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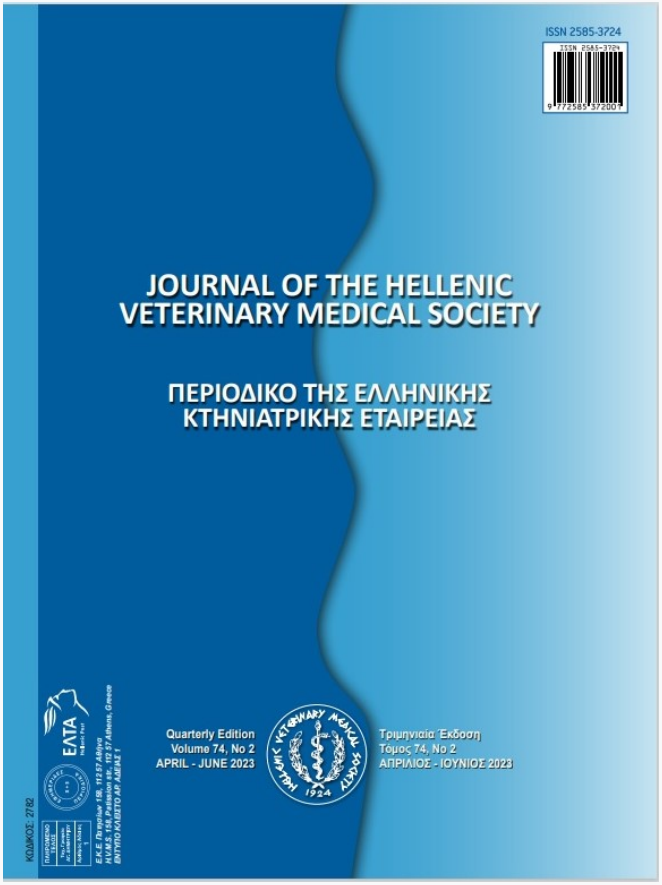

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Research article
Ερευνητικό άρθρο

Effects of caseous lymphadenitis agent (*corynebacterium pseudotuberculosis*) isolated from superficial abscesses of sheep on oxidative stress factors

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ABSTRACT: In this study, it was aimed to determine the incidence of *Corynebacterium pseudotuberculosis (C. pseudotuberculosis)* in caseous lymphadenitis cases in superficial lymph nodes of sheep and to evaluate its effect on oxidative stress factors. Thus, it was aimed to determine the diagnosability of superficial caseous lymphadenitis cases and to prevent the spread of diseases and related economic losses. A total of 103 sheep, 50 of which were healthy and 53 of which had caseous lymphadenitis, were evaluated in the study. Microbiological examinations were performed by taking 3-5 ml of pyogenic aspirate from the superficial lymph nodes of sheep with caseous lymphadenitis. Blood samples were taken from sheep with *C. pseudotuberculosis* isolated in microbiological examinations and the levels of oxidative stress factors were determined. *C. pseudotuberculosis* was isolated in 23 of the pyogenic aspirates of 53 sheep with caseous lymphadenitis. When sheep with C. pseudotuberculosis were compared with healthy sheep, it was determined that some antioxidant molecule levels (such as GSH-Px (14.62%, $p<0.001$), GSH (23.81%, $p<0.001$), SOD (4.70%, $p<0.001$), CAT (22.23%, $p<0.001$). The level of toxic malondialdehyde (MDA) (18.62%, $p<0.001$). As a result, it was determined that oxidative stress factors in sheep with superficial caseous lymphadenitis (caused by *C.pseudotuberculosis*) showed statistically differed significantly compared to sheep in the control group. Therefore, even if there is no specific marker for the diagnosis of superficial and visceral forms of caseous lymphadenitis, the levels of oxidative stress factors in suspected flocks may suggest disease risk. In addition, this study is very important in order to form a basis for future studies on this subject. Although it has been determined that these biochemical markers are not pathognomonic for caseous lymphadenitis, it would be more accurate to associate them with not only oxidative stress factors but also many other factors in future studies.

***Keywords*:** Oxidative stress; sheep; *C.pseudotuberculosis*; abscess

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INTRODUCTION

Caseous lymphadenitis is an infectious, zoonotic and chronic disease caused by *C. pseudotuberculosis* in sheep and goats (Baird and Fontaine, 2007; Windsor, 2011; Bastos et al., 2012; Ipek et al., 2012; Basbug et al., 2014; Tolu and Savas, 2014; Seyranoglu and Aslan, 2015; Ilhan, 2020). In addition to sheep and goats, the disease has also been reported to occur in cattle, horses, alpacas, buffalo, deer and camels (Dorella et al., 2006; Fontaine and Baird, 2008; Ipek et al., 2012; Ilhan, 2020).

Caseous lymphadenitis causes thick encapsulated abscesses in the lymph nodes and internal organs (especially in the lungs and mediastinal lymph nodes), containing creamy, yellow-green colored pus (Tolu and Savas, 2014; Akgül et al., 2018). The form of the disease that causes abscesses in the superficial lymph nodes and subcutaneous tissues is called external or superficial caseous lymphadenitis, and the form that causes abscesses in the internal organs is called internal or visceral caseous lymphadenitis (Ilhan, 2008; Ipek et al., 2012; Kumar et al., 2012; Basbug et al., 2014; Tolu and Savas, 2014; Oreiby, 2015; Akgül et al., 2018; Parin et al., 2018; Ilhan, 2020). Visceral caseous lymphadenitis is more likely to be seen in sheep than in goats. In cases of superficial caseous lymphadenitis, mostly skin and fleece deformities (decrease in fleece quality and yield) are formed, while progressive weight loss, respiratory disorders and recurrent rumen tympanis occur in cases of visceral caseous lymphadenitis (Al-Gaabary et al., 2002; Tolu and Savas, 2014; Oreiby, 2015). Caseous lymphadenitis cases may cause abortions in pregnant sheep. It rarely causes death, especially in young animals (Oreiby, 2015; Parin et al., 2018; Ilhan, 2020). Superficial caseous lymphadenitis is transmitted to healthy animals as a result of physical contact with agents scattered around as a result of rupture of abscess foci (Fontaine and Baird, 2008). Visceral caseous lymphadenitis is mostly transmitted to healthy animals by respiratory air because it causes lesions in the lung and mediastinal lymph nodes (Williamson, 2001; Fontaine and Baird, 2008).

C. pseudotuberculosis is a gram-positive bacterium in the form of coccoid or rod. It is a facultative intracellular bacterium without spores and capsules. *C. pseudotuberculosis* has two known virulence factors. One of them is phospholipase D and the other is mycolytic acid, which is found in excess in the cell wall (Songer, 1997; Baird and Fontaine, 2007; Slotte and Ramstedt, 2007; Sá Guimarães et al., 2011; Ipek et al., 2012; Oreiby, 2015; Akgül et al., 2018; Ilhan, 2020). Phospholipase D, which has the ability to break down ester bonds, is an exotoxin that damages the cell wall by breaking down sphingomyelin, one of the cell wall components. Mycolytic acid, on the other hand, coats the surface of the bacteria and protects it from macrophages and white blood cells. In addition, the cytotoxic effect of mycolytic acid can cause the death of macrophages (Songer, 1997; Baird and Fontaine, 2007; Slotte and Ramstedt, 2007; Windsor, 2011).

Oxidative stress is a situation in which the balance between free oxygen radicals and antioxidants in the body changes in favor of free oxygen radicals. With the increase in the concentrations of free oxygen radicals, lipids in the cellular membranes are oxidized, resulting in the formation of toxic products such as malondialdehyde (MDA). As a result, damage to cells can cause pathological disorders in the body. Inflammatory reactions or imbalances in body hemostasis can prevent the normal functioning of cells, limit antioxidant production and cause oxidative stress. This can increase plasma lipid peroxidation levels and increase oxidative stress in many tissues and organs such as erythrocyte, liver, kidney, etc. by changing antioxidant enzyme activities. (Seyranoglu and Aslan, 2015; Polat et al., 2021).

Early diagnosis of caseous lymphadenitis, which is common in many parts of the world and causes great economic losses, is very important in the herd. In this study, the effect of superficial caseous lymphadenitis agents isolated from sheep on oxidative stress was investigated. For this reason, it was thought that, by determining the levels of oxidative stress factors in suspected flocks, it could be a marker for the disease, although it is not a specific marker for the diagnosis of the superficial and visceral forms of caseous lymphadenitis, and could form a basis for future studies. In addition it is the first study to investigate the effect of abscesses formed in superficial lymph nodes due to *C. pseudotuberculosis* on oxidative stress, which reveals the importance of this study.

MATERIAL AND METHODS

Statement of ethics and formation of groups

The study was started after the approval of Inönü University Animal Experiments Local Ethics Committee with the document dated 02.12.2021 and numbered 2021/24-5. A total of 103 sheep, 50 healthy and 53 superficial caseous lymphadenitis were used

for this study. The healthy animals (control group) in the study were selected from the farms in Malatya region without caseous lymphadenitis disease. In addition, the sheep were included in the study after detailed clinical examinations of the animals in the herds where the disease was not present. The sick animals (Infected group) were selected from the animals with abscesses in the superficial lymph nodes in the farms suspected of the disease in the Malatya region. Healthy and infected animals were selected after performing routine examinations such as clinical and hematological examinations. Sheep with abscesses in the lymph nodes in the clinical examination constituted the infected group. Sheep with normal examinations constituted the control group. While healthy sheep (n=50) constituted the control group, sheep with caseous lymphadenitis (n=53) constituted the study group. The study continued over a period of 5 months.

Collection of samples

Abscess was suspected in animals with swelling on the superficial lymph nodes during clinical and/or ultrasonographic examination. It was decided to collect samples for microbiological analysis from animals whose abscess content (pus) was detected in the samples taken. The superficial lymph nodes that became abscessed due to caseous lymphadenitis (Figure 1a, 1b) were shaved and the area was disinfected with alcohol cotton. For microbiological analysis, 3-5 ml of pus was taken into sterile syringes by puncturing the abscess foci. The samples were brought to the microbiology laboratory.

To determine the levels of oxidative stress factors, 5-7 ml of blood was drawn into tubes containing 10% EDTA using the jugular veins of both healthy and sick sheep. The samples were brought to the biochemistry laboratory. ~~under the cold chain.~~

All the samples taken were delivered to the laboratories in the cold chain and by paying attention to all other storage and transportation rules in order for the study to continue properly and correctly.

Microbiological analysis

The contents taken from the superficial abscesses of sheep with suspected pseudotuberculosis were brought to the microbiology laboratory under aseptic conditions in the cold chain. For the isolation of bacterial agents from the pus content, after inoculation on 5% sheep blood agar *(Oxoid, Thermofisher Scientific, UK),* MacConkey agar *(Oxoid, Thermofisher Scientific, UK)* and selective media, they were left in aerobic and microaerophilic incubation at 37°C for 5-7 days. Gram staining was performed on the colonies that grew following incubation. Isolates obtained from abscess contents were tried to be determined by biochemical tests. *C. pseudotuberculosis* isolates detected by culture method were also identified by PCR (Polymerase Chain Reaction) due to the diversity in their biochemical properties. Genomic DNA extraction was performed by phenol/chloroform method. For

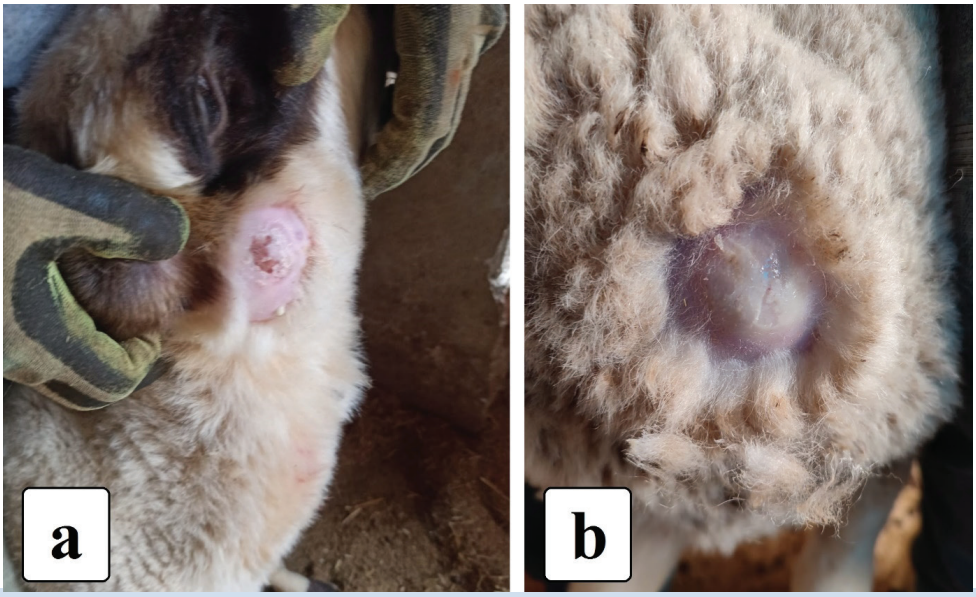

Figure 1. Abscess foci due to caseous lymphadenitis in superficial lymph nodes of sheep: retropharyngeal lymph node (a), prescapular lymph node (b).

this purpose, 300 µl of each culture produced in liquid broth was taken and transferred into 1.5 ml eppendorf tubes, then 300 µl of TNES buffer (20 mM Tris- HCI pH: 8.0, 150 mM NaCl, 10 mM EDTA, % 0.2 SDS) and 5 µl of Proteinase K were added to each tube in equal volume. The suspension was vortexed and incubated in a water bath for 2 hours at 56 ºC, then boiling was performed for 10 minutes for Proteinase-K inactivation. After boiling, 600 µl equal volume of phenol:chloroform:isoamylalcohol (25:24:1) was added, and carefully shaken by hand for 5 minutes, and then centrifuged at 13000 rpm for 10 minutes. After centrifugation, two layers formed in the eppendorf tube. The upper phase was transferred to another tube with the same numbers, without touching the lower phase (The lower phase is the dirty phase due to precipitation of protein residues). Pure ethyl alcohol 2.5 times the amount taken and 3 M Sodium acetate 1/10 were added, vortexed and kept at -20 ºC overnight. The suspension was centrifuged at 13000 rpm for 10 minutes and the supernatant removed, the resulting pellet was washed with 90% and 70% ethanol, respectively, and centrifuged at 13000 rpm for 5 minutes after each step. After centrifugation, the alcohol was removed and the pellet was left to dry. The dried pellet was diluted with 100 µl of sterile distilled water and kept at -20ºC to be used as target DNA in PCR. The PCR was performed in a Techne TC-512 gradient thermal cycler (Techne, Chelmsford, Essex, United Kingdom) in a total reaction volume of 50 µL containing 5 µL of 10X PCR buffer (750 mM Tris-HCl [pH 8.8], 200 mM $(NH_4)_2SO_4$, and 0.1% Tween-20), 5 µl 25 mM $MgCl_2$, 250 µM of each deoxynucleotide triphosphate, 1.25 U of Taq DNA polymerase (MBI Fermentas, Hanover, MD), 20 pmol of each of primer pairs, (F: ACCGCACTTTAGTGTGTGTG, R: TCTCTCACGCCGATCTTGTAT) and 5 µL of template DNA. PCR amplification was performed as described by Çetinkaya et al. 2002. PCR amplicons were detected by electrophoresis in 2% (w/v) agarose gel and stained with ethidium bromide and then visualized using an ultraviolet transilluminator. PCR products with a molecular size of 815 bp were considered as *C. pseudotuberculosis* (Cetinkaya et al., 2002).

Determination of oxidative stress factors and antioxidant levels

Blood samples were taken into EDTA tubes from the vena jugularis of sheep by means of an appropriate cannula, paying attention to the sterilization rules for biochemical analysis. To determine oxidative stress factors, blood samples in EDTA tubes were centrifuged at 3000 rpm for 15 minutes and plasma was obtained. Plasma was used to measure MDA level as a marker of lipid peroxidation. Whole blood was used for GSH and GSH-Px determination. Plasma separated EDTA blood samples were washed 3 times with saline (0.9% NaCl). After that, CAT and SOD activities and hemoglobin (Hb) levels were determined in erythrocytes.

The MDA level was measured according to the method of Placer et al. (1966) This method was based on the reaction of thiobarbituric acid (TBA) and MDA, one of the aldehyde products of lipid peroxidation. The MDA level was expressed as nmol/ml. GSH level was determined by a kinetic assay using a dithionitrobenzoic acid (DTNB) recycling method of Ellman et al. (1961) GSH levels were expressed as µmol/ml. CAT activity was carried out by using the Aebi's method (1984). The principle of the assay is based on the determination of the rate constant, k (dimension: k) of H_2O_2 decomposition. The reaction contained 50 mM potassium phosphate buffer and 10 mM H_2O_2 (as substrate) reaction was started by the addition of the sample. CAT activities were expressed as kat/g Hb. The GSH-Px activity was measured by the Beutler method (1984). GSH-Px catalyzes the oxidation of GSH to oxide glutathione (GSSG), using H_2O_2. The rate of formation of GSSG was measured by the glutathione reductase reaction. The SOD activity was tested by quantifying superoxide anion ($O2^{\bullet-}$) generated by xanthine and xanthine oxidases reacting with nitroblue tetrazolium (Sun et al., 1988). In the determination of hemoglobin level, Frankel et al. (1970) 's method was used. According to this, ferricyanide oxidizes Fe^{2+} in hemoglobin and converts it from +2 to +3 valuable iron and it converts into methemoglobin. Thereafter, potassium cyanide and cyanomethemoglobin, a stable pigment, are formed. The absorbance of cyanomethemoglobin is read at 546 nm.

Statistical analysis

The results were expressed as mean ± standard error (S.E.). Shapiro-Wilk normality test was used to determine whether the raw values of all the measured parameters showed normal distribution. As a result of this test, it was found that the values in all parameters showed normal distribution. Independent samples test (t-test) was used to test whether significant differences existed among the control and infected groups for MDA, GSH levels, CAT, GSH-Px, SOD activities. Statistical significance was accepted at a p-value <

0.05. Statistical Package for Social Sciences (SPSS)/ PC software program (Version 22.0; SPSS, Chicago, Illinois, USA) was used to perform the statistical analysis of the data.

RESULTS AND DISCUSSION

In the study, a single superficial lymph node abscess was detected in all animals from which abscess samples were taken. *C.pseudotuberculosis* was isolated in 23 of the 53 abscess contents examined microscopically (Figure 2a, 2b). While *C.pseudotuberculosis* was isolated alone in 16 of them, it was isolated together with other bacterial agents in 7 of them. Other bacterial agents isolated, respectively, are *S.aureus* (12/53), *P.auroginosa* (6/53), *Trueperella pyogenes* (4/53), *E.coli* (6/53) *Streptococcus spp.* (4/53) *Staphylococcus spp.* (6/53) types. It was determined that no bacteria grew in 5 of the samples. The agents isolated from the contents of superficial abscesses is presented in Table 1. The frequency of lymph nodes in which abscesses were detected due to caseous lymphadenitis are presented in table 2.

Figure 3 presents the levels of MDA, GSH; and the activities of antioxidant enzymes tested in our study, which are CAT, GSH-Px, and SOD. The levels is presented by the in healthy (control) and infected with *C. pseudotuberculosis* (23 infected animals). While MDA ($p < 0.001$) levels were determined to be higher in the control group compared to the infected group; GSH ($p < 0.001$) levels and CAT ($p < 0.001$), GSH-Px ($p < 0.001$) and SOD ($p < 0.001$) activities were determined to be low. The difference in MDA, GSH levels and CAT, GSH-Px, SOD activities between the two groups were 18.62%, 23.81%, 22.23%, 14.62% and 4.70%, respectively.

C. pseudotuberculosis is one of the most important bacterial agents that can cause abscesses in the superficial lymph nodes of sheep and goats. Al-Gaabary et al. (2009), reported that they isolated *C. pseudotuberculosis* from 90.07% of sheep and goats whose superficial lymph nodes were infected. Izgur et al. (1999) reported that they isolated *C. pseudotuberculosis* in 19 (46.3%) of 41 sheep they had clinically diagnosed with caseous lymphadenitis. Robaj et al. (2017) reported that they isolated *C. pseudotuberculosis* in 32 (84.2%) of 38 sheep from which they collected pyogenic aspirate from superficial lymph nodes. In this study, *C. pseudotuberculosis* was isolated from 23 (43.40%) of 53 sheep diagnosed with superficial caseous lymphadenitis. While *C. pseudotuberculosis* was isolated alone in 16 of 23 sheep, it was isolated together with other bacteria in 7 of them.

Except for *C. pseudotuberculosis*, many bacterial agents can be isolated from the aspirates obtained from abscesses formed in superficial lymph nodes. In the study of Izgür et al. (1999), it was reported that *Micrococcus spp* (19.5%), *Staphylococcus aureus* (7.3%), *Streptococcus epidermis* (4.8%) and *Pseudomonas aeruginosa* (7.3%) were isolated from sheep diagnosed with caseous lymphadenitis. Robaj et al. (2017) reported that they isolated *Staphylococcus aureus* (10.5%) and *Streptococcus pyogenes* (5.3%), except *C. pseudotuberculosis*, from pyogenic aspirates obtained from enlarged lymph nodes of sheep in Kosovo. In this study, *Staphylococcus aureus*

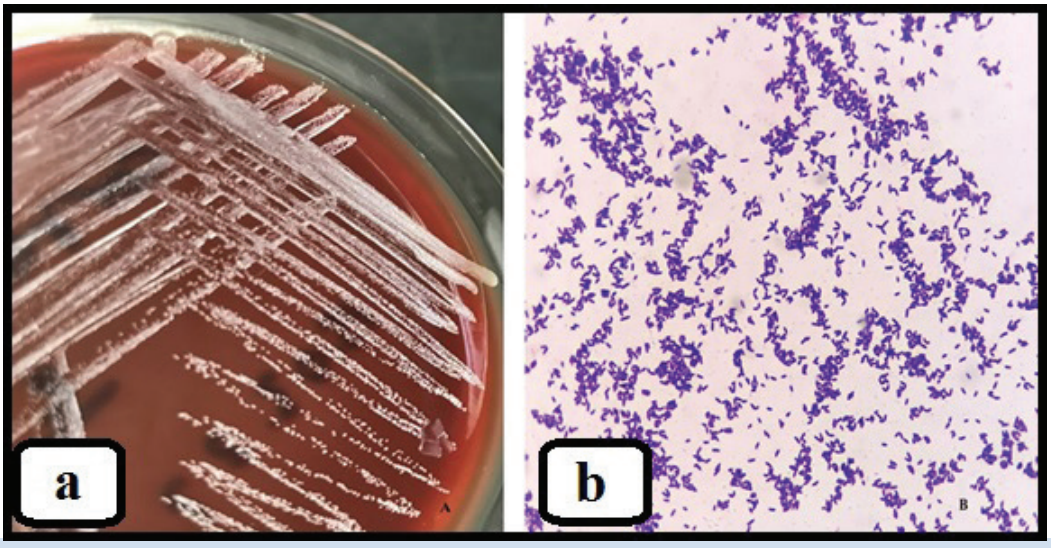

Figure 2. Colony morfology of *C.pseudotuberculosis* in Blood Agar (a), Microscopic view of *C.pseudotuberculosis* (b).

Table 1. Bacterial agents from superficial abscess contents

Sample Number	Isolated bacterial agent
1	*Pseudomonas auroginosa*
2	*Bacteria did not grow*
3	*Bacteria did not grow*
4	*Bacteria did not grow*
5	*Bacteria did not grow*
6	*Staphylococcus aureus + Pseudomonas auroginosa*
7	*Corynebacterium pseudotuberculosis*
8	*Corynebacterium pseudotuberculosis*
9	*Corynebacterium pseudotuberculosis + Staphylococcus aureus*
10	*Corynebacterium pseudotuberculosis*
11	*Corynebacterium pseudotuberculosis*
12	*Corynebacterium pseudotuberculosis*
13	*Staphylococcus aureus*
14	*Bacteria did not grow*
15	*Corynebacterium pseudotuberculosis*
16	*Staphylococcus aureus*
17	*Corynebacterium pseudotuberculosis*
18	*Corynebacterium pseudotuberculosis*
19	*Corynebacterium pseudotuberculosis + Staphylococcus aureus*
20	*Corynebacterium pseudotuberculosis*
21	*Pseudomonas auroginosa*
22	*Corynebacterium pseudotuberculosis*
23	*Corynebacterium pseudotuberculosis*
24	*Staphylococcus aureus + E.coli*
25	*Corynebacterium pseudotuberculosis + Staphylococcus aureus*
26	*Streptococcus spp.*
27	*Streptococcus spp.*
28	*Trueperella pyogenes*
29	*Staphylococcus aureus*
30	*Corynebacterium pseudotuberculosis*
31	*Staphylococcus aureus + Pseudomonas auroginosa*
32	*Staphylococcus spp*
33	*Corynebacterium pseudotuberculosis*
34	*Corynebacterium pseudotuberculosis +streptococcus spp.*
35	*E.coli*
36	*Staphylococcus spp*
37	*Corynebacterium pseudotuberculosis*
38	*Pseudomonas auroginosa*
39	*E.coli +Staphylococcus spp.*
40	*Staphylococcus aureus*
41	*Trueperella pyogenes*
42	*Corynebacterium pseudotuberculosis +streptococcus spp.*
43	*Trueperella pyogenes*
44	*Corynebacterium pseudotuberculosis*
45	*Corynebacterium pseudotuberculosis + Staphylococcus aureus*
46	*E.coli +Staphylococcus spp.*
47	*Corynebacterium pseudotuberculosis + Staphylococcus aureus*
48	*Staphylococcus aureus*
49	*Trueperella pyogenes*
50	*E.coli +Staphylococcus spp.*
51	*Corynebacterium pseudotuberculosis*
52	*Staphylococcus spp + E.coli*
53	*Psedomonas Auroginosa*

Table 2. The frequency of lymph nodes in which abscesses were detected due to caseous lymphadenitis

Lymph nodes	n (frequency)	%
Parotid	9	16.98
Retropharyngeal	23	43.39
Submandibular	14	26.42
Prescapular	7	13.21
Total		100

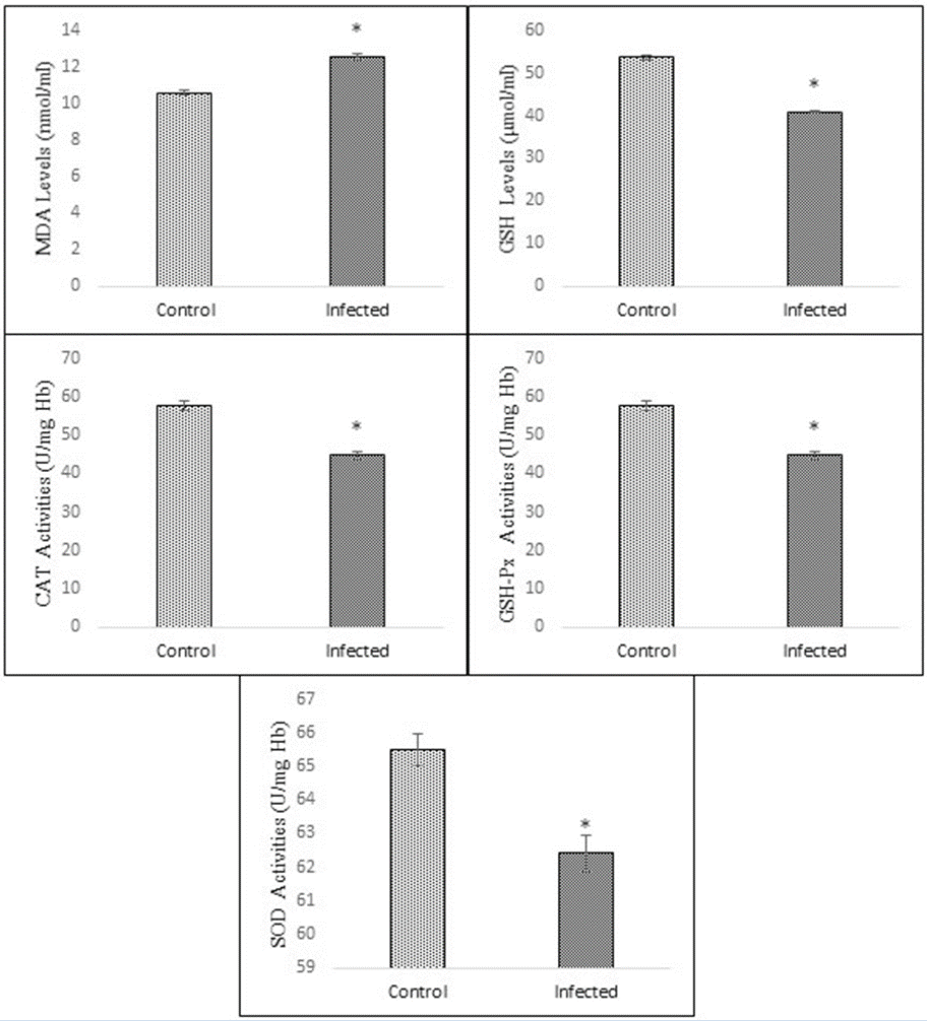

Figure 3. The relationship between oxidative stress factors in healthy sheep and sheep isolated from *C. pseudotuberculosis* (23 infected animals). Data are expressed as mean ± SEM. Symbols (*) show significant differences between the groups ($p < 0.001$).

(%22.64), *Pseudomonas auroginosa* (11.32%), *Trueperella pyogenes* (7.55%), *E.coli* (11.32%), *Streptococcus spp.* (7.55%) and *Stapylococcus spp* (11.32%) were isolated from sheep diagnosed with superficial caseous lymphadenitis.

In the external form of caseous lymphadenitis cases, abscesses can occur in many different lymph nodes. In many different studies, it was determined that the lymph nodes that were most affected by superficial caseous lymphadenitis cases showed variability. Zaitoun and Bayoumi (1994) reported that prescapular and prefemoral lymph nodes were more affected. Sheikh-Omar and Shah (1984) reported that retropharyngeal and mediastinal lymph nodes were more affected, while Mubarak et al. (1999) reported that parotid lymph nodes were more affected. On the other hand, Ilhan (2008) reported that mostly mandibular lymph node was affected in sheep they diagnosed with superficial caseous lymphadenitis. Al-Gaabary et al. (2009), reported that parotid lymph node in sheep and cervical lymph node in goats were more affected than caseous lymphadenitis cases. In this study, it was

determined that mostly parotid and cervical lymph nodes were affected in sheep diagnosed with caseous lymphadenitis clinically.

Oxidative stress is a situation in which the antioxidant capacity is insufficient due to the increase in the amount of free oxygen radicals in the body. In some inflammatory conditions and diseases, there is an increase in the production rate of free oxygen radicals (Seyranoglu and Aslan, 2015; Ertas and Kirmizigul, 2021; Polat et al., 2021). Free oxygen radicals cause lipid peroxidation in cell membranes and form toxic substances that are responsible for the pathogenesis of many tissue-damaged diseases (Seyranoglu and Aslan, 2015; Ertas and Kirmizigul, 2021; Polat et al., 2021). MDA is the most important lipid peroxidation product used in the evaluation of oxidative stress (Ertas and Kirmizigul, 2021). It has been reported that MDA levels increase in some trace element deficiencies, nutritional disorders, tumoral cases, and some parasitic and viral diseases (Ertas and Kirmizigul, 2021). Esmaeilnejad et al. (2014) reported that MDA levels increased significantly in their study on sheep with babesiosis. Again, Ertas and Kirmizigul (2021) reported that MDA levels increased in sheep with fasciolosis in their study. In this study, it was determined that the MDA levels of sheep with abscess formation in the superficial lymph nodes due to *C. pseudotuberculosis* were significantly increased (18.62%) compared to healthy sheep ($p<0.001$). In this study, the effect of abscesses encountered in the superficial lymph nodes of sheep due to C. pseudotuberculosis on the oxidative stress level was evaluated for the first time.

Antioxidants are defense mechanisms that neutralize the effect of free oxygen radicals in the body and prevent them from damaging cells (Ertas and Kirmizigul, 2021; Polat et al., 2021). There are many antioxidant molecules such as glutathione (GSH), glutathione peroxidase (GSH-pX), catalase (CAT) and superoxide dismutase (SOD) (Polat et al., 2021). However, due to the complexity of individual measurements of different antioxidant molecules, total antioxidant capacity (TAC) level can also be used in studies (Ertas and Kirmizigul, 2021). In this study, while determining the antioxidant levels, the levels of different antioxidant molecules were evaluated separately. In this study, it was aimed to make diagnosis of abscesses formed in superficial lymph nodes (due to *C. pseudotuberculosis*) and visceral form of pseudotuberculosis. For this purpose, it was thought that evaluating different antioxidant molecules one by one would be more effective.

In many studies (Esmaeilnejad et al., 2014; Bozukluhan et al., 2020; Ertas and Kirmizigul, 2021), it has been stated that antioxidant levels in sheep decrease significantly in disease states. There are studies reporting that the total antioxidant capacity of sheep with toxoplasmosis (Bozukluhan et al., 2020) and fasciolosis (Ertas and Kirmizigul, 2021) decreases significantly. Esmaeilnejad et al. (2014) reported that GSH-Px, SOD, CAT levels decreased significantly in sheep with Babesiosis. In this study, it was determined that there was a significant decrease in the levels of GSH, GSH-pX, CAT, SOD in sheep with abscesses in the superficial lymph nodes due to C. pseudotuberculosis. In this study, the highest decrease in antioxidant activities was detected in GSH (23.81%) and CAT (22.23%) molecules. Although it is not possible to say that the results of this study can be used as a biomarker for caseous lymphadenitis, it is noteworthy that it makes a difference in oxidative stress.

CONCLUSIONS

As a result, it was determined that abscesses formed in superficial lymph nodes due to C. pseudotuberculosis caused an increase in free oxygen radicals and a decrease in antioxidant activity. The increase in MDA level was determined as 18.62%. The antioxidant molecules with the highest decrease were determined as GSH (23.81%) and CAT (22.23%). Thus, it was thought that MDA, GSH and CAT values could be effective in the diagnosis of superficial caseous lymphadenitis. Our study is very important in that it will form an idea for the detailed studies to be done to determine the accuracy of this hypothesis and that it is the first study in this field.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

Aebi H (1984) Catalase. In vitro. Methods in Enzymol 105: 121-126. http://dx.doi.org/10.1016/S0076-6879(84)05016-3

Akgul G, Kahya Demirbilek S, Çelik ÖY, Irak K, Akgul MB, Mersin S (2018) Corynebacterium pseudotuberculosis Case in a Boer Goat X Turkish Hair Goat Crossbred. Erciyes Üniv Vet Fak Derg 15(1): 82-85.

Al-Gaabary MH, El-Sheikh WMA (2002) Epidemiological, clinical andpreventive studies on caseous lymphadenitis in sheep and goats at Gharbia governorate. In: 10th Sci. Cong. Fac. Vet. Med. Assiut University, Egypt, 402-417.

Al-Gaabary MH, Osman SA, Oreiby AF (2009) Caseous lymphadenitis in sheep and goats: Clinical, epidemiological and preventive studies. Small Rumin Res 87: 116-121. https://doi.org/10.1016/j.smallrumres.2009.10.008

Baird GJ, Fontaine MC (2007) Corynebacterium pseudotuberculosis and its role in ovine caseous lymphadenitis. J Comp Path 137: 179-210. https://doi.org/10.1016/j.jcpa.2007.07.002

Basbug O, Tuzcu N, Ercan N, Aydogdu U, Ograk YZ (2014) Serum vitamin D levels in sheep with caseous lymphadenitis. FÜ Sağ Bil Vet Derg 28(2): 77-80.

Bastos BL, Portela RWD, Dorella FA, Riberio D, Seyffert N, Castrao TLP, Miyoshi A, Oliveria SC, Meyer R, Azevedo V (2012) Corynebacterium pseudotuberculosis: Immunological responses in animal models and zoonotic potential. J Clin Cell Immunol S4: 1-15.

Beutler E (1984) Red cell metabolism. A manual of biochemical methods, 2nd ed. Pp. 160, Grune and Starton, New York.

Bozukluhan K, Merhan O, Kızıltepe S, Harmankaya A, Gökçe G (2020) Determination of oxidative stress and ceruloplasmin levels in sheep with toxoplasmosis. Van Vet J 31(2): 83-86. https://doi.org/10.36483/vanvetj.646976

Cetinkaya B, Karahan M, Atil E, Kalin R, De Baere T, Vaneechoutte M (2002) Identification of Corynebacterium pseudotuberculosis isolates from sheep and goats by PCR. Vet Microbiol 88(1): 75-83. https://doi.org/10.1016/s0378-1135(02)00089-5

Dorella FA, Pacheco LGC, Olivera SC, Miyashi A, Azevedo V (2006) Corynebacterium pseudotuberculosis: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet Res 37: 201-218. https://doi.org/10.1051/vetres:2005056

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95. http://dx.doi.org/10.1016/0006-2952(61)90145-9

Ertas F, Kirmizigul AH (2021) Investigation of oxidative stress and metabolic profile in sheep with fascioliasis. Atatürk Üniversitesi Vet Bil Derg 16(2): 204-210. https://doi.org/10.17094/ataunivbd.844383

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B, Malekinejad H, Jalilzadeh-Amin G, Arjmand J, Golabi M, Hajipour N (2014) Evaluation of antioxidant status, oxidative stress and serumtrace mineral levels associated with Babesia ovis parasitemiain sheep. Vet Parasitol 205: 38-45. http://dx.doi.org/10.1016/j.vetpar.2014.07.005

Fontaine MC, Baird GJ (2008). Caseous lymphadenitis. Small Rumin Res 76: 42-48. https://doi.org/10.1016/j.smallrumres.2007.12.025

Frankel S, Reitman S, Sonnen AC (1970) A textbook on laboratory procedure and their interpretation. Ch 10. Grand-Wohl's Clinical Laboratory Methods and Diagnosis. London. The CV Mosby Co, 403-404.

Ilhan Z (2020) In vitro antimicrobial susceptibility of corynebacterium pseudotuberculosis isolated from sheep with caseous lymphadenitis. Kocatepe Vet J 13(2): 267-271. https://doi.org/10.30607/kvj.738662

Ilhan FS (2008). Pathological observation on caseous lymphadenitis infection in sheep. YYÜ Vet Fak Derg 19(1): 23-28.

Ipek V, Akgül O, Kahraman MM, Öztürkoglu SI, Büyükcangaz E, Mecitoglu Z, Yılmazbaş Mecitoglu G (2012) Internal form of caseous lymphadenitis in a merinos sheep. Uludag Univ J Fac Vet Med 31(2): 71-76.

Izgür M, Akan M, Ilhan Z (1999) Microorganisms isolated from cases of caseous lymphadenitis. Ankara Univ Vet Fak Derg 46: 43-50.

Kumar J, Singh F, Tripathi BN, Kumar R, Dixit SK, Sonawane GG (2012) Epidemiological, bacteriological and molecular studies on caseous lymphadenitis in Sirohi goats of Rajasthan. Trop Anim Health Pro 44 (7): 1319-1322. https://doi.org/10.1007/s11250-012-0102-8

Mubarak M, Bastawrows AF, Abdel-Hafez MM, Ali MM (1999). Caseous lymphadenitis of sheep and goats in assıut farms and abattoırs. Assiut Vet Med J 42: 89-107.

Oreiby AF (2015). Diagnosis of caseous lymphadenitis in sheep and goat. Small Rumin Res 125: 160-166. http://dx.doi.org/10.1016/j.smallrumres.2014.11.013

Parin U, Kırkan Ş, Ural K, Savaşan S, Erbaş G, Gültekin M, Yüksel HT, Balıkçı C (2018) Molecular identification of Corynebacterium pseudotuberculosis in sheep. Acta Vet Brno 87: 3-8. https://doi.org/10.2754/avb201887010003

Placer ZA, Cushman L, Johnson BC (1966) Estimation of products of lipid peroxidation in biological fluids. Anal Biochem 16: 359-364. https://doi.org/10.1016/0003-2697(66)90167-9

Polat E, Han MC, Yılmaz S, Kaya E, Kayapinar SD, Coskun S, Yildirim A, Can UK (2021) The effect of hip dysplasia on some biochemical parameters, oxidative stress factors and hematocrit levels in dogs. Pol J Vet Sci 24(4): 473-478. https://doi.org/10.24425/pjvs.2021.139971

Robaj A, Hamidi A, Bytyqi H, Sylejmani D (2017) Frequency and antimicrobial susceptibility of bacterial isolates from caseous lymphadenitis in sheep in Kosovo. Bulg J Agric Sci 23(6): 1033-1036.

Sá Guimarães A, Borges do Carmo F, Pauletti RB, Seyffert N, Ribeiro D, Lage AP, Heinemann MB, Miyoshi A, Azevedo V, Gouveia AMG (2011) Caseous lymphadenitis: epidemiology, diagnosis, and control. The IIOAB Journ 2(2): 33-43.

Seyranoglu K, Aslan Ö (2015) Effects of vitamin e and selenium combination on oxidative response in lambs vaccinated against caseous lymphadenitis. Atatürk Üniversitesi Vet Bil Derg 10(3): 171-178. https://doi.org/10.17094/avbd.30833

Sheikh-Omar AR, Shah M (1984) Caseous lymphadenitis in sheep imported from Australia for slaughter in Malasia. Aust Vet J 61(12): 410-410. https://doi.org/10.1111/j.1751-0813.1984.tb07179.x.

Slotte JP, Ramstedt B (2007) The functional role of sphingomyelin in cell membranes. Eur J Lipid Sci Technol 109: 977-981. https://doi.org/10.1002/ejlt.200700024

Songer JG (1997) Bacterial phospholipases and their role in virulence. Trends Microbiol 5(4): 156-161. https://doi.org/10.1016/S0966-842X(97)01005-6.

Sun Y, Oberly LW, Ying LA (1988) Simple method for clinical assay of superoxide dismutase. Clin Chem 34: 497-500. https://doi.org/10.1093/clinchem/34.3.497

Tolu C, Savas T (2014) Variation in the frequency of superficial abscesses based on the caseous lymphadenitis (CLA) disease in goats. Hayvansal Üretim 55(1): 19-24. https://doi.org/10.29185/hayuretim.363903

Williamson LH (2001) Caseous lymphadenitis in small ruminants. Vet Clin N Am: Food Anim Pract 17: 359-371. https://doi.org/10.1016/s0749-0720(15)30033-5

Windsor PA (2011) Control of caseous lymphadenitis. Vet Clin Food Anim 27: 193-202. https://doi.org/10.1016/j.cvfa.2010.10.019

Zaitoun AM, Bayoumi AH (1994) Some epidemiological studies on ovine pseudotuberculosis. Assiut Vet Med J 31: 238-250. https://doi.org/10.21608/AVMJ.1994.185364