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Research Article

Investigation of Oxidative Stress Parameters in Brucella Infected Sheep and Goats

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ABSTRACT

Brucella infection is a common zoonotic disease in sheep and goats and is associated with various clinical signs and immune responses after infection. Oxidative stress is believed to have a significant impact on the pathophysiological mechanisms of this infection. The aim of this study was to investigate oxidative stress parameters in blood serum of *Brucella* infected sheep and goats. In the study conducted in Samsun province, 1831 sheep and 182 goat samples were examined; 30 *Brucella* positive sheep-goat sera and 30 healthy sheep-goat sera were used for biochemical analyses. In the samples, total thiol, native thiol, disulphide levels, native thiol/total thiol ratio, disulphide/native thiol ratio, disulphide/total thiol ratio, malondialdehyde (MDA) levels, and adenosine deaminase (ADA) activity were analyzed. According to our results, 450 of 1831 sheep samples (32.6%) and 71 of 182 goat samples (64%) were found to be positive. In the study conducted to investigate the effect of *Brucellosis* on oxidative stress parameters; total thiol (1374.84±506.61), native thiol (505.74±247.15), disulphide (434.54±230.77), MDA (2.01±0.65) and ADA (15.43±2.70) were found to be significantly higher in the *Brucella* group compared to total thiol (909.08±347.16), native thiol (360.34±156.18), Disulphide (274.37±127.092), MDA (1.45±0.42) and ADA (6.76±1.27) values in the control group (P=0.020, P=0.003, P=0.016, P=0.004, P<0.001). Disulphide/native thiol (112.54±94.37), disulphide/total thiol (30.37±79.14) values in *Brucella* group were determined higher than disulphide/native thiol (84.08±51.00), disulphide/total thiol (28.81±7.68) values in the control group, but no statistically significant difference was determined (P=0.287, P=0.572). In addition, native thiol/total thiol (39.25±18.29) value in the *Brucella* group decreased compared to native thiol/total thiol (42.37±15.37) value in the control group, but no statistically significant difference was determined (P=0.572). In conclusion, it was determined that oxidant-antioxidant balance was impaired and oxidative stress occurred in sheep and goats with *Brucella* infection. These findings reveal that *Brucella* infection in sheep and goats is closely associated with mechanisms of oxidative stress and tissue damage. Additionally, our study provides insights into the oxidative response in *Brucella* infection, enhancing our understanding of the disease's pathogenesis.

Keywords: *Brucella, goat, oxidative stress, serum, sheep*

Brucella ile Enfekte Koyun ve Keçilerde Oksidatif Stres Parametrelerinin İncelenmesi

ÖZET

Brucella enfeksiyonu, koyun ve keçilerde yaygın görülen ve çeşitli klinik belirtiler ile enfeksiyon sonrası immün yanıtlarla ilişkili bir zoonotik hastalıktır. Bu enfeksiyonun patofizyolojik süreçlerinde oksidatif stresin önemli bir rol oynayabileceği düşünülmektedir. Bu çalışmanın amacı, *Brucella* enfekte koyun ve keçilerin kan serumunda oksidatif stres parametrelerini incelemektir. Samsun ilinde yapılan araştırmada, 1831 koyun ve 182 keçi örneği incelenmiş; *Brucella* pozitif bulunan 30 koyun-keçi serumu ile sağlıklı 30 koyun-keçi serumu biyokimyasal analizler için kullanılmıştır. Toplanan örneklerde oksidatif stres parametreleri olan total tiyol, natif tiyol, disülfit, natif tyiol/total tiyol, disülfit/natif tiyol, disülfit/total tiyol, malondialdehit (MDA) düzeyleri ve adenozin deaminaz (ADA) aktivitesi analiz edilmiştir. Çalışma sonuçlarımıza göre; 1831 koyun örneğinin 450 tanesi pozitif (%32,6), 182 keçi örneğinin ise 71 tanesi (%64) pozitif olarak tespit edilmiştir. Brusellozun oksidatif stres parametreleri üzerindeki etkisini incelemek için yapılan çalışmada; *Brucella* grupta total tyiol (1374,84±506,61), natif tiyol (505,74±247,15), disülfit (434,54±230,77), MDA (2,01±0,65) ve ADA (15,43±2,70), kontrol grupta total tiyol (909,08±347,16), natif tiyol (360,34±156,18), disülfit (274,37±127,09), MDA (1,45±0,42) ve ADA (6,76±1,27) değerlerine göre anlamlı derecede yüksek bulunmuştur (P=0,020, P=0,003, P=0,016, P=0,004, P<0,001). *Brucella* grupta disülfit/natif tiyol (112,54±94,37), disülfit/total tiyol (30,37±79,14), kontrol grupta disülfit/natif tiyol (84,08±51,00), disülfit/total tiyol (28,81±7,68) değerlerine göre yüksek belirlenmiş, ancak istatistiksel olarak anlamlı fark belirlenememiştir (P=0,287, P=0,572). Ayrıca *Brucella* grubundaki natif tiyol/total tiyol (39,25±18,29) değeri ise kontrol grubundaki natif tiyol/total tiyol (42,37±15,37) değerine göre azalmış, ancak istatistiksel olarak anlamlı bir fark belirlenememiştir (P=0,572). Sonuç olarak, *Brucella* enfeksiyonu taşıyan koyun ve keçilerde oksidan-antioksidan dengesinin bozulduğu ve oksidatif stresin ortaya çıktığı tespit edilmiştir. Bu bulgular, koyun ve keçilerdeki *Brucella* enfeksiyonunun oksidatif stres ve doku hasarı mekanizmalarıyla yakından ilişkili olduğunu ortaya koymaktadır. Ayrıca, çalışmamız, *Brucella* enfeksiyonundaki oksidatif yanıt hakkında bilgiler sunarak, hastalığın patogenezine dair anlayışımızı güçlendirmektedir.

Anahtar kelimeler: *Brucella, keçi, koyun, oksidatif stres, serum*

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Introduction

Brucella infection is a zoonotic disease known for many years that causes significant losses by negatively affecting public health as well as animal health (Pappas et al., 2006; Nicoletti, 2010; Hull and Schumake, 2018). *Brucella* is a member of the Brucellaceae family in the order Rhizobiales, class Alphaproteobacteria. In the booklet published by the World Organisation for Animal Health (WOAH, 2022), 12 species associated with brucellosis were identified. Among these, *Brucella abortus* (cattle), *B. melitensis* (goat and sheep), *B. ovis* (sheep), *B. canis* (dog), *B. suis* (pig) and *B. neotomae* (common field mice, desert wood mouse) are known as classic species and show host specificity. In addition to these classical species, *B. ceti*, *B. inopinat, B. microti, B.* p*apionis, B. pinnipedialis,* and *B. vulpis* are known. Among the classical species, *B. melitensis*, *B. abortus* and *B. suis* are the main brucellosis agents causing serious infections and economic losses in both animals and humans. *B. abortus* usually causes disease in cattle and *B. suis* causes disease in pigs. This shows that brucellosis poses a significant threat to both animal and public health (Foster et al., 2007; Scholz et al., 2008; Scholz et al., 2010; Whatmore et al., 2014; Scholz et al., 2016; WOAH, 2022).

Among the *Brucella* species, especially *B. melitensis* is known as the main agent responsible for infections in sheep and goats and has three biovars, bv 1-3. This bacterial pathogen mainly causes abortion, arthritis, infertility, mastitis, orchitis and decreased milk yield in cattle, sheep, goats and pigs, and when it infects humans, it causes a febrile disease called brucellosis (Ko and Splitter, 2003; Pappas et al., 2006; Ica et al., 2012; Quintas et al., 2019; Freddi et al., 2021; WOAH, 2022). The most common routes of transmission of *Brucella* infection in sheep and goats are respiratory, digestive and genital tracts. Placenta, foetal fluids, milk and vaginal discharge of infected animals play an important role in the spread of this infection through direct contact. In addition, indirect transmission is also frequently observed due to the spread of these materials in the environment. Especially in crowded herds, infection can spread rapidly (Alton et al., 1988; Seleem et al., 2010). The diagnosis of brucellosis in sheep and goats is confirmed by serological and bacteriological tests in addition to clinical signs. Serological methods such as Rose Bengal Test, ELISA and complement fixation test are widely used as rapid and effective diagnostic tools in the field. These tests play an important role especially in screening infected herds and preventing the spread of the disease (Nicoletti, 1993; Díaz-Aparicio, 2013; WOAH, 2022).

The fact that brucellosis is a zoonotic disease directly concerns not only animal health but also human health. The infection can spread to humans through direct contact with infected animals or by consuming unpasteurized milk and dairy products. The fight against *Brucella* spp. is very difficult due to the rapid spread of *Brucella* spp. within the herd, long and costly protection and control programmes, and difficult treatment. Regular vaccination programmes and hygiene measures are of great importance to prevent and control the spread of infection (Corbel, 1997; Ko and Splitter, 2003; Pappas et al., 2006; Seleem et al., 2010; Godfroid et al., 2011; Olsen, 2014).

Brucella infections in sheep and goats lead to increased oxidative stress in the organism and during infection, the immune system produces reactive oxygen species (ROS) to destroy the pathogen. However, uncontrolled increase in ROS levels causes cellular damage by overcoming antioxidant defence mechanisms (Gutteridge, 1999; Tabakoğlu and Durgut, 2013; Erel and Neselioglu, 2014). Oxidative stress is defined as the disruption of the balance between antioxidants and oxidants and leads to tissue damage at cellular and molecular levels in disease processes. Nowadays, the evaluation of oxidative stress parameters is becoming increasingly important in determining the pathogenesis and prognosis of diseases (Kohen and Nyska, 2002; Azzam et al., 2012; Tabakoğlu and Durgut, 2013; Tanner et al., 2017). In particular, thiol-disulphide homeostasis stands out as a critical marker for monitoring cellular redox status. Thiol groups form an important part of antioxidant defence and respond to oxidative stress through reversible reactions with disulphide bonds. In this context, total thiol (TT), native thiol (NT), disulphide (Ds) and various thiol/disulphide ratios are the main parameters used in the evaluation of oxidative stress. Native thiol refers to the amount of native thiol present in cells, while total thiol includes both native thiol and thiol in disulphide bonds. Comparison of total thiol and native thiol levels gives important clues to understand the redox balance within the cell (Kohen and Nyska, 2002; Biswas et al., 2006; Erel and Neselioglu, 2014; Sato et al., 2014; Hudson et al., 2015; Kundi et al., 2015). Disulphide bonds are generated through the oxidation of thiol groups as a response to oxidative stress. In thiol-disulphide homeostasis, the ratios of disulphide levels provide valuable insights into the severity of oxidative stress. The native thiol/total thiol (NT/TT), disulphide/native thiol (Ds/NT), and disulphide/total thiol (Ds/TT) ratios serve as indicators of cellular impact under oxidative stress conditions. These ratios are widely recognized as key markers for assessing oxidative stress (Dominici et al., 1999; Kohen and Nyska, 2002; Biswas et al., 2006; Jones and Liang, 2009; Circu and Aw, 2010).

Malondialdehyde (MDA) is considered as one of the main indicators of lipid peroxidation. MDA, which is formed in the cell membrane during oxidative stress, occurs as a result of lipid peroxidation and is an important biomarker reflecting the degree of oxidative damage. MDA levels are a frequently used measurement method to determine oxidative stress (Kohen and Nyska, 2002; Nisbet et al., 2008; Tabakoğlu and Durgut, 2013; Aslan et al., 2017). The adenosine deaminase (ADA) enzyme plays a crucial role in regulating immune responses and cellular immunity. Given the involvement of oxidative stress in numerous inflammatory processes, ADA activity mea-

Table 1. Number of sheep and goats examined for *Brucella* and infection rates in Samsun Province

Total Number of Sample Taken	Average Sheep	Average Goat	Pozitive Sheep	Negative Sheep	Positive Goat	Negative Goat
2013	24.58(%)	39.01(%)	450	1381	71	111

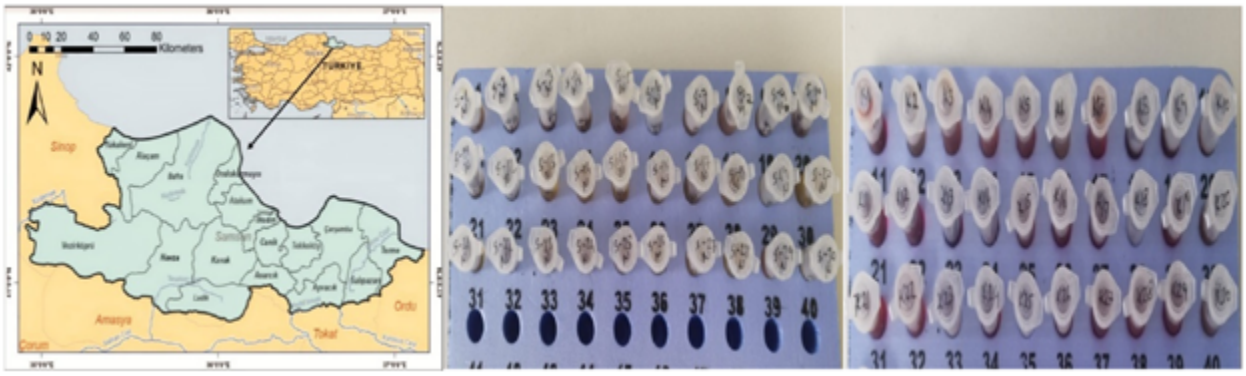

Figure 1. Representative map of Samsun Province and sheep and goat sera analysed

surement has become a valuable tool for assessing the severity of inflammation and monitoring disease progression (Haskó and Cronstein, 2004; Espinosa-Diez et al., 2015).

This study aimed to evaluate total thiol, native thiol, disulphide, disulphide/native thiol, native thiol/total thiol, disulphide/total thiol ratios, MDA levels, and ADA activity in *Brucella* infections affecting sheep and goats raised for livelihood in Samsun province.

Materials and Methods

Research and Publication Ethics

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments at the Samsun Veterinary Control Institute Directorate, as documented in the letter dated 12.11.2024 with the reference number 19572899/031-89.

Collection of Serum Samples

Serum samples from 1831 sheep and 182 goats were analysed for *Brucella* in Samsun (41.379°N, 36.0595°D) province (Table 1). Blood sera were firstly subjected to RBPT (Rose Bengal Plate Test) and then to CFT (Complement Fixation Test) to confirm suspicious samples and to determine antibody titres of positive samples.

The province where the serum samples were taken and the pictures of the samples are shown in Figure 1.

Brucella Rose Bengal Plate Test

Blood collected in tubes without additives was centrifuged at 3000 *g* for 3 min. The sera obtained were dropped 50 µl of serum on a clean white tile. Rose Bengal antigen (Vet-Vac, 10 ml, Pendik Veterinary Control Institute) was immediately added and mixed for 4 min. Sera exhibiting precipitation or clumping were classified as suspicious

Brucella Complement Fixation Test

Sera suspected of containing *Brucella*, *Brucella*-positive control sera (1 ml, 1/1200 titer, provided by the Pendik Veterinary Control Institute), and negative control sera were diluted 1:5 using veronal buffer diluent (VBD, Lot: KL0062). The sera were then heat-inactivated by placing them in a water bath at 58°C for 30 min. In a sterile U-bottom microplate, 25 µl of VBD was added to the wells in rows B through G. Next, 25 µl of the heat-inactivated sera were added to wells A, B, and H. A serial dilution was performed by transferring 25 µl of the mixture from row B to row G. Following this, 25 µl of *Brucella* antigen (Virion-Serion, Ref: 1297, Lot: SHF.BF, prepared at a working dilution of 1/30) was added to all the wells from rows A to G. Subsequently, 50 µl of complement (VBD, Lot: KL0016, working dilution 1/50) was added to each well and the plates were stored at +4°C overnight.

After 18 to 20 hours, the haemolytic system was prepared. Ambocceptor (Virion-Serion, Ref: 9002, Lot: KL0042) was diluted to a 1:2500 ratio using veronal buffer. Blood (10 mL) from *Brucella*-negative sheep was mixed with veronal buffer and centrifuged at 3000 g for 6 min. The resulting pellet of erythrocytes was then resuspended in veronal buffer to achieve a final concentration of 2%. This erythrocyte suspension was mixed with the ambocceptor solution in equal volumes (1:1 ratio) and incubated at room temperature for 30 min to prepare the haemolytic system.

The plates stored at +4°C were brought to room temperature for 30 min before 50 µL of the haemolytic system was added to each well. The plates were then incubated at 37°C in a water bath for 30 min and subsequently placed at +4°C for one hour. The results were interpreted as follows: the negative control wells must remain negative, the positive control should correspond to the specified titer of 1/1200, and wells in row H should show comple-

te haemolysis. The highest serum dilution showing 50% haemolysis was recorded as the titer.

Biochemical Analyses

Native thiol and Total thiol (Rel Assay) oxidative stress parameters in serum samples were measured using colorimetric test kits according to the procedure recommended in the kit. The measurements of the kits used were performed on an ELISA plate reader device. Disulphide value was calculated using the native and total thiol values obtained. MDA analysis was performed by the method reported by Yoshioka et al. (1979) and ADA activity was measured by Giusti method in a spectrophotometer (Giusti, 1974).

Thiol levels, both total and native, are determined using colorimetric test kits based on a specific procedure. In essence, the method involves reducing disulphide bonds to generate free thiol groups. Excess sodium borohydride, a reducing agent, is neutralized and removed using formaldehyde. Following this, thiol groups, including native thiols, react with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) to enable their measurement. The dynamic disulphide content is determined as half of the difference between total thiols and native thiols. From the measured values of native thiols (SH) and total thiols, disulphide (SS) levels, disulphide/total thiol ratio (SS/(SH+SS)), disulphide/native thiol ratio (SS/SH), and native thiol/total thiol ratio (SH/(SH+SS)) are calculated.

The MDA method is based on the fact that lipid content during the TBA reaction produces a stable red-pink colour with a minimum peak at 535 nm when heated at low pH and in the presence of TBA. Thiobarbituric acid reactive substances (TBARS) are assessed by quantifying the compounds produced during the reaction between malondialdehyde, a lipid peroxidation by-product, and thiobarbituric acid. The red-pink colour formation caused by the combination of MDA molecule and two TBA molecules is chromogen-induced. A small amount of MDA is formed during peroxidation and most of it is formed as a result of lipid peroxidation during the heating process following acidification of the medium.

Due to the reaction of ADA, adenosine (deoxyadenosine) is used as substrate for the measurement of ADA activity. ADA catalyses the formation of inosine (deoxyinosine) from adenosine. The resulting ammonia, together with sodium hypochlorite and phenol/nitroprusside, forms dark blue indophenol in alkaline solution. Sodium nitroprusside functions as a catalyst in the reaction. As a result, the ammonia concentration is directly proportional to the indophenol concentration, which is measured by absorbance.

Statistical Analysis

Statistical analyses were conducted using SPSS package program (Statistics 27, IBM SPSS Statistics) with a significance level set at $P<0.05$. The normality of the data was assessed using the Shapiro-Wilk test. For normally distributed data, an Independent T-test was performed. Pearson correlation analysis was used to examine the relationship between variables.

Results

A total of 2013 serum samples including 1831 sheep and 182 goat samples were analysed for *Brucella* spp. Of the 1831 sheep samples, 450 (32.6%) and 71 (64%) of 182 goat samples were positive (Table 1).

Positive blood sera were subjected to CFT test and titres are given in Table 2. Samples with a titer of 1/20 and above were considered positive. 247 (54.89%) of 450 sheep samples were 1/320, 71 (15.78%) were 1/160, 75 (16.67%) were 1/80, 40 (8.89%) were 1/40, 17 (3.77%) were 1/20 titer. 15 (21.13%) goat samples had 1/320 titer, 34 (47.89%) had 1/160 titer, 20 (28.17%) had 1/80 titer, 2 (2.81%) had 1/40 titer and no sample had 1/20 titer.

In the study, the values of some oxidative stress parameters were determined in a total of 60 samples, 30 *Brucella* positive blood serum samples and 30 *Brucella* negative blood serum samples taken from sheep and goats. Oxidative stress values of *Brucella* positive and control group are given in Table 3.

When Table 3 is examined, serum total thiol, native thiol, disulphide, MDA levels and ADA activity increased significantly in the *Brucella* group compared to the control group. Disulphide/native thiol and disulphide/total thiol levels also increased, but no statistically significant difference was found. Native thiol/total thiol levels decreased in the *Brucella* group compared to the control group, but no statistically significant difference was found.

The correlation data related to oxidative stress are provided in Table 4. When Table 4 was examined, it was determined that native thiol levels showed a significant positive correlation with total thiol, MDA levels and ADA activity as a result of the correlation analysis. While total thiol levels showed a significant positive correlation with disulphide levels and ADA activity, disulphide and MDA levels also showed a significant positive correlation with ADA activity.

Serum native thiol ($P=0.003$), total thiol ($P=0.020$) and disulphide ($P=0.016$) values of control group and *Brucella* group are given in Figure 2.

Native thiol/total thiol ($P=0.572$), disulphide/native thiol ($P=0.287$), disulphide/total thiol ($P=0.572$) values of the control group and *Brucella* group are given in Figure 3.

Serum ADA and MDA values of the control group and *Brucella* group are given in Figure 4.

Discussion

Although brucellosis has a low mortality rate, it is a well-established disease that causes significant direct and indirect losses due to abortions, decreases in milk

Table 2. Antibody titres of sheep and goats

Sheep Antibody titres		Goat Antibody titres	
1/320	54.89%	1/320	21.12%
1/160	15.78%	1/160	47.88%
1/80	16.67%	1/80	28.16%
1/40	8.89%	1/40	2.84%
1/20	3.77%	1/20	0%

Table 3. Total thiol, native thiol, disulphide, native thiol/total thiol (%), disulphide/native thiol (%), disulphide/total thiol (%), MDA and ADA levels (mean ± SD) in control and Brucellosis-infected sheep and goats

Parameters	Control Group	Brucellosis Group	P values
Total thiol (µmol / L)	909.08±347.16	1374.84±506.61	0.020
Native thiol (µmol / L)	360.34±156.18	505.74±247.15	0.003
Disulphide (µmol / L)	274.37±127.09	434.54±230.77	0.016
Native thiol/Total thiol (%)	42.37±15.37	39.25±18.29	0.572
Disulphide/Native thiol (%)	84.08±51.00	112.54±94.37	0.287
Disulphide/Total thiol (%)	28.81±7.68	30.37±7.9.14	0.572
MDA (mmol/L)	1.45±0.42	2.01±0.65	0.004
ADA (U/L)	6.76±1.27	15.43±2.70	<0.001

MDA; Malondialdehyde, ADA; Adenosine Deaminase

Table 4. Correlation table

Parameters	Native thiol	Total thiol	Disulphide	MDA	ADA
Native thiol		0.536***	0.096	0.355*	0.383*
Total thiol			0.892***	0.193	0.470***
Disulphide				0.381	0.349*
MDA					0.498***

* Correlation is significant at the 0.05 level P value
*** Correlation is significant at the 0.01 level P value

and meat yield, procedures applied for control, and the negative effects of its zoonotic nature on the health system (WOAH, 2022).

In the studies on sheep brucellosis in Türkiye, the seropositivity rate was determined as 2.6% in the state farms between 1952-1963, and this rate was found to be 5% in the studies conducted in Karacabey Harası between 1960-1970 (Eroğlu, 1989). In a sero-survey study conducted throughout Türkiye in terms of brucellosis, serum samples were taken from 30433 sheep by random sampling from four districts of each province throughout the country. All sera were first screened using the Rose Bengal Plate Test (RBPT), and positive samples were further confirmed with the Complement Fixation Test (CFT). It was reported that the prevalence rate in sheep was 1.97% (İyisan et al., 2000). In a study conducted by Yumuşak and Aksoy in Adıyaman, 92 (46.23%) of 199 sheep and 34 (44.15%) of 77 goats were seropositive (Yumuşak and Aksoy, 2014). In a study conducted in Hatay, 155 (33.5%) of 462 sheep were found to be seropositive for *Brucella* (Şahin and Yıldız, 2006). In our study, 450 of 1831 sheep samples (32.6%) and 71 of 182 goat samples (64%) were found to be positive. Our study was found to be in parallel with previous studies (İyisan et al., 2000; Şahin & Yıldız, 2006; Yumuşak and Aksoy, 2014).

Oxidative stress arises when the body's antioxidant defense mechanisms are insufficient to counteract excessive reactive oxygen or nitrogen species (RNS), leading to cellular damage and impairing vital cellular functions. Reactive oxygen species (ROS) serve as defense molecules crucial for the immune system's response to pathogens. However, elevated intracellular ROS levels can disrupt the oxidant-antioxidant equilibrium, contributing to oxidative stress, which is a key factor in the development of various diseases. This imbalance occurs when the generation of ROS or RNS surpasses the organism's antioxidant capacity. During infections, immune cells generate significant amounts of ROS and RNS to eliminate invading pathogens. However, these biochemical products may affect not only pathogens but also healthy

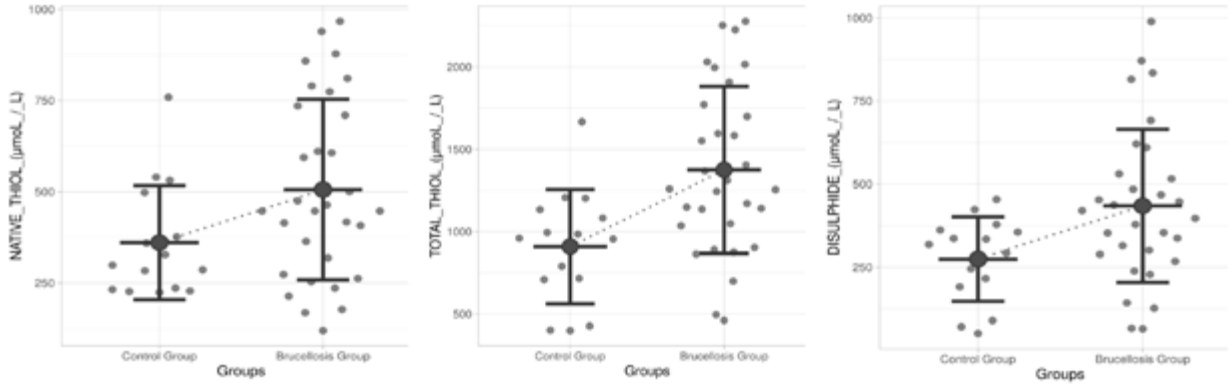

Figure 2. Serum native thiol, total thiol and disulphide values

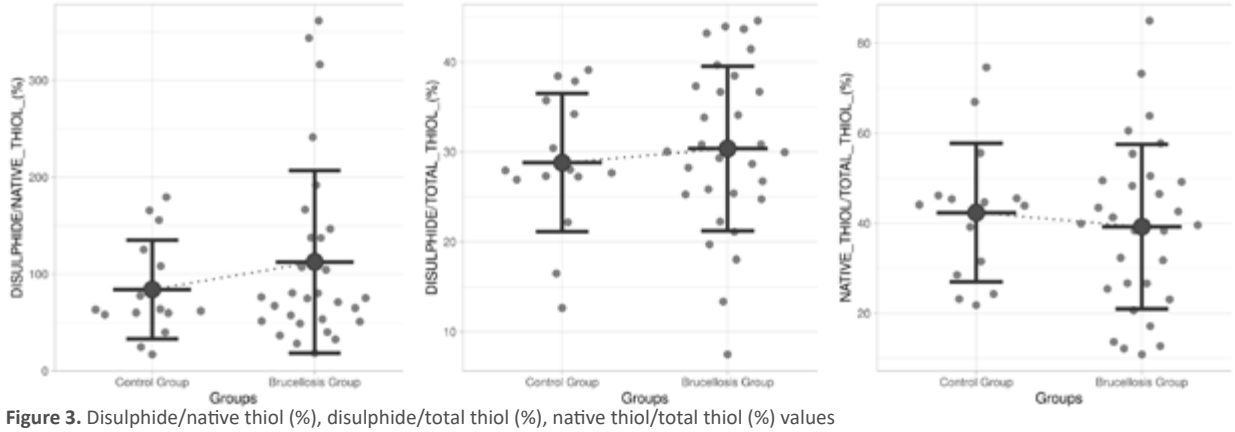

Figure 3. Disulphide/native thiol (%), disulphide/total thiol (%), native thiol/total thiol (%) values

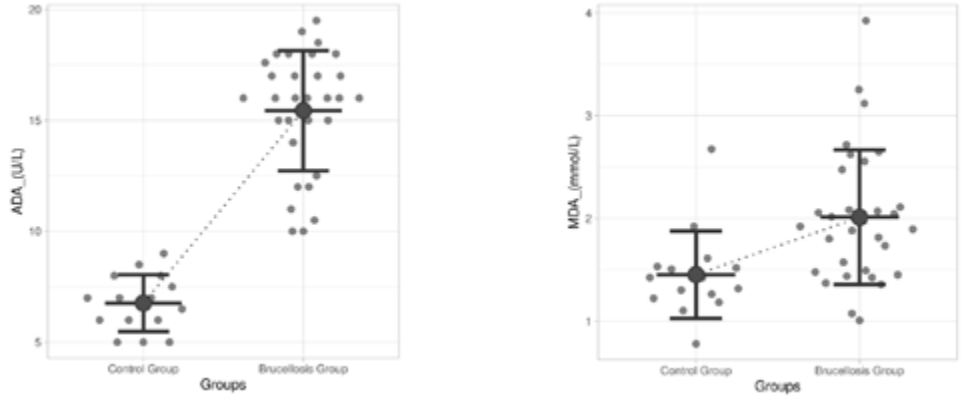

Figure 4. Serum ADA and MDA levels

cellular structures, leading to the initiation of pathological processes. In this context, oxidative response can be considered as an important biomarker indicating both the presence of pathogens and the activation of defence mechanisms against pathogens (Gutteridge, 1993; Valko, 2007; Ercan and Fidancı, 2012).

In this study, changes in the oxidative stress parameters total thiol, native thiol, native thiol/total thiol, disulphide, disulphide/native thiol, disulphide/total thiol, MDA and ADA and the presence of oxidative response in *Brucella* infected sheep and goats were investigated. Total thiol (P=0.020), native thiol (P=0.003), disulphide (P=0.016), MDA (P=0.004) and ADA (P<0.001) parameters in the *Brucella* group were significantly higher than the control group. Disulphide/Native thiol (P=0.287), Disulphide/total thiol (P=0.572) values determined higher in the *Brucella* group than in the control group, but no significant difference was detected. At the same time, the native thiol/total thiol ratio in the Brucella group decreased compared to the control group, but no significant difference was determined (P=0.572). Çenesiz et al. (2024) evaluated thiol disulphide homeostasis in their study on cattle with paratuberculosis. They determined that disulphide levels were higher in the paratuberculosis infected group compared to the control group and that the thiol disulphide balance was disrupted (Çenesiz et al., 2024). In a study conducted on cattle with pneumonia, Ertaş et al. (2023) evaluated the thiol disulphide balance before and after treatment, and no significant difference was determined in terms of disulphide values between the groups (Ertaş et al., 2023). In a study conducted by Adıgüzel and Merhan (2024) evaluating thiol/disulphide homeostasis in sheep infected with sheeppox

virus, disulphide levels increased in the healthy group compared to the infected group, but no significant difference was determined (Adıgüzel & Merhan, 2024). These findings indicate that oxidative stress, which increases as a result of infection, creates disruptive effects on the thiol-disulphide balance and the antioxidant defence system cannot respond adequately.

High levels of total thiol and native thiol can be considered as an indicator of the body's mechanism to cope with oxidative stress. Thiol groups play a protective role against oxidative damage by forming disulphide bonds against oxidants. The increase in disulphide levels observed in the *Brucella* infected group in our study can be considered as a reflection of this protective mechanism. Thiol-disulphide balance is considered as an indicator of oxidative stress in relation with the increase in free radical production (Erel & Neselioglu, 2014). Malondialdehyde level is a marker of lipid peroxidation and indicates the degree of oxidative damage. The increase in MDA level associated with *Brucella* infection confirms the oxidative destruction of lipids. In the MDA analysis performed before and after treatment in 50 individuals with acute brucellosis in Babol, Iran, the MDA level was 0.72±0.33 during *Brucella* infection and 0.46±0.48 after treatment (Bahnemiri et al., 2022). MDA concentration, an indicator of lipid peroxidation, increased significantly after *Brucella* inoculation and started to decrease to basal levels in plasma, liver and spleen from day 45. However, MDA level increased in the brain at the late stage of infection (Melek et al., 2006). ADA activity is considered as an important marker of immune response and plays a role in cellular immune response. The high level of ADA in the infected group in our study indicates that the cellular immune response increases during the fight against infection.

Conclusion

In this study conducted in Samsun province, it was determined that the seropositivity of brucellosis was high in sheep and goats. It can be said that *Brucella* infection causes deterioration in the antioxidant defence system by creating significant effects on oxidative stress markers. Increases in total thiol, native thiol, disulphide, MDA and ADA levels indicate the intensity of oxidative stress, while the low native thiol/total thiol ratio indicates that this stress has reached a level that exceeds antioxidant defence mechanisms. In conclusion, these findings demonstrate that *Brucella* infection in sheep and goats is closely related to mechanisms of oxidative stress and tissue damage. Additionally, our study sheds light on the oxidative response in *Brucella* infection, deepening our understanding of the disease's pathogenesis.

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Author contribution statement

RA, SÇ, AE and MÇ designed the research, RA, SÇ, BŞ, YK, AA, OT and NO performed the research study. RA prepared the manuscript with contributions from all coauthors.

Conflict of interest

The authors declare that they have no conflict of interest. The article "Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the Republic of Türkiye Ministry of Agriculture and Forestry. Neither the the Republic of Türkiye Ministry of Agriculture and Forestry can be held responsible for". This text is included in accordance with the recommendation of our Ministry.

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