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Intercellular adhesion molecule-1 (ICAM-1) and C-Reactive Protein (CRP) levels in Calves Naturally infected with *Mycoplasma bovis*

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Abstract

Mycoplasma bovis is one of the most important pathogens causing pneumonia worldwide. In this study, it was aimed to determine intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (CRP) levels in calves infected with *Mycoplasma bovis*. The animal material of the study consisted of 30 calves including 20 simmental calves with pneumonia due to *Mycoplasma bovis* at 2-4 months of age and 10 healthy calves at the same age and the race. Serum levels of intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (CRP) were determined in calves infected *M. bovis* and control group. Serum levels of intercellular adhesion molecule-1 (ICAM-1) and C-reactive protein (CRP) were found to be statistically higher in the calves with pneumonia than in the control group ($P<0.01$). A positive correlation between serum ICAM-1 concentrations and *Mycoplasma bovis* titers (increases in coefficient ratios) were statistically significant ($r=0.664$), indicating a positive correlation between serum ICAM-1 and CRP levels and titers of *Mycoplasma bovis* in the calves with pneumonia. As a result, it was observed that serum intercellular adhesion molecule-1 (ICAM-1) and C-Reactive Protein (CRP) concentrations were increased in infected pneumonia with *Mycoplasma bovis*. It is considered useful to consider these parameters in evaluating the prognosis of pneumonia cases of *Mycoplasma bovis* origin and the efficacy of applied treatment.

Keywords: Calf, *Mycoplasma bovis*, C-Reactive Protein, Intercellular adhesion molecule-1

INTRODUCTION

Mycoplasma bovis is a common reason of acute respiratory disease mostly affecting young calves (Abdeen et al.,2017). Bovine respiratory disease (BRD) continues to be the most economically significant disease in feedlots and one of the most important causes of morbidity and mortality in dairy calves and veal calves in veterinary medicine, animal husbandry, and animal welfare (Abdallah et al., 2016). *Mycoplasma bovis*-associated pneumonia occurs in cattle, including dairy and beef calves, beef cattle after arrival at a feedlot, and adults at any age (Hayman and Hirst 2003). *Mycoplasma bovis* is a particularly important cause of calf pneumonias (Nicholas,2004). Especially young calves under 4 months of age are at increased risk for pneumonia caused by *M. bovis* (Soehnlen et al., 2012). Mycoplasma infection is characterized by clinical symptoms of pleuritis and pneumonitis, mastitis, arthritis, keratoconjunctivitis, otitis, fever, asphyxia, depression and pathological symptoms (Hale et al., 1962; Pfutzner and Sachse, 1996; Sun et al., 2014). *Mycoplasma bovis* infection causes significant economic losses in calves such as body weight loss, medical treatment expenses and death in European countries and America. In Europe, it is reported that about 25-33% of pneumonic calf cases are stem from *Mycoplasma bovis*. Likewise, in the USA, it is stated that the rate of *Mycoplasma bovis* origin is increased up to 70% and the annual loss of *Mycoplasma bovis* is 140 million dollars (Tschopp et al., 2001; Nicholas and Ayling 2003; Caswel and Arehambauh, 2007).

The Intercellular Adhesion Molecule-1 (ICAM-1) has a central role in the regulation of cellular inflammatory responses. ICAM-1, which is expressed in endothelial cells, is one of the major cell surface glycoproteins that contribute to cell adhesion processes (Bevilacqua, 1993).

Acute phase proteins (APPs) play major roles in several aspects of the systemic reaction to inflammation, including the opsonization of several pathogens, the scavenging of potentially toxic substances and the overall regulation of different stages of inflammation (Petersen et al., 2004; Ceciliani et al., 2012). APPs are already used as markers of disease in veterinary clinical chemistry (Petersen et al., 2004). The first acute phase protein to be recognised, over fifty years ago (Tillett and Francis,1930), was C-reactive protein (CRP), which in man has become the most important analyte providing diagnostic information on the presence of inflammatory lesions, on the prognosis of the condition and on the response to treatment (Pepys and Baltz, 1983, Gabay and Kushner, 1999). CRP in man is a major APP, with its concentration increasing over 200 times from a low, virtually negligible, normal level (Eckersall, 2000).

The aim of this study was, therefore, to perform a systematic review/meta-analysis concerning selected ICAM-1 and CRP as diagnostic tools for calves infected naturally with *Mycoplasma bovis* when compared with the reference standard used to prognosis and treatment of *Mycoplasma bovis*.

MATERIALS AND METHODS

The animal material of this study; a total of 30 calves, including 10 simmental calves with pneumonia, 1-6 months of age and a control group of 10 healthy calves of the same age and descent, who were brought to the clinic of Van Yuzuncu Yil University Veterinary Medicine Internal Medicine clinic with respiratory system complaint. General clinical examinations of the study and control group of the animals were performed and the body temperature, respiration and heart frequency of the animals were determined. At the same time, the history of the animals were obtained whether they received any antibiotic treatment had been administered in the past. As a result of the anamnesis, antibiotic-applied calves were treated earlier and were not included in the study. In order to perform biochemical analyses, blood samples from vena jugularis were taken to anticoagulant free tubes from all the calves.

Biochemical analyses

For the analysis of the biochemical parameters, obtained blood samples were centrifuged (Rotofix32®-Hettich) at 3000 rpm. The resulting sera were stored at -20 °C until measurements were made. Serum Intercellular Adhesion Molecule 1 levels and serum C-reactive Protein (CRP) levels were determined by ELISA reader (DAS, Italy) according to the method described in commercial test kits.

As commercial test kits; Intercellular Adhesion Molecule 1 levels [ICAM-1 ELISA Kit, Catalog No. CK-E91670] and C-Reactive Protein (CRP) levels [C-Reactive Protein (CRP), ELISA Kit, Catalog No. CK-E91671] test kits were measured in an ELISA instrument as described in the procedure.

Mycoplasma bovis detection: It is determined by ELISA device (ELISA reader ®-DAS) in the obtained sera in accordance with the method described in the commercial test kits [Bio-X Diagnostics Mannheimia haemolytica® ELISA, Catalog No: BIO K 260/2]. From the obtained data according to the test kit, the coefficient of each sample was calculated with the given method. The coefficient values higher than 37% were regarded as positive and the coefficient values below 37% were regarded as negative.

Statistical Analysis

Descriptive statistics for the studied variables (characteristics) were presented as median, mean, standard deviation, minimum and maximum values. Mann-Whitney U test was performed to compare the groups for the variables. In addition, Spearman correlation coefficient was also calculated to determine between the variables. Statistical significance level was considered as 5% and SPSS (ver: 13) statistical program was used for all statistical computations.

RESULT

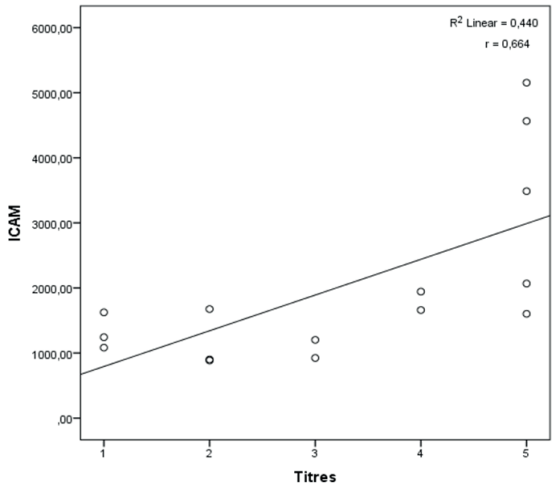
In calves infected with *Myoplasma bovis* had high fever (40.5-41°C), anorexia, dullness, respiratory distress according to clinical examination. It was determined that some patients extended their necks forward and that their nostrils were enlarged and their mouth was open.

In some patients, mucopurulent nasal discharge, breathing and wheezing sounds were detected and the pulse rate was determined as 100-120 beats/min. In some cases, rubbing sounds were detected, while in others, noises were heard by the stethoscope. In the lung percussion, pain in the thorax (especially the intercostal space) and pain and swelling in the joints were detected.

In the statistical analysis; ICAM-1 and CRP levels in *Mycoplasma bovis* infected calves were significantly higher than the same parameters of the control group.

Table1. ICAM-1 and CRP levels on control and *Myoplasma bovis*-infected calf

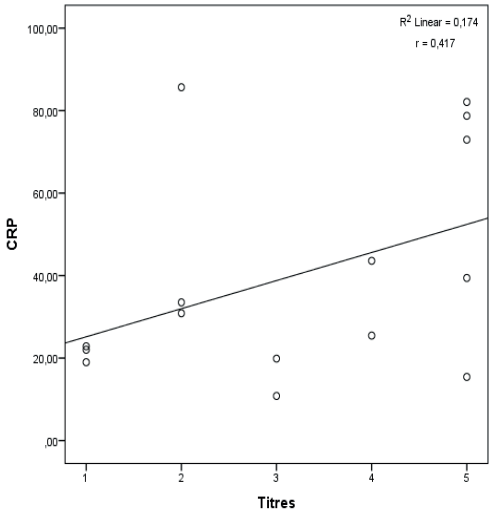
Parameters	Control	*Mycoplasma bovis*	**P**
ICAM -1 (ng/L)	611.37±320.99	2001.40±1334.21	0.001
CRP (ng/L)	22.99±18.71	40.16±26.34	0.046



Graphic 1. Correlation between ICAM-1 and Sample Coefficient in calves infected with *Mycoplasma bovis*

As a result of the analyses, when the coefficient value was between 45 and 55% the group was numbered as 1, between 65 and 75% was numbered as 2, between 75 and 85 as 3, between 85 and 90% was numbered as 4, and between 90 and 95% was numbered as 5 according to *Mycoplasma bovis* test kit.

Correlation analysis between ICAM-1 and CRP levels of the *Mycoplasma bovis*-infected calves and the factor titers (coefficient ratios) revealed a positive correlation between increases in ICAM-1 and CRP concentrations and titer increases in *Mycoplasma bovis* agent (increases in coefficient ratios). However, a statistically significant positive correlation (r=0.664) was found between increases in ICAM-1 concentrations of Mycoplasma bovis-infected calves and titers of *Mycoplasma bovis* agents (increases in coefficient ratios).



Graphic 2. Correlation between CRP and Sample Coefficient (Titers of the agents) in Mycoplasma bovis-infected Calves

DISCUSSION

It is assumed that one third of the losses caused by respiratory system diseases in the bovine industry are caused by *M. bovis* (Abdeen et al., 2017). *M. bovis* is capable of applying pathogenic effects on many organ systems, common clinical presentations contain arthritis, mastitis and otitis in calves (Horwood et al., 2014). Laboratory diagnosis is of great significance to the prevention and treatment of *M. bo-*

vis infection. Because *M. bovis* infection is often latent and the bacterium is rarely shed from healthy cattle, serological detection of *M. bovis* antibody, which can last for several months and can be detected at high levels by ELISA, is considered a more reliable method of diagnosis of *M. bovis* infection (Sun et al., 2014; Nicholas and Ayling, 2003).

The current study aimed at investigating the relationship between respiratory disorders in calves observed at ICAM-1 and CRP levels and titers of *Mycoplasma bovis* recorded at laboratory.C-reactive protein (CRP) in humans, dogs, pigs and horses is a major APP, revealing a rapid and pronounced increase in its serum concentration in response to infection or tissue injury (Ulutas et al., 2006). It is not clear whether CRP is an acute-phase reactant in ruminants or not; however, Schrodl and colleagues (1995) showed that CRP concentrations in cattle with bovine mastitis were 10 times greater than in healthy dairy cows. Similarly, Lee and colleagues (2003) reported that serum CRP levels had a correlation with the health condition of the diary herd. Vojtic and Krajne (2000) suggested that CRP was helpful in distinguishing the normal and pathological conditions in sheep. Likewise, in this study, the mean serum CRP level was significantly increased in calves with *Mycoplasma bovis*.

Ruminants are significantly different to other species in their acute phase response in that Hp is a major APP. In healthy cattle the serum Hp concentration is lower than 20 mg/L, but can pass the concentrations of 2 g/L within 2 days of infection. In cattle, Hp is effective in the diagnosis and prognosis of mastitis, enteritis, peritonitis, pneumonia, endocarditis, and endometritis (Petersen et al., 2004). Measurements of acute-phase proteins during infectious or inflammatory conditions are useful for diagnosis, prognosis and assessing the response to treatment (Eckersall, 2000).

The Intercellular Adhesion Molecule-1 has a central role in the regulation of cellular inflammatory responses (Bevilacqua, 1993). It is stated that in the patients with acute lung injury, levels of soluble intercellular adhesion molecule-1 were significantly higher in both edema fluid and plasma (median 938 and 545 ng/ml, respectively) from acute lung injury patients compared to hydrostatic edema patients (median 384 and 177 ng/ml, $P<0.03$ for both comparisons) (Calfee et al., 2009). In our study, serum ICAM-1 levels in calves infected with *Mycoplasma bovis* were significantly higher than in the control group. In addition, a positive correlation ($r=0.664$) was found between increased levels of ICAM-1 in *Mycoplasma bovis*-infected calves and titers of *Mycoplasma bovis* agents (increases in coefficient ratios).

As a result, *M. bovis* has an important role in influenza pneumonia and it was found that there is a positive correlation between titers of *M. bovis* agent and ICAM-1 and CRP concentrations. Changes in ICAM-1 and CRP concentrations (increases or decreases) are considered to be a consideration for these parameters in assessing the prognosis of *M. bovis*-associated pneumonia.

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