

Determination of serum amyloid A, haptoglobin and hepcidin levels in calves with endemic viral pneumonia

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Abstract: The aim of this study was to investigate serum amyloid A, haptoglobin and hepcidin levels in respiratory infections caused by various viral agents in calves. Pneumonia is an infectious or non-infectious disease affecting the lungs of all animals, and during the inflammation, blood levels of acute phase proteins increase rapidly. Heparin is a hormone that has many functions and is known as type II acute phase reactant. The blood samples were collected from a total of 20 calves to form the study group. The control group consisted of 10 healthy animals. Sera were analyzed with ELISA for antigen detection. According to the results, Bovine herpesvirus type-1 (BHV-1) was positive in 3 calves, Bovine viral diarrhoea virus (BVDV) was positive in 3 calves, Bovine respiratory syncytial virus (BRSV) was positive in 1 calf, and Bovine parainfluenza virus type 3 (BPI3) was positive in 1 calf. Adenovirus infection was detected in 5 animals together with other viruses. Mixed infections were observed in 12 calves. Haptoglobin, serum amyloid A and hepcidin values were measured in sera. According to the results, statistically significant differences were observed between the two groups for the haptoglobin ($P<0.01$), serum amyloid A ($P<0.05$) and hepcidin ($P<0.05$). It was concluded that these parameters can be used to support the diagnosis of respiratory infections of the calves.

Keywords: Calf, haptoglobin, hepcidin, serum amyloid A, viral pneumonia.

Yaygın viral pnömonili buzağularda serum amiloid A, haptoglobin ve hepsidin düzeylerinin belirlenmesi

Özet: Bu çalışmanın amacı, buzağularda çeşitli viral etkenlerinin neden olduğu solunum yolu enfeksiyonlarında serum amiloid A, haptoglobin ve hepcidin düzeylerini araştırmaktır. Pnömoni, tüm hayvanların solunum sistemlerini etkileyen, enfektif veya non-enfektif sebeplere bağlı olarak gelişen bir hastalık olup, yangı sırasında akut faz proteinlerinin kandaki değerleri hızla artmaktadır. Heparin; birçok fonksiyona sahip olan bir hormon olup aynı zamanda tip II akut faz reaktant olduğu bilinmektedir. Araştırmanın çalışma grubunu oluşturmak için, toplam 20 buzağıdan kan örnekleri alındı. Kontrol grubu ise, sağlıklı 10 hayvandan oluşturuldu. Kan serumları, antijen tespiti için ELISA testi ile tarandı. Elde edilen sonuçlara göre 3 hastada Bovine herpes virüs tip 1 (BHV-1), 3 hastada Bovine viral diarrhoea virus (BVDV), 1 hastada Bovine respiratory syncytial virüs (BRSV) ve 1 hastada Bovine parainfluenza virüs tip 3 (BPI3) pozitifliği. Adenovirüs enfeksiyonu 5 hayvanda diğer virüslerle birlikte tespit edildi. Oniki hastada miks enfeksiyon saptandı. Haptoglobin, serum amiloid A ve hepsidin değerleri toplanan serum örneklerinde ölçüldü. Sonuç olarak; iki grup arasında, haptoglobin ($P<0,01$), serum amiloid A ($P<0,05$) ve hepsidin ($P<0,05$) farkları istatistiksel açıdan önemli bulunarak, buzağuların solunum sistemi enfeksiyonlarının teşhisini desteklemede, belirtilen parametrelerin kullanabileceği saptandı.

Anahtar sözcükler: Buzağı, haptoglobin, hepsidin, serum amiloid A, viral pnömoni.

Introduction

Pneumonia is an infectious and non-infectious originated disease affecting lungs of all animals. There are many factors that lead the disease. It is a disease which causes serious financial losses especially in cattle farms. Pneumonia is the inflammation of the lung parenchyma. It is usually associated with inflammation of bronchioles and pleuritis. Clinically; it is characterized by fever, nasal discharge, increased respiratory rate, changes in depth and

characteristics of breaths, cough, abnormal breathing sounds in auscultation, lacrimation, depression and partial anorexia (2, 3). In dairy calf pneumonia; in acute outbreaks, many animals have been reported to be depressed and have a high fever. In some calves; there are visible signs in the respiratory system (22).

In the acute phase of the inflammation, proteins that show significant changes in blood levels are called acute phase protein (APP). Acute phase proteins are used to

assess the response of the body's immune system to inflammation or trauma (15, 19). Their secretion is regulated by the proinflammatory cytokines, in particular interleukin 6 (IL-6) (15). In addition to diarrhea, respiratory diseases are one of the major health problems affecting young dairy calves. In order to prevent outbreaks of disease, early detection, isolation and treatment of sick animals are important. The results obtained in several different studies have shown that APPs are useful for the detection and monitoring of respiratory diseases. In calves with respiratory tract diseases, the following APPs were determined: haptoglobin (Hp), fibrinogen (Fb), serum amyloid A (SAA), transferrin (Tf), lipopolysaccharide binding protein (LBP), α 1-acid glycoprotein (AGP), α 1-antitrypsin (α 1-AT), seromuroid (Sm), ceruloplasmin (Cp), albumin and α 2-macroglobulin (21).

Hepcidin is a hormone that has multiple functions and a peptide structure (14). Hepcidin has been studied in human and many animal species (mice, rats, pigs, fish, dogs) to date. In a study on healthy canine tissues, hepcidin was found to be secreted mostly in the liver and less in the lungs and kidneys but not in other tissues (9). Hepatic hepcidin production is under the influence of many stimulants such as low levels of iron and erythropoietic activation. Also some cytokines, especially IL-6, increase the level of hepcidin (13). Hepcidin also assists host defense due to its direct antimicrobial properties (8, 23). Inflammation states stimulate hepcidin production and increase its release, leading to a decrease in iron secretion from macrophages and a decrease in plasma iron levels (5, 16).

The aim of this study was to investigate serum amyloid A, haptoglobin and hepcidin levels in respiratory infections caused by various viral agents in calves.

Material and Methods

This research was carried out on the basis of the permission of Mehmet Akif Ersoy University Local Animal Ethics Committee dated 05.10.2016 and numbered 214. The research material consisted of Holstein breed calves from the farms in Burdur Province that were brought to Veterinary Hospital of Mehmet Akif Ersoy University. The owners of the animals in the study were informed about the applications. The blood samples were collected from the animals of the study group with high respiratory rate, wheezy respiratory, cough and seropurulent nasal discharge.

Blood samples were collected from a total of 20 calves according to the criteria mentioned above. These samples were gathered out of Holstein calves with 8 females and 12 males (aged 2 - 6 months). The control group consisted of 10 healthy calves (aged 2-6 months) from another farm. Complete blood counts of the calves

were performed in both groups (Diatron Abacus Junior Vet Hematology Analyzer, S / N 130702 model).

The blood samples were coagulated, and their sera were separated in the cooled centrifuge at 4000 rpm for 5 min. The resulting serum samples were transferred to Eppendorf tubes (1.5 ml) evenly using micropipette. The tubes were recorded with their sample numbers, and stored at -20°C until used.

Twenty blood serum collected from suspected animals with clinical signs of pneumonia were analyzed with ELISA test for the detection of causative agents antigens (Bio-X Diagnostics S.A ELISA kit for serodiagnosis of Bovine herpesvirus type-1 (BHV-1)), Bovine viral diarrhea virus (BVDV), Bovine respiratory syncytial virus (BRSV), Bovine parainfluenza virus type 3 (BPI3) and Adenovirus 3. Sera were diluted 1/100 before inoculation. The samples were analyzed with ELISA according to the manufacture's recommendations. In the ELISA reader, the percentages of the agents in the blood were determined numerically. The results were evaluated according to the evaluation of the agent positivity given in the kit procedure. Bovine specific haptoglobin, serum amyloid A and hepcidin ELISA (Enzyme-Linked Immunosorbent Assay) kits (MyBioSource, San Diego (USA)) were used for the measurement of their levels in sera of study and control group animals. In order to increase the reliability of the study, serum samples were run in duplicate.

Statistical analysis: The obtained data were evaluated by the 2-Sample t test method between the study and control groups. Data were given as mean and standard error. P value of <0.05 was considered significant.

Results

Agent positivity evaluation intervals are shown in Table 1. According to the results, BHV-1 was positive in 3 calves, BVDV was positive in 3 calves, BRSV was positive in 1, and BPI3 was positive in 1 calf. Mixed infections were determined in 12 calves (Table 2).

The common symptoms of calves were cough, wheezing, seropurulent respiratory nasal discharge and depression. The clinical findings of calves in study and control groups are given in Table 3. While there was no difference in the body temperature between the two groups, the number of respiratory ($P<0.01$) and pulse rate ($P<0.05$) of the calves in study group were found to be higher than in those in control group. When the hematological parameters of the control and study groups were evaluated, the difference between the values of leukocyte ($P<0.01$), monocyte ($P<0.01$), neutrophils ($P<0.01$), and hematocrit ($P<0.05$) were found to be significant. No significant difference was found between lymphocyte, erythrocyte, hemoglobin and platelets values ($P>0.05$) (Table 4).

Table 1. Agent positivity evaluation intervals.

	0	+	++	+++	++++	+++++
BHV-1	V≤30	<V≤67	<V≤104	<V≤141	<V≤178	178<
BVDV	V≤20	<V≤40	<V≤60	<V≤80	<V≤100	100<
BRSV	V≤20	<V≤40	<V≤60	<V≤80	<V≤100	100<
BPI3	V≤20	<V≤40	<V≤60	<V≤80	<V≤100	100<
Adeno 3	V≤20	<V≤33	<V≤56	<V≤79	<V≤102	102<

V: Value, +: Strength of positivity.

BHV-1: Bovine herpesvirus type 1; BVDV: Bovine viral diarrhea virus; BRSV: Bovine respiratory syncytial virus; BPI3: Bovine parainfluenza virus type 3; Adeno 3: Adenovirus type-3.

Table 2. Percentages of pneumonia agents in calves of study group.

Calves (No)	BHV-1 (% agent)	BVDV (% agent)	BRSV (% agent)	BPI3 (% agent)	Adeno3 (% agent)
1	17.2	22.9	36.77 ⁺	65.62 ⁺⁺⁺	15.34
2	30.5	36.3	59.16 ⁺⁺	10.61	15.6
3	119.0	170.2 ⁺⁺⁺⁺	101.1 ⁺⁺⁺⁺	108.7 ⁺⁺⁺⁺	81.6 ⁺⁺⁺⁺
4	20.7	16.4	58.38 ⁺⁺	55.3 ⁺⁺	52.1 ⁺⁺
5	96.7 ⁺⁺	9.09	16.44	15.3	10.5
6	22.9	161.3 ⁺⁺⁺⁺	70.94 ⁺⁺⁺	69.1 ⁺⁺⁺	16.3
7	15.6	11.7	9.5	31.97 ⁺	13.9
8	17.6	32.88 ⁺	23.05 ⁺	19.07	15.9
9	100.7 ⁺⁺	13.54	15.11	10.6	138.3 ⁺⁺⁺⁺
10	31.0	84.319 ⁺⁺⁺⁺	85.72 ⁺⁺⁺	103.3 ⁺⁺⁺⁺	56.7 ⁺⁺
11	32.0	156.25 ⁺⁺⁺⁺	98.61 ⁺⁺⁺	110.6 ⁺⁺⁺⁺	108 ⁺⁺⁺⁺
12	26.3	86.45 ⁺⁺⁺⁺	21.22	14.5	10.9
13	18.8	167.6 ⁺⁺⁺⁺	67.27 ⁺⁺⁺	14.5	18.9
14	36.9 ⁺	7.74	18.8	20.1	7.4
15	82.1 ⁺⁺	18.8	17.27	17.3	11.9
16	129.6 ⁺⁺⁺⁺	168.3 ⁺⁺⁺⁺	68.61 ⁺⁺⁺	80.7 ⁺⁺⁺	15.8
17	20.6	45.6 ⁺⁺	17.2	18.87	12.9
18	11.1	58.08 ⁺⁺	13.38	20.16	9.22
19	15.5	30.75 ⁺⁺	25.33 ⁺	33.8 ⁺⁺	19.5
20	19.7	85.11 ⁺⁺⁺⁺	54.83 ⁺⁺	77.7 ⁺⁺⁺	13.7

+ : Strength of positivity.

Table 3. Comparison of clinical findings of calves in control and study groups.

Parameters	Study group (n= 20) Mean±SD	Control group (n= 10) Mean±SD	P
Respiratory rate/ min	72.8±4.6	34.8±1.6	<0.001
Pulse rate/ min	101.9±4.4	84.1±3.3	0.005
Body temperature/ °C	38.49±0.21	38.64±0.10	0.504

Table 4. Mean values of some hematological parameters of calves in control and study groups.

Parameters	Study group (n=20)	Control group (n=10)	P
WBC (10 ⁹ /l)	18.42±2.07	9.185±0.691	<0.001
LYM (10 ⁹ /l)	6.22±0.70	6.60±0.56	0.674
MON (10 ⁹ /l)	0.963±0.2	0.092±0.011	<0.001
NEU(10 ⁹ /L)	11.24±1.6	2.73±0.39	<0.001
RBC (10 ¹² /l)	8.38±0.5	9.17±0.32	0.192
HGB(g/dl)	8.35±0.43	8.77±0.39	0.480
HCT (%)	26.17±1.7	30.0±1.2	0.036
MCV(fl)	31.75±1.2	33.60±1.0	0.250
PLT (10 ⁹ /l)	923±393	707±51	0.593

WBC: Leukocyte, LYM: Lymphocyte, MON: Monocytes, NEU: Neutrophil, RBC: Erythrocyte, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean cell volume, PLT: Platelets.

Table 5. Statistical evaluation of haptoglobin, hepcidin and serum amyloid A values in both groups.

Parameters	Study group (n=20)	Control group (n=10)	P
Haptoglobin (ng/ml)	197±20	1.00±1.6	<0.001
Serum amyloid A (ng/ml)	4.89±1.2	0.66±0.73	0.005
Hepcidin (ng/ml)	0.833 ±0.055	0.073±0.800	0.005

A moderate difference was found between hepcidin and serum amyloid A values of the control and study groups ($P<0.05$). The most significant difference was observed in the haptoglobin ($P<0.01$). The values of haptoglobin, hepcidin and serum amyloid A were showed in Table 5.

Discussion and Conclusion

In this study, while there was no difference in body temperature between the two groups, the number of breath and pulse rates of the calves in study group per minute was found to be higher than in those in control group ($P<0.01$, $P<0.05$, respectively). Our results were similar to the results of Yılmaz and Gökçe (24). The lack of fever in the animals in the study group is thought to be due to the passing of the viremia period.

When the hematological parameters of the animals in the study and control groups were evaluated, it was determined that leukocytosis was related to acute respiratoric inflammation due to infection and it was found to be similar to the other study findings (24). There was a difference in neutrophil counts ($P<0.01$) and hematocrit values ($P=0.036$), but no significant difference was found in other parameters. Since no bacterial agents were investigated in the present study, secondary bacterial infection is highly possible.

Acute phase proteins have been accepted as a valuable indicator in determining the importance of pathological conditions in humans and animals. In cattle, SAA and Hp take place at the forefront of important acute-phase proteins, but their responses differ from individual animals.

In the case of viral and bacterial infections, acute phase response can be differ. For example, acute phase response to bacterial infections is reported to be more severe than viral infections in bovine mastitis (6).

Alsemgeet et al. (1) reported that haptoglobin was not detected in healthy cows and newborn calves. In the same study serum amyloid A level was found to be lower in healthy calves compared to adult cows, whereas in calves with inflammatory diseases; it has been reported to be significantly high. Niine et al. (17) reported that SAA and Hp levels were significantly increased in calves with *Giardia* and *Cryptosporidium* infections compared to healthy calves. Kabu et al. (12) reported a statistically significant increase in the concentrations of serum Hp and

SAA in water buffaloes with dermatosis compared to the control group. Sahinduran et al. (20) reported that serum Hp and SAA concentrations, in cattle with BVD and BHDV-1 in single and dual infection, have shown statistically significant increase compared to healthy cattle. In the other study, significant increases were observed in serum Hp and SAA concentrations in calves suspected from Bovine respiratory disease (10). Also significant increase in the SAA concentration had been observed in calves with enteritis and pneumonia compared to the control group (11). Coşkun et al. (4) reported that SAA and Hp levels in both of the blood serum and the bronchoalveolar lavage were higher in calves with bronchopneumonia than in healthy calves. In our study, SAA and Hp levels were significantly higher in calves with pneumonia than in healthy calves ($P<0.05$ and $P<0.00$, respectively). It was thought that the increase in the values in these parameters was the result of acute phase response. These results are in agreement with above mentioned studies. Especially in cattle, SAA and Hp levels were found to be a suitable marker for site conditions in early detection of the diseases with viral pneumonia. In addition, the levels of these parameters were higher in patients with BVDV and in animals with mixed infections in this study.

Hepcidin plays a fundamental role in the regulation of iron metabolism. Hepcidin is an acute phase protein that is synthesized to restrict the body's iron stores and to prevent iron being requisitioned by invading microorganisms (18) and inflammation states stimulate hepcidin production (5). Hepcidin also helps host defense due to its direct antimicrobial properties (8, 23). Erkilic et al. (7), reported that serum hepcidin levels were very high in calves suspected of septicemia, and these levels decrease drastically after treatment. In our previous study on 56 cattle with single and dual infection (BHV-1, BVDV), hepcidin levels were compared. It was found that there was a significant difference between the serum hepcidin concentrations of cattle with single and dual infection and the control group of cattle (20). The results of this study were similar to the above mentioned study and the difference between serum hepcidin concentration of calves in study group and serum concentrations of calves in control group was significant ($P<0.05$).

Considering all three parameters (SAA, Hp and hepcidin) in our study, haptoglobin level was higher than

the other two parameters. Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with mastitis, pneumonia, enteritis, peritonitis, endocarditis, abscesses, endometritis and other natural or experimental infectious conditions (6). Accordingly, haptoglobin which is important acute phase protein in bovine, can have more importance in respiratory infections in calves.

As a conclusion, hepcidin, serum amyloid A and especially haptoglobin were found to be significantly increased in calves with viral pneumonia.

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Conflict of Interest

The authors declare that there have no conflict of interests.

References

1. **Alsemgeest SPM, Kalsbeek HC, Wensing T, et al** (1994): Concentrations of serum amyloid-a (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Vet Quart*, **16**, 21-23.
2. **Ames TR** (1997): Dairy Calf Pneumonia. The disease and its impact. *Vet Clin North Am Food Anim Pract*, **13**, 379-391.
3. **Bryson DG, McNulty MS, McCracken RM** (1983): Ultrastructural features of experimental parainfluenza type 3 virus pneumonia in calves. *J Comp Pathol*, **93**, 397-414.
4. **Coskun A, Guzelbektes H, Simsek A, et al** (2012): Haptoglobin and SAA concentrations and enzyme activities in bronchoalveolar lavage fluids from calves with bronchopneumonia. *Revue Méd Vét*, **163**, 615-620.
5. **Coyne DW** (2011): Heparin: clinical utility as a diagnostic tool and therapeutic target. *Kidney Int*, **80**, 240-244.
6. **Eckersall PD, Bell R** (2010): Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet J*, **185**, 23-27.
7. **Erkilic EE, Erdogan HM, Ogun M, et al** (2016): Relationship between hepcidin and oxidant/antioxidant status in calves with suspected neonatal septicemia *Vet World*, **9**, 1238-1241.
8. **Falzacappa MVV, Muckenthaler MU** (2005): Heparin: iron-hormone and anti-microbial peptide. *Gene*, **364**, 37-44.
9. **Fry MM, Liggett JL, Baek SJ** (2004): Molecular cloning and expression of canine hepcidin. *Vet Clin Path*, **33**, 223-227.
10. **Joshi V, Gupta VK, Bhanuprakash AG, et al** (2018): Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. *Microb Pathogenesis*, **116**, 33-37.
11. **Kabu M, Elitok B, Kucukkurt I** (2016): Decetion of serum amyloid-A concentration in the calf clinically diagnosed with pneumonia, enteritis and pneumoenterites. *Ciência Rural*, **46**, 293-299.
12. **Kabu M, Sayın Z** (2016): Concentrations of serum amyloid A, haptoglobin, tumour necrosis factor and interleukin-1 and -6 in Anatolian buffaloes naturally infected with dermatophytosis. *Vet Med-Czech*, **61**, 133-135.
13. **Kemma E, Pickkers P, Nemeth E, et al** (2005): Timecourse analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood*, **106**, 1864-1866.
14. **Krause A, Neitz S, Magert HJ, et al** (2000): LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett*, **480**, 147-150.
15. **Murata H, Shimada N, Yoshioka M** (2004): Current research on acute phase proteins in veterinary diagnosis. *Vet J*, **168**, 28-40.
16. **Nemeth E, Valore EV, Territo M, et al** (2003): Heparin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood*, **101**, 2461-2463.
17. **Niine T, Peetsalu K, Nieminen M, et al** (2017): Giardia and Cryptosporidium infections in neonatal reindeer calves: Relation to the acute phase response. *Comp Immunol Microb*, **54**, 45-50.
18. **Orro T, Jacobsen S, LePage JP, et al** (2008): Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. *Vet J*, **176**, 182-187.
19. **Petersen HH, Nielsen JP, Heegaard PMH** (2004): Application of acute phase protein measurements in veterinary clinical chemistry. *Vet Res*, **35**, 163-187.
20. **Sahinduran Ş, Kale M, Kıyıcı R, et al** (2017): Some acute phase proteins and hepcidin levels in single and dual infection with BVD and BHV-1. *MAKÜ Sag Bil Enst Derg*, **5**, 115-123.
21. **Svensson C, Lundborg K, Emanuelson U, et al** (2003): Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Prev Vet Med*, **58**, 179-197.
22. **Waltner-Toews D, Martin SW, Meek AH** (1978): Dairy calf management, morbidity and mortality in Ontario Holstein herds. II: Age and seasonal patterns. *Prev Vet Med*, **4**, 125-135.
23. **Wessling-Resnick M** (2010): Iron Homeostasis and the Inflammatory Response. *Annu Rev Nutr*, **30**, 105-122.
24. **Yılmaz O, Gökçe G** (2017): Sığırlarda enfeksiyöz solunum sistemi hastalıkları kompleksinde (brdc) klinik, hematoloji, biyokimya, oksidatif stres, akut faz proteinler üzerinde araştırmalar. *Ataturk Üniversitesi Vet Bil Derg*, **12**, 34-44.