

Serum amyloid A, haptoglobin and ceruloplasmin levels before and after treatment in cattle with dermatophytosis

Research Article

ABSTRACT

The aim of this study is to determine the changes in serum amyloid A (SAA), haptoglobin (Hp) and ceruloplasmin (Cp) levels, before and after treatment in cattle with dermatophytosis. The animal material of the study consisted of a total of 30 cattle, 20 with dermatophytosis and 10 healthy, of different ages and genders. Blood was taken from the sick animals twice, before and after the treatment, and once from the healthy animals, and their serum was separated. The obtained sera were stored at -20 °C until the Hp, SAA and Cp levels were measured. Sick animals were vaccinated with Trichoben® (Interhas, TR) twice, with an interval of 14 days. When the pre-treatment and post-treatment serum Hp, SAA and Cp values of the sick animals were statistically compared, it was determined that the pre-treatment values were significantly higher ($P < 0.001$) than the post-treatment values. Similarly, when the values of pre-treatment and control animals were compared, it was seen that the difference was significant ($P < 0.001$). When the serum SAA, Hp and Cp values of the animals in the post-treatment and control groups were compared, it was determined that the difference was statistically insignificant ($P > 0.05$). As a result, it was determined that SAA, Hp and Cp values, which are positive acute phase proteins, increased significantly in cattle with dermatophytosis and these values decreased with treatment. According to this result, it was concluded that serum SAA, Hp and Cp values are important biomarkers in the evaluation of the prognosis of the disease in cattle with dermatophytosis.

Keywords: Dermatophytosis, bovine, serum amyloid A, haptoglobin, ceruloplasmin

Introduction

Dermatophytosis (ringworm, trichophytosis) is a group of superficial fungal infections of the skin, hair, feathers and nails rich in keratin, and is an important zoonotic infection limited to inanimate cornified tissues. The disease not only affects the skin but also causes stress in animals, weight loss, decrease in milk yield, growth retardation, and due to these effects, it is economically important in our country (Kırmızıgül et al., 2008, Aslan et al., 2010).

Dermatophytes first affect the inanimate layer of the skin called the stratum corneum and cause inflammatory reactions at the infection site by secreting keratinase enzyme. Infection and inflammatory reactions, which usually start in the head (60%) or neck (30%) region, are mostly in the form of increased local temperature, redness, swelling and first ring-shaped, asbestos-looking alopecia (Aslan et al., 2010, Cafarchia et al., 2010, Lakshmipathy and Kannabiran 2010, Yılmaz et al., 2010, Paksoy et al., 2013, Bhikane et al., 2015).

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Neslihan Şeliman^{1a}
Erdoğan Uzlu^{2b}
Ali Haydar Kırmızıgül^{1c}

¹Department of Internal Medicine, Faculty of Veterinary Medicine, University of Kafkas, Kars, Türkiye

²Department of Internal Medicine, Faculty of Veterinary Medicine, University of Balıkesir, Balıkesir, Türkiye

ORCID-

^a[0000-0003-0993-2093](https://orcid.org/0000-0003-0993-2093)

^b[0000-0002-3064-6633](https://orcid.org/0000-0002-3064-6633)

^c[0000-0002-9283-1391](https://orcid.org/0000-0002-9283-1391)

Correspondence

Erdoğan UZLU
uzlu@hotmail.com

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In cattle herds, dermatophytosis usually proceeds enzootically. The disease is caused by fungi of the *Trichophyton*, *Microsporum* and *Epidermophyton* genus, also called dermatophytes in cattle. *Trichophyton* species are mostly responsible for infections in animals, followed by *Epidermophyton* and *Microsporum species* (Kırmızıgül et al., 2008, Aslan et al., 2010, Lakshminpathy and Kannabiran 2010).

Acute phase proteins (APP) are blood proteins synthesized by hepatocytes in acute phase response (APR). The acute phase response, is a non-specific, complex reaction of the organism against to homeostasis that occurs as a result of inflammation, tissue damage, infection, neoplastic growth or immunological disorders (Merhan and Özcan 2010). Acute phase proteins are classified into two groups as positive and negative acute phase proteins. Positive APPs (haptoglobin, serum amyloid A, ceruloplasmin, alpha 1-acid glycoprotein, C-reactive protein, and fibrinogen) are substances in glycoprotein structure released by the stimulation of inflammatory cytokines from hepatocytes and are proteins with increased serum levels. Negative APPs (albumin, transferrin, retinol binding protein) are structural plasma proteins commonly found in blood (Merhan and Özcan 2010, Sevgisunar and Şahinduran, 2014). An increase in the synthesis of some plasma proteins (C-reactive protein/CRP, Ceruloplasmin/Cp, Haptoglobin /Hp) is observed during APR. By looking at the plasma or serum levels of these proteins, an important idea that has clinical value in terms of diagnosis and prognosis of the disease can be obtained (Ulutaş and ark. 2007).

Hp is the most important acute phase protein in which two α , two β -polypeptide chains are linked by disulfide bonds and increases approximately 8 hours after inflammatory stimuli. The primary function of Hp is to bind free hemoglobin released from erythrocytes. Hp-hemoglobin compound slows down the growth of bacteria. Hp is one of the important

parameters used to determine the severity of the inflammation. Although the functions of serum amyloid A (SAA) are still being investigated, it has been reported that it may have functions such as transport of cholesterol to hepatocytes, suppression of fever, inhibition of oxidative destruction of neutrophil granulocytes, stimulation of calcium mobilization by monocytes, endotoxin detoxification, inhibition of lymphocyte and endothelial cell proliferation. Cp is an important protein involved in the transport of copper in the blood and is one of the acute phase proteins used to understand the severity of inflammation in disease states. Cp prevents further damage to tissues by collecting oxygen radicals (Ulutaş et al., 2007, Karapehlivan et al., 2007; Sevgisunar and Şahinduran, 2014, Tothova et al., 2014).

In this study, it was aimed to determine the changes in some acute phase proteins in cattle with dermatophytosis and to investigate the changes that will occur in these proteins after vaccination against to dermatophytosis.

MATERIALS AND METHODS

All animal material of this study; A total of 30 cattle were brought to the animal hospital of the veterinary faculty from the livestock enterprises in Kars and its villages with the complaint of dermatophytosis or for the purpose of general health check-up. In the dermatophytosis (Group I) group, a total of 20 cattle, 7-24 months old, 7 *Brown Swiss* crosses and 13 *Simmental* crosses, 10 females and 10 males, were included. In the control group (Group II), 10 healthy cattle of the same age range, of different sexes were used. For general examination, blood was drawn from the animals in the first group twice, before and after the treatment. From the healthy animals in the second group, blood was drawn once during the general examination. The blood samples were centrifuged at 3000 rpm for 10 minutes and their serums were separated. These serum samples obtained were stored at -20°C until the SAA, Hp and Cp values were measured. The animals in the

first group were administered 10 ml of Trichoben[®] (Interhas, TR) vaccine for therapeutic purposes and the vaccine was repeated for the second time with an interval of 14 days. In addition, animals were checked in weekly periods following drug administration and their recovery processes were observed. Blood samples were taken again from Group I, 28 days after repeat vaccination.

The lesioned areas of cattle in Group I were cleaned with cotton soaked in 70% alcohol before scraping. Then, skin scrapings were taken from the lesioned areas by a sterile scalpel. The samples were first processed with 10% KOH and after the skin samples were prepared, they were examined under the microscope. The appearance of typical spores in the examination was evaluated as positive in terms of dermatophytosis. The scrapings taken from the border areas of the lesioned and healthy skin of the cattle were incubated for 2-6 weeks at 32°C in an aerobic humid environment by sowing with "Sabouraud Dextrose Agar (SDA)" horizontal stub method. The morphologies of the fungal colonies that grew during the incubation period were examined with the naked eye and stereomicroscope.

According to the "k" clause of the 8th paragraph of the 8th article of the relevant regulation, the all-examination materials evaluated in this study were obtained from the samples taken from the animals during the routine examination, with the permission of the animal owners.

Acute Phase Protein Analysis

SAA was determined with an ELISA-based assay, Phase SAA Assay Kit (Tridelta, Development Ltd, Ireland), and Hp was determined Spectrophotometrically Phase Haptoglobulin Assay Kit (Tridelta, Development Ltd, Ireland), Cp concentration was determined by Richterich and Colombo pH

5.6 and at 546 nm. It was measured spectrophotometrically with the "p-phenylenediamine oxidase activity method" defined as.

Statistical Analysis

Statistical analyzes were done with SPSS-20.0 windows program. The analysis of variance method was used in repeated measurements to determine the changes in the parameters determined before and after the treatment in animals in the control and Group I. $P < 0.05$ and less were considered significant in statistical evaluations.

RESULTS

It was observed that dermatophytosis lesions were located on the head in 11, neck in 5, inguinal region in 2 and dorsal region in 2 of the cattle included in the study. The lesions were "asbestos-looking, scaly, dry and ring-shaped" which classic symptoms for the disease are. As a result of microscopic examinations and cultures made from scrapings taken from the lesioned areas, it was determined that all of the factors were *Trichopyton verrucosum*.

As a result of the study, changes in serum SAA, Hp and Cp values of animals in group I and group II are given in *Table 1*. When the SAA, Hp and Cp values of the animals in Group I were compared (before and after the treatment), the statistical difference between these values was found to be significant ($P < 0.001$). In group I, all three parameters were found to be decrease compared to pre-treatment (*Figure 1,2,3*). When the SAA, Hp and Cp values obtained from the cattle in the first group before the treatment were compared with the healthy cattle in the group II, the difference was determined to be statistically significant ($P < 0.001$). When the after-treatment values obtained from the cattle in the first group were compared with the values of the healthy animals in group II, the difference between the

results was found to be statistically insignificant ($P>0.05$).

Table 1 SAA, Hp and Cp values of animals in the group I and II.

Parameters	Group I (n=20)		Group II (n=10) ($\bar{x}\pm Sx$) (min-max)	P
	Before Treatment ($\bar{x}\pm Sx$) (min-max)	After Treatment ($\bar{x}\pm Sx$) (min-max)		
SAA ($\mu\text{g/dL}$)	96.44 \pm 174.38 ^A	8.02 \pm 20.78 ^B	8.02 \pm 19.27 ^B	0.001
Hp ($\mu\text{g/dL}$)	0.21 \pm 0.53 ^A	0.06 \pm 0.12 ^B	0.07 \pm 0.10 ^B	0.001
Cp	18.13 \pm 34.28 ^A	8.96 \pm 16.87 ^B	8.47 \pm 14.53 ^B	0.001

^{A, B} Groups with different letters on the same line are statistically significant.

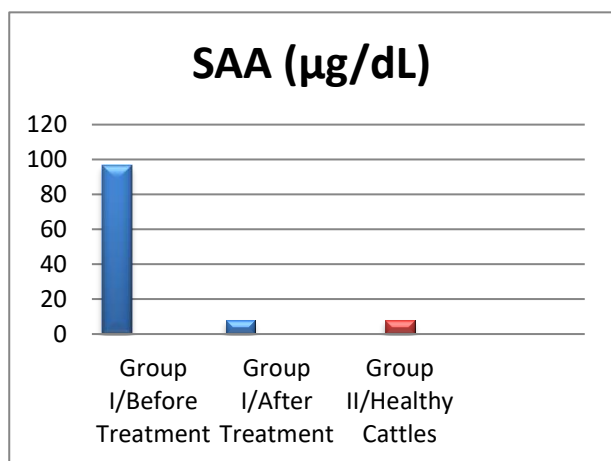


Figure 1. The change in SAA values of Group I cattle “before and after treatment” and Group II healthy cattles.

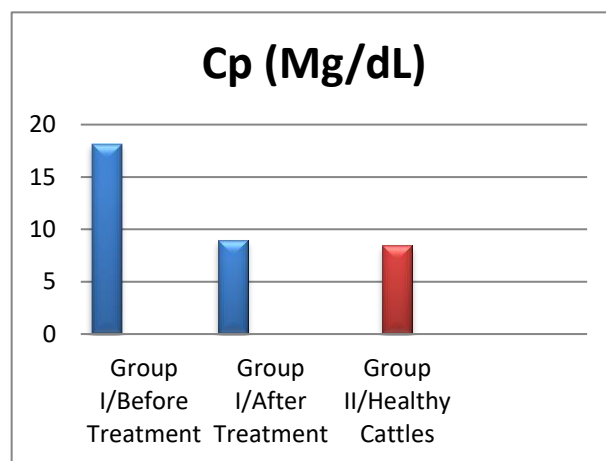


Figure 3. The change in Cp values of Group I cattle “before and after treatment” and Group II healthy cattles.

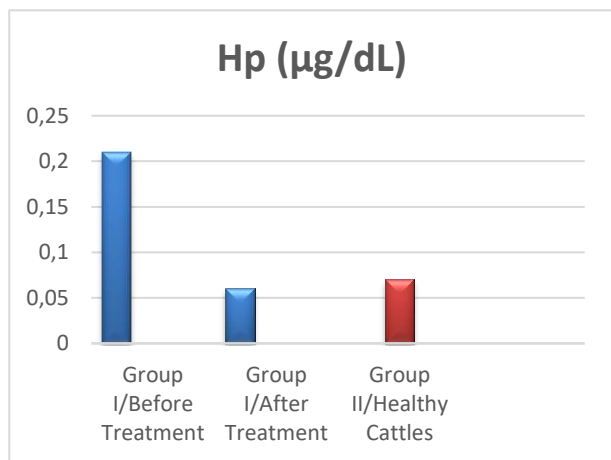


Figure 2. The change in Hp values of Group I cattle “before and after treatment” and Group II healthy cattles.

DISCUSSION AND CONCLUSION

Dermatophytosis is characterized by keratinized thickening of the epithelial layer and shedding of infected hairs in cattle. It is known that dermatophytosis, which is common in the world, causes diseases of varying severity in humans and animals and it is stated to be of zoonotic importance. The disease causes loss of live weight, deterioration of skin quality, growth retardation in animals and it causes serious economic losses as the export of sick animals is prohibited. Dermatophytosis is clinically characterized by raised, ring-shaped alopecia with asbestos-like surface (Aslan et al., 2010, Cafarchia et al., 2010, Lakshmipathy and Kannabiran 2010, Yilmazer et al., 2010, Paksoy et al., 2013, Bhikane et al., 2015). The lesions

determined clinically in this study were similar to those reported by the researchers; it was characterized by ring-shaped, asbestos-like and alopecia.

Significant changes in APP concentrations have been reported by investigators in most inflammatory conditions and diseases diagnosed in animals. However, there are limited studies investigating APPs in dermatological diseases of ruminants (Çitil, 2003, Ulutaş et al., 2007, Kabu and Sayın, 2016).

Serum amyloid A, also called apolipoprotein, although its functions are not fully revealed, in inflammatory events; it is an important AFP with 7 known isoforms in cattle, whose functions such as suppression of fever, inhibition of oxidative destruction of neutrophil granulocytes, inhibition of lymphocyte and endothelial cell proliferation have been demonstrated by researchers (Sevgisunar and Şahinduran 2014, Tothova et al., 2014). Studies examining SAA in dermatological cases in ruminants are very rare. In a study conducted in Anatolian buffaloes, it was reported that SAA increased but it was also determined that the SAA value was not adequately examined in dermatophytosis cases in cattle (Kabu and Sayın, 2016). In the aforementioned study conducted by the researchers, it was reported that the SAA value obtained from buffaloes with dermatophytosis was found to be quite high compared to the control group consisting of healthy buffaloes, while it was determined that the SAA value, we obtained in our study showed statistically similar results with this study ($p < 0.001$). These results are explained by many researchers as increasing the SAA value by being affected by inflammatory phenomena or infections (El-Bahr and El-Deeb, 2013, Kabu and Sayın, 2016). In our study, it was thought that the high SAA value we obtained from cattle with dermatophytosis was due to inflammation caused by

dermatophytosis, in line with what the researchers reported.

Haptoglobin is one of the most important acute phase proteins and shows a significant increase approximately 8 hours after inflammatory stimuli (Sevgisunar and Şahinduran, 2014; Karaca and Akgül 2016). The primary function of Hp is to bind the free hemoglobin released from erythrocytes and slow down the growth of potential pathogens through this compound. Because of this and many reasons such as its anti-inflammatory properties, Hp is one of the important parameters used to determine the severity and health status of the inflammations (Sevgisunar and Şahinduran 2014, Tothova et al., 2014). There are still not enough studies investigating serum Hp values in cattle in different cases and physiological conditions (Chan et al., 2004, Debski et al., 2016). Studies investigating Hp values in skin problems in different animal species or specifically dermatophytosis cases in ruminants are very limited. Researchers reported that this difference in Hp value, which was determined to be significantly increased in buffaloes with dermatophytosis compared to healthy animals, was also statistically significant ($p < 0.001$). In our study, it was determined that the high Hp value we obtained from cattle with dermatophytosis was similar to the Hp values obtained previously from buffaloes with dermatophytosis and dogs with skin problems. Consistent with other researchers who reported that Hp is a very important AFP in understanding the severity of fire, it was concluded that the high Hp value we obtained in group I at the beginning of our study was also caused by severe dermatophytosis (Ulutaş et al., 2007, Kabu and Sayın, 2016). It was determined that the difference between the Hp values we obtained in our study was statistically significant ($P < 0.001$).

Ceruloplasmin is a very important AFP that is primarily synthesized in the liver but can also be

produced in extrahepatic areas, is involved in the transport of copper in the blood and can be used for diagnosis in most disease states. Cp is also an AFP that increases to prevent damage by collecting oxygen radicals in tissues damaged by inflammatory events (Ulutaş et al., 2007, Sevgisunar and Şahinduran, 2014, Tothova et al., 2014). In a limited number of studies examining the Cp value in dogs with skin problems, it was reported by researchers that Cp was determined to be higher than in healthy dogs (Charlton et al., 2002, Ulutaş et al., 2007). Arslan et al. (2008), in a study they conducted in cattle exposed to environmental stress, found that the Cp value decreased after in the treatment compared to before the treatment and last Cp levels that did not show any statistical difference with the control group consisting of healthy animals. In our study, it was determined that the after-treatment Cp values obtained in cattle diagnosed with dermatophytosis decreased which was statistically significant ($P < 0.001$). It was determined that the Cp values obtained from Group I after the treatment were very close to the values of the control group and there was no statistical difference between these two values, similar to what the researchers reported (Ulutaş et al., 2007, Arslan et al., 2008). These increases in Cp values obtained from cattle with dermatophytosis were consistent with those reported by the limited number of researchers who evaluated AFPs in animal inflammatory diseases. It was concluded that this increase may be due to intense inflammation caused by dermatophytosis and damage to skin cells and the resulting increased Cp amount.

As a result, some AFPs, which were found to be increased in cattle with dermatophytosis in this study, decreased with the initiation of the successful treatment process following Trichoben® (Interhas, TR) application. This result we obtained was thought to be related to the reduction of stress, cellular healing of the skin, clinical improvement and disappearance of inflammatory symptoms. By this study, the

SAA, Hp and Cp values obtained from cattle with dermatophytosis before and on the 14th day after vaccination have not been evaluated and reported by other researchers before. In addition, the present study clearly reveals that SAA, Hp and Cp values are significantly affected in dermatophytosis cases in cattle. It is also concluded that the results obtained from the present study will contribute to future research in inflammatory processes or diseases in which AFP values in cattle or other animal species will be examined, through hypotheses and results.

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Ethical declaration: In accordance with the "Clinical applications for diagnostic and therapeutic purposes" mentioned in Article 1 of the "Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees" published in the Official Gazette No. 28914 in 2014, all examination materials evaluated in this study were obtained from the samples taken for diagnostic purposes during routine health examinations from cattle brought to KAU Veterinary Faculty Animal Hospital, Internal Diseases Polyclinic by animal owners at different times, again with the permission of animal owners. During the study period, the animals did not receive any additional medical treatment other than standard medical practices.
Conflict of interest: There is no conflict of interest between the authors.

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