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Evaluation of Some Biochemical Parameters In Saanen Goats Naturally Infected With Mycoplasma agalactiae

Sezai ARSLAN
Department of Internal Medicine, Faculty of Veterinary Medicine, Tekirdag Namik Kemal University, Tekirdağ, Türkiye.
https://orcid.org/0000-0001-9537-6024
✉: sarslan@nku.edu.tr

ABSTRACT

Mycoplasma agalactiae in sheeps and goats causes significant economic losses worldwide. The disease can cause mastitis, arthritis, ophthalmitis and less commonly abortion. This study aimed to reveal what kind of changes the disease causes in routine biochemical parameters and PCT and CRP levels in naturally infected goats. The material of the study consisted of fifteen naturally infected with Mycoplasma agalactiae and nine healthy (control group) female goats in a private commercial enterprise. M. agalactiae was diagnosed by a growth inhibition test in milk and blood serum. Serum biochemical analysis was performed by autoanalyzer. In the statistical study conducted between the control and infected groups, a significant decrease was found in albumin levels and A/G ratio in the infected group, and a significant increase in globulin, chlorine and potassium levels. No significance could be determined in levels of ALT, AST, D.BIL, T.BIL, BUN, Creatinine, Urea, Glucose, Na, P, CRP and PCT. As a result, it was revealed that PCT is not an important biomarker in goats with Mycoplasma agalactiae.

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ÖZET

Koyun ve keçilerde Mycoplasma agalactiae, dünya çapında önemli ekonomik kayıplara neden olmaktadır. Hastalık, mastitis, artrit, oftalmitis ve daha az yaygın olarak abort yapabilir. Bu çalışmanın amacı, doğal olarak enfekte olan keçilerde hastalığın rutin biyokimyasal parametreleri ile PCT ve CRP seviyelerinde meydana gelen değişiklikleri ortaya koymaktır. Çalışmanın materyalini, özel bir ticari işletmede doğal olarak Mycoplasma agalactiae ile enfekte olmuş 15 ile 9 adet sağlıklı (kontrol grubu) dişi keçi oluşturmaktadır. M. agalactiae, süt ve kan serumunda Growth Inhibition Test ile teşhis edildi. Serum biyokimyasal analizi otomatik analizör ile yapıldı. Kontrol ve enfekte gruplar arasında yapılan istatistiksel çalışmada, enfekte gruplarda albümin seviyelerinde ve A/G oranında önemli bir azalma, globulin, klorin ve potasyum seviyelerinde ise önemli bir artış tespit edildi. ALT, AST, D.BIL, T.BIL, BUN, kreatinin, üre, glukoz, Na, P, CRP ve PCT seviyelerinde anlamlılık belirlenemedi. Sonuç olarak, Mycoplasma agalactiae enfeksiyonlu keçilerde PCT'nin önemli bir biyobelirteç olmadığı ortaya konuldu.

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INTRODUCTION

Contagious agalactia is a common disease that is seen in sheeps and goats worldwide and causes significant economic losses (Corrales et al., 2007; Kumar et al., 2014; Jaÿ & Tardy, 2019). The main microorganism of contagious agalactia in sheeps and goats is *Mycoplasma agalactiae (M. agalactiae)*. However, Contagious agalactia is caused by *Mycoplasma*

mycoides subsp. mycoides (large colony type) (MmmLC) and *Mycoplasma capricolum subsp. capricolum* (Mcc) (Bergonier et al., 1997). Contagious agalactia causes severe mastitis, arthritis, ophthalmitis (Kusiluka & Kambarage, 1996) and sometimes respiratory disease (Bergonier et al., 1997). Less frequently, it may cause abortion during advanced pregnancy (Bergonier et al., 1997). Clinical signs can be observed 5 to 7 days after inoculation of infective organisms or 60 days after infective organisms are removed from the environment. Clinical cases are most common in spring (Anderson et al., 2002). The organism is excreted with milk, urine, faeces, eye and nasal discharge for months, and this may be a source of infection for other animals in the herd (Plummer & Plummer, 2012). Mortality rate can be detected up to 20% in untreated animals. (Smith & Sherman, 2009).

In recent years, procalcitonin (PCT) has become a promising new biomarker for the early detection of systemic bacterial infections (Cleland & Eranki, 2022). A correlation between PCT and the severity of sepsis has been observed, and PCT-levels are low or only slightly elevated in patients without bacteremia or systemic infection (Assicot et al., 1993; Durnaś et al., 2016). The concentration of procalcitonin (PCT) increases in bacterial infections, but not in viral infections (Moulin et al., 2001; Matur et al., 2023). Parasitic infections such as malaria (Al-Nawas & Shah, 1997; Uzzan et al., 2006) and babesiosis (Arslan et al., 2018) are also expressed (Hausfater et al., 2007). C-reactive protein (CRP) is an acute-phase protein and a non-specific marker of systemic inflammation (Black et al., 2004; Kang et al., 2009). In individuals with acute disease, cytokines, particularly interleukin-6, stimulate hepatic CRP production and their rising plasma levels (Bock, 2011). Plasma CRP level is increased in various diseases, mostly bacterial infections (Bock, 2011). CRP rises rapidly with the onset of inflammation and decreases with healing (Pincus et al., 2011). CRP is often applied for the detection and preliminary classification of latent infections, as bacterial infections can induce much higher levels of CRP than viral ones (McPherson, 2011).

In a study conducted on goats infected with *M. agalactiae* (Kızıl & Ozdemir, 2006), it was reported that body temperature, pulse and respiratory rates increased, but rumen contractions decreased in clinical examination of infected goats. In a biochemical examination, it has been reported that detection of increasing levels of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity in plasma, whereas decreasing levels of plasma total protein, albumin and glucose concentrations. Moreover, no significant changes were detected in PCV, Hb, alkaline phosphatase (AP) and γ-glutamyl transferase (GGT) activity, sodium, potassium and chloride concentrations.

Biochemical reports on *M. agalactia* in goats are inconclusive. There are no studies on PCT levels in goats with *Mycoplasma agalactia*e. This study aimed to determine the changes in clinical and biochemical values including CRP and PCT in goats naturally infected with *Mycoplasma agalactia*e.

MATERIALS and METHODS

Ethical Statement

The ethical approval of this study was obtained from the Local Ethic Committee of Animal Experiments at Tekirdag Namik Kemal University (T2021-576).

Animals and Clinical Examination

The study material consisted of 24 female Saanen goats (aged 3-6 years), 15 naturally infected with *M. agalactia* (infected group) and 9 healthy (control group). All goats in the study were bred on a private commercial farm in Kırklareli district of Türkiye with the same feeding, housing and management conditions. None of these animals had been vaccinated against *M. agalactiae*. On clinical examination, sick animals had symptoms of mastitis, arthritis, and keratoconjunctivitis specific to contagious agalactia. Control animals had no history of symptoms or signs associated with contagious agalactia.

Microbiological Procedure

Blood samples were taken from the vena jugularis into sterile tubes (Kaygısız & Sönmez, 2018; Akkose, 2020). Sera was obtained by centrifugation of blood samples at 3000 rpm for 20 minutes. Some of the serum samples, milk samples and some internal organs of the dead animals were transported to Mycoplasma National Reference Laboratory of Pendik Veterinary Control and Research Institute under freezing conditions. Some of the serum samples were also stored at -20°C for biochemical analysis. A growth inhibition test in milk and blood serum and latex agglutination test for *Mycoplasma capricolum subspecies capripneumoniae* was performed for the diagnosis of *M. agalactiae* (TCGTHB, 2014). In addition, the indirect ELISA test (IDvet, France) for the detection of antibodies to *M. agalactiae* in the sera of the infected and healthy goats was performed according to the manufacturer's instructions for use.

Biochemical Parameters

Serum Sodium (Na), potassium (K), chlorine (Cl), phosphorus (P), Calcium (Ca^{+2}), Total protein (TP), Albumin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Direct bilirubin (D.BIL), Total bilirubin (T.BIL), Urea (U), Creatinine (CR),

Glucose (GLU) and C-reactive protein (CRP) concentrations were analyzed with an autoanalyzer (Roche, Hitachi cobas® 6000, biochemistry analyzer, Germany/Japan). Serum procalcitonin (PCT) levels were measured with an immunoassay autoanalyzer (Roche/Hitachi cobas® e411, Germany/Japan). Serum Globulin (G) values were obtained by subtracting the serum albumin value from the serum Total protein value for each goat and Albumin/Globulin (A/G) ratios were calculated for each goat.

Table 1. Biochemical parameters in control and naturally infected goats
Çizelge 1. Kontrol ve doğal olarak enfekte keçilerde biyokimyasal parametreler

Parameters	Units	Infected Group (X±Sx)			Control Group (X±Sx)			P
TP	g dL^{-1}	7.43	±	0.30	6.84	±	0.16	0.174
ALB	g dL^{-1}	2.69	±	0.1	3.30	±	0.04	0.000
G	g dL^{-1}	4.74	±	0.27	3.55	±	0.18	0.001
A/G	-	0.6	±	0.04	0.94	±	0.05	0.000
ALT	U L^{-1}	15.07	±	3.67	14.89	±	1.54	0.892
AST	U L^{-1}	89.07	±	22.00	84.89	±	10.60	0.601
D.BIL	mg dL^{-1}	0.02	±	0.02	0.02	±	0.01	0.893
T.BIL	mg dL^{-1}	0.14	±	0.04	0.13	±	0.01	0.285
BUN	mg dL^{-1}	12.33	±	1.76	12.33	±	0.50	1.000
CRE	mg dL^{-1}	0.38	±	0.08	0.38	±	0.04	0.938
UREA	mg dL^{-1}	26.24	±	4.00	26.33	±	0.91	0.946
GLU	mg dL^{-1}	49.67	±	4.40	50.00	±	4.27	0.858
K	mmol L^{-1}	3.52	±	0.18	3.37	±	0.20	0.035
Na	mmol L^{-1}	140.07	±	2.09	140.67	±	1.32	0.449
Cl	mmol L^{-1}	97.63	±	2.11	95.63	±	0.52	0.003
P	mg dL^{-1}	4.78	±	1.32	4.97	±	0.74	0.699
CRP	mg L^{-1}	0.05	±	0.07	0.03	±	0.02	0.414
PCT	ng mL^{-1}	<0.02			<0.02			1.000

Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistics 24 statistical software. The normality of data was analyzed with the Shapiro-Wilk test. Non-parametric Mann-Whitney U and parametric t-tests were used for comparing the groups. Statistical significance was determined as $p<0.05$.

RESULTS and DISCUSSION

In the herd, clinical findings such as loss of appetite, depression, fatigue, diarrhoea, mastitis, arthritis and keratoconjunctivitis were widely seen in the goats. The infection started with mastitis characterized by increased udder heat, pain and swelling, followed by udder atrophy and a decrease in the amount and quality of milk production. In carpal and tarsal joints, swelling and pain were observed in affected goats. Also, mucopurulent exudation, conjunctivitis, corneal opacity and keratitis were detected in the eyes. Respiratory signs ranged from mild cough to dyspnea.

M. agalactiae was isolated and identified from milk and serum samples. *Mycoplasma capricolum subspecies capripneumoniae* could not be detected by latex agglutination test. While antibodies against *M. agalactiae* were detected in the affected goats with the indirect ELISA test, they could not be detected in the healthy goats (control group).

Serum biochemical parameters are presented in Table 1. It was detected that a significant decrease in albumin levels and A/G ratio and a significant increase in globulin, chlorine and potassium levels in the infected group. No significance was detected in ALT, AST, D.BIL, T.BIL, BUN, Creatinine, Urea, Glucose, Na, P, CRP and PCT levels when comparing the infected and healthy goats.

In studies on other mycoplasma species (Mondal et al., 2004; Shah et al., 2017), total serum protein and albumin were found to be significantly decreased. In this study, we have found that a decrease in the amount of albumin in infected goats compared to the control group and the normal levels of liver enzymes may be due to malabsorption disorder of hypoalbuminemia or malnutrition as a result of anorexia due to the disease. Decrement in albumin level was consistent with previous similar studies (Mondal et al., 2004; Kızıl & Ozdemir, 2006). Increment in globulin level in the infected group may be due to the increase in the antibodies of the organism against the infection. Similar biochemical changes have also been reported by Mondal et al. (2004). In a previous study (Mondal et al., 2004), a decrease in the A/G ratio was detected, and in this study, we found a decrease in the A/G ratio in infected goats. This decrease may have occurred due to the increase in the amount of globulin in serum samples.

Although Kızıl and Ozdemir (2006) could not detect a significant difference in potassium and chlorine

levels, an increase in potassium and chlorine levels has been found in infected goats in this current study. This has been suggested that it may be due to metabolic acidosis following diarrhoea. It has been reported that there are increases in AST and ALT in caprine mycoplasmal pneumonia (Mondal et al., 2004), increasing levels of ALT in Mycoplasma mycoides subsp. (Shah et al., 2017), and increasing levels of AST and LDH in goats infected with *M. agalactiae* (Kızıl & Ozdemir, 2006). In this current study, we could not detect significant changes in AST and ALT levels. This suggests that the agent does not cause a significant failure in the liver. This finding is similar to the study conducted by Rosendal (1981), in which no liver changes were detected in experimentally induced mycoides in goats.

There was no significant difference in PCT levels in goats infected with mycoplasma spp. compared to the control. No study has been found on the effect of *M. agalactia*e on PCT levels in goats. In humans, it has been stated that the PCT levels do not increase in Mycoplasma and Chlamydia infections, which are among atypical bacteria (Self et al., 2017; Saleem, 2019). In the current study, no increase in CRP levels was detected in infected goats. There is no literature available on CRP in goats with *M. agalactiae*. Studies have reported that CRP may not be an acute phase protein in goats (Maudsley et al., 1987; Pathak & Agrawal, 2019).

CONCLUSION

As a result, the absence of increased levels of PCT and CRP in goats with *M. agalactiae* infection can be attributed to the immune response exhibited by the goats. Furthermore, a decrease in albumin levels and an increase in globulin, chlorine, and potassium levels were observed. Therefore, evaluating these parameters is likely to be clinically beneficial in managing the disease.

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Contribution of Authors

The concept of the study, sample collection, analysis, and writing stages were conducted by SA.

Conflict of Interest

The author declares that they have no potential conflict of interest regarding the authorship and/or publication of this article.

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