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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

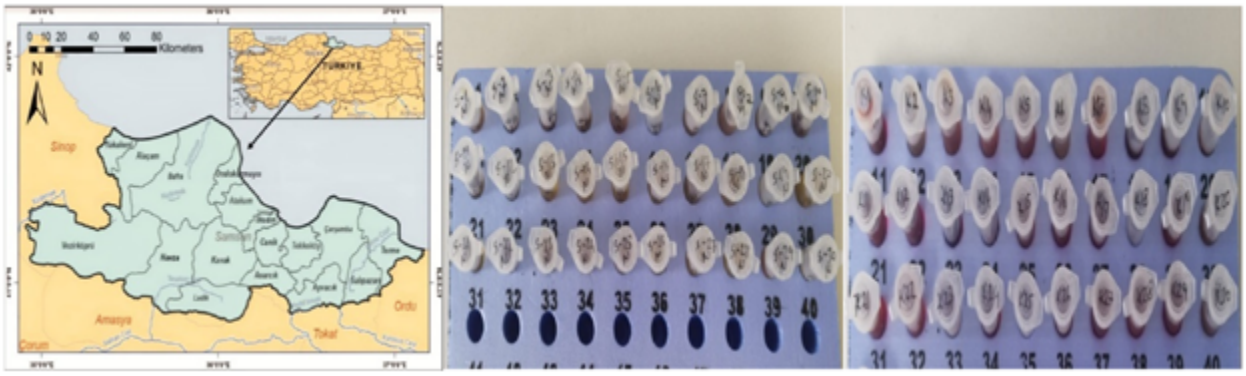

Figure 1. Representative map of Samsun Province and sheep and goat sera analysed

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

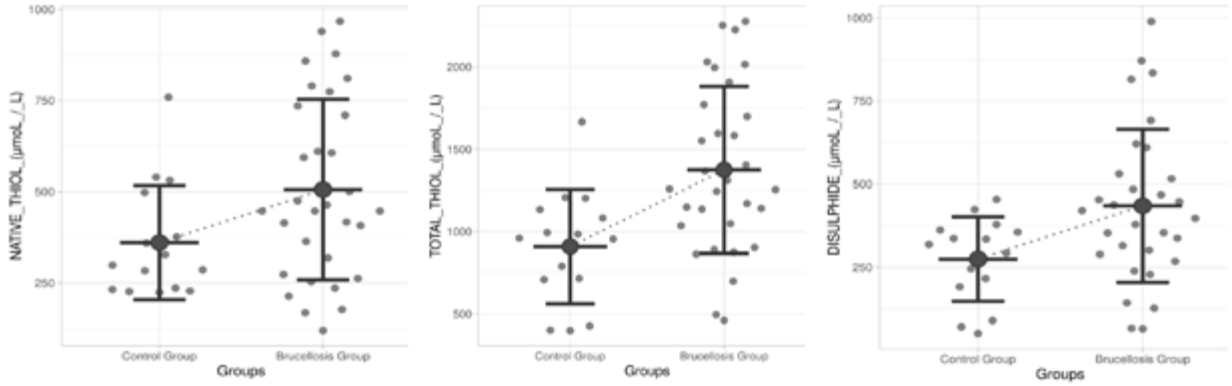

Figure 2. Serum native thiol, total thiol and disulphide values

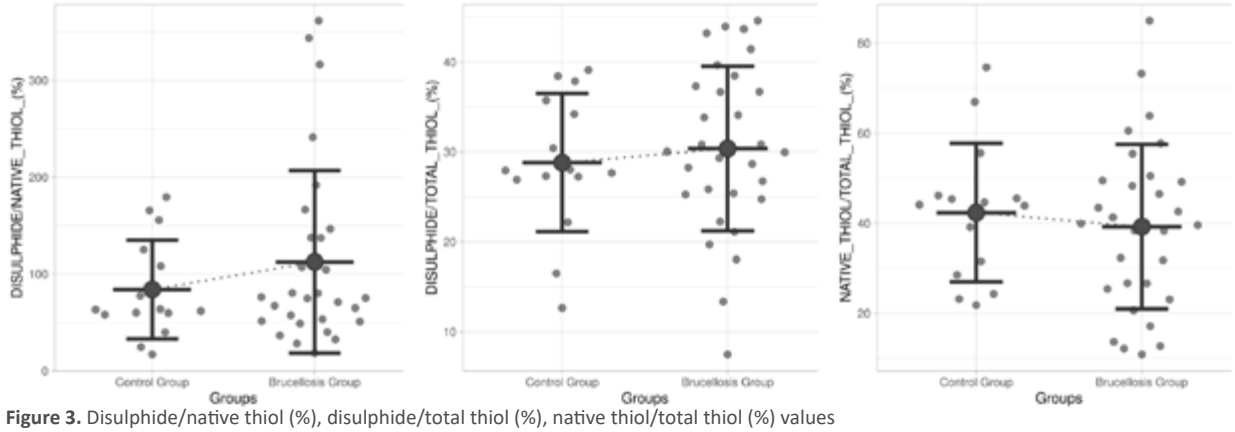

Figure 3. Disulphide/native thiol (%), disulphide/total thiol (%), native thiol/total thiol (%) values

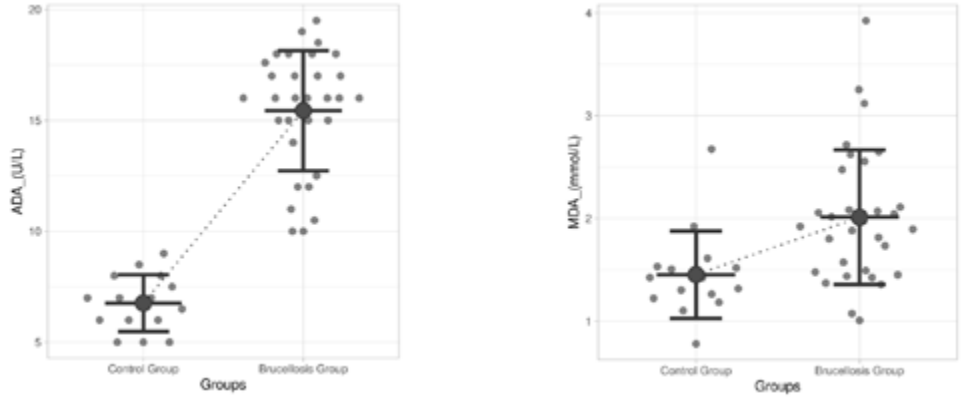

Figure 4. Serum ADA and MDA levels

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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Research Article

Investigation of the Gastroprotective Activity of Propolis Extracted in Different Solvents

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ABSTRACT

Propolis is a product produced by honeybees that collects the resins of plants. Secondary metabolites, including phenolic compounds, which are found in the resin of plants and are incorporated into propolis, give propolis many biological properties. Propolis exhibits various properties such as antibacterial, antiviral, antifungal, antioxidant, immunomodulator, anti-inflammatory, antiulcer and wound healing accelerator. The properties vary depending on the source of the plant, season, altitude, climate zone and extraction solvent. The present study investigated and compared the effects of different propolis extracts prepared using alcohol, water, dimethyl sulfoxide (DMSO), and olive oil on the indomethacin-induced gastric ulcers in nine group of Balb/C mice (n=8). Epithelial loss, erosion, bleeding, edema, inflammatory cell infiltration, and mean clinical scores results were evaluated statistically between different propolis extracts compared to omeprazole. Since the histopathological results were remarkably similar, the general assessment was made with the mean clinical score. It was found that DMSO and olive oil extracts of propolis had gastroprotective effects similar to omeprazole. In contrast, hydro-alcohol and water extracts did not show significant differences compared to their solvents and gastroprotective activity. In conclusion, it was determined that the olive oil extract of propolis, which is especially suitable for direct consumption, has the potential to be used as a gastroprotective.

Keywords: *Apitherapy, honeybee, gastroprotective, DMSO, olive oil, propolis*

Farklı Çözücülerde Ekstrakte Edilen Propolisin Mide Koruyucu Etkinliğinin Araştırılması

ÖZET

Propolis bal arılarının bitkilerin reçinelerini toplayarak oluşturdukları bir üründür. Bitkilerin reçinesinde bulunan ve propolise geçen içerisinde fenolik bileşenlerinde bulunduğu sekonder metabolitler propolise birçok biyolojik özellik kazandırır. Propolisin antibakteriyel, antiviral, antifungal, antioksidan, immunmodülatör, antiinflamatuvar, antiülser ve yara iyileşmesini hızlandırıcı gibi birçok özelliği bulunmaktadır. Bu özellikler çevredeki bitki örtüsü, mevsim, yükseklik, iklim kuşağı ve ekstraksiyon çözücüsüne göre değişkenlik göstermektedir. Bu çalışmada, hidro-alkolik, su, dimetilsülfoksit (DMSO) ve zeytinyağı kullanılarak hazırlanan farklı propolis ekstraktlarının her grupta 8 adet Balb/C fare olacak şekilde 9 grupta indometazin ile indüklenen gastrik ülser üzerindeki etkileri araştırılmış ve karşılaştırılmıştır. Epitel kaybı, erozyon, kanama, ödem, inflamatuar hücre infiltrasyonu ve ortalama klinik skor sonuçları, omeprazole kıyasla farklı propolis ekstraktları arasında istatistiksel olarak değerlendirildi. Histopatolojik sonuçlar birbiri ile benzer olduğu için genel değerlendirme ortalama klinik skoru ile yapılmıştır. DMSO ve alkol ekstrakt propolislerin omeprazole benzer gastroprotektif etkilerinin olduğu hidro-alkolik ve su ekstraktının ise kendi çözücülerine göre önemli fark oluşturmadığı ve gastroprotektif etkinlik göstermediği belirlenmiştir. Sonuç olarak, özellikle doğrudan tüketime uygun olan propolisin zeytinyağı ekstraktının gastroprotektif olarak kullanılabilme potansiyeli olduğu belirlenmiştir.

Anahtar kelimeler: *Apiterapi, bal arısı, mide koruyucu, DMSO, zeytinyağı, propolis*

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Introduction

Gastric ulcer is one of the most common diseases of the upper digestive system (Ramakrishnan and Salinas, 2007; Abumunaser, 2021; Ruiz-Hurtado et al., 2021a). While ulcerative lesions in the stomach are primarily observed in the curvature part, ulcerative lesions can also be observed in all gastric tissues from the pylorus to the cardia (Malfertheiner et al., 2009). Among the etiological factors of peptic ulcer, endocrine disorders, acute and chronic renal damage, and various neoplasms of gastric origin have been reported (Kuna et al., 2019). Apart from these etiological factors, the most common factor causing gastric ulcers is the use of non-steroidal and steroid-derived drugs. The pathophysiology of gastric ulcer is caused by disorders of mucosal protective mechanisms such as mucus, bicarbonate, prostaglandin (PG) synthesis in the stomach and duodenum, microcirculatory problems and disruption of the acid-pepsin balance, which can damage the mucosa. As a result of these factors, the gastric mucosal barrier is weakened, and acid release is increased, resulting in damage to the gastric epithelium (Ramakrishnan and Salinas, 2007; Abumunaser, 2021; Ruiz-Hurtado et al., 2021a). Propolis has been revealed to have many biological activities such as antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory and antiulcer (Stojanović et al., 2020). Various studies have also found that propolis has effects such as accelerating the osteogenic process and increasing regeneration in various tissues such as bone, cartilage and dental pulp (Ekeuku and Chin, 2021). In addition, propolis is also known to have a protective effect against gastric ulcers, one of the most common diseases of the digestive system (de Barros et al., 2007; Abd El-Hady et al., 2013; Ruiz-Hurtado et al., 2021a).

Many methods are used to treat peptic ulcers. However, these methods can cause adverse effects on the patient (Ruiz-Hurtado et al., 2021a). Therefore, many natural preventive and therapeutic alternatives are used. Propolis, used in traditional and complementary medicine, is one of the most important natural products used for this purpose. Propolis is a resinous substance, sticky, with a distinctive odour and varying in colour from light to dark brown, produced by worker bees when they bring nectar collected from growing parts of trees such as leaves, buds, branches and shoots to the hive, where it undergoes biochemical changes with wax and various enzymes they secrete. Propolis is an important bee product that has been used in traditional medicine since ancient times to treat many diseases (Sorucu, 2019; Stojanović et al., 2020).

The effect of propolis is due to the active substances such as phenolic compounds in propolis. Many factors affect the presence of these active substances in propolis (Stojanović et al., 2020). In addition, the extraction method and solvent selection affecting the presence of these phenolic substances in the final product are fundamental (Kekeçoğlu and Sorucu, 2021). While ethanol is the most preferred solvent, solvents such as water, methanol, methylene chloride, dichloromethane, lactic acid, hexane, ethyl acetate, acetone, olive oil, β-cyclodextrin, dimethyl sulfoxide, propylene glycol, ethyl acetate and chloroform are also frequently used in various studies. The different solvents used significantly affect the pharmacological properties of propolis since they cause a chemical alteration in the soluble active compounds (Oruç et al., 2023).

Although the gastroprotective activity of propolis has been demonstrated, only a study has been found to investigate the gastroprotective effect of propolis extracted in different solvents against gastric ulcers (Ruiz-Hurtado et al., 2021a; Sahin et al., 2023). The study compared the effects of ethanol and water extract propolis (Sahin et al., 2023). The present study aimed to investigate and compare the protective effects of propolis extracted with four different solvents (water, alcohol, DMSO, olive oil) against indomethacin-induced gastric ulcers in mice models.

Material and Methods

Propolis Extraction

The raw red propolis used in the study was purchased from Muğla (Apitonic-Bee Happy Beekeeping). The propolis was homogenised by grinding it into powder with a grinder. 150 g of propolis was weighed for each extraction, and 450 ml of each extraction solvent (ultrapure water, cold-pressed olive oil, 50% DMSO and 70-30% ethanol-water) was added. The mixtures were shaken in an orbital shaker for one week and filtered through Whatman No1 filter paper to obtain extracts (Kekeçoğlu and Sorucu, 2021; Sorucu and Oruç, 2019). Propolis was taken 1 ml into tared tubes, solvents were evaporated, and resin ratios were determined. The extracts were stored at +4 C until the experimental work.

Animals and Experimental Design

The study was conducted with the approval of Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee under approval number 2022/04 (Date of approval 30/05/2022). A total of 72 male BALB/c mice with a live weight of 15-25 g were used in the study. The mice were provided by the Experimental Animal Application and Research Centre of Muğla Sıtkı Koçman University. The experimental study was carried out in this centre under conditions suitable for mice.

Mice were randomly divided into nine groups of eight animals each. The propolis extracts were applied at 100 mg resin/kg. (Ruiz-Hurtado et al., 2021b).

Since the ulcer-causing potential of indomethacin is higher than other NSAIDs, the preparation was chosen to create an ulcer model. The groups were first treated with propolis extracts and one hour later given indomethacin at 100 mg/kg to induce ulceration (de Barros et al., 2007).

The groups were formed, and treatments were administered via oral gavage as follows.

- Group 1 (OMP): Omeprazole was administered at 30 mg/kg as a positive control.

- Group 2 (WEP): Propolis dissolved in ultrapure water was administered (100 mg/kg).
- Group 3 (W): Ultrapure water (ELGA) 1 ml was administered as a negative control (equal volume WEP).
- Group 4 (EWEP): Ethanol (Merck)-water (70%-30%) extract of propolis was administered (100 mg/kg).
- Group 5 (EW): Ethanol-water (70%-30%) was administered as a negative control (equal volume EWEP).
- Group 6 (DMSOEP): DMSO (Tekkim)-water (50%-50%) propolis extract was administered (100 mg/kg).
- Group 7 (DMSO): DMSO water (50%-50%) was administered as a negative control (equal volume DMSOEP).
- Group 8 (OOEP): Olive oil (cold-pressed Memecik olive oil from Milas) propolis extract was administered (100 mg/kg).
- Group 9 (OO): Olive oil was administered as a negative control (equal volume OOEP).

The volume of solvents (W, EW, DMSO, OO) used for the negative control was the same as that of propolis extracts given for the experiment. Mice were euthanised by cervical dislocation under anaesthesia with 10 mg/kg xylazine hydrochloride (Rompun®, Bayer, 23.32 mg/ml, Germany) followed by 70 mg/kg ketamine hydrochloride (Ketalar®, Parke-Davis, 50 mg/ml, Germany) one hour after indomethacin administration, and their stomachs were removed and sent for macroscopic histopathological examination in 10% formaldehyde (de Barros et al., 2007; Ruiz-Hurtado et al., 2021b)

Histopathologic Analysis

Stomach samples were fixed in 10% formaldehyde solution. After fixation, the tissues were processed through an alcohol and xylol series and embedded in paraffin blocks. Sections of 3-5 µm thickness were transferred from the paraffin blocks to microscope slides, stained with haematoxylin-eosin, and then examined microscopically. In the histopathological examination, the groups were scored semiquantitatively, with slight modifications, for (1) epithelial cell loss (score: 0-3), (2) hemorrhage (score: 0-3), (3) inflammatory cell infiltration (score: 0-3), (4) lamina propria mucosal erosions (score: 0-3), (5) edema (score: 0-3). The scoring was determined as follows: 0: none, 1: light, 2: medium, 3: violent (Yang et al., 2017).

Statistical Analysis

Statistical analyses of the results were performed with the software Minitab 21.0.1. The Mann-Whitney U test was used to compare the means of the each groups. The assessment of significance levels was evaluated according to $P \leq 0.05$.

Results

The pathological results of the study were evaluated as a positive control of omeprazole, and the solvent of each propolis application was a negative control. Scores of mean histopathological results are given in Table 1 and Figure 1.

Pathological examination revealed that epithelial loss was lowest in the OMP group and highest in the EEP group among the treatment groups (Table 1 and Figure 1).

Mild epithelial loss was observed in the groups. It was seen that the OMP group was the most successful in treatment with less epithelial loss, followed by the DMSO group with more homogeneous results. With regard to bleeding, there were significant findings in the alcohol groups, whereas no bleeding was observed in the WEP group. In the OOEP, DMSOEP and OMP groups, bleeding was observed in only one animal, indicating that these groups also successfully prevented bleeding after the WEP group. The EEP group had mild to moderate inflammatory cell infiltration, whereas mild infiltration was observed in the other groups (Table 1 and Figure 2-3).

Similarly, erosion and edema were more prominent in the EEP group than in the others. When all the results were evaluated, it was determined that the scores closest to the treatment group (OMP) were found in the DMSOEP and OOEP groups, respectively. In the negative control groups (W, EW, OO, DMSO), the most prominent findings regarding all histopathological changes were observed in the EW group. In contrast, the conclusions of the OO and DMSO groups were observed to be attenuated (although not as therapeutically). It was also determined that epithelial loss in the EW group was violent, bleeding persisted, the inflammatory response continued significantly, although not very severely, and edema, particularly, was found to be violent (Table 1 and Figure 1).

In the statistical analysis of the pathological results with the mean clinical scores, it was found that the results closest to the OMP treatment group were DMSOEP and OOEP, and there was no statistical difference between them. The other treatments were found to have a negative significant difference compared to OMP, which means that they had no therapeutic effect. Both clinical scoring and pathological examination results show that DMSOEP and OOEP treatments have gastroprotective efficacy in indomethacin-induced gastric ulcers. In addition, EWEP treatment has been found to have adverse effects on epithelial loss, erosion, edema formation and bleeding (Table 1 and Figure 1).

Discussion

Gastric ulcer is a significant health problem that causes gastrointestinal complications such as bleeding and perforation in both humans and animals caused by various drugs, chemicals and stress (Ruiz-Hurtado et al., 2021a). Therefore, many studies have been conducted to determine the gastroprotective effect (De Barros et al., 2008; Ruiz-Hurtado et al., 2021a). In gastroprotective studies, the efficacy of NSAID drugs such as indomethacin or acetic acid was evaluated by using many natural agents such as propolis before ulcer formation (Liu et al., 2002; Mohafez et al., 2010; Pillai et al., 2010; El-Ghazaly et al., 2011; Abd El-Hady et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Badriyya et al., 2021; Boeing et

Table 1. Pathological results of gastric tissue [Mean (SD)]3

Treatment groups2	Mean clinical scores	Epithelial loss	Bleeding	Inflammatory cell infiltration	Erosion	Edema
OMP (+)	2.13 (0.84)	0.50 (0.54)	0.13 (0.35)	0.88 (0.35)	0.00 (0.00)	0.75 (0.46)
WEP	3.38 (1.06)	1.13 (0.35)	0.00 (0.00)	1.00 (0.00)	0.00 (0.00)	1.25 (0.89)
W(-)	5.13 (3.14)	1.25 (0.46)	0.63 (1.06)	1.63 (0.74)	0.50 (0.76)	1.13 (0.64)
EEP	7.13 (2.70)	1.63 (0.74)	1.25 (0.89)	1.63 (0.52)	1.25 (0.71)	1.50 (0.93)
WE (-)	9.38 (2.45)	2.13 (0.64)	1.63 (1.19)	1.88 (0.35)	1.13 (0.64)	2.38 (0.74)
DMSOEP	2.75 (1.04)	1.00 (0.54)	0.13 (0.35)	1.00 (0.00)	0.00 (0.00)	0.63 (0.52)
DMSO (-)	3.25 (2.25)	0.88 (0.84)	0.38 (0.52)	0.88 (0.35)	0.13 (0.35)	1.00 (0.76)
OOEP	2.88 (1.36)	0.88 (0.84)	0.13 (0.35)	0.88 (0.35)	0.25 (0.46)	0.88 (0.35)
OO (-)	4.38 (2.26)	1.25 (0.71)	0.38 (0.52)	1.00 (0.54)	0.50 (0.76)	1.25 (0.71)
P values1						
WEP	0.020	0.015	0.334	0.312	1.000	0.180
W(-)	0.010	0.010	0.227	0.022	0.008	0.201
EEP	0.010	0.010	0.005	0.004	0.002	0.060
WE (-)	0.010	0.004	0.004	0.002	0.001	0.001
DMSOEP	0.205	0.080	0.997	0.335	1.000	0.619
DMSO (-)	0.030	0.030	0.278	0.028	0.564	0.438
OOEP	0.199	0.030	0.998	0.798	0.146	0.554
OO (-)	0.020	0.031	0.279	0.590	0.040	0.116

[1]Statistical comparison of positive control omeprazole with other treatments.
[2]Omeprazole: OMP, water extract propolis: WEP, only water: W, Hydro-alcoholic extract propolis: EEP, only water-ethanol: WE, dimethyl sulphoxide extract propolis: DMSOEP, only dimethyl sulphoxide: DMSO, olive oil extract propolis: OOEP, only olive oil: OO, (+): positive treatment control, (-): negative treatment control, Standard deviation: SD, Pooled Standard deviation: P-SD
[3]n = 8 per treatment group

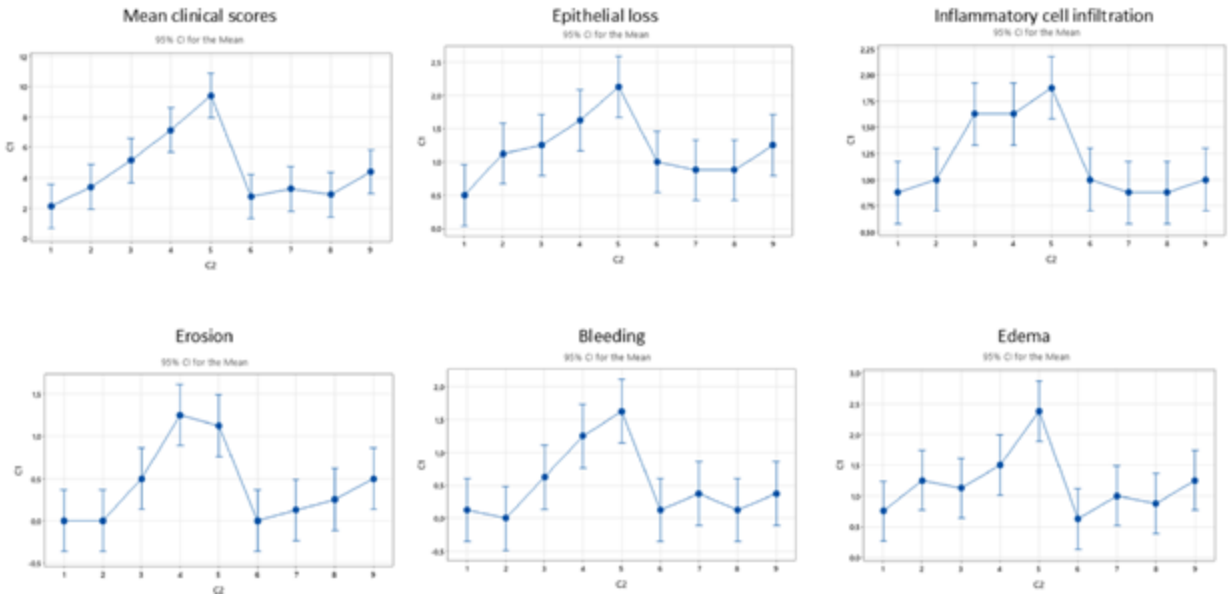

Figure 1. Means of pathology scoring results of the gastric tissue.

The pooled standard deviation is used to calculate the intervals. C1 is mean of pathological scores ± SD, C2 is treatment groups. Omeprazole: OMP, water extract propolis: WEP, only water: W, Hydro-alcoholic extract propolis: EEP, only water-ethanol: WE, dimethyl sulphoxide extract propolis: DMSOEP, only dimethyl sulphoxide: DMSO, olive oil extract propolis: OOEP, only olive oil: OO, (+): positive treatment control, (-): negative treatment control.

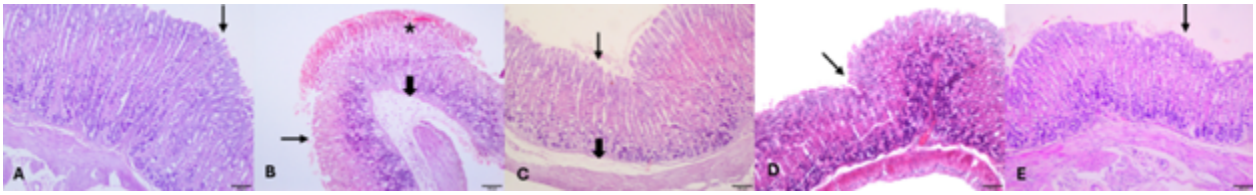

Figure 2. The histopathology of the treatments of propolis extracts and omeprazole.

A: Olive oil extract propolis, B: Hydro-alcoholic extract propolis, C: Water extract propolis, D: Dimethyl sulphoxide extract propolis E: Omeprazole, Arrow; epithelium (mucosa), Star; bleeding areas, Bold arrow; edema and cell infiltration (H&E staining, 20x).

al., 2021; Ruiz-Hurtado et al., 2021b; Boeing et al., 2023; Oyetayo et al., 2023; Sahin et al., 2023). The evaluations are compared with the solvent of the extract used with gastroprotective drugs such as proton pump inhibitor or H2 receptor antagonist, etc. and their gastroprotective activities are studied. (Liu et al., 2002; Pillai et al., 2010; Mohafez et al., 2010; El-Ghazaly et al., 2011; Abd El-Hady et al., (2010) Indian propolis similar to cimetidine. In the current study, no difference was found between propolis extracted with 70% ethanol, as in other studies, and the solvent ethanol only, except for preventing edema formation. In addition, the lowest gastroprotective effect of the four extracts was observed in the EEP when compared to omeprazole, the positive control treatment.

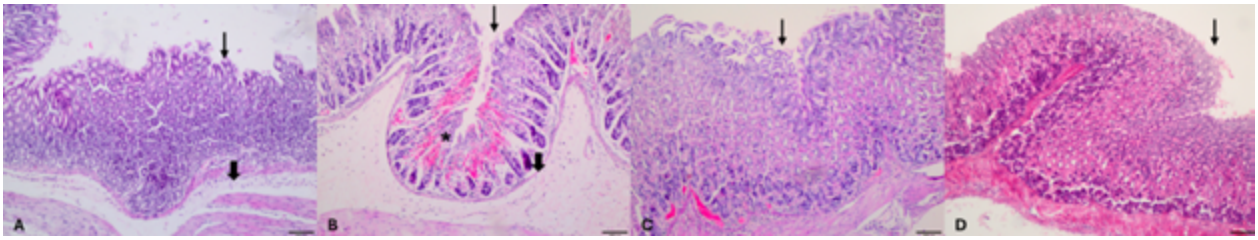

Figure 3. The histopathology of the negative controls.

A: Only olive oil, B: Only water-ethanol, C: Water, D: Only dimethyl sulphoxide, Arrow; epithelium (mucosa), Star; bleeding areas, Bold arrow; edema and cell infiltration (H&E staining, 20x).

et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Badriyya et al., 2021; Boeing et al., 2021; Ruiz-Hurtado et al., 2021b;Boeing et al., 2023; Oyetayo et al., 2023; Sahin et al., 2023). In the present study, similar to previous studies, the gastroprotective activity of four different propolis extracts was evaluated in mice using the same model. Previous studies have examined the efficacy of propolis extracted with 70% ethyl alcohol and water or propolis lyophilised after extraction with alcohol and then dissolved in water (Pillai et al., 2010; El-Ghazaly et al., 2011; Boeing et al., 2021; Sahin et al., 2023). Another study examined the efficacy of propolis extracted with acetone by dissolving in water after lyophilisation (Oyetayo et al., 2023). In all previous studies, a significant gastroprotective activity of ethanol-extracted propolis was observed (Liu et al., 2002; Mohafez et al., 2010; Pillai et al., 2010;Abd El-Hady et al., 2013; Costa et al., 2020; de Mendonça et al., 2020; Boeing et al., 2021; Ruiz-Hurtado et al., 2021b; Boeing et al., 2023; Sahin et al., 2023). Studies investigating the gastroprotective activity of propolis extracted with ethyl alcohol have shown remarkable similarities with conventional antiulcer drugs, which are Boeing et al., (2023) Brazilian red propolis, de-Mendonça et al., (2020) Brazilian red propolis, Oyetayo et al., (2023) Nigerian propolis and Ruiz-Hurtado et al., (2021b) Mexican propolis similar to omeprazole, Boeing et al., (2021) Brazilian red propolis similar to carbenoxolone, Costa et al., (2021) Brazilian green propolis similar to omeprazole, ranitidine and carbenoxolone, Abd-El Hady et al., (2013) Egyptian propolis similar to ranitidine, Mocam et al., (2024) Cameroonian propolis similar to sucralfate, Pillai Similarly, the least gastroprotective effect was observed in the EEP in the total clinical score examination, and no difference was found with the group that was administered 70% ethanol only. Many studies claim that the gastroprotective effect of propolis is due to the phenolic compounds it contains (Costa et al., 2020; de Mendonça et al., 2020; Ruiz-Hurtado et al., 2021b). Although previous studies have shown that the highest phenolic compounds were also obtained in alcohol extract propolis, the least effect was determined in EEP in present study (Kekeçoğlu and Sorucu, 2021). Badriyya et al. (2021) non-alcoholic commercial propolis was used in a study in mice against aspirin-induced gastric ulcers and showed a significant protective effect. While most studies have been carried out in rats, the present study was conducted in mice, similar to the study by Badriya et al., (2021). In addition, El-Ghazly et al., (2011) determined that water extract of propolis applied to rats against indomethacin-induced ulcers had as much protective activity as lansoprazole. In the present study, the gastroprotective activity of the water extract propolis was slight but not as strong as omeprazole. There was no statistical difference between WEP and W in terms of gastroprotection in preventing bleeding and edema. On the other hand, gastroprotective activity was observed, and there was a statistically significant difference between WEP and W in the total clinical score evaluation. Şahin et al. (2023) determined that water and ethanol extract propolis had gastroprotective effects and water extract propolis was more effective, which was the only study compared with different solvents. The fact that the water extract was

better than the ethanol extract corroborates our results. In the current study, DMSOEP and OOEP were found to be the best solvents in terms of gastroprotective effects on the stomach. In the statistical analysis, although some parameters were not found to be different with solvents when histopathological evaluations were performed separately, a significant statistical difference occurred in the overall clinical score evaluation. Although DMSO, one of these solvents, is not widely used due to some toxic effects, propolis extracted in olive oil is essential for safe use in terms of stomach protection.

Conclusion

The present study investigated and compared the gastroprotective activity of propolis extracted with various solvents. The results showed that the water extract of propolis showed a slight effect, and the ethanol extract did not. Although the gastroprotective activity of propolis is due to its phenolic compounds, the ineffectiveness of the ethanolic extract in which these substances are highly concentrated is a situation that needs to be clarified. This situation can be explained by the fact that alcohol also accelerates ulcer formation and even creates an ulcer model. However, the reason why the phenolic compounds it contains are ineffective here should be investigated. DMSO and olive oil extracts were found to be more effective than other solvents. In addition, both propolis extracts were found to have as much of a gastroprotective effect as omeprazole. In addition, the compounds in DMSO and olive oil extracts that have this effect need to be studied in the future. In conclusion, although DMSO, one of these solvents, is not widely used due to some toxic effects, propolis extracted in olive oil is important for safe use in terms of stomach protection.

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Author contribution statement

Concept and design: A.S, O.B., A.A., A.T; Data collection or processing: A.S., A.A.; Analysis or interpretation: A.A.; Literature search: A.A, O.B.; Writing: A.A, O.B, A.T.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Identification and Antifungal Susceptibility of Candida Species in Canine Oral Flora

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ABSTRACT

In this study, the presence of *Candida* species was investigated in 60 oral swab samples collected from dogs. The samples were cultured on *Candida* chromogenic agar and incubated at 30°C for 48 hours. At the end of the incubation period, growth was observed on 30 (50%) of the plates and 57 *Candida* colonies were isolated. These colonies exhibited growth as single or multiple agents on the chromogenic agar. Species identification was performed based on the colour patterns displayed on the chromogenic agar, leading to detected of six distinct *Candida* species. Among the isolates, 16 (28.1%) were identified as *Candida krusei*, 10 (17.5%) as *Candida glabrata*, 10 (17.5%) as *Candida parapsilosis*, 9 (15.8%) as *Candida utilis*, 7 (12.3%) as *Candida tropicalis*, and 5 (8.8%) as *Candida albicans*. To confirm their identification at the molecular level, all isolates were verified as belonging to the *Candida* genus using PCR with ITS3-ITS4 primers. The antifungal susceptibility of the isolates (n=57) was assessed using the disk diffusion method. The results revealed that the isolates exhibited resistance to miconazole (43.8%), ketoconazole (26.3%), flucytosine (100%), and fluconazole (93.1%), while showing high sensitivity to nystatin (93.1%). This study highlights the presence of *Candida* species in the oral flora of dogs and underscores the emergence of antifungal resistance among these isolates. The findings suggest that the presence of *Candida* species in the oral microbiota of dogs could pose a potential health risk to both animals and humans, particularly in immunocompromised individuals.

Keywords*: Antifungal, Candida, chromogenic agar, dog, identification.*

Köpek Oral Florasındaki Candida Türlerinin İdentifikasyonu ve Antifungal Duyarlılıkları

ÖZET

Bu çalışmada, köpeklerden toplanan 60 oral sürüntü örneğinde *Candida* türlerinin varlığı araştırıldı. Örnekler *Candida* kromojenik agarına ekildi ve 30°C'de 48 saat inkübe edildi. İnkübasyon süresinin ardından, petrilerin 30'unda (%50) üreme gözlendi ve 57 *Candida* kolonisi izole edildi. Bu koloniler kromojenik agarda tekli veya çoklu etken olarak üreme gösterdi. Tür tanımlaması, kromojenik agarda görüntülenen renk farklılıklarına göre yapıldı ve altı farklı *Candida* türü tespit edildi. İzolatlar arasında 16 (%28,1) *Candida krusei*, 10 (%17,5) *Candida glabrata*, 10 (%17,5) *Candida parapsilosis*, 9 (%15,8) *Candida utilis*, 7 (%12,3) *Candida tropicalis* ve 5 (%8,8) *Candida albicans* olarak tanımlandı. Moleküler düzeyde tanımlanmalarını doğrulamak için tüm izolatların ITS3-ITS4 primerleri ile PCR kullanılarak *Candida* cinsine ait olduğu doğrulandı. İzolatların (n=57) antifungal duyarlılığı disk difüzyon yöntemi kullanılarak değerlendirildi. Sonuçlar izolatların mikonazole (%43,8), ketokonazole (%26,3), flusitozine (%100) ve flukonazole (%93,1) direnç gösterirken, nistatine (%93,1) yüksek duyarlılık gösterdiğini ortaya koydu. Bu çalışma, köpeklerin ağız florasında *Candida* türlerinin varlığını vurgulamakta ve bu izolatlar arasında antifungal direncin ortaya çıktığını vurgulamaktadır. Bulgular, köpeklerin ağız mikrobiyotasında *Candida* türlerinin varlığının, özellikle bağışıklık sistemi baskılanmış bireylerde hem hayvanlar hem de insanlar için potansiyel bir sağlık riski oluşturabileceğini düşündürmektedir.

Anahtar kelimeler: *Antifungal, Candida, identifikasyon, köpek, kromojenik agar*

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Introduction

The oral microbiota of both humans and animals consists of a diverse array of bacteria and yeasts (Willis and Gabaldon, 2020). Dogs, like humans, harbor a variety of yeasts in their oral mucosa, and their colonisation patterns and pathogenic potential require further investigation. Despite the critical role of the oral microbiota in maintaining canine health, research on the isolation and correct identification of yeasts began in the 20th century (Brito et al., 2009). The oral microbiota in dogs is highly diverse and complex, making it challenging to fully characterize. Fungal colonization of the oral cavity in canines is primarily associated with yeasts of the genera *Candida* spp., *Malassezia pachydermatis*, *Trichosporon* spp., *Rhodotorula* spp. and *Geotrichun* spp. with yeasts of the genus *Cryptococcus* being isolated less frequently (Jin and Lin, 2005; Bentubo et al., 2010; Brilhante et al., 2018). The genus *Candida* exhibited a high prevalence, constituting 82.2% of the isolated yeast profile. Notably, *Candida zeylanoides*, a rare species even in humans, was isolated from the oral mucosa of dogs, suggesting that this fungus may represent a new "ecological niche" in canine microbiota, with potential for opportunistic pathogenicity (Navarro et al., 2020).

Out of a total of 100.000 yeast species, approximately 200 are deemed pathogenic, with 50 of them commonly associated with mycoses. Yeasts are responsible for most mycoses in humans and animals, with *Candida*, *Cryptococcus*, *Malassezia*, and *Trichosporon* being the most notable genera (Watkinson et al., 2015). Pathogenic yeasts may induce anorexia and adipsia in animals, leading to compromised immunity and clinical complications (Navarro et al., 2020). Yeasts are symbiotic inhabitants of the canine microbiota, but they can become pathogenic under certain conditions and cause discomfort and pain (Jadhav and Pal, 2006; Brito et al., 2009).

Candida is a fungus that can form pseudo hyphae and reproduce through budding, with a size of 5µ and an oval or round shape depending on its subspecies and environmental conditions. Hippocrates and Galen first described it as an oral lesion in the 4th century BC. In 1839, Bernhard Rudolph Konrad von Langenbeck obtained an organism from the oesophageal mucosa of a patient who had died of typhus and initially thought it was a parasite that caused typhus, which he called "Typhus Leichen" (typhus bodies) (Talay and Odabaş, 2002). Finally, in 1923, Roth Berkhout demonstrated that this organism was not a species of Monilia and proposed that it be named "*Candida*". In the 1800s and early 1900s, there was some confusion and misclassification in naming, but the binomial classification of *Candida albicans* was fully adopted in 1954. Recently, there have been increasing reports of higher incidences of non- *albicans Candida* species mentioned above (Williams and Lewis, 2011). In addition to *C. albicans*, other important species isolated from clinical infections include *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, and *C. tropicalis* (Patil et al., 2015). The significant phenotypic variability observed in *Candida* species, and the rise in antifungal resistance among strains, has emerged as a major clinical concern (Navarro et al., 2020).

The prolonged use of antibiotics and a high intake of carbohydrates in the diet may destroy or suppress the competitive bacterial microbiota, disturbing its balance with the host organism and allowing for the excessive growth of yeasts (Lacaz et al., 2002). Senility, likely exacerbated by poor oral hygiene practices in dogs throughout their lifetime and other predisposing factors as mentioned earlier, is considered a significant condition predisposing to periodontal disease. A study involving stray dogs found that animals over 4 years of age were more susceptible to developing this disease, ranging from mild gingivitis to severe periodontitis (Paula et al., 2021).

Early detection of potentially pathogenic yeasts associated with these organisms in the oral microbiota is crucial, as it provides additional clinical assistance for disease diagnosis and treatment (Kurtzman et al., 2011).

The incidence of yeast mycoses has significantly increased, rendering it a significant public health concern, particularly in systemic clinical conditions and hospital-acquired infections. Antifungal medications used in human and veterinary medicine exhibit specific characteristics concerning their chemical structure and mechanism of action, directly or indirectly interfering with the fungal cell and producing fungistatic or fungicidal effects (Paula et al., 2021).

The main classes of antifungal agents used for the treatment of invasive fungal infections include polyene antifungals (such as amphotericin B), azoles (including fluconazole, voriconazole, ketoconazole, itraconazole, and posaconazole), pyrimidines (such as 5-fluorocytosine), and echinocandins (such as caspofungin and micafungin) (Pappas et al., 2009). This study, investigated the presence of *Candida* species in 60 samples of canine oral swabs. The aim of this study was to diagnose the *Candida* species present in the oral flora of dogs and to determine the antifungal susceptibility of these species.

Materials and Methods

Samples

A total of 60 healthy dogs (32 females and 28 males) were randomly selected for the study from a sample of dogs presented to various veterinary clinics in Aydın. Sterile oral swab samples were obtained from different regions of the oral cavity, including the gingival mucosa, dental biofilm, and periodontal sulcus (Santin et al., 2013). The samples were transported under cold chain conditions (+4°C) to the Faculty of Veterinary Medicine Microbiology Laboratory at Aydın Adnan Menderes University for further analysis.

Phenotypic Identification

The oral swab samples were inoculated onto HiCrome™ Candida Differential Agar (Hi-Media, India) for the isolation of *Candida* spp. and incubated at 30°C for 48 hours. Gram staining was performed on the colonies grown on the chromogenic agar, and they were identified as *Candida* spp. based on their size, shape, and

budding characteristics observed in the Gram staining, before being subjected to colony typing. The colonies identified as *Candida* spp. were further identified based on the colour of their colonies on chromogenic agar: *C. albicans*-light green, *C. tropicalis*-metallic blue, *C. glabrata*-cream-white, *C. krusei*-purple, *C. parapsilosis*-light purple, and *C. utilis*-light pink (Brito et al., 2009).

Genotypic Identification

The colonies identified as *Candida* spp. were subcultured onto Sabouraud Dextrose Agar (Hi-Media, India) and incubated at 30°C for 48 hours. DNA was extracted from purified colonies using a MagAttract HMW DNA extraction kit (Qiagen, Netherlands). PCR analyses using ITS3 and ITS4 primers were performed for molecular identification of *Candida* spp. The field strain confirmed as *C. albicans* by Sanger sequencing method was used as positive control and sterile deionized water was used as negative control. For this purpose, Taq Premix (2x) 10 µl, ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (100 pmol) 0.2 µl each, $MgCl_2$ (50 mM) 0.5 µl, 5 µl DNA, and ddH_2O were added to a total volume of 20 µl for each sample. For amplification, thermal cycling was performed using thermal cycler with an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 4 minutes. Following the PCR analysing, the resulting amplicons were electrophoresed on a 2% agarose gel containing ethidium bromide at 80V for 40 minutes. Bands between 250-500 bp on the gel in the imaging system (Vilber Lourmat, Germany) were considered *Candida* spp. (Fujita et al., 2001).

Antifungal Susceptibility Test

The susceptibility of *Candida* isolates identified phenotypically and genotypically was evaluated using the disk diffusion method. Colonies were inoculated into Brain Heart Infusion Broth (Hi-Media, India) and adjusted to a density of 0.5 MacFarland. The isolates with their densities adjusted were inoculated onto Mueller Hilton Agar No.2 (Hi-Media, India) and the indicated disks were placed, followed by incubation at 30°C for 48 hours (CLSI, 2018). In this study, resistance to three different antifungal groups was investigated, namely the polyene macrolide group, the azole group, and the pyrimidine group. The active substances used were nystatin (100 U) from the polyene macrolide group; ketoconazole (10 µg) and miconazole (10 µg) from the imidazole class of the azole group; fluconazole (10 µg) from the triazole class of the azole group; and flucytosine (1 µg) from the pyrimidine group.

Results

In this study, 60 oral swab samples from dogs were inoculated onto HiCrome™ Candida Differential Agar (Hi-Media, India). The *Candida* colonies were identified based on the colours they produced on chromogenic agar. After incubation, colony growth was observed in 30 (50%) of the petri dishes containing samples taken from female dogs, with colony growth being detected in 17 (53.1%) of these samples, and in 13 (46.4%) of the male dogs' samples. Six different *Candida* species were diagnosed from the 57 colonies obtained. Among the isolated colonies, 16 (28.1%) were purple, 10 (17.5%) were white-cream, 10 (17.5%) were light purple, 9 (15.8%) were light pink, 7 (12.3%) were metallic blue and 5 (8.8%) were light green in colour. The colonies with a purple colour were identified as *C. krusei*, white-cream as *C. glabrata*, light purple as *C. parapisilosis*, light pink as *C. utilis*, metallic blue as *C. tropicalis*, and light green as *C. albicans* (Figure 1).

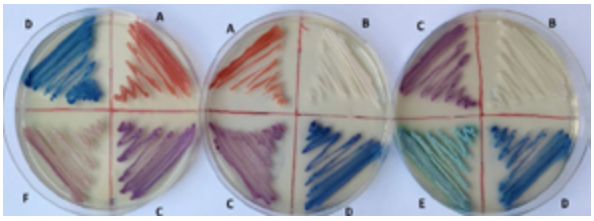

Figure 1. *Candida* colonies colours and morphologies on Candida chromogenic agar.
A. *Candida utilis*; B. *Candida glabrata*; C. *Candida krusei*; D. *Candida tropicalis*; E. *Candida albicans;* F. *Candida parapsilosis*

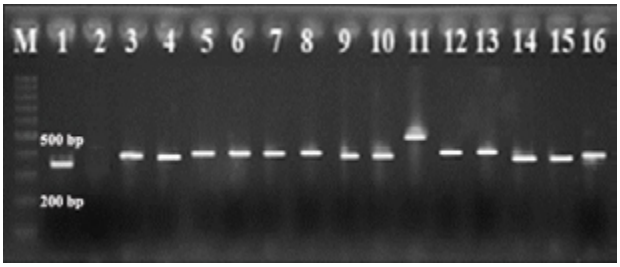

Figure 2. ITS3-ITS4 PCR analysis electrophoresis image of *Candida* species.
M: Marker; 1: Positive control; 2: Negative control; 3-16: *Candida* spp. isolates

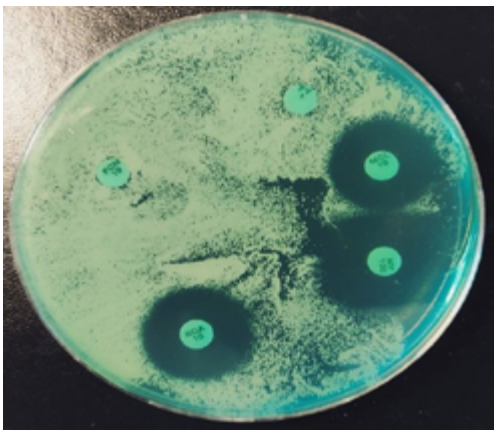

Figure 3. Imagine of the antifungal disk diffusion method performed on Mueller Hinton Agar.
FCN: Flucytosine; FY: Fluconazole; MCL: Miconazole; NY: Nystatin; KCA: Ketoconazole

The growth patterns of *Candida* colonies on chromogenic agar were observed to be either single or multiple. Fourteen petri dishes exhibited growth of a single *Candida* species, 8 petri dishes exhibited growth of two *Candida* species, 5 petri dishes exhibited growth of three *Candida* species, and 3 petri dishes exhibited growth of four *Candida* species (Table 1).

Table 1. Distribution of the isolated *Candida* isolates.

Sample number	Age	Gender	*C. krusei*	*C. parapsilosis*	*C. glabrata*	*C. utilis*	*C. tropicalis*	*C. albicans*
1	2	Female	+		+	+		+
2	2	Female	+		+		+	+
3	2.5	Male	+	+		+	+	
4	2.5	Female	+		+			+
5	2.5	Female		+		+		+
6	2.5	Female	+		+	+		
7	2	Female			+	+	+	
8	2	Male		+	+	+		
9	2	Female	+		+			
10	2.5	Male	+		+			
11	2	Male	+				+	
12	2	Male	+				+	
13	2	Female		+		+		
14	2.5	Male		+		+		
15	2.5	Female	+					+
16	3	Female			+	+		
17	2.5	Female	+					
18	2.5	Female	+					
19	2.5	Male	+					
20	2	Female		+				
21	2	Male		+				
22	2.5	Male		+				
23	2.5	Female		+				
24	2.5	Female		+				
25	2	Female		+				
26	2	Male		+				
27	1.5	Male		+				
28	2	Female					+	
29	2	Male					+	
30	2.5	Male			+			

Fifty-seven Candida isolates were subjected to phenotypic identification, and PCR analyses revealed that all of them produced bands in the 250-500 bp range (Figure 2). Antifungal susceptibility testing was performed on 57 isolates that were genotypically confirmed as *Candida* spp. (Figure 3). It was observed that four isolates identified as *C. krusei* exhibited resistance to all tested antifungal agents. Resistance to the pyrimidine group was detected in 100% of the isolates (n=57). Among the azole antifungal groups, the triazole class demonstrated the highest resistance rate (93.1%), whereas the polyene macrolide group exhibited the highest sensitivity (93.1%). An analysis of antifungal susceptibility based on active compounds revealed that all isolates (n=57) were 100% resistant to flucytosine, while 93.1% (n=53) were resistant to fluconazole, 43.8% (n=25) to miconazole, and 26.3% (n=15) to ketoconazole. In contrast, 93.1% (n=53) of the isolates were susceptible to nystatin. Notably, four isolates resistant to nystatin were identified as *C. krusei*. Additionally, all isolates were determined to have developed resistance to at least two antifungal agents.

Discussion

The present study provides critical insights into the prevalence and antifungal susceptibility of *Candida* species isolated from the oral flora of dogs, with notable implications for both veterinary medicine and public health. The findings highlight the diversity of *Candida* species in canine oral samples, the high prevalence of antifungal resistance, and potential zoonotic risks.

In this study, *C. krusei* (28.1%) was identified as the most prevalent species, contrasting with other studies such as

Navarro et al. (2020), who reported *C. albicans* as the dominant species (39.5%). Interestingly, our findings show *C. albicans* as the least frequent isolate (8.8%), which may be attributable to differences in geographical location, sampling techniques, or the health status of the dogs studied. Furthermore, the distribution of species such as *C. parapsilosis* (17.5%) and *C. tropicalis* (12.3%) aligns with studies by Brito et al. (2009), who reported similar prevalence rates in canine oral samples, though with minor variations. These discrepancies may reflect environmental factors, host-specific microbiota dynamics, or methodological differences between studies.

The detection of antifungal resistance in this study raises significant concerns. Notably, all isolates resisted at least at least two antifungal agents, with 100% resistance observed against flucytosine. This is consistent with findings by Olabode et al. (2016), who reported high flucytosine resistance rates in *Candida* species isolated from dogs. Resistance to fluconazole was also alarmingly high (93.1%), corroborating findings by Brilhante et al. (2015), who documented fluconazole resistance in *C. tropicalis* isolates from animal sources. The high prevalence of fluconazole resistance may be linked to the widespread use of azoles in both human and veterinary medicine, potentially contributing to cross-resistance in *Candida* populations. This underscores the need for judicious use of antifungal agents and implementing antimicrobial stewardship programs in veterinary settings.

Interestingly, *C. krusei* isolates in this study exhibited resistance to all antifungal groups, highlighting their inherent resistance to certain antifungal agents, particularly azoles. This finding is concerning, as *C. krusei* is known for its reduced susceptibility to conventional antifungal treatments (Navarro et al., 2020). The observation that nystatin retained the highest sensitivity (93.1%) among the antifungal agents tested is promising, particularly for managing infections caused by *Candida* species in veterinary practice. Similar findings by Yurayart et al. (2013) reinforce the utility of polyenes like nystatin as effective antifungal agents for resistant *Candida* strains.

The identification of multiple *Candida* species in single samples suggests a complex fungal microbiota within the canine oral cavity. This polymicrobial nature of *Candida* infections has previously been documented by Živković et al. (2013), who found mixed infections in dogs with stomatitis. In our study, 43.3% of the samples exhibited polymicrobial growth, with up to four *Candida* species isolated from a single sample. This diversity has significant clinical implications, as co-infections may contribute to antifungal resistance and complicate treatment strategies.

From a zoonotic perspective, the findings underscore the potential risk of cross-species transmission of resistant *Candida* strains. Dogs, as companion animals, share close physical contact with humans, facilitating the exchange of microorganisms. Previous studies (Kobayashi et al., 2008) have highlighted the potential for zoonotic transmission of *Candida* species, particularly in immunocompromised individuals. In this context, the high prevalence of antifungal resistance observed in this study warrants further investigation into the role of dogs as reservoirs of resistant fungal pathogens.

Despite the comprehensive nature of this study, certain limitations should be acknowledged. The sample size, although sufficient to identify trends, may not fully represent the broader canine population. Additionally, the lack of clinical data on the health status of the dogs limits the ability to correlate *Candida* prevalence and antifungal resistance with specific host factors. Future studies should aim to include larger sample sizes, incorporate detailed clinical histories, and investigate molecular mechanisms underlying antifungal resistance.

Conclusion

In conclusion, this study highlights the diversity and antifungal resistance of *Candida* species in dog's oral flora, with important implications for both veterinary and public health. The high resistance rates observed underscore the urgent need for antimicrobial stewardship and the development of alternative antifungal strategies. Given the close interaction between humans and dogs, the zoonotic potential of resistant Candida strains cannot be ignored. Further research is needed to better understand the dynamics of cross-species fungal transmission and resistance evolution.

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Author contribution statement

Concept: OD, HTYD; Design: OD, HTYD; Data Collection or Processing: OD, HTYD, YS; Analysis or Interpretation: OD, HTYD, SK; Literature Search: OD, HTYD, YS, SK; Writing: OD, HTYD.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Evaluation of Oxidative Stress Parameters in Leptospira Infected Sheep

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ABSTRACT

Leptospirosis is a bacterial infection with zoonotic character which is widespread all over the world, especially in humid and temperate regions and poses a serious threat to public health. In this study, 94 female sheep blood sera sent to Samsun Veterinary Control Institute Directorate with suspicion of leptospirosis were firstly subjected to ELISA (Enzyme Linked Immunosorbent Assay) test for leptospirosis antibodies. total thiol, native thiol, disulphide, native thiol/total thiol, disulphide/native thiol, disulphide/total thiol, malondialdehyde (MDA) levels and adenosine deaminase (ADA) activity were evaluated in the samples.

According to the results of the ELISA test performed in our study, 16 female sheep serum samples were found to be positive for *Leptospira interrogans* ser. hardjo, while 78 samples were found to be negative. To evaluate the effect of leptospirosis on oxidative stress parameters, 15 positive serum samples and 15 negative serum samples taken from healthy sheep were used as control group. According to the results of biochemical analyses, total thiol, native thiol, disulphide, native thiol/total thiol, disulphide/native thiol, disulphide/total thiol values in the *Leptospira* group were not significantly different from those in the control group. However, MDA values and ADA activity in the *Leptospira* group were significantly higher than those in the control group ($P<0.001$). As a result, it was determined that oxidant-antioxidant balance was disturbed in sheep infected with *Leptospira* and oxidative stress occurred as a result. Our findings suggest that MDA and ADA activity can be used as a biomarker in the diagnosis of leptospirosis and may give an idea about the severity of the disease.

Keywords: *Blood sera, Leptospira, Oxidative stress, Sheep.*

Leptospira ile Enfekte Koyunlarda Oksidatif Stres Parametrelerinin Değerlendirilmesi

ÖZET

Leptospirozis tüm dünyada özellikle de nemli ve ılıman bölgelerde yaygın olarak görülen halk sağlığını ciddi boyutta tehdit eden zoonotik karakterli bakteriyel bir enfeksiyondur. Bu çalışmada Samsun Veteriner Kontrol Enstitüsü Müdürlüğüne leptospirozis şüphesi ile gönderilen 94 adet koyun kan serumu öncelikle leptospirozis antikorları yönünden ELISA testine tabi tutuldu. Alınan numunelerde oksidatif stres parametrelerinden toplam tiyol, natif tiyol, disülfit, natif tiyol/toplam tiyol, disülfit/natif tiyol, disülfit/toplam tiyol, MDA düzeyleri ve ADA aktivitesi değerlendirilmiştir.

Çalışmamızda yapılan ELISA testi sonuçlarına göre 16 adet koyun serum örneği *Leptospira interrogans* ser. hardjo yönünden pozitif olarak tespit edilirken, 78 adet örnek ise negatif olarak tespit edilmiştir. Leptospirozisin oksidatif stres parametreleri üzerindeki etkisini değerlendirmek amacıyla 15 adet pozitif serum örneği ve kontrol grubunu oluşturmak amacıyla sağlıklı koyunlardan alınan 15 adet negatif serum örneği kullanıldı. Biyokimyasal analiz sonuçlarına göre; *Leptospira* gruptaki toplam tiyol, natif tiyol, disülfit, natif tiyol/toplam tiyol, disülfit/natif tiyol, disülfit/toplam tiyol değerleri kontrol gruptaki değerler ile karşılaştırıldıklarında anlamlı düzeyde bir değişiklik bulunamamıştır. Fakat *Leptospira* gruptaki MDA değerleri ve ADA aktivitesi kontrol gruptaki değerlere göre anlamlı derecede yüksek bulunmuştur ($P<0.001$). Sonuç olarak *Leptospira* ile enfekte koyunlarda oksidan-antioksidan dengesinin bozulduğu ve bunun sonucunda oksidatif stresin meydana geldiği belirlendi. Bulgularımız MDA ve ADA aktivitesinin leptospirozis tanısında bir biyobelirteç olarak kullanılabileceğini ve hastalığın şiddeti hakkında fikir verebileceğini düşündürmektedir.

Anahtar kelimeler: *Kan serumu, Koyun, Leptospira, Oksidatif stres.*

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Introduction

Leptospirosis is a zoonotic infection that is common worldwide and has serious negative effects on the economies of countries indirectly as well as direct effects on human and animal health (Ellis, 2014; Haake and Levett, 2015). The infection is caused by *Leptospira* species with high genetic diversity (Reller et al., 2014; Picardeau, 2017). *Leptospira* is a Gram-negative aerobic, double-membraned, periplasmic endoflagellum, slender, hook-shaped and highly motile bacterium in the spirochete family (Picardeau, 2017). *Leptospiras*, which are classified as pathogenic, intermediate and saprophytic according to their different levels of pathogenicity in humans and animals (Adler and Moctezuma, 2009) currently have 87 species (LPSN, 2024) and around 300 antigenic serovars (Levett, 2014). Pathogenic *Leptospira* species are the most common causes of leptospirosis worldwide. On the other hand, *Leptospira* species classified as intermediate are potential pathogens that can cause mild infections. Saprophytes are widespread in the environment and are not pathogenic (Guglielmini et al., 2019; Vincent et al., 2019). Leptospirosis remains a serious public health problem in temperate, subtropical and tropical climates due to its wide range of hosts, including humans, domestic and wild animals, which can be reservoir and incidental hosts (Picardeau, 2017; Bertelloni et al., 2019). Reservoir hosts are asymptomatic carriers who excrete *Leptospira* into the environment in urine. The most common cause of leptospirosis is accidental contact with *Leptospira*-infected urine (Adler and Moctezuma, 2009). Therefore, the epidemiology of *Leptospira* is often closely related to the reservoir host of the *Leptospira* serovar (Blasdell et al., 2019). For example, the reservoir hosts of *L. icterohaemorragie* are mice, *L. pomona* are pigs, *L. bratislava* are pigs and horses, *L. canicola* are dogs and *L. hardjo* are cattle and sheep. (Arent et al., 2013; Arent et al., 2015; Arent et al., 2016; Arent et al., 2017a, Arent et al., 2017b). Clinical manifestations of leptospirosis in humans can range from febrile conditions such as mild influenza to severe and fatal symptoms such as internal organ haemorrhages and multiple organ failure. In the acute phase of leptospirosis, patients have symptoms such as renal failure, jaundice, severe pulmonary haemorrhage syndrome (SPHS) and meningitis (Haake and Levett, 2015). Leptospirosis in sheep shows symptoms such as high fever, severe weakness, haemorrhagic and anaemic syndrome in the acute period (Adler and Moctezuma, 2009; Ellis, 2014), in the chronic phase, it causes abortion, premature pup mortality, infertility and severely reduced milk production (Ellis, 1994; Lilenbaum et al., 2009). This zoonotic disease infects approximately 1 million people annually and causes 60000 deaths (Costa et al., 2015). However, the absence of specific symptoms of leptospirosis is thought to cause confusion with other infectious diseases with similar symptoms and thus underdiagnosis. Considering the complex epidemiological cycle of leptospirosis, the development of more sensitive and specific diagnostic methods for the management of acute infections and the detection of subclinically infected reservoir hosts is critical for disease control. Direct detection of active infection is performed by microbiological and molecular methods such as culture, histopathological examination, immune histochemistry and nucleic acid amplification tests (NAAT). Serological methods such as microscopic agglutination test (MAT), ELISA and lateral flow methods are used for the detection of *Leptospira spp.* antibodies. The diagnosis of leptospirosis should be based on potential exposure, clinical presentation, laboratory values such as hemogram and biochemistry, and the results of multiple tests (Nally et al., 2020; Philip et al., 2020). The roles of direct and indirect diagnostic methods in detecting the disease are interdependent. While direct detection methods are more sensitive in the acute phase of infection, indirect, i.e. serological detection methods have higher sensitivity in the chronic phase. A combination of direct and indirect diagnostic methods is necessary to establish the true prevalence of infection in specific host species. For serological and molecular diagnostic methods to be used correctly and effectively, factors such as which *Leptospira* species and serovars will be investigated, the characteristics of the epidemic and the affected species should be well known. In this way, more precise results can be obtained, supported by advanced methods such as whole genome sequencing. Isolation of *Leptospirae* presents technical challenges due to the complexity of non-selective media, the slower growth of *Leptospirae* compared to other organisms that may contaminate the media, and the need for dark field microscopy to reliably assess culture positivity. Recently developed selective media in culture approaches (Chakraborty et al., 2011) and suitable media for sensitive organisms have partly overcome these difficulties (Hornsby et al., 2020).

New diagnostic tests are needed for rapid and accurate diagnosis of acute infections. Initiating interventions such as antibiotics and supportive treatment early in the disease is critical in treating the infection. Improved diagnostic methods for surveillance and control in animals are needed to assess the effectiveness of eradication of renal tubular or genital infections following antimicrobial therapy (Ellis et al., 1985). For this reason, in recent years, many researchers have been investigating the usefulness of oxidative stress parameters in the diagnosis of infectious and non-infectious diseases. Oxidative stress plays an important role in the pathophysiology of many diseases that threaten animal health such as sepsis, mastitis, acidosis, ketosis, enteritis, pneumonia, respiratory and joint diseases (Lykkesfeldt and Svendsen, 2007; Celi, 2011). Microorganisms such as bacteria, viruses (Valyi-Nagy and Dermody, 2005) and parasites (Stocker et al., 1985) can increase the production of reactive oxygen radicals (ROS) by disrupting metabolic processes in host cells. Leptospirosis is a toxin-mediated infection that causes lipid peroxidation because of the cytotoxic effect of the membrane lipopolysaccharide in the structure of *Leptospira* bacteria on the host cell (Kim et al., 1997).

Therefore, oxidative stress is thought to play a role in the pathogenesis of leptospirosis.

The aim of this study was to evaluate the effect of leptospirosis on oxidative stress parameters in sheep.

Materials and Methods

Calculation of Sample Size

In this study, the sample size was calculated using the formula proposed by Daniel W.W. based (Daniel, 1999) on a 95% confidence level, an estimated prevalence (P) of 6%, and a 5% margin of error (d = 0.05), the minimum required sample size was determined to be 92.

Study Material

The material of our study consisted of 94 female sheep blood serum samples sent to Samsun Veterinary Control Institute Directorate with suspicion of Leptospirosis (showing haematuria and icterus findings during clinical examination) between 2023-2024. This study was carried out with the permission of the Local Ethics Committee for Animal Experiments of Samsun Veterinary Control Institute Directorate with the letter dated 28.01.2025 and numbered 19572899/031-92.

Serological Analysis

Serum samples were tested for *Leptospira* with a commercial antibody ELISA test kit (BT LAB Sheep *Leptospira* IgG Antibody Test Kit). The test kit and serum samples were kept at room temperature for 30 minutes before starting the test. 50 µl of negative control was added to the negative control well, 50 µl of positive control was added to the positive control well, 40 µl of sample diluent and then 10 µl of serum sample were added to the wells to be used for samples and mixed thoroughly. The prepared plate was incubated at 37°C for 30 minutes. After the first incubation, the plate was washed 5 times with washing solution. After washing, 50 µl HRP (Horse Radish Peroxidase) conjugate was added to each well of the plate and the plate was incubated again at 37°C for 30 minutes. After incubation, washing was performed again. 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well of the plate and incubated at 37°C for 10 min in the dark. After this final incubation of the assay, 50 µl of stop solution was added to each well of the plate and the optical density (OD) values of the wells were read at 450 nm on an ELISA reader (Mindray MR-96A). The OD values obtained were evaluated as positive or negative according to the evaluation criteria specified in the test kit below,

Cutoff Value=Negative Control value + 0.15

OD Value<Cutoff value is negative,

OD Value≥Cutoff value was considered positive.

Biochemical Analysis

Native thiol (Rel Assay) 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 spectrophotometric method described by Erel and Neselioglu (2014) was used for measurement. In this method, the disulphide bonds formed because of oxidation were converted to optional thiol groups by $NaBH_4$/sodium borohydride. Unused sodium borohydride was removed by binding with formaldehyde. The amount of native thiol and the amount of total thiol formed by the reduction of disulphide bonds were measured with DTNB (5,50-dithiobis-(2 nitrobenzoic acid) chromogen at 412 nm in ELISA plate reader. When calculating the amount of disulphide, native thiol was subtracted from total thiol and half of the difference was taken.

Malondialdehyde (MDA) analysis was measured by the method reported by Yoshioka et al (Yoshioka et al., 1979). The lipid content in the test thiobarbituric acid (TBA) reaction was carried out by the formation of a stable red-pink colour with a minimum peak at 535 nm when heated at low pH and in the presence of TBA. Red-pink colour, chromogen formed by the combination of an MDA molecule and two TBA molecules. Some of the MDA was formed during peroxidation, the majority was formed by the breakdown of LPO during the heating phase after acidification of the medium. The test was performed spectrophotometrically. Adenosine deaminase (ADA) activity was measured by Giusti method (Giusti, 1974). Using the ammonium ion released from adenosine by enzyme action, the formation of indophenol complex because of Bertholet Reaction and measurement of this complex at 623 nm in a spectrophotometer device was carried out according to the principle.

Statistical Analysis

All statistical calculations of the data were performed using SPSS statistics 27.0 (IBM SPSS Inc, Chicago, IL, USA) package programme. Shapiro-Wilk test was used to evaluate whether the data were normally distributed. It was determined that the data showed normal distribution. T test was used to determine the differences between groups in all data. $P<0.05$ was considered statistically significant.

Results

As a result of the ELISA test performed on 94 female sheep blood sera, 16 (17.02%) serum samples were positive for *L. interrogans ser. hardjo* and 78 (82.98%) were negative. As a result of the biochemical analyses performed on 15 *Leptospira* positive serum samples and 15 *Leptospira* negative control group; Total thiol, native thiol, disulphide, native thiol/total thiol, disulphide/native thiol, disulphide/total thiol values in the *Leptospira* group were not significantly different from the values in the control group. However, MDA activity and ADA in the *Leptospira* group were significantly higher than the values in the control group ($P<0.001$). The positivity and negativity of the samples are given in Table 1, oxidative stress parameter results are given in

Table 1. Positive and negative status of the samples

Total Number of Samples	Number of Positive Samples	Number of Negative Samples
94	16 (17.02%)	78 (82.98%)

Table 2 and measurement graphs of these parameters are given in Figures 1, 2 and 3.

Table 2. Oxidative stress parameter values of *Leptospira* positive and control groups*

Parameters	Healthy Group	Leptospirosis Group	P values
Total thiol (µmoL/L)	246.63±64.06	264.57±49.98	0.449
Native thiol (µmoL/L)	112.81±29.20	99.98±33.60	0.338
Disulphide (µmoL/L)	66.91±30.59	82.34±24.07	0.180
Native thiol/Total thiol (%)	47.98±17.00	38.04±11.19	0.097
Disulphide/Native thiol (%)	65.32±36.84	91.74±39.57	0.111
Disulphide/Total thiol (%)	26.00±8.50	30.97±5.59	0.097
Malondialdehyde (µmol/mL)	0.96±0.20	1.91±0.53	<0.001
Adenosine deaminase (U/L)	6.45±1.21	10.77±2.72	<0.001

*Data are presented as mean ± standard error of the mean (SEM).

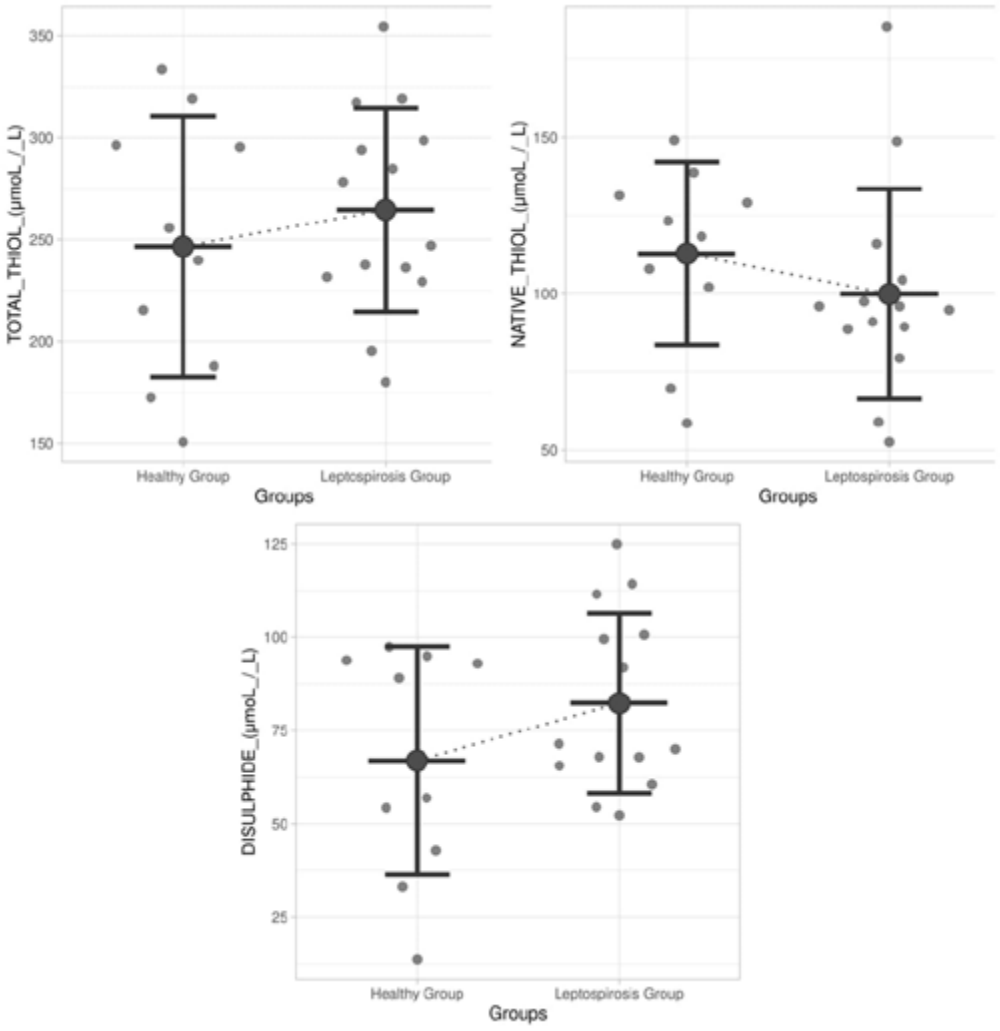

Figure 1. Serum native thiol, total thiol and disulphide values

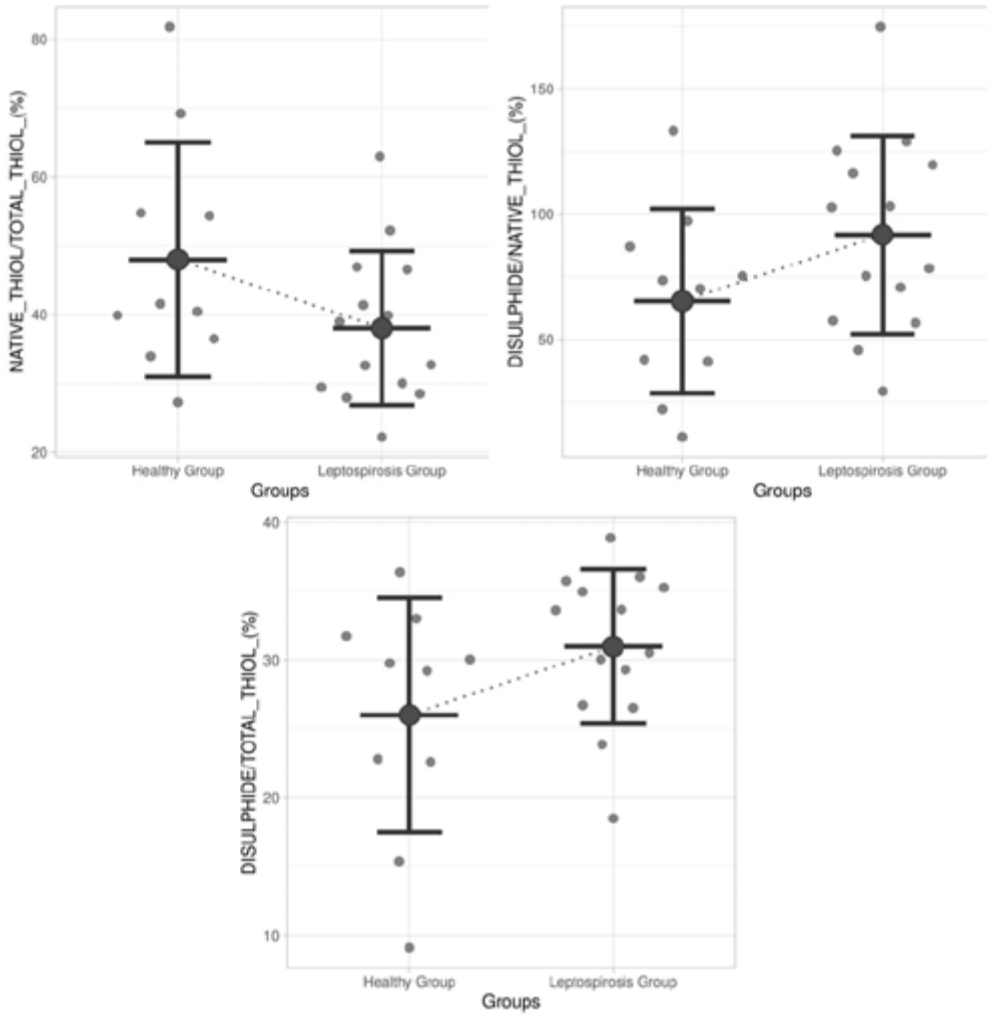

Figure 2. Serum native/total thiol (%), disulphide/native thiol and disulphide/total thiol (%) values

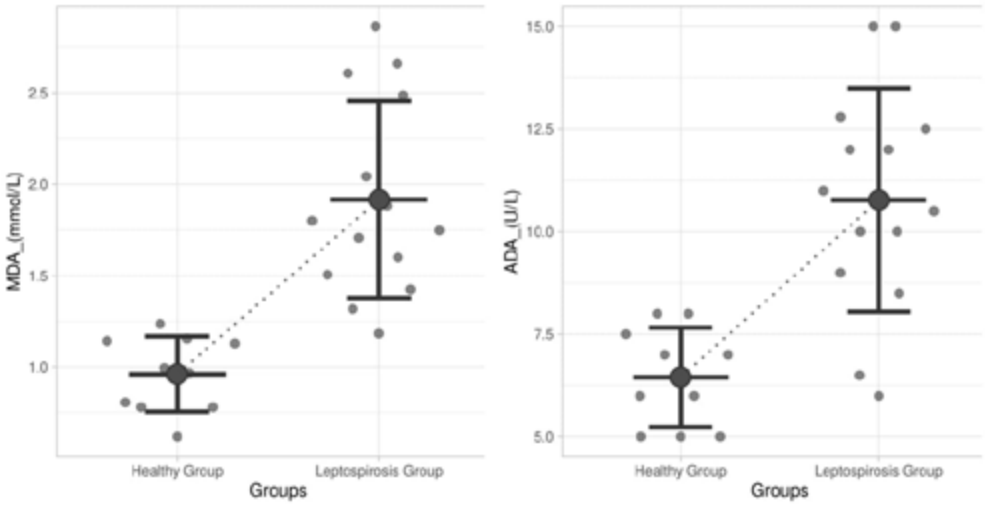

Figure 3. Serum MDA and ADA values

Discussion

The incidence of leptospirosis, which has zoonotic character and seriously threatens human and animal health in the world and in our country, varies from country to country and from region to region. There are studies on the prevalence and seropositivity of leptospirosis in our country. The seropositivity rate reported in serological studies in cattle is between 14% and 38.6%. Although there are not many serological studies on leptospirosis in sheep in our country, the rates reported in a few serological studies are between 2.9% and 8% (Harran et al., 2022). In our study, the seropositivity rate was found to be 17.02%. Our seropositivity rate was higher than other studies in our country. This may be because the mate-

rials used in our study were obtained from sick sheep showing symptoms of leptospirosis. The seropositivity rate reported in serological studies in cattle in European countries is between 0% and 100% (Sohm et al., 2023). In serological studies conducted in different countries around the world, it has been reported that the seropositivity rate of leptospirosis in sheep is between 0% and 60.7% (Antoniolli et al., 2024). It is thought that the reason for such differences between the results of the studies may be differences such as climate, geographical region, sampling times, selection methods of the samples and the reasons for taking the samples.

Diagnostic methods of bacterial, viral, fungal and tumoral diseases are updated every day. Oxidative stress parameters have started to be used as an important guiding marker for the diagnosis and prognosis of these diseases. There are many studies proving that oxidative stress parameters can be used as biomarkers in the diagnosis of leptospirosis and in understanding the extent of the damage caused by this disease in tissues and organs and consequently in determining the treatment method. The most researched of these parameters is MDA, which is formed because of lipid peroxidation and has mutagenic and toxic effects (Esterbauer et al., 1990). Once formed, MDA can be degraded by different enzymes, particularly mitochondrial aldehyde dehydrogenase, or it can interact with proteins and nucleic acids to form various compounds that damage DNA-protein cross-links and biomolecules (Marnett, 1999; Voitkun and Zhitkovic, 1999). MDA has been used as a biomarker to determine oxidative stress in various biological samples such as blood, serum and urine from sick humans and animals affected by a wide range of diseases including non-infective diseases such as cancer, cardiovascular, pulmonary, neurodegenerative diseases and infective diseases such as brucellosis, tuberculosis and leptospirosis (Merendino et al., 2003; Del Rio et al., 2005). The detection of end products such as MDA in infective diseases proves that lipid peroxidation has an important role in the prognosis of such diseases (Busch and Binder, 2016). The membrane polysaccharide in the structure of *Leptospira* bacteria causing leptospirosis causes lipid peroxidation because of its cytotoxic effect on the host cell (Kim et al., 1997) When considered, it is evaluated that MDA may have the potential to be an important marker in the diagnosis of leptospirosis. For this reason, there are various studies evaluating MDA levels in animals infected with leptospirosis. In previous studies, MDA levels were found to be statistically significantly higher in the *Leptospira*-infected group compared to the control group, as in our study (Erdoğan et al., 2008; Gazioğlu and Güvenç 2015; Niroomandi et al., 2022). Other studies and the findings obtained in our study strengthen the opinion that MDA can be used as a biomarker in the diagnosis of leptospirosis.

Adenosine deaminase is a very important enzyme of purine metabolism. Its function in purine metabolism is to irreversibly reduce adenosine and deoxyadenosine to inosine and deoxyinosine, respectively (Franco et al., 1997). ADA is found in all cell types, but higher ADA activity has been found in lymphoid tissues, thymus and peripheral lymphocytes. In addition, another important function of ADA is the differentiation, maturation and proliferation of T lymphocytes (Franco et al., 1997; Cordero et al., 2001). Considering the damage caused by *Leptospira* in the blood vessels and cells of the host, we believe that ADA can be used as a biomarker in the diagnosis of leptospirosis. However, there are not many studies evaluating the activity of ADA in leptospirosis infection. In the literature review conducted within the framework of this study, 2 studies evaluating the activities of ADA in leptospirosis infection were found. The first of these was the study conducted in cattle by Atakişi et al. In this study, ADA activity in the *Leptospira*-infected group was found to be statistically higher than in the control group as in our study (Atakişi et al., 2014). However, in the experimental study of Tonin et al. (2012) on mice, ADA activity in the infected group was found to be statistically lower than in the control group, contrary to the results of our study and the study of Atakişi et al (2014). Although ADA activity was found to be lower in the infected group in one of the three studies in contrast to the others, the statistically different result from the control group suggests that ADA may be a potential biomarker in the diagnosis of this disease. However, we believe that more studies should be conducted on the evaluation of leptospirosis infections of ADA.

Thiol-disulphide balance plays a crucial role in antioxidant defence, immune response, regulation of enzyme activity and apoptosis (Valko et al., 2006). There are studies evaluating thiol disulphide balance in infective diseases of viral and bacterial origin such as Crimean-Congo haemorrhagic fever, acute tonsillopharyngitis and brucellosis. In these studies, it was reported that thiol-disulphide balance was disturbed, and this parameter could be used as a biomarker in the diagnosis of these infective diseases. However, no study was found in which thiol-disulphide balance was evaluated in *Leptospira* infection. Although we could not detect a significant difference in thiol-disulphide levels in the control and infective groups, we believe that further studies should be conducted in this field.

Conclusion

Scientific studies and the findings obtained in this study showed that MDA and ADA can be used as biomarkers in the diagnosis of *Leptospira* infections. Studies evaluating oxidative stress parameters in *Leptospira* infections were mostly performed in cattle and to a lesser extent in horses and dogs. In the literature review conducted within the framework of this study, no study evaluating oxidative stress parameters in *Leptospira* infections in sheep was found. For this reason, we believe that scientific studies should be carried out in this field. In addition, we could not find any study evaluating the thiol-disulphide balance in *Leptospira* infections other than our study. We think that the evaluation of this important parame-

ter, which has been proven to be used as a biomarker in the diagnosis of infective and non-infective diseases, in further studies will contribute to the literature.

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Author Contribution Statement

All authors are responsible for the entire content of this article and have approved its submission.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Review

Effect of Gamma Irradiation on the Microbial Load and Quality of Foods

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ABSTRACT

Foodborne pathogenic microorganisms pose a significant public health issue worldwide, while post-harvest food losses are also considered one of the leading causes of hunger and malnutrition globally. In the food industry, irradiation technology, particularly used as an alternative to thermal processes and regarded as an environmentally friendly method, plays a crucial role in addressing food insecurity and foodborne diseases worldwide. Food irradiation is a non-thermal, technical process in which food is exposed to ionizing or non-ionizing radiation (such as UV, visible light, infrared, radio waves) at specific doses. The irradiation process, which does not involve high temperatures, preserves the food's nutritional value, freshness, and sensory properties (texture, colour, taste, and flavour) because it does not damage the structure of food components. The basic principle is that when the irradiation source hits the food, excitation and ionization occur, which inhibits DNA synthesis in living organisms. This effect is primarily used to inhibit the growth of pathogenic microorganisms. Gamma irradiation technology is effective in inhibiting both important foodborne pathogens (e.g. *Escherichia coli* O157, *Campylobacter jejuni*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp.), and microorganisms reducing food quality. Even low doses (up to 10 kGy, the safe dose limit) affect target groups of microorganisms. This review discusses the role and applicability of irradiation technology in ensuring the microbiological quality of foods.

Keywords: *Food irradiation, food safety, gamma irradiation*

Gama Işınlama Yönteminin Gıdaların Mikrobiyal Yükü ve Kalitesi Üzerine Etkisi

ÖZET

Gıda kaynaklı patojen mikroorganizmalar dünya çapında önemli bir halk sağlığı sorunu oluştururken, hasat sonrası meydana gelen gıda kayıpları da dünya çapında açlığın ve yetersiz beslenmenin önde gelen nedeni olarak değerlendirilmektedir. Gıda endüstrisinde özellikle ısıl işlemlere alternatif olarak kullanılan ve çevre dostu yöntem olarak kabul edilen ışınlama teknolojisi dünyadaki gıda güvensizliği ve gıda kaynaklı hastalık sorunlarının çözümünde önemli bir rol oynamaktadır. Gıda ışınlaması, gıdanın iyonlaştırıcı özelliği olan veya olmayan radyasyonlara (UV, görünür ışık, kızılötesi, radyo dalgaları gibi) belirli dozlarda maruz bırakılmasıyla gerçekleşen, ısıl olmayan teknik bir işlemdir. Yüksek sıcaklık uygulaması içermeyen ışınlama işlemi, gıda bileşenlerinin yapısına zarar vermediği için, gıdaların besleyiciliğini, tazeliğini ve duyusal özelliklerini (doku, renk, tat ve aroma) koruyabilmektedir. Temel prensip olarak, ışınlama kaynağının gıda maddesine çarpmasıyla uyarım ve iyonlaşma meydana gelmekte ve bu durum canlılarda DNA sentezini engellemektedir. Bu etki özellikle patojenik mikroorganizmaların gelişmesini engellemek için kullanılmaktadır. Gama ışınlama teknolojisi, *Escherichia coli* O157, *Campylobacter jejuni, Listeria monocytogenes, Staphylococcus aureus* ve *Salmonella* spp. gibi tehlikeli hastalıklara neden olan ve gıdaların kalitesine zarar veren mikroorganizmaları/patojenleri inhibe edebilen bir teknoloji olup, düşük dozları bile (10 kGy güvenli doz limiti) hedef mikroorganizma gruplarını etkilemektedir. Bu derlemede, ışınlama teknolojisinin gıdaların mikrobiyolojik kalitesini sağlamadaki rolü ve uygulanabilirliği ele alınmıştır.

Anahtar kelimeler: *Gıda* ışınlama*, gıda güvenliği, gama* ışınlama

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Introduction

Food safety is a critical issue for human health, encompassing the entire process from farm to fork, including foodborne diseases. Foodborne diseases pose a threat to human health all over the World, and cause problems in even in developed countries with modern food processing and distribution systems (Arapcheska et al., 2020). Despite the implementation of safety practices such as modern technologies, good manufacturing practices, risk assessments, quality control, and hygiene management, there is still an increase in the number of foodborne diseases and poisonings as of today. Especially in recent years, with the significant and remarkable developments in technology and globalization in the food market, new preservation methods such as irradiation, ozonation, cold plasma, high pressure applications to minimize the risk of microbiological contamination in unprocessed or processed products are constantly being investigated (Prokopov and Tanchev, 2007; Amit et al., 2017). Irradiation, one of the comparably new food preservation methods, is a technique used for various purposes such as extending the shelf life of fresh or processed foods, preserving ready-to-eat meat products when appropriate additives are not used, reducing losses caused by chemical or microbiological spoilage, preventing post-harvest damage, protecting fruits, vegetables, grains and legumes from insect infestations during storage, preventing sprouting in tuber vegetables as potatoes, eliminating pathogenic microorganisms in meat, fish, poultry, seafood and spices. It is also called cold pasteurization as it does not involve heat treatment (Pillai and Pillai, 2021).

Types of Radiation Used in Food Preservation

In food preservation, gamma rays, accelerated electron beams, and X-rays can be used. Among these, the most commonly used in the food industry are the gamma rays. Gamma rays are generated by sources such as Cobalt-60 (Co-60) and Cesium-137 (Cs-137). Cs-137 is not recommended for food irradiation due to its high solubility in water, which may cause environmental damage. Co-60, on the other hand, is widely used in food irradiation since it is insoluble in water and has almost no harm to the environment (Ravindran and Jaiswal, 2019). Accelerated electron beams are not favoured in food applications because of their low penetration depth, and strong effect on atoms and molecules, such as breaking of double helix structures, and formation of highly reactive free radicals (Edae, 2023; Mshelia et al., 2023). X-rays have high penetration and dose rates, enabling shorter irradiation periods for foods. X-rays, with a penetration depth of 25 cm, are commonly used to irradiate packaged food products. The minimal environmental impact of X-rays, their effectiveness, and the possibility of direct installation on commercial processing lines have proven their applicability in microbial control (Mshelia et al., 2023).

Gamma Irradiation Application

In gamma irradiation, chromosomes (DNA), the carrier of the genetic code is targeted. Gamma irradiation affects macro and micromolecules in microorganisms, leading to various chemical changes (Eugster et al., 2018). The primary lesions caused by ionizing radiation in intracellular DNA are chemical damages to purine and pyrimidine bases, as well as to the deoxyribose sugar. In general, double strand breaks are formed at a rate of 5-10% of the single strand break rate induced by ionizing radiation. However, most microorganisms can repair their single strand breaks. To survive, microorganisms must be able to repair DNA damage quickly. Therefore, some microorganisms have different repair mechanisms that involve a variety of enzymes. If there are too many lesions in the DNA molecule for the microorganism to handle, replication is halted (Ajibola, 2020).

There are three terms, which have been introduced into the terminology for microbial inactivation: radurization, radicidation, and radappertization. Radurization (low dose) involves the application of a radiation dose ranging from 0.1 to 1 kGy, which reduces microbial or insect load that cause food spoilage, inhibits respiration in fruits and vegetables, delays ripening, and extends the quality and shelf life of food products. Radicidation (medium dose) refers to a radiation dose in the range of 1-10 kGy. Through radicidation, the number of pathogens such as *Salmonella* spp. and *L. monocytogenes* in foods can be reduced or they can be totally eliminated. Radappertization (high dose) is a radiation treatment applied at doses above 10 kGy to kill almost all microorganisms present in food. Radappertization is used to eliminate resistant bacteria and spores (Indiarto et al., 2023). The doses applied and the food products treated are summarized in Table 1 (Arapcheska et al., 2020). The use of gamma irradiation for meat, seafood, poultry, spices, and vegetable seasonings has been approved by the Food and Drug Administration (FDA). According to the World Health Organization (WHO), exposure of food to doses up to 10 kGy is generally considered safe and does not pose a microbiological or toxicological hazard to human health. However, radiation doses above 10 kGy may cause deterioration in sensory properties such as colour, texture, and taste of the foods (Jeong et al., 2020).

There is a negligible temperature increase after irradiation as 0.36°C at a dose of 1 kGy. The absorption of the maximum permissible dose for food irradiation (10 kGy) results in a temperature increase of only 2.4°C on the food. Irradiation at doses below 10 kGy results in less chemical modification than processes such as heating (Arvanitoyannis et al., 2009; Bashir et al., 2021).

The Effect of Irradiation on Microorganisms

The resistance of microorganisms to gamma radiation depends on the ability of their DNA repair enzymes to repair single strand breaks. Strains lacking this ability are more radiosensitive than others. There is considerable variability in radiation resistance of microorganisms. For example, yeasts are more resistant to radiation than molds. Single stranded DNA viruses are more sensitive to

Table 1. Irradiation at various doses and its applications (Arapcheska et al., 2020)

Dose Level	Purpose	Food Products
Low Dose (<1 kGy)	Prevention of sprouting killing insects and larvae in wheat, flour, fruits, and vegetables after harvest, slowing down the ripening process, and the elimination of certain harmful parasites associated with food.	Potatoes, onions, garlic, ginger, bananas, mangoes and non-citrus fruits, cereals and legumes, dried vegetables, dried fish and meat, fresh pork
Medium Dose (1-10 kGy)	Significant reduction or elimination of specific microorganisms and parasites that cause food spoilage, and reduction or elimination of many pathogenic microorganisms.	Strawberry, grapes, dried vegetables, fresh or frozen seafood, fish, raw or frozen poultry and meat
High Dose (>10 kGy)	Sterilization of food for special uses, such as meals for immunocompromised patients, elimination of certain viruses that cause diseases.	Sterilized food for immunocompromised patients

radiation than double stranded DNA viruses. The higher radiation resistance of Gram-positive bacteria compared to Gram-negative bacteria highlights the importance of peptidoglycan in bacterial resistance to gamma radiation (Harrell et al., 2018). Environmental factors, such as moisture content, temperature during irradiation, presence or absence of oxygen, and whether the food is fresh or frozen, also influence microbial resistance to irradiation (Gradini et al., 2019). Additionally, bacterial sensitivity to irradiation depends on the growth phase. In general, cells in the exponential growth phase are more sensitive to ionizing radiation compared to microbial cells in the latent or stationary phase (Arapcheska et al., 2020). Certain spore-forming and toxin-producing bacteria, such as *Bacillus* spp. and *Clostridium* spp., are more resistant to irradiation than non-spore-forming species. Therefore, radiation doses below 10 kGy can only reduce the number of spores. However, irradiation can be applied in combination with other preservation methods, such as freezing, to prevent spore formation (Mshelia et al., 2023).

The Effect of Irradiation on Meat and Meat Products

Irradiation of meat is an alternative food preservation method to traditional techniques such as salting, curing, smoking, drying, canning, cooking, cooling, freezing, modified atmosphere packaging, and high-pressure applications. This preservation method is advantageous because it causes minimal physical changes to the food, does not require the use of additives, and does not involve thermal processing. Studies have shown that while macro nutrients in meat are not significantly altered, some vitamin levels can be affected by irradiation. Meat contains water-soluble B vitamins such as thiamine, riboflavin, niacin, pyridoxine, biotin, cobalamin, choline, folic acid, and pantothenic acid. There are little fat-soluble vitamins in meat. Beef contains around 1 µg of vitamin A per gram of fat, negligible amounts of vitamins D, E and K. Fat-soluble vitamins are generally more stable under irradiation compared to water-soluble vitamins. For extending the shelf life of fresh or frozen red meat and poultry products, irradiation doses of 3.0 kGy are recommended, while 7.0 kGy doses are advised to reduce pathogenic microorganisms. Studies have shown that irradiation with 3.0 kGy gamma rays reduces the growth of mesophilic bacteria, coliforms, and *Staphylococcus aureus* in beef (Jayathilakan et al., 2015; Indiarto et al., 2023). According to the Food Irradiation Regulation in Türkiye, maximum irradiation dose for poultry, red meat, and their products (fresh or frozen) is set at 7.0 kGy (FIR, 2019). Poultry meat and products can be contaminated with pathogens such as *Campylobacter*, *Salmonella* spp., and *E. coli* O157. Low-dose irradiation can inactivate over 90% of the bacteria present in meat. The microbiological quality of poultry is significantly improved with irradiation doses of 1.0 to 2.0 kGy (Singh and Singh, 2020).

Rahimi et al. (2013) studied the effects of irradiation at doses of 2, 5, 7 and 10 kGy on ground beef, showing that gamma irradiation reduced the number of microorganisms in all irradiated ground beef samples (20 samples, up to day 10) at 2, 5, 7 and 10 kGy, which also extended the shelf life of the products. They reported that irradiation at high doses (more than 7 kGy) has a negative effect on some organoleptic properties such as color, as well as on parameters affecting meat quality such as flavor and fat oxidation. Therefore, they recommended the use of low doses (less than 5 kGy) to reduce the microbial load of minced meat. In another study, the effects of gamma irradiation and frozen storage on improving the shelf life of turkey breast meat were evaluated, irradiation was applied at doses of 0.5, 2 and 4 kGy, and it was reported that the number of mesophilic bacteria decreased by about 5 log units and Salmonella was not detected in samples irradiated at 4 kGy (Jouki, 2013). Zhao et al. (2017) applied irradiation at various doses (0.5, 1.5, 3, 4, 6 and 8 kGy) for pasteurization of beef jerky and reported that 4 kGy is an appropriate dose for pasteuriza-

tion of jerky. It was reported that deterioration in color, taste and texture occurred with the increase in dose and these deteriorations were associated with the increase in free radicals. In a study comparing the effect of irradiation on sporulating bacteria with the addition of nitrite, cooked hams were irradiated at doses of 1.5, 3, 4.5 and 6 kGy. It was reported in the study that the level of nitrite required to inhibit *Clostridium sporogenes* spores was equivalent to a gamma ray dose of 3 kGy. With 3 kGy irradiation dose, it has been shown that microbiological safety can be ensured and the sensory quality of the product can be improved while inhibition is achieved (Silva et al., 2020).

Akhter et al. (2021) investigated the synergistic effect of low dose (1 kGy) gamma irradiation combined with natural antimicrobial (nisin and sodium nitrate) and antioxidant agents (rosemary and BHT) on the quality of sheep meat, in this study it was reported that rosemary extract and nisin application along with low dose irradiation were the most effective natural alternatives to maintain the quality of meat emulsions. In another study investigating the effect of irradiation on various bacteria, 2, 4 and 6 kGy doses of irradiation were applied to ostrich meat and the reduction in the number and inhibition of some bacteria were observed. The results of the study showed that the irradiation dose of 4 kGy was effective in the inhibition of bacteria and the number of mesophilic bacteria, coliform bacteria*, S.aureus* and psychrophilic bacteria decreased at this dose. At the same time, *Salmonella* spp. and *E. coli* were eliminated at this dose (Mashak and Abbasi, 2023).

Effect of Irradiation on Spices

Spices and herb seasonings are among the most irradiated food products for microbial decontamination on commercial scale. Spices are prone to microbial contamination during harvesting, processing, and storage. Spices that are often dried in outdoor are susceptible to contamination by pathogenic microorganisms such as *Salmonella* spp., *Clostridium perfringens*, *Bacillus cereus*, as well as molds and insects. Irradiation of spices with doses ranging from 3.0 to 10.0 kGy has been established as a reliable method to enhance microbiological safety. Irradiation of spices is practiced on a commercial scale in over 20 countries such as USA, Mexico, Vietnam, Thailand, India, Australia, New Zealand, Pakistan, South Africa, Malaysia, Indonesia. Irradiation of potatoes, onions and spices were the first food items approved for domestic marketing by health authorities of India in 1994 (Roberts, 2016; Kyung et al., 2019; Singh and Singh, 2020).

Cruz-Zaragoza et al. (2011) examined the effects of low doses of irradiation on the total number of mesophilic bacteria and showed that 0.5 kGy dose irradiation on coriander led to a 99.9% reduction in the total number of mesophilic bacteria. In a study on the effect of microbial load in red pepper, gamma irradiation was used at doses of 2, 4 and 6 kGy and D_{10} values of total mesophilic bacteria were determined as 2.66 kGy in gamma irradiation. At 6 kGy irradiation, it was reported that the bacterial population decreased, yeast, mold and coliform bacteria were completely inhibited and sensory properties were also preserved at this irradiation dose (Jung et al., 2015). Sadecka et al. (2018) conducted a similar study on black pepper and reported that 5 kGy irradiation dose was sufficient to control total microbial load. When they examined the irradiation dose in terms of organoleptic properties and essential oil components, it was reported that no physical and chemical problems occurred up to 10 kGy. In a study that also tested irradiation doses above 10 kGy on spices with high bacterial load, fennel and cinnamon were irradiated at doses of 2.5, 5, 7.5, 10 and 15 kGy and the change in microbial load was observed. Proving again that doses of 10 kGy and above are sufficient to reduce bacterial load, the study also reported that a dose of 5 kGy is sufficient to eliminate mold and yeast growth. 7.5 kGy dose of irradiation can be considered as a suitable dose for decontamination and microbial protection of these spices, as well as for increasing their antioxidant capacity (Ahmed and Hassan, 2023).

Labelling of Irradiated Foods

The Radura symbol, shown in Figure 1, is derived from the word 'radurization', which is a combination of the words 'radiation' and 'durus', the Latin term for permanent (Mshelia et al., 2023). The Radura symbol is typically green and resembles a plant within a circle, with the upper half of the circle is dashed. In the symbol, the central dot represents the radiation source, while the two segments (the leaves) represent the biological shield provided to protect workers and the environment (Ehlerman, 2009 ; Gonçalves et al., 2011).

Figure 1. Radura Symbol (Mshelia et al., 2023)

Conclusion

The development of new technologies is crucial for ensuring consumers access to healthy and nutritionally rich functional foods, which are increasingly in demand. Technologies such as irradiation, which do not require

thermal processing or the use of chemical substances to obtain safer products, are also considered environmentally friendly methods. Understanding the principles of these technologies, inactivating not only vegetative cells but also spores, and optimizing production conditions are crucial, which makes ongoing research essential. Using different radiation sources, and dose levels according to the type and composition of foods minimizes potential negative effects on food quality. Irradiation, with its ability to reduce numbers of or eliminate food-borne pathogens, can be combined with other food preservation methods both to improve hygiene conditions, and to ensure higher quality and safer end products. The possibility to apply irradiation to packaged products eliminates the contamination risks that may arise post-processing, giving it a significant advantage over other preservation techniques. However, the acceptance of irradiated foods by consumers has not yet reached the desired level yet. The main reason for this is consumer bias and suspect that irradiated foods may be radioactive. In addition to lack of consumers awareness, high cost of gamma irradiation applications is one of the main reasons why this method found limited application in food products. Still, irradiation is gaining increasing acceptance each year despite consumer concerns about irradiated foods.

Author contribution statement

All authors accepted responsibility for the entire content of this review and approved its submission. The concept of the review was made by all authors. This review was written by HD. HD conducted the literature search of the review. Critical reading of the article was conducted by DB. DB performed the interpretation of this review.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Case Report

Clinical Findings of The Coexistence of an Extraluminal Multiple Vaginal Leiomyoma with Ovarian Cystadenoma in a Dog

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A B S T R A C T

In this report, we present a case of a dog diagnosed with both vaginal leiomyosarcoma and ovarian cystadenoma, occurring concurrently with perioperative clinical findings. A twelve-year-old intact Siberian Husky bitch was brought to our clinic due to constipation and urination difficulties attributed to a perineal mass that had been present for approximately one month. Although no tissue mass was visible protruding from the vagina, perineal swelling was remarkable, and the dog exhibited mild leucocytosis and anemia. Vaginal cytology showed densely keratinized superficial cells, indicating high oestrogen levels. Ultrasonography of the perineal and abdominal areas revealed a solid mass without internal blood flow and numerous cystic reflections in the left ovary. During surgery, after removing the two solid masses via episiotomy, an ovariohysterectomy was performed. Histopathological examination confirmed the diagnoses of vaginal leiomyosarcoma and ovarian cystadenoma. Following the operations, the patient regained normal urination and defecation and recovered without complications or recurrence. As a conclusion, this case report highlights the importance of considering concurrent reproductive tract tumours in geriatric bitches presenting with perineal swelling and urinary difficulties, emphasizing the need for thorough diagnostic evaluation and surgical intervention for successful management and recovery.

Keywords: *Genital tumours, vagina, ovaries, female dogs.*

Bir Köpekte Eşzamanlı Görülen Ekstraluminal Vajinal Leiomyoma ve Ovaryan Kistadenomanın Klinik Bulguları

Ö Z E T

Bu raporda bir köpekte eşzamanlı olarak görülen vajinal leiomyosarkom ve ovaryan kistadenoma olgusuna ait perioperatif klinik bulgular sunulmuştur. Kısırlaştırılmamış 12 yaşlı Sibirya kurdu cinsi dişi köpek, yaklaşık bir aydır mevcut olan perineal kitleye bağlı kabızlık ve idrar yapma güçlüğü şikayetiyle kliniğimize getirildi. Vajinadan dışarı çıkan bir kitle görülmemesine rağmen, perineal şişlik belirgindi ve köpekte hafif lökositoz ile anemi tespit edildi. Vajinal sitolojide, yüksek östrojen düzeyine işaret eden yoğun keratinize süperfisiyal hücreler gözlendi. Perineal ve abdominal bölgenin ultrasonografik incelemesinde, damarlaşma içermeyen solid bir kitle ve sol ovaryumda çok sayıda kistik yapı tespit edildi. Operasyon sırasında, epizyotomi ile iki solid kitle çıkarıldıktan sonra ovariohisterektomi uygulandı. Histopatolojik inceleme sonucunda vajinal leiomyoma ve ovaryan kistadenoma tanılarını konuldu. Operasyon sonrası dönemde hasta, normal idrar ve dışkılama fonksiyonlarını yeniden kazandı ve herhangi bir komplikasyon veya nüks gelişmeden iyileşti. Sonuç olarak, bu olgu sunumu, perineal şişlik ve idrar yapma güçlüğü şikayetiyle gelen geriatrik dişi köpeklerde eşzamanlı üreme sistemi tümörlerinin göz önünde bulundurulmasının önemini vurgulamakta ve başarılı yönetim ve iyileşme için kapsamlı bir tanısal değerlendirme ile cerrahi müdahalenin gerekliliğini ortaya koymaktadır.

Anahtar kelimeler: *Genital tümör, vajina, ovaryum, dişi köpek.*

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Introduction

Canine vaginal tumours, which constitute approximately 2.4–3% of all neoplasms in dogs, are primarily leiomyomas. These tumours arise from smooth muscle cells and can potentially develop in any organ containing connective tissue or mesenchymal components (McEntee, 2002; James et al., 2012; Singh et al., 2014). They are typically characterized as non-invasive, slow-growing masses with no metastatic potential to other organs (Klein, 2007; Devereaux and Schoolmeester, 2019).

In contrast to the high incidence of vaginal tumours, ovarian tumours are very rare accounting for only 1.2% of all tumours identified in dogs. Another genital neoplasm observed in older bitches is cystadenoma, which originates from ovarian surface epithelial cells and has a higher prevalence among all ovarian tumours (40-50% of cases), usually detected unilaterally. Their growth is most commonly papillary or cystic, and they are generally progressive and asymptomatic (Schlafer and Miller, 2007; Carreira and Pires, 2016; White and Brearly, 2018). Due to the increase in ovarian size, these tumours are often incidental findings during routine spaying procedures, abdominal scans, or necropsies (Carreira and Pires, 2016).

The coexistence of reproductive tumours located in different portions of the genital system has been reported previously (Serin et al., 2006; Ozmen et al., 2008; Ferré-Dolcet et al., 2020; Brodzki et al., 2023). Some cases are more likely attributable to the coincidence of independent, age-related pathological processes. Nevertheless, many studies suggest that oestrogen-secreting tumours or ovarian follicular cysts may play a role in the pathogenesis of reproductive tract leiomyomas due to the high incidence of vaginal tumours in intact bitches. Researchers have described leiomyomas in dogs as hormone-dependent neoplasms that do not occur in spayed females (MacLachlan and Kennedy, 2002). Bitches spayed at an early age are not prone to developing leiomyomas, and in cases where leiomyomas are present, the condition tends to regress following spaying (Sathya and Linn, 2014; Dhoke et al., 2016).

In this report, we present the clinical, ultrasonographic, and histopathologic findings of coexistent vaginal leiomyoma and ovarian cystadenoma in a 12-year-old bitch, which was successfully treated with a combined surgery approach consisting of episiotomy and ovariohysterectomy.

Case

A 12-year-old Siberian Husky breed bitch with notable perineal swelling was referred to the Animal Hospital of the Aydın Adnan Menderes University, due to constipation and difficulty urinating for two weeks. In the patient's history, this painless swelling had been present for one month, but she had been experiencing issues with defecation and urination for the past two weeks.

Her body condition and appetite were reported as normal by the owners. In clinical parameters, her rectal temperature, pulse, and respiration were normal, and within healthy limits. The perineal area appeared enlarged. During the clinical examination of the rounded swelling, no ulceration or infectious lesions were found on its surface (Figure 1).

When performing a vaginal touche to explore the possible intraluminal findings, no mass formation or vaginal discharge was noticed, except for mild vulvar oedema. The vaginal cytology sample, stained with Giemsa stain, showed a significant estrogenic effect, with the dominance of the cornified and anuclear superficial epithelial cells. According to the owners, her proestrus bleeding was not as intense as before and lasted only a couple of days. No cyclic abnormalities related to prolonged estrus were recorded. Blood analysis revealed mild leucocytosis and anaemia (WBC:19.75×10^3 /µL; RBC: 4.54x19.75×10^{12} /µL; HCT: 33%; PLT: 85).

After the clinical examination, B-mode and Doppler ultrasonographic assessments of her perineal area were performed. Initially, the imaging revealed a mass, allowing for differentiation between a perineal tumour, cyst, or hernia formation. Color Doppler imaging showed no detectable blood flow within the mass. Transdermal sonography revealed a solid mass without internal cyst or blood flow, well-encapsulated. Abdominal ultrasonography showed that the left ovary contained several cysts, with diameters exceeding 25 millimeters (Figure 2).

Surgery was performed under general anesthesia using xylazine [2 mg/kg of Xylazine HCL (Rompun %2, Bayer, Germany)] and ketamine [10 mg/kg of Ketamine HCL (Alfamine %10, Alfasan, Holland)]. First, the perineal tumour was removed via an episiotomy. Following urinary catheterization and aseptic preparation of the surgical site, an episiotomy incision was made on the tumour, with careful attention to the urinary meatus. Small haemorrhages were controlled with sealing and ligation. After incising the capsule of the swelling, two solid, firm, spherical masses were detected and easily removed without any hemorrhage (Figure 3).

They were fixed in 10% v/v formalin and sent immediately to a private pathology laboratory for histopathological examinations. The excision site was then closed. The soft tissues were sutured with USP 2/0 polyglactin 910 (Vicryl®, Ethicon, Netherlands), and the skin was closed with a 2/0 silk suture (Silk®, Kruuse, Denmark). In the second part of the surgery, the routine ovariohysterectomy method was performed. Both ovaries and uterine horns were removed by ligation. Dressing of the sutures in both areas were performed with crystalline for a week. Systemic antibacterial injections with enrofloxacin (5 mg/kg) were prescribed for 7 days postoperatively. The owner reported that constipation began to improve 48 hours after the surgery. By the following week, the dog had recovered and did not develop postoperative complications. After 10 days, the bitch had fully recovered from the surgical wound, and a follow-up examination

Figure 1. Preoperative appearance of the perineal swelling

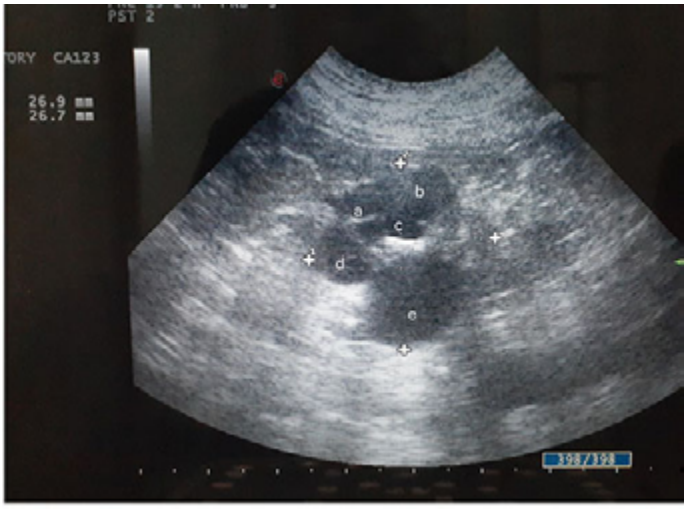

Figure 2. Ultrasonography of left ovary showed increased ovarian size (calipers) and multicystic areas (a,b,c,d)

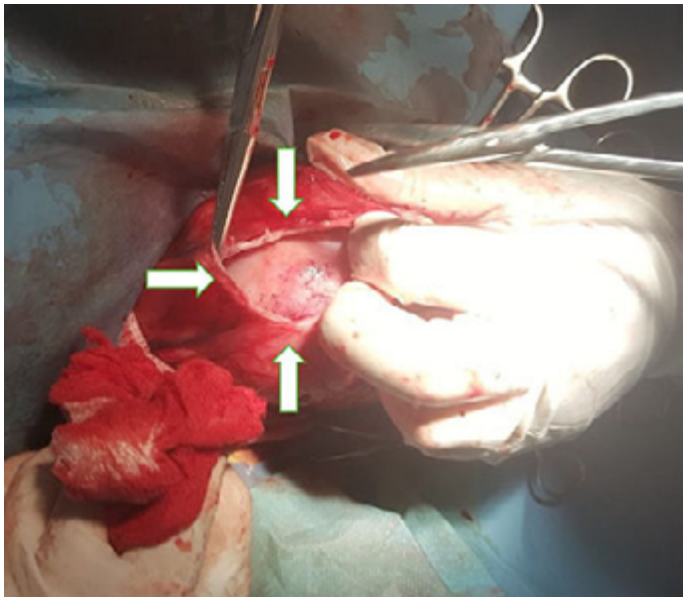

Figure 3. Excision of the fibrous tumour capsule (white arrows)

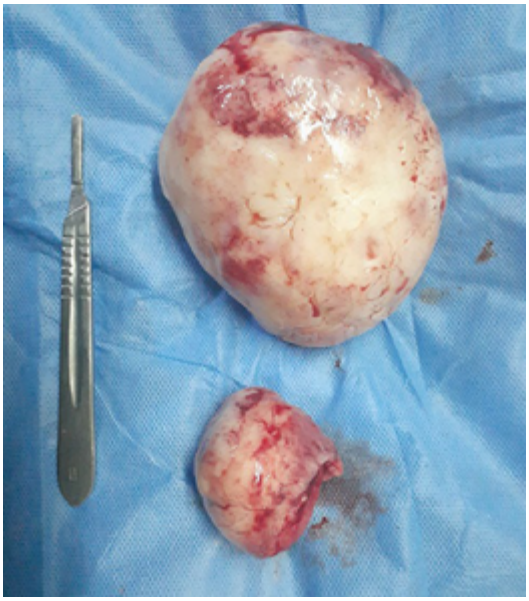

Figure 4. Solid and separate tumoural masses

after 2 months revealed no recurrence of the tumour.

Histopathological examinations revealed vaginal leiomyoma and ovarian cystadenoma (A-Pathology Laboratory; Protocol number: V207365-20). Two pieces of solid white material with regular borders (9 and 4 cm in diameter) were examined (Figure 4), All sections were composed of tumoural areas, with many sections showing tumour parenchyma consisting of well-differentiated cells with spindle-shaped oval nuclei, no atypia, and no mitosis. The cells were seen crossing each other in the bundles, without any signs of malignancy.

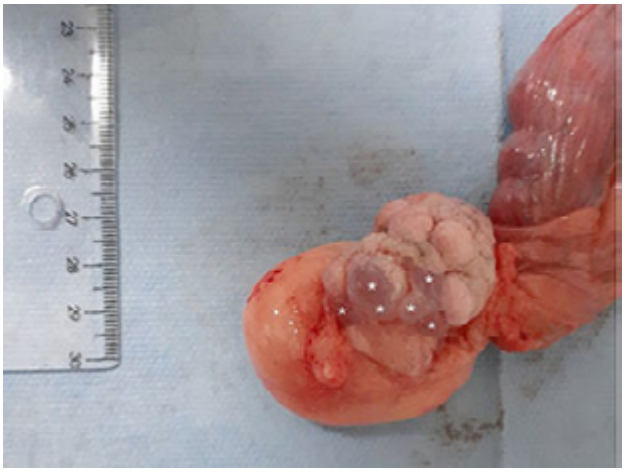

Figure 5. Cysts on the left ovary (white stars)

Yellow-brown ovarian material (38x3x2 cm) with a multicystic area measuring 3 cm in diameter (Figure 5) was also examined. The samples taken showed fibrocytic stroma, adenoid, and cystic papillary proliferations of varying diameters.The tumour cells were cuboidal, with well-differentiated euchromatic nuclei. Based on these histopathological findings, cystadenoma was diagnosed.

Discussion

In nulliparous bitches, the risk of neoplasms in the caudal genital system increases with age. Among vestibulovaginal tumours in dogs, leiomyomas are the most frequently reported, accounting for 80–90% of cases. and it has been confirmed that their growth is associated with several ovarian disorders, particularly those that result in high oestrogen levels (Yuefei et al., 2012; White and

Brearley, 2018).

Clinically, these masses are often described as well-encapsulated, single or multiple, painless swellings localized either intravaginally or extraluminally in the perineal region (White and Brearley, 2018). In certain cases, masses concealed beneath dense fur may remain undetected until they cause difficulty with urination or defecation. Consequently, a thorough perineal examination, including vaginal palpation, is recommended during annual clinical evaluations, particularly for older bitches (Saikia et al., 2018; Erdoğan, 2022).

Large intraluminal tumours may protrude through the vulva and, in some instances, extend cranially and caudally into the uterus and vagina (Dhoke et al., 2017). In contrast, extraluminal tumours are more commonly associated with perineal swelling, as observed in our case. In prolonged tumour cases, the growth of connective tissue leads to increased firmness. Additionally, large tumours can cause clinical complications such as bladder irritability, rectal pressure, tenesmus, and obstructed labour (Kang and Holmberg, 1983; Akkuş et al., 2016; Umamageswari et al., 2016). According to previous reports, defecation difficulties resulting from rectal pressure caused by the firm mass were observed in the present case.

In cases where a suspicious vaginal mass is identified, further diagnostic investigations are warranted to assess for concurrent ovarian abnormalities, which may contribute to the growth of vaginal tumours. Ovarian cysts and tumours, in particular, are frequently overlooked until they reach advanced stages and are often incidentally identified during routine spaying procedures (Carreira and Pires, 2016). Therefore, clinicians should conduct a thorough evaluation to investigate coexisting reproductive tract tumours in older females. Although some common symptoms of vaginal leiomyoma (such as recurrent vaginal secretions, prolonged proestrus bleeding, mate acceptance, or high serum estrogen results) were not in the present case, a remarkable estrogenic effect was detected in vaginal cytology. Additionally, abdominal scans revealed round, multiple cystic formations in the left ovary, and mild leucocytosis was recorded.

Ovarian tumours are typically detected in older, intact bitches, with an average age of onset ranging from 10.9 to 11.2 years (Feldman and Nelson, 2004). In the present case, the tumour was identified in a 12 years old bitch, aligning with previous observations.

Cystadenomas originate from the rete ovarii, generally affecting one ovary and, very rarely, both. They consist of multiple thin-walled cysts filled with transparent fluid (Herron, 1983). Epithelial neoplasms account for approximately 40–50% of all ovarian tumours and predominantly exhibit either a papillary (adenoma or adenocarcinoma) or cystic (adenoma or adenocarcinoma) growth pattern. Ovarian tumours are classified based on their ultrasonographic appearance as solid, solid with a cystic component, and cystic (Diez-Bru et al., 1998; Yotov et al., 2005). In this case, the ultrasonographic findings indicated a cystic pattern.

Ovarian diameters exceeding 25 millimeters and cystic anechoic areas are typical indications for spaying operations. In suspected ovarian abnormalities, the presence of cornified and anuclear superficial epithelial cells in vaginal cytological samples reflects the high serum uestrogen level (Root Kustritz et al., 2010). Periodic gynaecological examinations in older intact female dogs, including monitoring ovarian activity and measuring total ovarian dimensions, play a crucial role in the early diagnosis of various gynaecological pathologies (Erdoğan, 2022). Regarding the estrogenic stimulation caused by ovarian cysts in the development of vaginal leiomyomas, a combined approach of tumour excision and ovariohysterectomy was preferred to prevent the recurrence of vaginal leiomyomas. The primary treatment for vaginal leiomyomas involves surgical excision of the mass (Klein, 2007). In cases involving larger masses, an episiotomy may be necessary to facilitate proper removal (Rollon et al., 2008; Nelissen and White 2012). Iatrogenic damage to the urethra or accidental injury to other perineal structures is a potential surgical complication that can significantly impact the patient's quality of life. Therefore, preoperative urethral catheterization is recommended to minimize the risk of injury and improve surgical outcomes. The second surgical approach is spaying to prevent and control further tumour growth (McEntee, 2002; MacLachlan and Kennedy 2002; Yotov et al., 2005). In this case, guided by urethral catheterization, two extraluminally masses were successfully removed via episiotomy without any surgical complications. Based on ultrasonographic findings of the left ovary, ovariohysterectomy was performed as a secondary surgery to reduce the likelihood of similar tumoural growth. No mass recurrence or genital disorders were detected during the next six months postoperative follow-up.

Conclusion

In conclusion, this case report points out that perineal mass related to the genital tract can cause deteriorating daily functions, necessitating additional genital assessments for optimal surgical management, particularly in the presence of concurrent ovarian disorders. Although no cyclic disorder, vaginal discharge, or abdominal distension was observed, a definitive diagnosis was achieved through detailed abdominal ultrasonography and vaginal cytology. Based on these findings, the surgical management of vaginal leiomyoma in bitch should involve a combination of tumour resection and ovariohysterectomy, with careful consideration to prevent tumour recurrence.

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Author contribution statement

Clinical Examinations and Surgery, Literature Search, Writing G.E.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

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