DETERMINATION OF ACUTE PHASE PROTEIN LEVELS IN CATTLE WITH PARATUBERCULOSIS

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Aim - Paratuberculosis leads to economic losses due to loses body weight, decrease in milk yield and fertility problems. Diagnosis of this disease is of great importance for both economic and public health. However, there is a definite method for the diagnosis of this disease. In this study, serologically in tagged paratuberculosis in cattle detection of acute phase proteins from serum haptoglobin (Hp) , Serum amyloid-A (SAA), ceruloplasmin (Cp), C-reactive protein (CRP), albümin (alb) and total protein (TP) is intended to identify changes in the level.
Material and Method: In study, 30 paratuberculosis positive cattle formed test group and 30 healthy cattle formed control group. Serologically diagnosis for paratuberculosis used ELISA (IDEXX Paratuberculosis screenging ab test). SAA, Hp, CRP, Cp, albumin and Tp of acute phase proteins level were measured for each blood sample for all of groups.
Results: All analysis of results showed that SAA ($p<0.001$), Hp ($p<0.001$), CRP ($p<0.01$) ve Cp ($p<0.001$) level was higher in cattle with paratuberculosis than in healthy cattle. The significant decreaase in the levels of total protein and albümin in cattle with paratuberculosis ($p<0.001$).
Conclusion: While in cattle with paratuberculosis an increase in the levels of acute phase proteins such as serum SAA, Hp, CRP and Cp was showed, albümin and total protein levels decreased. It was considered that the measurement of acute phase proteins was useful in the evaluation of health in cows.

Keywords - Acute phase protein; bovine; Mycobacterium paratuberculosis; paratuberculosis We would like to thank University of Ondokuz Mayis BAP for support . It was summary from the master thesis.

I. INTRODUCTION

Mycobacterium avium spp. paratuberculosis (MAP) causes Johne disease, which is a chronic granulomatous infection in gastrointestinal system in cattle, in many countries (Hermon-Taylor, 2001; Kennedy et al., 2001). MAP was isolated from daily milks, drinking water, various food sources and carcasses. That is why, it was reported that humans can easily be exposed to MAP (Beumer et al. 2010, Meadus et al. 2008). Also, it is a pathogen which is associated to Crohn disease in humans (Riis et al. 2005; Pickup et al. 2005). Even though MAP causes diseases in many animal species, the mechanism of the disease is not fully understood (Schleig et al., 2005). The diagnosis of the paratuberculosis cannot made precisely. The most used method is ELISA. However, this method has disadvantages. Therefore, alternative methods are sought to increase the accuracy of the diagnosis. In the cattle with paratuberculosis, knowing the changes of acute-phase proteins will be beneficial for diagnosing and controlling the disease.

II. MATERIAL METHOD

A total of 400 cattle blood, which is got from the cattle farms in Samsun Region aseptically consists the material of the study. After the removal of the serums of the taken blood, they were conserved in 80 ºC until the analyses are made. The diagnosis of paratuberculosis in cattle is determined with ELISA method. 30 of the taken samples were found paratuberculosis positive and they consisted our study group. The serums which are healthy and paratuberculosis negative are taken as control group.

Paratuberculosis Diagnosis in Cattle

The serum samples were tested with paratuberculosis using commercial antibody ELISA kit (IDEXX Mycobacterium paratuberculosis Antibody Test Kit, Montpellier SAS, FRANCE). The microplate was placed in ELISA reader (Mindray MR-96A) and the density of the each well determined as 450 nm. The percentage result for the obtained OD values were calculated with the following formula.

$$\text{RESULT (\%)} = \frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}}$$

According to the formula above, from the obtained results; 55% and above considered as positive, 45-55% considered as suspected and 45% and below considered as negative.

The Analysis of Acute-Phase Proteins

It is measured with commercial ELISA test kits, SAA (Tridelta Development Ltd. Ireland Cat No: TP-802), Hp (Tridelta Development Ltd. Ireland Cat: No. TP-801), CRP (Tridelta Development Ltd. Ireland Cat No: TP-80 Con) and ELISA reader DAS, according to the modified Ravin method which is a Cp spectrophotometric method (Thermo Scientific ™ Genesys 10S UV-Vis.) . The amount of albumin and total protein in serums were measured in autoanalyzer with using commercial test kits (Audict kit, Autolab AMS Srl, Autoanalyzer, Netherlands) with a colorimetric method.

III. **FINDINGS**

The most common findings in cattle with paratuberculosis are diarrhea and weight loss. In our study too, as clinical findings recurrent diarrhea, weight loss and loss of yield were observed in the animals which consist our study group. The ones in the control group are the cattle which were healthy in clinical terms.

Some acute-phase proteins were searched in the bloods of the 30 paratuberculosis positive cattle and the paratuberculosis negative cattle. The values of serum acute-phase proteins of healthy and paratuberculosis cattle are given in Table 3.
Serum Amyloid A was found higher in the paratuberculosis cattle (369,9 ± 17,0 ng/mL) compared to healthy cattle (253,1 ± 82,7 ng/mL) ($p<0.001$).

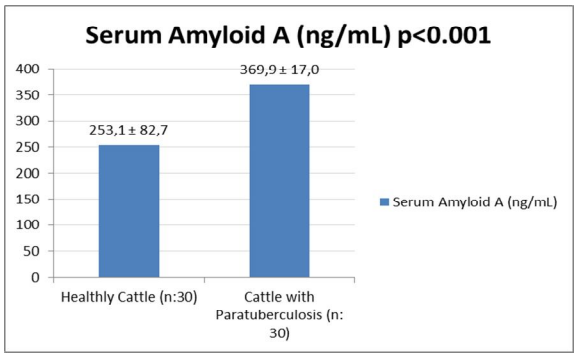


Figure 1. Serum amyloid-A amount in the serums of healthy and paratuberculosis animals

Serum haptoglobin was multiplied itself more than double in paratuberculosis cattle (0,820 ± 0,615 mg/mL) compared to healthy cattle (0,354 ± 0,177 mg/mL) ($p<0.001$).

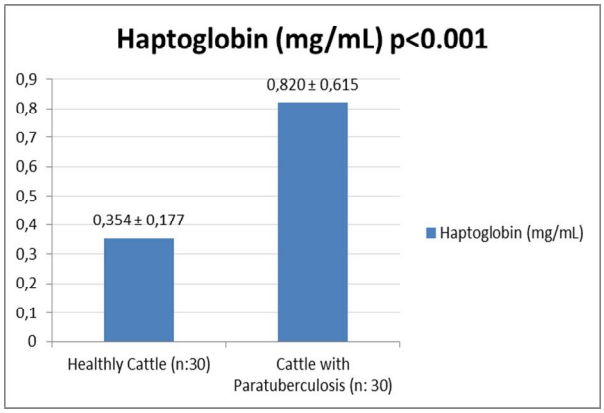


Figure 2. Serum Haptoglobin amount in the serums of healthy and paratuberculosis animals

C-reactive protein was found higher in the paratuberculosis cattle (0,260 ± 0,121 µg/mL) compared to healthy cattle (0,1885 ± 0,0571 ng/mL) ($p<0.01$).

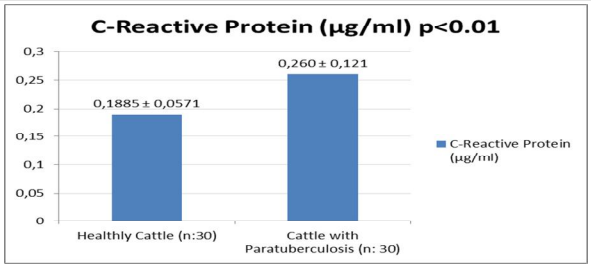


Figure 3. C-Reactive Protein amount in the serums of healthy and paratuberculosis animals

Ceruloplasmin was found almost twice as more in paratuberculosis cattle (33,7 ± 12,3 mg/dl) compared to healthy cattle (18,08 ± 5,43 mg/dl) ($p<0.001$).

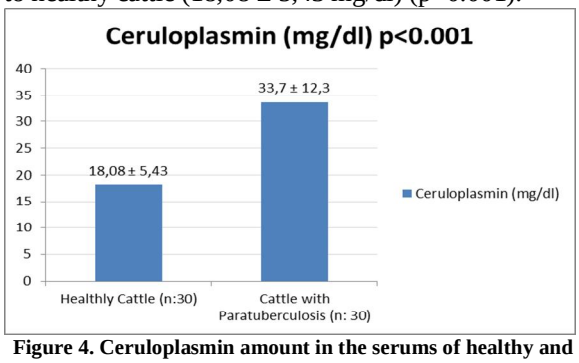


Figure 4. Ceruloplasmin amount in the serums of healthy and paratuberculosis animals

The albumin was lower in the paratuberculosis cattle (2,977 ± 0,699 mg/dl) compared to healthy cattle (3,713 ± 0,407 mg/dl) ($p<0.001$).

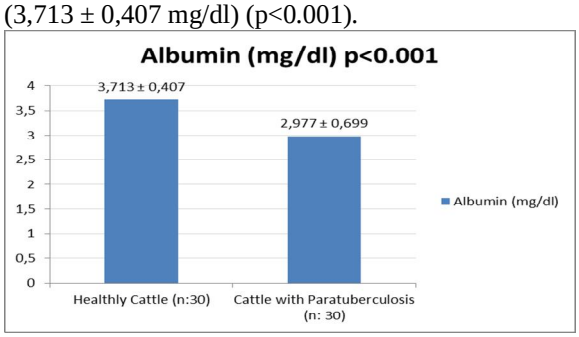


Figure 5. The albumin amount in the serums of healthy and paratuberculosis animals

Serum total protein amounts were lower in paratuberculosis cattle (26,95 ± 4,73 mg/dl) compared to healthy cattle (59,3 ± 12,7 mg/dl) ($p<0.001$).

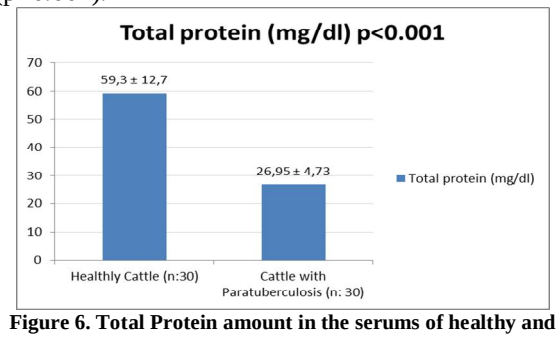


Figure 6. Total Protein amount in the serums of healthy and paratuberculosis animals

DISCUSSION

Paratuberculosis is a chronic infectious disease which shows itself with granulomatous inflammation in intestines and regional lymph nodes. The typical finding is chronic or episodic diarrhea which cannot treated. The disease causes live weight loss, decrease in milk yield, decrease in benefiting from the feed, decrease in fertility and increase in the frequency of mastitis in ruminants. The disease is usually results with death with excessive weight loss and general weakness. The symptoms of the disease can be seen in cattle, however in some cases, the cause of disease locates in mucosa and lymph nodes therefore makes these animals permanent carriers. Also, it threatens the public health with its role in Crohn's disease's etiology in humans (Engstrand, 1995). In the diagnosis of the disease, even though there is no certain diagnostic method, the use of assistive methods can help the veterinarian (Ganheim et al., 2007; Mecitoğlu and Demir, 2012).

SAA, Hp, CRP and some other acute-phase proteins were found beneficial for evaluating health in humans and some domestic animals. The importance of acute-phase proteins are the usage of them in veterinary clinical biochemistry as nonspecific variables for imaging the inflammation activity (Gruys et al., 2005).

An effective diagnosis is important for the success of the disease control program. Therefore, additional biochemical markers are necessary for the control of paratuberculosis in animals (El-Deeb et al., 2014). Knowing the changes in the amounts of serum acute-phase protein in paratuberculosis cattle, is expected to help the diagnostic tests which are performed for the paratuberculosis diagnosis in cattle.

Before, Pazarçeviren (2008) in his study, found that serum haptoglobin level is increased in the diarrheal calves (Pazarçeviren 2008). In our study too, serum haptoglobin level was found higher in infective cattle (0,820 ± 0,615 mg/mL) compared to healthy cattle (0,354 ± 0,177 mg/mL).

And serum SAA was found higher in paratuberculosis cattle (369,9 ± 17,0 ng/ml) compared to healthy cattle (253,1 ± 82,7 ng/mL). In the study, which is conducted by Hajimohammadi et al. (2013), an important increase is observed in diarrheal calves' serum Hp and SAA amounts compared to healthy calves. The results of these studies show similarity to the present study results.
C-reactive protein has important roles in defense against infection, clearance of the damaged tissues, prevention of autoimmunization and the regulation of inflammatory response. It was shown majorly in humans and dogs and less in horses, pigs, cats and ruminants. This reactant is not used widely in cattle like it is used in other species, because it is not quite clear that CRP is an acute-phase protein for cattle (Murata et al., 2004). There are less studies about CRP in domestic animals. Petersen et al. reported that in inflammatory situations CRP does not change in cattle (Petersen et al., 2004). CRP mostly related with lactation and it is reported that CRP level in milk in mastitis cows are increased significantly compared to healthy cows (Schrödl et al., 1995). While the CRP increase was not observed in cattle, in this study, serum CRP level was found significantly higher in paratuberculosis cattle (0,260 ± 0,121 µg/ml) compared to healthy cattle (0,1885 ± 0,0571 µg/ml).
The CRP results in the present study shows similarities to the change in CRP levels which is obtained from the similar studies in which the inflammatory diseases were studied.

In our study, ceruloplasmin level was found 18,08±5,43 mg/dl in healthy cattle. However, this level increased 33,7±12,3 mg/dl in paratuberculosis cattle ($p<0.001$). Similarly, in diarrheal calves, serum ceruloplasmin level was found higher compared to healthy calves (Pazarçeviren, 2008). In the other study conducted with goats, serum ceruloplasmin levels in mixed helminth infected goats did not show any significant change compared to control groups (Ulutaş et al., 2008).

In a study, which is conducted with camels, serum albumin level is significantly decreased in paratuberculosis camels compared to healthy camels (El-Deeb et al., 2014). Similarly, in our study too, albumin amount was found lower in infective group (2,977±0,699 mg/dl) compared to control group (3,713±0,407 mg/dl). This decrease could be caused by hypoproteinemia. The significant decrease in albumin may be caused by albumin leakage into the damaged tissue.

Total protein amount was found lower in infective group (26,95±4,73 mg/dl) compared to control group (59,3±12,7 mg/dl). Similarly, total protein levels were found lower too, in paratuberculosis camels compared to healthy camels (El-Deeb et al., 2014). The decreases in serum albumin and total protein levels in the present study show similarities to the results of the other studies.

In cattle, the most important acute-phase proteins are known as SAA, Hp and Cp. It is reported that haptoglobin, SAA and Cp serum levels increase in cattle differently when there is a surgical trauma, mastitis, inflammation due to bacteria and helminth, and determining the amounts of these proteins can be beneficial in diagnosis, prognosis, the control of treatment effectiveness and animal health. And CRP is considered as an acute-phase protein which is not much changeable. In paratuberculosis animals, along with the other acute-phase proteins, the increase in

CRP shows that it can be beneficial in the diagnosis and prognosis of paratuberculosis. It is thought that these values will be beneficial in the diagnosis and prognosis of this disease.

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