

## Evaluation of Serum Amyloid-A Levels in Clinically Detected Pneumonia in Goats

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### ABSTRACT

In this study, 20 goats between the ages of 0-24 months were used. After clinical, systemic, and hematological examinations, goats were divided into two groups. Clinically diagnosed with pneumonia (n=10) were the study group, whereas healthy goats (n=10) were formed as the control group. Auscultation was performed in the clinical examination. Blood samples from Vena jugularis in the hematological study, White Blood Cell (WBC), Red Blood Cell (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH); Mean Corpuscular Hemoglobin Concentration (MCHC) concentrations were performed. In blood serum, Total Protein (TP), Albumin (ALB), Serum Amyloid-A (SAA) concentrations were measured. As a result, the values of WBC and SAA are statistically higher in goats with pneumonia compared with the control group. As for RBC, Hb, Hct, MCV, MCH, MCHC, TP, ALB concentrations, no statistical difference was found between the two groups. As a result, we think SAA is the most valuable parameter to evaluate the course of the disease and prognosis along with hematologic, biochemical, and clinical findings in veterinary medicine.

**Keywords:** Pneumonia, Goat, Serum Amyloid-A

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### Klinik Olarak Pnömoni Tespit Edilen Keçilerde Serum Amiloid-A Düzeylerinin Değerlendirilmesi

### ÖZ

Sunulan çalışmada 0-24 aylık 20 adet keçi kullanıldı. Keçilerin klinik, sistemik ve hematolojik muayeneleri yapıldıktan sonra onarlı iki gruba ayrıldı. Klinik olarak pnömoni teşhisi (n=10) konulan keçiler çalışma grubuna, sağlıklı keçiler ise kontrol (n=10) grubuna dahil edildi. Klinik muayene sırasında oskültasyon yapıldı. Vena jugularisten alınan kan örneklerinden hematolojik muayenede; Lökosit (WBC), Eritrosit (RBC), Hemoglobin (HGB), Hematokrit (HCT), Eritrosit Hacmi (MCV), Eritrosit Ortalama Hemoglobin Miktarı (MCH), Eritrosit Ortalama Hemoglobin Yoğunluğu (MCHC) konsantrasyonları ölçüldü. Kan örneklerinden elde edilen serumlarda; Total Protein (TP), Albumin (ALB) ve Serum Amiloid-A (SAA) konsantrasyonu ölçüldü. Yapılan çalışma sonucunda pnömonili keçilerde WBC ve SAA konsantrasyonları, kontrol grubuna göre istatistiksel açıdan yüksek tespit edildi. RBC, HGB, HCT, MCV, MCH, MCHC, TP, ALB konsantrasyonlarında gruplar arasında bir fark tespit edilmedi. Sunulan çalışma sonuçları dikkate alındığında SAA'nın veteriner hekimlik alanında, hematolojik, biyokimyasal, ve klinik bulgularla beraber hastalığın prognozunu değerlendirilmesinde önemli bir parametre olduğunu düşünmekteyiz.

**Anahtar Kelimeler:** Pnömoni, Keçi, Serum Amiloid-A

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## INTRODUCTION

Respiratory infections are common in livestock. However, pneumonic pasteurellosis is the most common and widely prevalent disease among respiratory system infections in ruminants. Typically, the infection is highly contagious and mostly fatal. Thus, it causes serious economic losses in the livestock sector. The devastating impact of the disease on small ruminant breeding is also evident, and it leads to major deaths due to acute epidemics. Along with this fact, in terms of farm management, the use of chemotherapeutic drugs and intensification of vaccination programs in disease-intensive businesses cause high financial costs almost every year (Davies et al. 1997, Daniel et al. 2006, Mohamed and Abdelsalam, 2008).

Acute Phase Response (APR) is a response that develops following infective cases, inflammatory reactions, immunological problems, traumatic or neoplastic events, and it has been reported that this response develops with systemic and metabolic changes (Gruys et al. 1994, Petersen et al. 2004). Most of the acute phase proteins (APP) have been examined thoroughly for human medicine and are now being used routinely in the diagnosis and prognosis of most diseases. It is considered that APPs can have important usage areas in animal health. In the light of these facts, although APPs have different importance for various animal species, it is a fact that not enough studies have been done in this area, so APPs cannot be used in the field of animal health on the scale of routine tests (Gökçe and Bozukluhan 2009, Eckersall and Bell 2010). Recent studies have presented that Haptoglobin (Hp) and Serum Amyloid A (SAA) are among the essential acute phase proteins for ruminants and  $\alpha$ 1 acid glycoprotein (AGP) is of moderate importance (Eckersall and Bell 2010). The ratio of APPs in plasma concentrations depends on the severity and activity of the inflammatory reaction, and the determination of the number of APPs in the blood circulation can provide information about the current inflammatory reaction. It has been observed that serum or plasma Haptoglobin (Hp) concentration increases in cattle in natural or experimentally induced infective conditions after inflammatory or traumatic events (Alsemgeest 1994, Heegard et al. 2000, Fisher et al. 2001). Studies have reported that Hp rates in cattle are a very important variable in the diagnosis of bacterial (Skinner et al. 1991) and viral (Höfner et al. 1994) infections and that the concentration of Hp increases substantially in these infections. It has been reported that in some infections such as Bovine Viral Diarrhea (BVD), Mannheimia haemolytica infection, Pasteurella multocida infection, mastitis, metritis, and hepatic lipidosis, changes in serum Hp rates can be an important parameter for APR (Petersen et al. 2004, Eckersall and Bell 2010).

In this study, it was aimed to determine serum amyloid A levels in clinically healthy goats with pneumonia and to determine the relationship between these levels and routine hematological and biochemical parameters.

## MATERIAL AND METHOD

### Animal Material and Clinical Examinations

0 to 24-month-old 20 goats coming from a private business within the borders of Afyon province were used. Then, the goats with respiratory problems were detected in the businesses, and anamnesis was gathered. Following the anamnesis, the goats diagnosed with pneumonia underwent routine systemic clinic, body temperature, heart and respiratory rate, mucus membrane, and lung auscultation examinations; then, the goats diagnosed with clinical pneumonia formed the disease group (Group P n=10). The Control Group (Group C, n=10) underwent routine systemic clinic, body temperature, heart and respiratory rate, mucus membrane, and lung auscultation examinations and formed by the goats presenting no pathologic conditions. The research was sanctioned by Afyon Kocatepe University Animal Experiments Local Ethics Committee (49533702/145).

Blood samples were obtained from the V. Jugularis of goats in both groups, in accordance with the appropriate technique, into a dry biochemistry tube (red-capped) for serum and EDTA tubes for plasma and hematological assessments. In blood samples, WBC (White Blood Cell), RBC (Red Blood Cell), Hemoglobin (HGB), Hematocrit (HCT), MCV, MCH, and MCHC measurements were made hematologically (Compteur Analyseur d'Hematologie MS9-3). Blood samples were centrifuged at 5000 g for 10 minutes at room temperature. Serums were kept at  $-20^{\circ}\text{C}$  until the measurement phase. Total Protein (BIOLABO SA TP Test Kit), Albumin (BIOLABO SA ALB Test Kit) measurements were analyzed in ELISA Reader (Awareness Technology, Inc. U.S.A. ChemWell) using commercial kits. Serum Amyloid A (Cusabio Biotech CO., LTD. Goat serum amyloid A Test Kit) was measured using commercial kits in ELISA (Chromate 4300 Microplate Reader / Awareness Technology).

In order to statistically analyze the study, the normal distribution test was performed with the Kolmogorov-Smirnov test, and it was observed that the data were not homogeneously distributed. Considering the sampling number, the Mann-Whitney U test was used for intergroup comparisons for each parameter. The level of significance was calculated as  $p < 0.05$ .

## RESULT

Serum Amyloid A (ng/ml) levels were evaluated in the presented study. Serum amyloid A concentration was statistically ( $p < 0.001$ ) higher in the pneumonia group compared to the control group. Serum Amyloid A concentration was determined as  $3381.17 \pm 178.97$  (mean  $\pm$  SE) in the P group, while it reached up to  $719.56 \pm 133.01$  (mean  $\pm$  SE) levels in the study group and was higher than the control group. (Table 1)

In the present study, serum T. Protein and Albumin levels were evaluated among biochemical parameters. Serum T. Protein concentration was found as  $85.70 \pm 11.24$  (mean  $\pm$  SE) in the control group and  $90.01 \pm 8.05$  (mean  $\pm$  SE) in the pneumonia group. Serum Albumin concentration was  $27.00 \pm 4.49$  (mean  $\pm$

SE) in the control group and  $25.53 \pm 4.34$  (mean  $\pm$  SE) in the study group. (Table 1)

In our study, WBC ( $\times 10^9/L$ ) concentration was found to be significantly higher ( $p < 0.001$ ) in the Pneumonia group ( $P: 20,71 \pm 5.66 \times 10^9/L$ ) compared to the control group ( $F: 10.22 \pm 2.46$ ) in the hematological examination. Again, in the P group, MCV ( $14.08 \pm 1.42$ ) and MCH ( $5.47 \pm 0.60$ ) values were statistically significant ( $p < 0.05$ ) compared to the Group C (MCV;  $12.76 \pm 1.08$ , MCH;  $4.95 \pm 0.30$ ). (Table 2)

In the evaluation of RBC ( $\times 10^{12}/L$ ), HGB (g/dl), HCT (%), and MCHC concentrations, there was no statistically significant difference ( $P > 0.05$ ) in goats with pneumonia compared to the control group. (Table 2)

**Table 1.** SAA, TP and Alb Concentrations (Mean $\pm$ SEM) in Pneumonia and Control Groups

Parameter	Control	Pneumonia	P
SAA	719,56 $\pm$ 133,01	3381,17 $\pm$ 178,97	P<0.001
T protein	85,70 $\pm$ 11,24	90,01 $\pm$ 8,05	P>0,05
Albumin	27,00 $\pm$ 4,49	25,53 $\pm$ 4,34	P>0,05

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 2.** Hematologic Parameters Concentrations (mean  $\pm$  SE) in Pneumonia and Control Groups

Parameter	Control	Pneumonia	P
WBC	10,22 $\pm$ 2.46	20,71 $\pm$ 5.66	P<0.001
RBC	12,79 $\pm$ 1,39	12,59 $\pm$ 1,84	P>0,05
HGB	6,70 $\pm$ 0,75	6,81 $\pm$ 0,86	P>0,05
HCT	17,06 $\pm$ 2,13	17,05 $\pm$ 1,83	P>0,05
MCV	12,76 $\pm$ 1,08	14,08 $\pm$ 1,42	P<0.05
MCH	4,95 $\pm$ 0,30	5,47 $\pm$ 0,60	P<0.05
MCHC	39,33 $\pm$ 2,38	39,76 $\pm$ 3,07	P>0,05

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

## DISCUSSION

Serum amyloid A (SAA) is among the group of apolipoproteins, the main functions of which are to bind, transport, and excrete lipoproteins. This protein also takes part in the immune response through neutrophil and macrophage activation or elimination of coliform bacteria. Further immune-related functions are to participate in monocyte chemotaxis, to inhibit

Phagocyte oxidative burst, lymphocyte and endothelial cell proliferation, and to stimulate the migration and adhesion of T cells. Furthermore, SAA is in charge of detoxifying endotoxins and regulating phagocytosis during inflammation and infection. The fact that SAA concentration increases in farm animals in case of trauma, viral infections, and physical stress has been confirmed (Murata et al., 2004, Jensen and Whitehead 1998, Bolanos-Garcia and Miguel, 2002). SAA belongs to the large APP group in cattle and

small ruminants (Cray et al.2019). In our study, serum amyloid A concentration was statistically higher ( $p < 0.001$ ) in the pneumonia group in comparison to the control group. Serum Amyloid A concentration was determined as  $3381.17 \pm 178.97$  (mean  $\pm$  SE) in the P group, while it reached up to  $719.56 \pm 133.01$  (mean  $\pm$  SE) levels in the study group, which was more elevated compared to the control group.

Acute-phase protein studies are scarce not only on goats but also on sheep, and reference ranges have not been fully determined. In an experimental survey of goats, with subcutaneous turpentine administration, the SAA concentration value, which was  $4.88 \text{ mg / L}$  before the injection, reached up to  $107.8 \text{ mg / L}$  level in 24 hours (22-fold increase). Researchers have reported that SAA is a valuable indicator in determining inflammation in goats (Gonzalez et al., 2008). *Corynebacterium pseudotuberculosis* was applied to goats experimentally; SAA concentration ( $17.85 \pm 0.91 \text{ pg / mL}$ ) increased more rapidly and significantly (Jeber et al. 2016). The authors reported that this increase is due to the fact that hepatocytes and adipocytes produce SAA that is an acute phase marker that can respond swiftly to infections (Odah et al., 2018). Gonzalez. et al. (2011) found the changes in haptoglobin level to be significant in their experimental pregnancy toxemia study on goats, while the changes in SAA concentration were not statistically significant. SAA value was determined as  $0.92 \text{ } \mu\text{g / mL}$  in a study conducted on 55 goats to determine reference ranges of acute-phase proteins in healthy goats. SAA value in goats was determined as  $0.46\text{-}0.50 \text{ mg / L}$  in the measurements carried out with different ELISA kits (Czopowicz M. et al., 2017). In the measurements made in sheep diagnosed with *Pasteurella*, the SAA level was found higher in comparison to the control group. In their study on goats, they took samples from the farms where goats were vaccinated against *Pasteurella* and where the vaccination was not applied; both goats with *pasteurella* and healthy ones from these farms formed the study group. While the SAA was  $33.84 \text{ pg/ml}$  in the normal vaccinated group and  $35.43 \text{ pg/ml}$  in the vaccinated pneumonia group; The SAA was  $34.12 \text{ pg/ml}$  in the normal unvaccinated group and  $49.67 \text{ pg/ml}$  in the unvaccinated pneumonia group. In our study, SAA concentration was determined as  $719.56 \pm 133.01 \text{ ng/ml}$  in goats in the control group, while the SAA concentration in goats with pneumonia was determined as  $3381.17 \pm 178.97 \text{ ng/ml}$ . As is the case in all other studies, we also think that the high value of SAA concentration in our study was caused by inflammation due to pneumonia in goats. Heegaard et al. (2000) reported that haptoglobin was closely linked to the duration of the acute phase response, the severe clinical findings of pneumonia, and the extend of lung consolidation; however, SAA answered to infection most swiftly.

In the presented study, in terms of serum TP (C:  $85,70 \pm 11,24$ , P:  $90,01 \pm 8,05$ ) and Alb (C:  $27,00 \pm 4,49$ , P:  $25,53 \pm 4,34$ ) concentration, there was no statistically significant ( $P > 0,05$ ) increase in Pneumonia group compared to the control group. In the study by Gürgöze and Gökalp (2018), Total protein values were reported as  $5.27 \pm 1.99 \text{ g / dl}$  in Aleppo goats and as  $6.75 \pm 1.10 \text{ g / dl}$  in Ankara Angora goats. In another study conducted on goats, it was reported that the serum TP concentration of healthy goats was in the range of  $6.30\text{-}8.65 \text{ g / dL}$  (İriadam 2004). In a study on Desi goats, TP was  $7.29 \text{ g/L}$ , and Alb was  $4.77 \text{ g/L}$  (Balamurugan et al., 2015); in another study on Iranian goats, total protein was  $3.294\text{-}7.460 \text{ g/dl}$ , and albumin was  $2.315\text{-}3.926 \text{ g/dl}$  (Omidi et al., 2018). In other studies, TP and Alb values are quite different for goats. The reason for this is that the goat breeds, ages, genders, and physiological periods used in the study are different (Balamurugan et al., 2015; İriadam, 2004; Omidi et al., 2018; Piccione et al., 2010). In our study, the average values of goats in both groups were determined in this range. In the presented study, it was determined that the increase in serum Alb and TP concentrations in goats in both groups was not statistically significant.

The study found WBC concentration within reference ranges in the control group (Merck Manual, 2021) (WBC:  $10,22 \pm 2.46$ ) while it was statistically higher ( $p < 0,001$ ) in the Pneumonia group (WBC:  $20,71 \pm 5.66$ ) in comparison to the control group. In our research, WBC concentration was found within the reference ranges in the control group (Merck Manual, 2021). In a field study on Kilis goats, WBC values were determined as  $6,92\text{-}10,97 \times 10^3/\text{mm}^3$  (İriadam. 2004). In another study on goats, the WBC value in the control group was found as  $10.3 \times 10^3/\mu\text{L}$  (Jarikre et al., 2016). These findings are compatible with our control group. In our study, the WBC value in the Pneumonia group ( $20,71 \pm 5.66$ ) was above the normal values. In other studies, it has been reported that WBC count has increased significantly in goats with pneumonia, and leukocytosis, which is caused by a relative increase of neutrophil granulocytes, has happened due to the body's reaction against respiratory system infection (Daramola et al., 2005, Ezeasor and Emikpe., 2015, Maina et al., 2015, Jarikre et al., 2016). In this study, the increase in WBC count in goats who were clinically diagnosed with pneumonia and that there is a statistical significance compared to the control group are consistent with the literature.

In the hematological study conducted on Gaddi goats, Rastog ve and Singh (1990) found RBC value as  $20.43 \times 10^6/\text{mm}^3$ , hemoglobin count as  $11.1 \text{ g/dl}$ , hematocrit value as  $\%31$ , WBC value as  $12.96 \times 10^3/\text{mm}^3$ , and the percentage of white blood cells as 18-31 of neutrophils, 1-4 of eosinophils, 0-1 of basophils, 55-78 of lymphocytes and 3-7 of monocytes. They calculated the mean red blood cell

volume (MCV) as 18.43, the mean red blood cell hemoglobin (MCH) as 5.1 pg, and the mean red blood cell hemoglobin concentration (MCHC) as 30.4%. In the same study, it was stated that hemoglobin and hematocrit values were characteristically higher than other breeds, and MCV was low. In another study conducted in Turkey, goats within different age ranges (6-month and 3-year-old) were examined haematologically, and the results were found as in the following: RBC value as  $10.92\text{--}21.50 \times 10^6/\text{mm}^3$ , WBC value as  $6.92\text{--}10.97 \times 10^3/\text{mm}^3$ , hemoglobin count as 8.40–10.72 g/dl, hematocrit value as %21.66–37.60, MCV as 19.83–17.48, MCH as 7.69–4.98 pg and MCHC as %37.38–28.45 (İriadam, 2004). In our study, the values for the control and pneumonia groups were found as in the following: RBC C:  $12.79 \pm 1.39$  P:  $12.59 \pm 1.84$  ( $P > 0.05$ ), WBC C:  $10.22 \pm 2.46$  P:  $20.71 \pm 5.66$  ( $p < 0.001$ ), HGB C:  $6.70 \pm 0.75$  P:  $6.81 \pm 0.86$  g/dl ( $P > 0.05$ ), HCT C:  $17.06 \pm 2.13$  P:  $17.05 \pm 1.83$  ( $P > 0.05$ ), MCV C:  $12.76 \pm 1.08$ , P:  $14.08 \pm 1.42$  ( $p < 0.05$ ), MCH C:  $4.95 \pm 0.30$  P:  $5.47 \pm 0.60$  pg ( $p < 0.05$ ), and MCHC C:  $39.33 \pm 2.38$  P:  $37.38\text{--}28.45$  ( $P > 0.05$ ). Even though there was a statistical difference among the control group in terms of MCV and MCH values, not only they were within the reference ranges (Merck Manual, 2021) but also were parallel to the control groups of other studies on goats (İriadam, 2004, Daramola et al., 2005, Jarikre et al., 2016).

## CONCLUSION

Considering the presented study results, it was determined that the increase in SAA concentration in serum obtained from goats in the pneumonia group accompanied the clinical symptoms of naturally infected animals, and SAA was found in minimal concentrations in the control group, which was formed by the healthy animals. Considering the results, it is thought that measuring serum amyloid A concentration as a routine would be beneficial to determine the infection severity, to choose a suitable treatment, to watch the efficiency of the selected treatment method, and would be helpful for detecting animals that show no clinical symptoms and have a subclinical course during the herd health screening in terms of veterinary medicine.

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