase proteins with inflammation.

Paraoxonase enzyme acts as an antioxidant synthesized from the liver, kidneys, and intestines. PON1 is found in combination with high-density lipoprotein in plasma and plays a role in preventing oxidation of plasma lipoproteins (Deveci et al. 2018, Kuru et al. 2020). It is protective against lipid peroxidation caused by free radicals on cell surfaces (Aguiar et al. 2012; Çevik et al. 2012; Giera et al. 2012). In recent years, studies have increased that PON1 may be an indicator of inflammation and disease (Torrente et al. 2019; Kuru et al. 2020; Ruggerone et al. 2020). In literature reviews, not much information is available regrading PON1 in ASP. In this study, decreased PON1 activity may be due to exacerbated lipid peroxidation caused by inflammation or infection in calves with ASP. In addition, PON1 enzyme activity may be reduced as a result of protecting against oxidation, such as negative acute phase protein in ASP-induced inflammation or infection.

Iron as an essential trace element is a necessary for many microorganisms as well as for allowing haemoglobin to enter its structure and sustain oxidation events. In an unbalanced diet, acute or chronic infections, chronic liver diseases and inflammation, serum Fe and UIBC levels may decrease and thus TIBC level decreases (Kaneko et al. 2008). Bactericidal activity increases with a decrease in serum Fe concentrations in many diseases or inflammations (Kuru et al. 2015; Merhan et al. 2017; Bozukluhan et al. 2018). The iron in the host is used for cellular growth during bacterial infections. In addition, as a defence mechanism, a large amount of iron is drawn from tissue fluids to prevent bacterial growth (Yılmaz and Gökçe 2017). The decrease in serum Fe concentration and accordingly, in TIBC level, was found to be significant in our study. This decrease may probably be due to an acute-phase response to infection and/or unbalanced nutrition due to loss of appetite caused by infection.

There may be significant changes in leukocytes in respiratory system diseases, especially in those caused by bacteria (Gülersoy and Şen 2017). Total leukocyte count was found to be higher in calves with fibrinous pneumonia (İder 2019). Increased WBC due to possible infection in ASP in our study was similar to other studies. In addition, the increase in lymphocytes and granulocytes is a strong indicator of inflammation as a positive glutaraldehyde test. Studies in cattle with pneumonia have reported increased hemoglobin and red blood cell count (Šoltésová et al. 2015; Yılmaz and Gökçe 2017). In the present study, respiratory distress in calves with ASP caused hypoxia. We think that induction of erythropoiesis as a result of compensation mechanisms and polycythaemia developed due to hypoxia. As a result of polycythaemia, red blood cell and haemoglobin counts were found to be high in the ASP group in our study.

In conclusion, serum neopterin, PCT, and TSA levels were found to be increased in calves with aspiration pneumonia. Procalcitonin and neopterin correlated positively with TSA and WBC, and negatively with Fe and PON1. We believe that the evaluation of these biomarkers together with the decrease in iron and PON1 can provide important diagnostic and prognostic information regarding the severity of the disease.

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Conflict of Interest

The authors declare no conflicts of interest.

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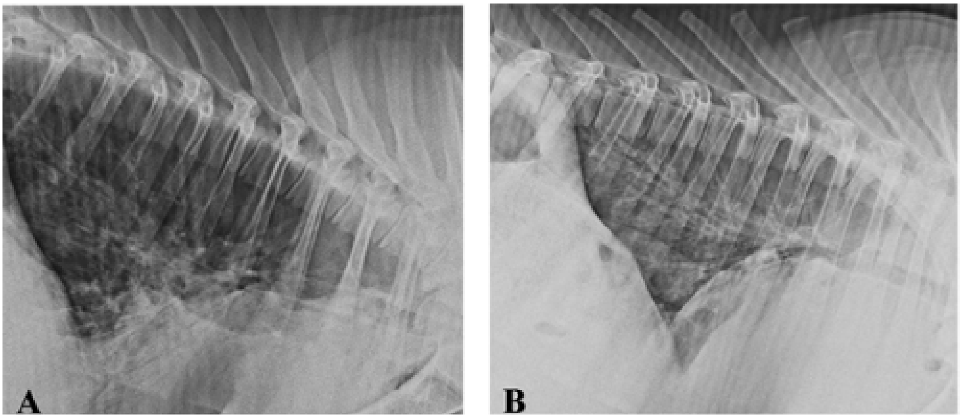

Fig. 1. A - Latero-lateral radiograph of a calf in the aspiration pneumonia group. Increased opacity in the cranial lobes of the lung due to consolidation. The consolidation rate in the bronchi decreased caudally, and the severe opacity was replaced by radiolucent parts containing air. B - Latero-lateral radiograph of another calf in the aspiration pneumonia group. Increased opacity observed in the cranio-ventral lobes of the lung; it was observed that the opacity towards the caudal lobes continued with a decrease.

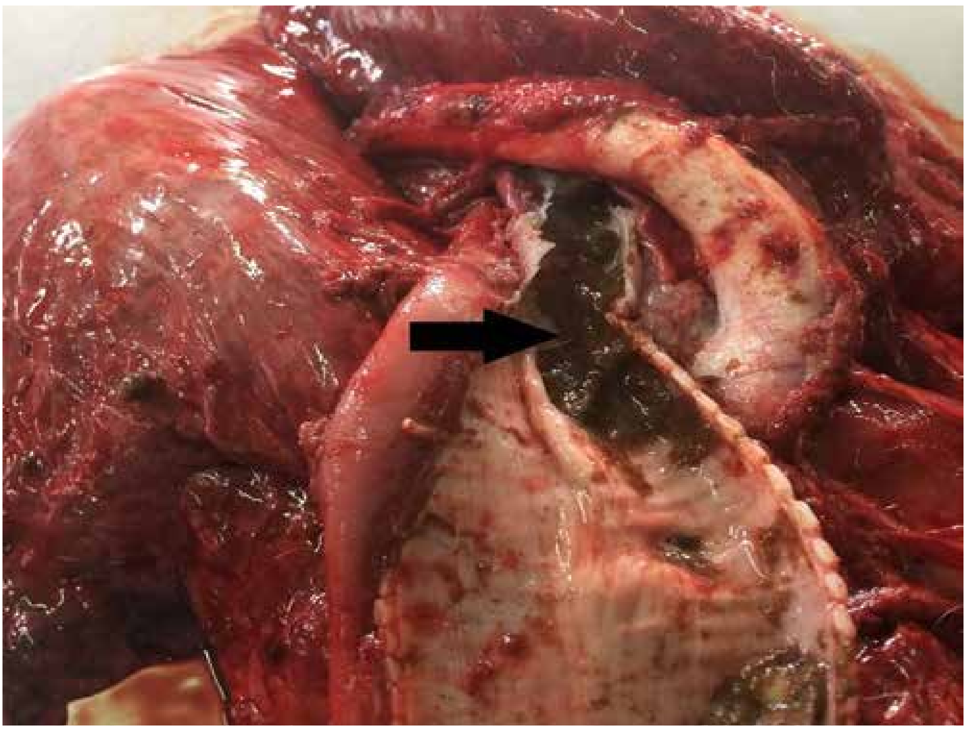

Fig. 3. Brownish foreign material aspirated in the trachea (black arrow)

Plate II

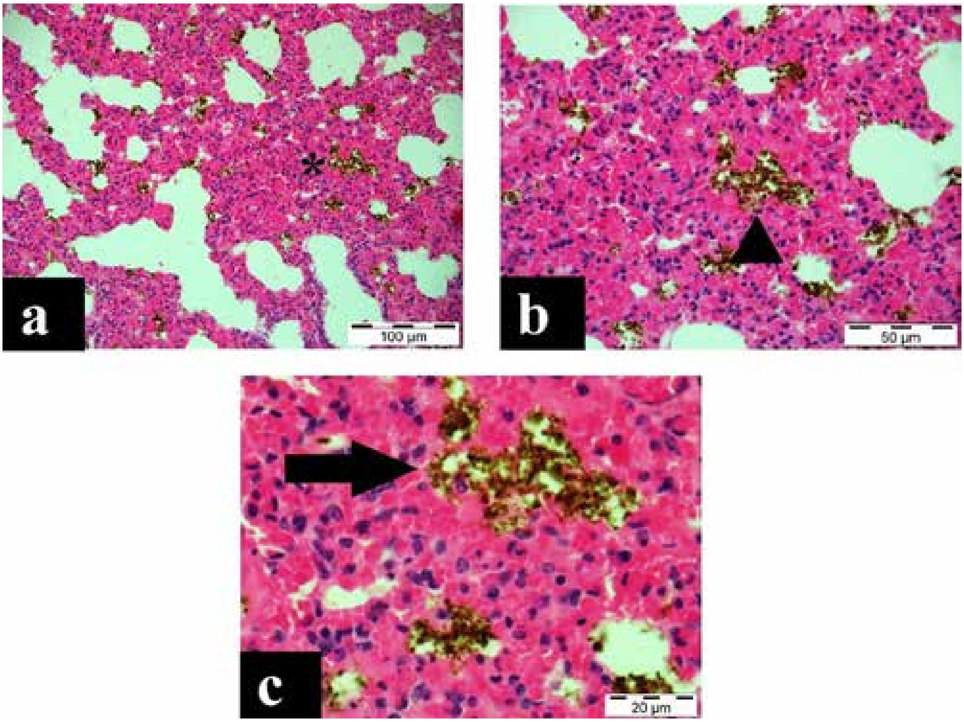

Fig. 4. A - Lung tissue, foreign material in alveoli (star), H&E, bar = 100 µm. B - Higher magnification, foreign material (arrowhead), H&E, ba r = 50 µm. C - Higher magnification, yellow-brownish foreign material in alveolar lumen (arrow), H&E, bar = 20 µm