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Effects of Sulfur Supplementation on Thyroid Hormones in Angora Goats Fed With A High-Nitrate Diet[#]

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ABSTRACT

This study was to aimed for determination the effect of high dietary nitrate intake and inorganic sulfur supplementation on thyroids hormones in Angora goats. In this study, eighteen male Angora goats aged 14 months were divided into three groups (n = 6): control group fed with a basal diet, nitrate group fed with a basal diet supplemented with 1500 ppm nitrate, and nitrate + sulfur group fed with a basal diet supplemented with 1500 ppm nitrate and 1.8% sodium sulfate. On days 45, 90, 135, and 180 of the study, the concentrations of the thyroid-stimulating hormone (TSH), total and free triiodothyronine (TT_3 and FT_3), and tetraiodothyronine (TT_4 and FT_4) were measured in the serum samples. On day 180, except for TT_4, the serum TSH and total and free T_3 and free T_4 concentrations were higher ($P<0.05$) in the nitrate + sulfur group than in the control and nitrate groups. This study suggested that Angora goats could tolerate a feed containing 1500 ppm nitrate with respect to the thyroid hormones, and inorganic sulfur might serve as a natural source for alleviating the negative effects of the high-nitrate diet on the thyroid gland in a dose-dependent manner.
Keywords: Angora goat, Nitrate, Sulfur, Thyroid hormones

Yüksek Nitratlı Diyetle Beslenen Ankara Keçilerinde Tiroid Hormonları Üzerine Kükürt İlavesinin Etkileri[#]

ÖZ

Bu çalışma, yüksek diyetli nitrat alımı ve inorganik kükürt ilavesinin Ankara keçilerindeki tiroid hormonlarına etkisini belirlemek amacıyla yapıldı. Çalışmada, yaklaşık 14 aylık yaştaki 18 erkek Ankara keçisi 3 gruba bölündü: kontrol grup bazal diyetle beslendi, nitrat grubu 1500 ppm nitrat ilave edilen bazal diyetle beslendi ve nitrat+kükürt grubu 1500 ppm nitrat ve %1.8 sodyum sülfat ilave edilen bazal diyetle beslendi. Çalışmanın 45., 90., 135. ve 180. günlerinde serum örneklerinde tiroid uyarıcı hormon (TSH), total ve serbest triiyodotironin (TT_3 ve FT_3) ve tetraiyodotironin (TT_4 ve FT_4) düzeyleri ölçüldü. 180. günde, TT_4 hariç, serum TSH, total ve serbest T_3 ve serbest T_4 konsantrasyonları nitrat+kükürt grubunda kontrol ve nitrat grubundan daha yüksekti ($P<0.01$). Bu çalışma, Ankara keçilerinin tiroid hormonları dikkate alındığında 1500 ppm nitrat içeren bir beslemeyi tolere edebileceğini ve inorganik kükürdün doza bağlı olarak tiroid hormonları üzerine yüksek nitratlı diyetin olumsuz etkilerini hafifletmek için doğal bir kaynak görevi görebileceğini önermektedir.
Anahtar Kelimeler: Ankara keçisi, Kükürt, Nitrat, Tiroid hormonları

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INTRODUCTION

The use of high-nitrate fertilizers to increase agricultural production can lead to accumulation of nitrates/nitrites in soil, plants, and drinking water sources, and thus cause a potential health risk for humans and animals (Jordao et al. 2002, Mensinga et al. 2003). Generally, forage containing up to 1000 ppm nitrate is considered safe for ruminants, but forages containing 1200-1500 ppm nitrate cause chronic nitrate poisoning in the ruminants (Kaya and Akar 1989). Previous studies demonstrated that ruminants consuming 800-2000 ppm nitrate daily through water and forage showed subacute and chronic toxicity symptoms (Bartik and Piskač 1981, Pirinçci and Acet 1984). Although nitrates are relatively nontoxic for the ruminants, nitrites are highly toxic. In ruminants, under normal conditions, nitrate is first reduced to nitrite (Chow and Hong 2002, Takahashi et al. 1998), and then to ammonia by the microorganisms in the rumen. However, because the conversion of nitrate into nitrite is faster than the reduction of nitrite to ammonia by the microorganisms, a high nitrate intake through water and diet leads to the accumulation of nitrite in the rumen (Cheng et al. 1988).

Goitrogenic agents such as nitrate, perchlorate, and thiocyanate act as competitive inhibitors of the Na^+/I^- symporter (Jahreis et al. 1986). Therefore, nitrate reduces inorganic iodine uptake by the thyroid gland and, thus, intrathyroidal iodine concentration. When the animals consume water or a diet with high nitrate levels, blockage of the Na^+/I^- transport protein decrease the synthesis of thyroid hormones (Kostogrys ve ark. 1989, Nlend ve ark. 1999). Previous studies indicated that high nitrate intake decreased the binding of active iodine to Na^+/I^- transport protein and thyroid gland activity in humans and animals (Bruning-Fann and Kaneene 1993, Eskiocak et al. 2005, Simon et al. 2000).

Studies on small ruminants revealed that chronic nitrate toxicity can decrease thyroid hormone concentration in sheep (Georgiev et al. 1987) and goats (Simon et al. 2000). Although a few studies exist on the thyroid hormones in Angora goats (Keçeci and Keskin 2002, Puchala et al. 2001) to date no study has demonstrated the effects of high dietary nitrate intake on the thyroid hormones in Angora goats producing mohair.

Sulfur is a constituent of several biomolecules such as proteins, nucleic acids, and sulfur-containing coenzymes. It is particularly required in ruminants for microbial synthesis of sulfur-containing amino acids (cysteine, cystine, and methionine), thiamine, biotin, and enzymes (Carneiro et al. 2000, Takahashi et al. 1989). Moreover, nitrate supplementation increased sulfur requirements in the diet to improve microbial synthesis of sulfur-containing amino acids in the rumen. The nitrogen/sulfur ratio in the diets of ruminants is 10/1 (National Research Council, 1981). Several studies reported that adding easily digestible carbohydrates or sulfur to diet decreased the nitrite accumulation due to an increased amount of ammonia used by bacteria (Burrows et al. 1987, Takahashi et al. 1998, Takahashi et al. 1989). Given that nitrate is rapidly converted into nitrite by microorganisms in the rumen, sulfur supplementation to a high-nitrate diet may promote conversion rate of nitrite into ammonia and also decrease the accumulation of nitrite in the rumen. Takahashi et al. (1989) suggested that sulfur supplementation to the diet of ruminants decreases the formation of nitrite by microorganisms in the rumen.

In spite of the widespread investigation of nitrate intoxication in animals, no study to date has determined the effects of inorganic sulfur supplementation on the thyroid hormones in Angora goats fed with a high-nitrate diet. Therefore, the aim of the present study was to investigate the long-term effects of sulfur supplementation to a high-nitrate diet on thyroid hormones in Angora goats.

MATERIAL and METHODS

Animals

This study was performed on 18 male Angora goats aged 14 months. The study lasted for 180 days. The animals were provided by the Department of Anatolian Agricultural Enterprises Directorate and kept in the farm of the Afyon Kocatepe University Breeding Research Center under the same feeding and maintenance conditions during a 10-day adaptation period. The animals were equally divided into three groups (n = 6): control group fed with a basal diet, experimental nitrate group fed with a basal diet supplemented with 1500 ppm nitrate, and nitrate + sulfur group fed with a basal diet supplemented with 1500 ppm nitrate and 1.8% sodium sulfate. The animals were fed twice daily with dry alfalfa at 1% of body weight and 0.57 kg/day concentrate and provided water *ad libitum* through the experimental period. The experimental protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine (168-AKÜHEK-66-07).

Biochemical Analysis

Blood samples were collected from the jugular vein before the morning feeding on days 45, 90, 135, and 180 of the experiment. Sera were obtained by blood centrifugation (3000 rpm, 10 min, 4°C) and stored at -20°C until analysis. The serum TSH level was estimated using the commercial kits (DiaMetra, Milano, Italy, Ref: DKO013). Serum total triiodothyronine (Diagnostic Systems Laboratories, USA (DSL)-10-3100S), FT_3 (DSL-10-41100), TT_4 (DSL-10-3200), and FT_4 (DSL-10-40100) concentrations were determined by specific enzyme-linked immunosorbent assays.

Statistical Analysis

The statistical differences between the control and experimental groups were evaluated using one-way analysis of variance and Tukey post-hoc tests (SPSS for Windows 11.5.0, SPSS, IL, USA). The data were expressed as mean ± standard deviation. A difference in the mean values with $P < 0.05$ was considered to be significant.

RESULTS

Data obtained on thyroid hormones during the experimental period are shown in Table 1. On comparing the control group with the nitrate group, serum TSH and FT_4 levels were found to increase on days 90 and 135, respectively. However, no negative effect of nitrate on thyroid hormones was observed in all the groups at the end of the experimental period. On day 180, except for TT_4, the serum TSH and total and free T_3 and free T_4 concentrations were generally higher ($P < 0.05$) in the nitrate + sulfur group than in the control and nitrate groups.

Table 1. Serum TSH, TT_3,TT_4, FT_3 and FT_4 concentrations during the experimental period in all groups (X ± SEM)

Parameters	Day	Control	Nitrate	Nitrate+Sulfur	P-value
TSH(mlU/L)	45.	2.47 ± 0.95	1.77 ± 0.62	1.81 ± 0.75	0,782
	90.	1.14 ± 0.15^{b}	3.31 ± 0.30^{a}	4.20 ± 0.89^{a}	0,004
	135.	1.33 ± 0.37^{b}	1.69 ± 0.49^{b}	3.98 ± 0.84^{a}	0,014
	180.	1.42 ± 0.44^{b}	1.46 ± 0.46^{b}	3.52 ± 0.61^{a}	0,016
TT_3 (ng/dL)	45.	103.22 ± 11.49	135.84 ± 19.31	127.49 ± 12.14	0,296
	90.	94.67 ± 8.40^{b}	131.10±14.61^{ab}	196.88 ± 29.95^{a}	0,008
	135.	131.75 ± 19.12	115.19 ± 9.61	170.11 ± 16.29	0,065
	180.	112.22 ± 7.62^{b}	107.85± 15.94^{b}	193.20 ± 21.87^{a}	0,003
TT_4 (µg/dL)	45.	25.14 ± 7.40	19.27 ± 3.09	22.51 ± 5.69	0,768
	90.	20.93 ± 4.53	28.75 ± 6.87	14.25 ± 3.79	0,180
	135.	16.00 ± 2.94	19.40 ± 6.74	12.04 ± 2.85	0,533
	180.	27.16 ± 8.16	17.92 ± 3.10	15.34 ± 3.81	0,332
FT_3 (pg /mL)	45.	2.20 ± 0.12	2.36 ± 0.28	2.26 ± 0.14	0,840
	90.	2.00 ± 0.12	2.36 ± 0.42	3.02 ± 0.43	0,154
	135.	2.13 ± 0.35	2.34 ± 0.12	3.04 ± 0.41	0,138
	180.	2.19 ± 0.10^{b}	2.26 ± 0.20^{b}	3.20 ± 0.42^{a}	0,037
FT_4 (ng/dL)	45.	0.53 ± 0.07	0.55 ± 0.07	0.70 ± 0.14	0,450
	90.	0.47 ± 0.04^{b}	0.67 ± 0.10^{ab}	0.90 ± 0.09^{a}	0,009
	135.	0.50 ± 0.05^{b}	0.71 ± 0.05^{a}	0.84 ± 0.06^{a}	0,002
	180.	0.69 ± 0.10^{b}	0.61 ± 0.08^{b}	1.46 ± 0.13^{a}	0,000

a,b: Differences among groups indicated with different letters in the same row are significant ($P < 0.05$).

DISCUSSION

Although the exact mechanism underlying the influence of high nitrate intake on thyroid hormones is unknown, a high nitrate intake by animals may alter the production and secretion of thyroid hormones (Kostogrys et al. 1989). The decreased concentrations of the circulating thyroid hormones may indicate hypothyroidism induced by nitrate toxicity (Jahreis et al. 1986). In the present study, high nitrate intake did not affect the thyroid hormone concentrations in the blood of goats except for samples on day 90 for TSH concentration. The present results indicated that adding 1500 ppm nitrate to diet would not have any negative effects on the thyroid hormones in Angora goats. When the diet contained 1500 ppm nitrate, the nitrate concentration increased in the ruminal fluid, but its concentration remained unchanged in the blood of Angora goats (Ozdemir et al. 2014). This finding suggested that 1500 ppm nitrate could be used as a nitrogen source for protein synthesis by microorganisms in the rumen and had no effect on Na^+/I^- transport protein affecting intrathyroidal iodine concentration (Jahreis et al. 1986, Kostogrys et al. 1989) in Angora goats. In the present study, the blood TT_3 concentration was below the levels (232-252 ng/dL), while the blood TT_4 concentration was above the levels (11.1 and 15.1 μg/dL), reported in Angora goats by previous studies (Keçeci and Keskin 2002, Puchala et al. 2001). The discrepancies in the blood TT_3 and TT_4 concentrations among this and other studies on Angora goats might be due to the differences in the systemic conversion of T_4 into T_3.

The present results showed that sulfur supplementation to a high-nitrate diet increased serum TSH and FT_3 and FT_4 and TT_3 concentrations in Angora goats. Sulfation of thyroglobulin, the thyroid hormone precursor in the thyroid gland, is a major pathway for thyroid hormone synthesis. TSH regulates both thyroglobulin sulfation and thyroid hormone synthesis (Nlend et al. 1999). A previous study (Ozdemir et al. 2014), reported that adding sulfur to a high-nitrate diet decreased the plasma nitrate concentration, but the change was not statistically significant. This suggested that nitrate in the diet with sulfur was increasingly used by microorganisms in the rumen to produce bacterial proteins, and thus sulfur supplementation to a high-nitrate diet decreased the circulating nitrate concentration. However, in the nitrate + sulfur group, an increase in thyroid hormones might be associated with a decrease in plasma nitrate levels due to sulfur. This result indicated that sulfur supplementation to a high-nitrate diet might be an important factor against the negative effects of nitrate on the production and secretion of thyroid hormones in a dose-dependent manner. No study to date has explored the relationship between sulfur and thyroid hormone metabolism in ruminants. Therefore, changes in circulating TSH, T_4, and T_3 concentrations may not interfere with the ability of sulfur supplementation to stimulate the production and secretion of thyroid hormones in Angora goats.

In conclusion, this study suggested that Angora goats were able to tolerate a feed containing 1500 ppm nitrate with respect to the thyroid hormones and sulfur might serve as a natural source for alleviating the negative effects of the high-nitrate diet on the thyroid gland in a dose-dependent manner. However, more advanced studies are needed to elucidate the relationship between sulfur metabolism and thyroid hormone metabolism.

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RESEARCH ARTICLE

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Van, Muş, Siirt ve Diyarbakır İllerinde Sığırlarda Anaplasmosis'in Seroprevalansı

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ÖZ

Rikettsiales dizisi, Anaplasmatacae ailesindeki Anaplasma türlerinin meydana getirdiği anaplasmosis, tropik ve subtropik iklim bölgelerindeki memeli hayvanlarda görülen enfeksiyöz bir hastalıktır. Sığır anaplasmosisi genellikle *Anaplasma marginale* ile ilişkilendirilir ve bu hastalık hem kan emici sinekler ile mekanik hem de keneler ile biyolojik yolla nakledilir. Bu çalışma Van, Muş, Siirt ve Diyarbakır İllerinde sığırlarda Anaplasma spp. seroprevalansının araştırılması amacıyla yapılmıştır. Serum örnekleri ticari cELISA kiti ile Anaplasma'ya karşı gelişen antikorlar yönünden analiz edilmiştir. Çalışma sonucunda incelenen 182 sığırın 52'sinde (%28,6) Anaplasma spp. antikorları bakımından seropozitiflik saptanmıştır. Dişi sığırlarda seroprevalans %29.3, erkeklerde ise %27.1 olarak belirlenmiş ve bu farklılık istatistiksel açıdan önemsiz bulunmuştur. İstatiksel olarak sığırlarda yaş gruplarına göre farklı seropozitiflik saptanmış olup, en yüksek oran 3-5 (%45.1) yaş arasındaki hayvanlarda bulunmuştur. Ayrıca çalışma merkezleri arasında en yüksek seropozitiflik %78.7 oranı ile Siirt ilinde belirlenmiştir. Sonuç olarak, Van, Muş, Siirt ve Diyarbakır illerinde sığırlarda subklinik ve kronik Anaplasma enfeksiyonlarının varlığı ortaya konulmuştur.
Anahtar Kelime: Anaplasmosis, Sığır, cELISA, Seroprevalans

Seroprevalance of Anaplasmosis in Cattle in Van, Muş, Siirt and Diyarbakır Provinces

ABSTRACT

Anaplasmosis, caused by the genus Anaplasma related to the family Anaplasmataceae the order Rickettsiales, is an infectious disease occurs in mammals in tropical and subtropical climatic regions. Bovine anaplasmosis is usually associated with *Anaplasma marginale* infection in cattle and its can be transmitted both mechanically by biting flies or and biologically by ticks. The purpose of this study was to investigate the presence of Anaplasma spp. in cattle in Van, Mus, Siirt and Diyabakir provinces. cELISA was used to detect specific anti-Anaplasma spp. antibodies in the serum samples. 52 (28.6%) of the 182 asymptomatic cattle were seropositive against Anaplasma. The prevalence of anaplasmosis in female and male cattle was found as 29.3% and 27.3%, respectively and this difference was not found significant. Seropositive rate was statistically differ among the age groups of cattle and the highest seropositive rate was found in 3-5 years. Moreover, the highest seropositive rate of study sites was determined in Siirt as 78.7%. As a result, this is serologic survey for subclinical and chronic Anaplasma spp. infections performed on cattle in Van, Mus, Siirt and Diyabakir province.
Keywords: Anaplasmosis, Cattle, cELISA, Seroprevalans

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GİRİŞ

Sığırlarda anaplasmosis, intraeritrositik riketsiya grubunda yer alan *Anaplasma marginale*, *A. centrale, A. bovis (Ehrlichia bovis) ve A. phagocytophilum* (*E. phagocytophila*, *E. equi* ve HGE etkeni) tarafından oluşturulan enfeksiyöz bir hastalıktır. Bu türler arasında özellikle *A. marginale* oldukça yüksek patojeniteye sahiptir. Anaplasmosis sığırlarda anemi, ikterus, yüksek ateş gibi klinik semptomlarla seyretmekte olup verim kaybı, abort ve ölümlere yol açarak özellikle tropik ve subtropik iklim bölgelerinde ciddi ekonomik kayıplara neden olmaktadır (Sevinç 2004). Anaplasmosis sığırlara vektör keneler tarafından biyolojik olarak nakledilirken bazı kan emici sinekler de (Tabanus ve Psorophora spp.) Anaplasma türlerini mekanik olarak nakledebilmektedirler. Ayrıca enjektörler, kulak küpeleri, boynuz kesme veya kastrasyon ekipmanları gibi kanla kontamine cisimler de sığırlar arasında anaplasmosisin mekanik olarak bulaşmasına sebep olabilmektedir. Biyolojk nakilde ise, çeşitli kene türleri (*Argas persicus*, *Ornithodoros lahorensis*, *Rhipicephalus annulatus*, *R. decoloratus*, *R. microplus*, *R. bursa*, *R. sanguineus*, *R. simus, Dermacentor albipictus*, *D. andersoni*, *D. occidentalis*, *D. variabilis*, *Hyalomma excavatum*, *Ixodes ricinus*) görev almaktadır (Gökçe ve ark. 2013). Bu kene türlerinden *I. ricinus*, *R. annulatus*, *R. bursa*, *H. excavatum*, *A. persicus* ve *O. lahorensis*' in Doğu ve Güneydoğu Anadolu bölgesinde görüldüğü bildirilmiştir (Taşcı 1989, Sayın ve Dumanlı 1982, Dumanlı 1983, Güler ve ark. 1993, Özer ve Aydın 1996, Arslan ve ark. 1999, Akdemir 2002).

Bu hastalığın Dünya'daki durumuna bakıldığında özellikle Amerika Kıtasında oldukça yaygın olduğu görülmektedir. Türkiye'de ise sığırlarda anaplasmosis konusunda yeterli çalışma bulunmamaktadır. Gökçe ve ark. (2013) 188 sığır üzerinden yaptıkları serolojik bir çalışmada *A. marginale* antikorlarını %52.1 olarak bildirmektedir. Yapılan bir başka çalışmada 484 sığırın 287'sinin serolojik olarak *A. marginale* yönünden pozitif olduğu bildirilmektedir (Ekici ve Sevinç 2011). Birdane ve ark. (2006) 645 sığırın % 55.35'ini A.marginale yönünden seropozitif bulmuşlardır. Son yıllarda bu alanda sınırlı sayıda da olsa moleküler çalışmaların varlığı dikkati çekmektedir. Bu çalışmalarda Türkiye'de sığırlarda *A. marginale*, *A. centrale* ve *A. pahagocytophilum*'un varlığı ortaya konulmuştur (Aktaş ve ark., 2009; 2011). Karadeniz bölgesinde yapılan bir moleküler çalışmada toplanan kenelerin %6.56'sı *A. centrale* yönünden pozitif bulunmuştur (Aktaş ve ark. 2012).

Anaplasmosis' in teşhisinde, yüksek ateş (41ºC), progresif anemi, sarılık gibi genel klinik semptomlar şüpheleri artırabilmektedir. Hastalık bu semptomlarla sığırlarda görülen başka enfeksiyöz hastalıklara benzerlik gösterebilmektedir. Ancak akut dönemde enfeksiyonun teşhisi perifer kandan yapılmış ve giemsa ile boyanmış preparatların mikroskobik muayenesi ile eritrositler içerisinde mavi-mor renge boyanmış etkenlerin görülmesiyle yapılabilmektedir. Latent hayvanlarda mikroskobik bakı tanı için yeterli olamamaktadır. Taşıyıcı hayvanların teşhisi, Anaplasma türüne karşı şekillenen spesifik antikorların veya parazite ait DNA'nın tespit edilmesi ile mümkündür. Kompetitif ELİSA yöntemi, sensivitesi ve spesifitesinin oldukça yüksek olması ve enfeksiyondan 6 yıl sonra bile teşhis koyabilme özelliğinden dolayı son yıllarda özellikle epidemiyolojik çalışmalarda güvenilir bir şekilde kullanılmaya başlanmıştır (Torioni de Echaide ve ark. 1998, Kocan ve ark. 2000, Woldehiwet 2010, Ekici ve Sevinç 2011).

Bu çalışma Doğu ve Güneydoğu Anadolu'nun bazı illerindeki (Van, Muş, Siirt ve Diyarbakır) sığırlarda Anaplasma etkenlerine karşı oluşan antikorların varlığının saptanması ve sığır anaplasmosisi üzerine epidemiyolojik verilerin elde edilmesi amacıyla yapılmıştır.

MATERYAL ve METOT

Hayvan materyali ve kan örnekleri

Çalışmanın materyalini, 2016-2017 yılları arasında Van, Muş, Siirt ve Diyarbakır'da meraya çıkmış ve sağlıklı görünümlü toplam 182 sığır oluşturmuştur. Hayvanlara ait bilgiler (yaş, cinsiyet, ırk) hayvan sahiplerinden alınmış ve kaydedilmiştir. Rastgele seçilen toplam 182 sığırın vena jugularis'inden tekniğine uygun olarak 5 ml'lik steril EDTA'lı (di-sodium ethylenediamine tetra-acetate) tüplere kan alınmış ve örnekler soğuk zincirde taşınarak Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Parazitoloji Anabilim Dalı Laboratuvarı'na getirilmiştir. Alınan bu kan örnekleri 1500 rpm' de 10 dak. santrifüj edildikten sonra serumları ayrılarak analiz gününe kadar -20ºC'de saklanmıştır.

c-ELISA Testi

Çalışma için ticari kompetetif ELISA (C-ELISA) kiti kullanılmıştır. C-ELISA testi, üretici firmanın test prosedürüne göre yapılmıştır (Anaplasma antibody test kit, C-ELISA, catalog number: 282-2VMRD-USA). Kompetitif ELISA yönteminin prensibi antijen antikor reaksiyonuna dayanır.

ELISA Sonuçlarının Değerlendirilmesi

Test sonucu, spektrofotometrik olarak 630 nm filtre absorbanslarında okunmak suretiyle belirlenmiştir. Pozitif kontrol ve örneklerin değerlendirilmesi test prosedüründe belirtilen (İnhibisyon yüzdesi, %I=100-[(Serum O.D. X 100)

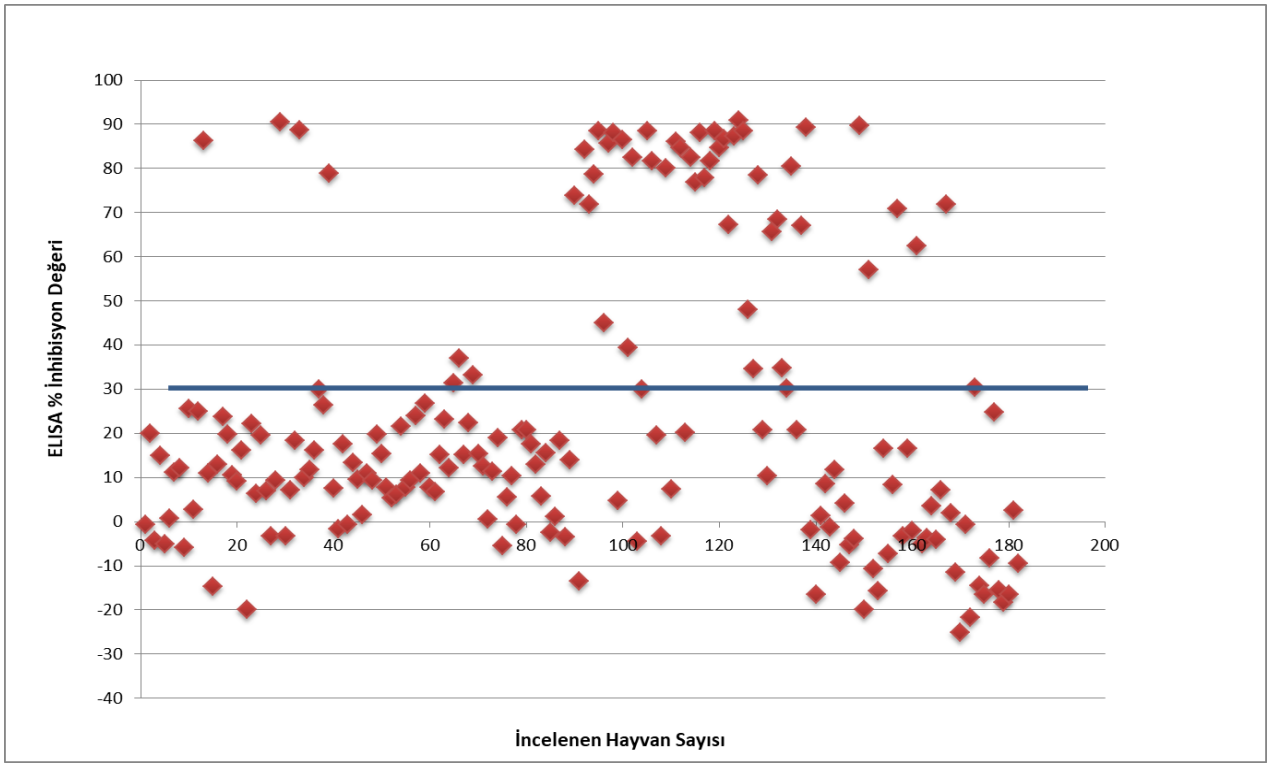
: (Ortalama Negatif kontrol O.D)] formül ile yapılmıştır. Bu aşamada negatif kontrol optik dansite (OD) 0.40-2.10 aralığında alınmıştır. Pozitif kontrol hesaplamalar sonucunda %30'a eşit ve büyük olarak kabul edilmiştir. İnhibisyonun %30' a eşit ve daha fazla olduğu örnekler pozitif, diğerleri ise negatif olarak değerlendirilmiştir.

İstatiksel Analizler

Sonuçların istatiksel değerlendirilmesinde sığırlarda Anaplasmosis prevalansı ile yaş, cinsiyet, ırk ve yer faktörlerinin ilişkisi, Pearson's Chi Square testiyle araştırılmıştır. İstatistik hesaplamaları SPSS 22 programı kullanılarak yapılmıştır.

BULGULAR

Bu araştırmada 4 farklı merkezden (Van, Muş, Siirt, Diyarbakır) toplanan 182 sığıra ait serum örneği Anaplasma antikorlarının varlığı yönünden serolojik olarak incelenmiştir. Çalışma kapsamında incelenen örneklerin %28.6'sı (52/182) Anaplasmosis yönünden seropozitif bulunmuştur. ELISA ile incelenen örneklerde saptanan inhibisyon değerleri Şekil 1'de gösterilmiştir.



Şekil 1: İncelemesi yapılan sığırlarda cELISA ile saptanan inhibisyon değerlerinin (% I) dağılımı
Figure 1: Distribution of inhibition values (% I) detected by cELISA in the examined cattle

Sığırlarda cELISA sonuçlarına göre Anaplasmosisin yerleşim yerlerindeki dağılımı Tablo 1'de verilmiştir. İncelenen hayvanlarda en yüksek pozitiflik %78.7 oranıyla Siirt ilinde görülmüş, bunu Diyarbakır (%15.2), Van (%10.9) ve Muş (%7) illeri izlemiştir (p=0.000). Çalışmada saptanan pozitifliklerin yaş, ırk ve cinsiyete göre dağılımları Tablo 2 'de verilmiştir. cELISA testi sonuçlarına göre seropozitiflik oranı dişi sığırlarda %29.3 (36/123), erkek sığırlarda %27.1 (16/59) olarak tespit edilmiştir. Sığırlarda cinsiyet durumuna göre seropozitiflik oranları karşılaştırılmış ve seropozitif hayvanlar arasında cinsiyet açısından istatistiksel olarak anlamlı bir fark bulunmamıştır (p>0,05; p=0,764). Anaplasmosis seropozitifliği en fazla %45.1 oranı ile 3-5 yaş arasındaki sığırlarda, en az %9.3 ile 2 yaş ve altı sığırlarda tespit edilmiştir. 3-5 yaş arasındaki hayvanların seropozitiflik oranı ile diğer yaş gruplarının seropozitiflik oranları arasında istatistiksel olarak anlamlı bir fark tespit edilmiştir (p=0.000). En yüksek anaplasmosis seropozitifliği

Holstein ırkı sığırlarda görülmüş (%48.4), bunu yerli (%27.3) ve melez ırkı (%21.6) takip etmiştir (p=0.020).

Tablo 1: Sığırlarda anaplasmosisin yerleşim yerlerine göre yaygınlığı
Table 1: The prevalance of cattle anaplasmosis according to localities

Yerleşim Yeri	Muayene Edilen Sığır Sayısı	Seropozitif	
		Sığır sayısı	%'si
Van	46	5	10.9
Muş	43	3	7
Siirt	47	37	78.7
Diyarbakır	46	7	15.2
Toplam	**182**	**52**	**28.6**

Tablo 2: Sığırlarda anaplasmosis prevalansının yaş, cinsiyet ve ırkla ilişkisi
Table 2: The correlation of the anaplasmosis prevalence in cattle with age, sex and race

Epidemiyolojik Veriler	İncelenen Sığır Sayısı	Seropozitif Sığır Sayısı	%
Yaş*			
≤2	43	4	9.3
3-5	71	32	45.1
≥6	68	16	23.5
Toplam	**182**	**52**	**28.6**
Irk**			
Melez	74	16	21.6
Holstein	31	15	48.4
Yerli	77	21	27.3
Toplam	**182**	**52**	**28.6**
Cinsiyet***			
Erkek	59	16	27.1
Dişi	123	36	29.3
Toplam	**182**	**52**	**28.6**

*P=0.000, **P=0.020, ***P>0.05

TARTIŞMA

Anaplasmosis evcil hayvan ve insanlarda görülen, klinik seyri hafiften ölümcüle kadar değişebilen riketsiyal bir hastalıktır. *A.marginale*, *A. centrale*, *A. bovis*, *A. ovis*, *A. platys* ve *A. phagocytophilum* olmak üzere 6 önemli Anaplasma türü içerisinde sığırları etkileyen en patojen tür *Anaplasma marginale*'dir. *A. marginale*, eritrosit içerisinde, 0.3-1.0 mikron büyüklüğünde olup özellikle eritrosit duvarında veya duvara yakın bir bölgede yerleşim gösterirken, *A. centrale*, eritrositlerin merkezinde veya merkeze yakın olarak bulunur. Anaplasma türlerinin konak hücrelerine tutunmalarında ve enfeksiyon oluşturma kabiliyetlerinde majör yüzey antijenleri önemli rol oynamaktadır. *A. marginale*'de; MSP1a, MSP4, MSP5, MSP1b, MSP2 ve MSP3 olmak üzere altı adet merozoit yüzey antijeni bulunmaktadır. *Anaplasma marginale* yüzey antijenlerinden MSP5, tek bir gen tarafından kodlanmakta ve diğer yüzey antijenlerine göre farklı coğrafik bölgelerdeki izolatların ayrımında stabil bir genetik marker olarak kullanılabilmektedir (Kocan ve ark. 2003). Bu nedenle bu antijen ve antijene spesifik monoklonal antikor kullanılarak geliştirilmiş olan kompetitif ELISA (cELISA), anaplasmosisin teşhisinde kullanılan son derece duyarlı ve özgül bir testtir (Torioni de Echaide ve ark. 1998, Corona ve Martinez 2009, Corona ve ark. 2009). Bu çalışmada Van, Muş, Siirt ve Diyarbakır illerinde yetiştirilen sığırlarda Anaplasma spesifik antikorların varlığını araştırmak için cELISA testi kullanılmıştır.

Akut anaplasmosisin teşhisi mikroskobik muayene ile kolayca yapılabilirken, subklinik veya kronik enfeksiyonlarda düşük parazitemi nedeniyle hastalığın saptanması oldukça zor olmaktadır (Shompole ve ark. 1989). Böyle hayvanların teşhisinde serolojik testlerden (IFA ve cELISA testleri vb.) ve moleküler tekniklerden yararlanılmaktadır (Dik ve Sevinç 2002). Irak'ta ELISA testi ile 184 sığırın 24' ünde (%13.04) *A. marginale* antikorları belirlenmiş olup, seropozitif

hayvanlar PZR-RFLP (Restriksiyon Parça Uzunluğu Polimorfizmi) ile analiz edilmiş, genetik karakterizasyon çalışmaları sonucunda ise 20'sinin *A. marginale* olduğunu tespit edilmiştir (Jassem ve Agaar 2015). Atif ve ark. (2013) tarafından Pakistan'ın kuzey kesiminde (Sargodha, Khushab ve Rawalpindi) 1050 sığırdan toplanan kan örneklerinin 326 'sında (%31.05) cELISA testi ile *A. marginale* antikorları tespit edilmiştir. Tembue ve ark. (2011) Mozambik'te indirekt ELISA yöntemiyle inceledikleri sığırların %76.5' ini *A. marginale* enfeksiyonu yönünden pozitif bulmuşlardır. Hornok ve ark. (2007) Macaristan'da cELISA testi ile incelenen sığırların %80.8'inin (21/26) Anaplasma antikorları yönünden seropozitif olduklarını belirlemiş ve 12 seropozitif örneğin 4'üne sekans analizi yapılarak *A. marginale* olduğunu tespit etmişlerdir. Malezya'da 267 sığırın cELISA yöntemiyle incelenmesi sonucu 212'sinin (%74.9) *A. marginale* yönünden pozitif olduğu tespit edilmiştir (Pong ve Nik Him 2012).

Türkiye'de anaplasmosis konusunda yeterli çalışma bulunmamaktadır. Yapılan az sayıdaki çalışmada mikroskobik, serolojik ve son yıllarda moleküler olarak özellikle vektör kenelerde anaplasmosis araştırılmıştır. Selçuk ve ark. (2015) Bursa yöresinde klinik muayenelerinde anaplasmosisden şüphelenilen 61 sığırın %45.9' unda cELISA testi ile Anaplasma enfeksiyonu yönünden seropozitiflik belirlemişlerdir. Birdane ve ark. (2006) 645 sığır üzerinde yaptığı bir araştırmada sığırların mikroskobik olarak %34.11'nin, serolojik olarak ise %55.35'nin *A. marginale* yönünden pozitif olduğunu rapor etmişlerdir. Yapılan bir başka çalışmada sığırlarda anaplasmosis yönünden serolojik olarak cELISA ve IFA testlerinin karşılaştırılması yapılmıştır (Ekici ve Sevinç 2011). Bu çalışmada testlerin spesifitesi %100 bulunurken, sensivitesini ise cELISA için %87.3, IFA için %90.3 olarak bildirmişlerdir. Araştırıcılar cELISA testinin kullanımının kolay olması, ucuz olması ve özel laboratuvar şartlarına ihtiyaç duyulmaması nedeniyle tavsiye etmektedirler. Aktaş ve ark. (2011) Karadeniz bölgesinde 6 ilde 389 sığır üzerinde yaptıkları moleküler çalışmada Anaplasma enfeksiyonların %9 olarak bulmuşlardır. Açıcı ve ark. (2016) tarafından Karadeniz bölgesinde yapılan bir çalışmada 270 sığırın 102' si (37.8) *A. marginale* enfeksiyonu yönünden pozif bulunmuştur. Gökçe ve ark. (2013) Kars yöresindeki sığırlarda cELISA yöntemiyle *A. marginale* seroprevalansını %52.1 (98/188) oranında bildirmişlerdir. Doğu ve Güneydoğu Anadolu'nun bazı illerinde yapılan bu çalışmada incelenen 182 sığırın 52'si (%28.6) Anaplasma antikorlarının varlığı yönünden pozitif bulunmuştur. Çalışmaların yapıldığı bölgeler arasındaki coğrafik ve iklimsel farklılıklar, vektör kene popülasyonu ve enfeksiyonun yayılmasında rol oynayan rezervuar konak yoğunluğu Anaplasma enfeksiyonlarının görülme sıklığını doğrudan etkileyen faktörlerdir (Ahmadi-Hamedani ve ark. 2009, Altay ve ark. 2014). Bu sebeplere bağlı olarak hastalığın yayılışı bölgeden bölgeye değişiklik gösterebilir.

Tembue ve ark. (2011), *Anaplasma spp.* antikorlarını dişi sığırlarda %76,6, erkeklerde ise % 76,4 olarak saptamışlar ve cinsiyete bağlı enfeksiyon oranlarında istatistiksel anlamda bir farklılık olmadığını kaydetmişlerdir. Benzer şekilde bu çalışmada dişi sığırlarda %29,3, erkeklerde ise %27,1 oranında *Anaplasma spp.* antikorları tespit edilmiş ve cinsiyetler arasındaki farklılık istatistiksel açıdan önemsiz bulunmuştur ($p>0.05$). Bu çalışmada ırklara göre anaplasmosis'in dağılışı, en yüksek holstein ırkı sığırlarda (%48,4) saptanmış olup, bunu yerli (%27,3) ve melez (%21,6) ırkları izlemiştir. Irklar arasında, holstein ırkında saptanan enfeksiyon oranı, yerli ve meleze göre istatistiksel olarak önemli bulunmuştur ($p<0.05$). Benzer şekilde Belal ve ark. (2014), holstein ırkı sığırlardaki enfeksiyon oranını (%30) yerli ırklardan (%20.4) daha yüksek bulmuşlardır.

Birdane ve ark. (2006)'nın İç Ege bölgesindeki sığırlarda yaptıkları bir çalışmada; *A. marginale* seroprevalansı 3-4 yaşlı sığırlarda %58.21; 4 yaşından büyük sığırlarda %82.07 olarak bulunmuştur. Bangladeş'te 398 sığırın mikroskobik muayene yöntemiyle incelenmesi sonucunda 102'sinin (%25.82) *Anaplasma spp.* yönünden pozitif olduğu ortaya konulmuş, enfeksiyon oranının da yaşın ilerlemesine paralel olarak artış gösterdiği tespit edilmiştir (Belal ve ark. 2014). Atif ve ark. (2013), *A. marginale* enfeksiyonunu 4 yaş üstü sığırlarda (% 48.6), 2-4 (% 21.2) ve 1-2 (% 14.7) yaş arası sığırlara oranla daha yaygın bulmuşlardır. Benzer şekilde bu çalışmada ELISA ile seropozitif bulunan sığırların %9.3'ü (2/69) 2 yaş ve altı grubunda, % 45.1'inin de 3-5 yaş grubunda olduğu belirlenmiştir. Enfeksiyonun yayılışının nispeten yaşlı sığırlarda daha yüksek olması, parazitin inkubasyon süresinin uzun olması ile konak-parazit ilişkisinde yaşlı sığırların muhtemelen vektörlere daha uzun süre maruz kalmasıyla açıklanmaktadır (Jonsson ve ark. 2008). Yaptığımız bu çalışmada Anaplasmosis enfeksiyonu en yüksek olarak Siirt ilinde %78.7 oranında bulunurken, en düşük Muş ilinde (%7) bulunmuştur. Bu durum bölgesel iklim, kene türleri farklılıkları ve kan emici sineklerin görülme sıklığından kaynaklanabilir. Bu çalışmada kan örneği alınan sığırların hiçbirinde anemi, ateş, sarılık gibi klinik anaplasmosis belirtileri saptanmamasına karşın, nispeten yüksek oranda Anaplasma seropozitifliği tespit edilmiştir. Bu sonuç sığırların kronik olarak enfekte veya taşıyıcı olduklarını göstermektedir (Kocan ve ark 2000).

SONUÇ

Bu araştırma, Van, Muş, Siirt ve Diyarbakır illerinde sığırlarda Anaplasmosis enfeksiyonunu ortaya koyan epidemiyolojik bir ön çalışmadır. Elde edilen bu veriler daha sonra bu alanda yapılacak olan çalışmalara literatür katkısı sağlayacaktır. Antikor tespitine dayalı serolojik teşhis metotlarının, incelenen örneklerde etkeni her zaman tam olarak teşhis edememesi nedeniyle, serolojik muayene sonuçlarının PZR gibi DNA tabanlı yöntemlerle desteklenmesi veya doğrulanmasının gerekli olduğu kanısındayız.

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RESEARCH ARTICLE

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Effects of Curcumin on A Renal Ischemia/Reperfusion Injury in Rats

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ABSTRACT

This study aimed to investigate the biochemical and histopathological effects of curcumin on Renal Ischemia/Reperfusion Injury. Twenty-four female Wistar rats were used in the study and divided into control (C), sham, ischemia/reperfusion (I/R), and curcimin (CUR) groups (n=6). Laparotomy was performed under general anesthesia in I/R and CUR groups than the left renal pedicle was dissected it was clamped. Then, 1-h ischemia and 6-h reperfusion were applied. 500 mg/kg Curcumin was given intraperitoneally to the CUR group after ischemia application. MPO, IMA, MDA, NO, SOD, GPx, AOS, urea, and creatinine levels were measured in serum samples. MPO, MDA, NO, SOD, GPx, and AOS were also measured in tissue samples. The histopathological examination was performed. Serum and tissue AOS levels were significantly higher in the CUR group than in the I/R group (P<0.05). Tissue NO levels were significantly lower in the CUR group than in the I/R group (15.30±5.41 and 4.8±1.37, respectively) (P<0.05). Histopathological scores were also significantly lower in the CUR group than in the I/R group (P<0.05). The results showed that curcumin prevented I/R damage by decreasing oxidative stress in serum and tissue samples in rat renal I/R model.
Keywords: Curcumin, Renal Ischemia/Reperfusion, Rats.

Ratlarda Renal İskemi/Reperfüzyon Hasarında Curcumin'in Etkileri

ÖZ

Bu araştırmada, Renal İskemi/Reperfüzyon Hasarı Modelinde Curcumin'in etkilerini biyokimyasal ve histopatolojik olarak araştırmak amaçlanmıştır. Çalışmada ratlar kontrol (C), sham, iskemi repürfüzyon (I/R) ve curcumin (CUR) grubu olmak üzere (n=6) toplam 24 dişi Wistar Rat kullanılmıştır. Genel anestezi altında Grup I/R ve CUR da laparatomi uygulanarak sol böbrek pedikülü diseke edilmiş ve böbrek arteri klempe edilerek 1 saat iskemi ve 6 saat reperfüzyon gerçekleştirilmiştir. Grup CUR da 500 mg/kg Curcumin iskemi sonrası intraperitonal olarak verilmiştir. Serum MPO, IMA, MDA, NO, SOD, GPx, AOS , üre ve kreatinin ölçümleri gerçekleştirilmiştir. Doku örneklerinde MPO, MDA, NO, SOD, GPx, AOS ölçülmüştür. Histopatolojik inceleme ile böbrek dokusunda I/R hasarı skorlanmıştır. Serum ve doku AOS Grup I/R Grup CUR ile karşılaştırıldığında istatistiksel önemi olacak şekilde Grup CUR'de yüksel bulunmuştur (p<0.05). Doku NO düzeyi I/R grubu ile karşılaştırıldığında Grup CUR'da istatistiksel önemi olacak şekilde düşük bulunmuştur (15,30±5,41, 4,8±1,37) (p<0.05). Histopatolojik skorlamada Grup CUR, Grup I/R ile karşılaştırıldığında istatistiksel olarak anlamlı olacak şekilde düşük bulunmuştur (p<0.05). Sonuç olarak; bu araştırmada Curcumin'in Renal I/R hasarı modelinde serum ve doku örneklerinde oksidatif stresi azaltarak I/R hasarını önlemede olumlu etkisi olduğu ve histopatolojik inceleme ile de Curcumin'in bu olumlu etkisi desteklenmiştir.
Anahtar kelimeler: Curcumin, Renal İskemi/Reperfüzyon, Rat.

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INTRODUCTION

Acute kidney failure (AKF) is a clinical syndrome characterized by a rapid disruption of normal functions of the kidney for various reasons. This syndrome is characterized by the accumulation of nitrogenous waste materials and the deterioration of fluid and electrolyte balance as a result of a rapid decline in glomerular filtration rate (GFR) over hours or day by day (Adewusi and Afolayan, 2009; Atthe et al., 2009; He et al., 2011; Tanrıverdi and Karadağ, 2010; Uslu et al., 2009). Renal functions usually improve with an effective treatment in patients whose kidney functions were normal before the development of acute renal failure (ARF). This situation is the most important characteristic of AKF distinguishing it from chronic kidney failure (Tanrıverdi and Karadağ, 2010).

The most common cause of ARF is ischemia. Ischemic ARF occurs due to conditions such as kidney transplantation, cardiovascular surgical interventions, hemorrhage, sepsis, dehydration, and trauma (Guan et al., 2009; Kumar et al., 2009).

The pathophysiology of Ischemia/Reperfusion Injury in the kidney is quite complex. Activation of neutrophils involves the release of ROS and other inflammatory mediators such as adhesion molecules and cytokines. Studies have shown beneficial effects of some agents in combating IRH. For example, doxycycline has been shown to be effective in reducing proinflammatory cytokine levels (İhtiyar et al., 2011; Kucuk et al., 2009). Leptin has shown to reduce tumor necrosis factor alpha levels and increase nitrite levels (Erkasap et al., 2004). Levosimendan acts as an antioxidant due to nitric oxide–related mechanism (Grossini et al., 2012), and iloprost shows the same effect by suppressing lipid peroxidation (Döslüoglu et al., 1993). Finally, ascorbic acid sweeps out all the free radicals and acts as an antioxidant (Korkmaz and Kolankaya, 2009).

Curcuma longa is a plant belonging to the Zingiberaceae family. It is commonly found in India and China. The local paramedical use of curcumin has been studied by many researchers in recent years (Pandya et al., 2000). Curcumin (CUR) has anti-inflammatory, immunomodulatory, anti-tumoral, and anti-psoriatic effects due to its antioxidant properties.

This study investigated the effect of CUR on the rat ischemia/reperfusionc (I/R) injury model. All biochemical and antioxidant parameters in serum and tissue specimens were measured, and changes in electrolyte levels and histopathology samples were examined.

MATERIALS and METHODS

A total of 24 female Wistar rats weighing 250–350 g were used. In this study raised under the same environmental conditions were used. For at least one week prior to surgery, the animals were housed in standard cages in a pathogen-free environment with free access to food (until 2 h before the anesthetic procedure) and water and with a 12-hour light/dark cycle. The animals were randomly separated into four groups, each containing six rats. All the procedures were performed according to the accepted standards of the Guide for the Care and Use of Laboratory Animals. The study was started with the approval of Afyon Kocatepe University, Local Animal Experiment Commity dated: 08.11.2016 with the number of 137-16.

Study design

Control group (C group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline and sacrificed after 6 h.

Sham group (S group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline 1 h before laparotomy and their blood and tissue samples were collected 6 h after laparotomy.

Ischemia/reperfusion group (I/R group) (n = 6): The rats in this group were injected intraperitoneally with 2 mL physiological saline 1 h before laparotomy and their blood and tissue samples were collected after 6-h reperfusion following 60-min renal ischemia.

Curcumin group (CUR group) (n = 6): The rats in this group were injected intraperitoneally with 500 mg/kg CUR(Sigma Life Science, Lot: SLBN7214V, MO, USA) dissolved in ethanol after laparotomy, and their blood and tissue samples were collected after 6-h reperfusion following 60-min renal ischemia.

Anesthesia procedure

General anesthesia was performed intramuscularly using 8 mg/kg xylazine HCl (Alfazine, Ege-Vet, Turkey) and 80 mg/kg ketamine HCl (Alfamine, Ege-Vet, Turkey).

Renal ischemia/reperfusion procedure

Laparotomy was performed with midline incision in rats in the I/R and CUR groups. After each renal pedicle was found, renal artery occlusion was achieved using microvascular clamps (Bulldog). Fading in the kidneys after using clamps was detected as a sign of occlusion. Subsequently, the abdomen, which was temporarily closed with silk sutures, was opened after 60 min. The clamps were removed, and the color change in the kidney was

observed. The abdomen was temporally closed by using 5 mL intra-abdominal Ringer lactate and sewing up the laparotomy line with 4.0 silk sutures. The rats were allowed to awaken and anesthetized after 6-h reperfusion once their intracardiac blood and kidney tissue samples were taken. Finally, the rats were sacrificed by exsanguination from the abdominal aorta.

Preparation of kidney tissue samples and protein determination

Kidney tissue samples were homogenized in 1:10 w/v of 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. . The homogenate was centrifuged in a refrigerated centrifuge at 15,000 rpm for 15 min to obtain supernatants. Subsequently, protein levels were determined using Lowry method (Lowry et al., 1951), followed by the determination of SOD, GPx, malondialdehyde (MDA), and NO levels and antioxidant activity.

Determination of serum ischemic modified albumin levels with myeloperoxidase (MPO), GPx, MDA, SOD, NO, and antioxidant status in renal tissue samples and serum

Myeloperoxidase (MPO) [Sunred Rat MPO Enzyme-Linked Immunosorbent Assay (ELISA) Kit, Cat. No. 201-11-0575, China], GPx (Cayman Chemical Company, ELISA Kit Cat No. 703102, USA) and antioxidant assay (Cayman Chemical Company, ELISA Kit Cat. No. 709001, USA) levels in the tissue samples and serum were determined using a commercially available ELISA kit. The absorbance of the color formed by the reaction of MDA with thiobarbituric acid [known as Draper and Hadley method (1990) (Draper and Hadley, 1990) measured at 535 nm. The SOD activity was determined using the method reported by Sun et al. (1988) (Sun et al., 1988) , which was based on the inhibitory effect of SOD on nitroblue tetrazolium reduction of superoxide anions by xanthine/xanthine oxidase system. Nitric oxide levels were determined according to the method reported by Miranda et al. (2001) (Miranda et al., 2001). The samples were deproteinized by diluting them at a ratio of 1/3 with 10% TCA (Trichloroacetic acid) before measurement.

The ischemia-modified albumin (IMA) level in the serum was determined using the commercial dual-antibody sandwich ELISA kit (Sunred Rat Myeloperoxidase MPO ELISA Kit Cat. No. 201-11-1672, China).

Histopathological examination

The kidneys of the rats that underwent necropsy were fixed in buffered neutral 10% formaldehyde solution. They were trimmed after 48 h and moved into the trays for tissue attachment. The tissues were traversed through the series of alcohol and xylene applications and blocked in paraffin. The blocks were sliced into 4- to 4-μm sections using a microtome and placed on the slides. The sections were stained with hematoxylin–eosin (HE) technique and examined under a light microscope. The changes in the kidneys were evaluated according to the criteria given in Table 1.

Table 1. Degree of kidney histopathology
Tablo 1. Böbrek Histopatolojisinin Derecelendirilmesi

Degree	Damage	Pathological definition
0	Absent	Normal tubule
1	Mild	Mild swelling, loss of brush border edge
2	Moderate	Massive swelling, middle vacuolization
3	Middle	Shrinkage in the nucleus, severe vacuolization
4	Severe	Necrotic, apoptotic cells, basal membrane rupture
5	Necrosis	Complete necrosis of the tubule

Statistical analysis

Data were presented as means±standard deviation (S.D.) values. The Kruskal–Wallis H test was used as a nonparametric test to determine changes during the biochemical and electrolyte analysis of oxidative stress. In addition, the chi-square test was used for comparing the variables obtained from the pathological analysis. The data obtained in the study was analyzed using the Statistical Package for Social Sciences (SPSS, 18.0 software, USA). Differences were considered statistically significant when $p<0.05$.

RESULTS

The levels of MPO, IMA, MDA, NO, SOD, GPx, AOS, urea, and creatinine in the serum samples were measured in control, sham, I/R, and CUR groups in this study. Serum and tissue AOS levels were significantly higher in the CUR group than in the I/R group ($P < 0.05$). (Table 2). MPO, MDA, NO, SOD, GPx, and AOS levels were measured in the kidney tissue. Tissue NO levels were significantly lower in the CUR group than in the I/R group (15.30 ± 5.41 and 4.8 ± 1.37, respectively) ($P < 0.05$). (Table 3). At the end of

the study, serum K^+, Ca^{2+}, Na^+, and Cl^- levels were measured in the venous blood samples taken from all groups before sacrified. Serum K^+ levels in I/R grup were higher than in CUR Grups (8.69 ± 0.84 vs 6.93 ± 1.51) ($P<0.05$). The results are shown in Table 4.

Histopathological scores were also significantly lower in the CUR group than in the I/R group (83.3%=Degree 3, 0%=Degree 4, 6.7% =Degree 5 in the CUR group vs 50%, 33,3%, 16,7% in the I/R grup, respectively) (Pearson chi-square test: $P = 0.001$ (Table 5), (Fig 1a,b,c,d).

DISCUSSION

This study investigated whether the intraperitoneal application of curcumin at a dose of 500 mg/kg in rats had beneficial effects in correcting renal I/R Injury. Studies on the physiopathology of I/R injury have reported that ROS produced from the damaged tissue after reperfusion induces the release of proinflammatory cytokines by stimulating macrophages. These cytokines trigger an inflammatory response, increasing tissue damage

(Pompermayer et al., 2005). ROS which is released after neutrophil migration in ischemic tissue, protease, elastase, MPO, and proinflammatory cytokines increase tissue damage (Kettle and Winterbourn, 1997).

In the present study, both serum level and activity of MPO (an indicator of tissue neutrophil activation) in the kidney tissue increased in the control group compared with the sham and I/R groups. In contrast, MPO activity decreased in the CUR group.

IMA has been identified as a new marker of inflammatory diseases in recent years (Ellidag et al., 2013). IMA activity has been found to be high in high oxidative stress–related, inflammatory diseases (Roy et al., 2006) (Roy et al., 2006). It has been reported that the IMA level elevates within minutes after ischemia, remains high for 6–12 h, and returns to a normal level within 24 h (Anwaruddin et al., 2005).

In this study, the IMA level in the I/R group was found to be statistically significantly higher after 6 h of reperfusion compared with that in the CUR group. On the contrary, the IMA level measured at the end of the study was lower in the CUR group than in the control group.

Clinical trials have shown a major role of ROS in the pathogenesis of renal I/R Injury (Sancaktutar et al., 2014) (Sancaktutar et al., 2014). Lipid peroxidation is a complex phenomenon initiated by the removal of a hydrogen atom from a methylene group placed between two unsaturated bonds in lipid molecules. This results in the formation of a new carbon-centered lipid free radical. Lipid peroxides or hydroperoxides are formed from this new lipid free radical in the presence of oxygen. These end products are converted into MDA, which is a relatively more stable end product and can be used as a marker for lipid peroxidation (Slater, 1988).

In this study, MDA levels in kidney tissue were significantly elevated in both I/R and CUR groups compared with the control and sham groups. However, MDA level in the CUR group was determined to be lower than that in the I/R group. However, the serum I/R level was lower in the I/R group than in the CUR group.

Tubular cells normally do not produce NO. Ischemic damage increases intracellular NOS outflow in the tubular cells. Ischemia in the tubular cells has been shown to induce peroxynitrite formation by increasing NO and superoxide production (Yaqoob et al., 1996).

The NO level in the kidney tissue was found to be statistically significantly lower in the CUR group compared with that in the I/R group. On the contrary, NO level was significantly higher in the I/R group than in the control group.

The antioxidant properties of curcumin have been shown to reduce oxidative stress and tissue destruction in the heart and brain and also I/R injury in the liver (Thiyagarajan and Sharma, 2004). Curcumin acts as an antioxidant by inhibiting the conversion of XD into XO, lipid peroxidation, and ROS in the ischemic environment. Additionally, it reduces lipid peroxidation by increasing the activity of the enzymes such as curcumin catalase, superoxide dismutase, and glutathione peroxidase (Miquel et al., 2002).

In this study, the difference in the serum SOD and GPx levels were found to be insignificant between the I/R and CUR groups. However, the SOD level in the kidney tissue was found to be significantly higher in the CUR group compared with that in the I/R group. Although GPx was found to be lower in both serum and kidney tissues in the I/R group, serum GPx level was found to be higher in the CUR group than in all other groups. AOS in the serum and kidney tissues was found to be lower in the I/R group compared with that in the CUR group.

Table 2. Measurement results of biochemical and oxidant/antioxidant parameters in serum.
Tablo 2. Serum biyokimya ve oksidan/antioksidant parametrelerin ölçüm sonuçları.

Group	MPO (ng/mL)	IMA (ng/mL)	MDA (nmol/mL)	NO (µmol/L)	SOD (U/mL)	GPx [nmol/(min · mL]	AOS (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control (*n* = 6)	3.43 ± 0.94	23.73 ± 3.91[a]	1.56 ± 0.34	3.76 ± 0.34	1.21 ± 0.16	43.50 ± 5.46	0.3 ± 0.02[b]	49.42 ± 7.14[a]	0.31 ± 0.09[a]
Sham (*n* = 6)	3.87 ± 0.43	26.97 ± 1.16	1.86 ± 0.39	3.49 ± 0.41	1.35 ± 0.35	43.83 ± 10.3	0.26 ± 0.02[bc]	45.38 ± 5.37[a]	0.29 ± 0.04[a]
I/R (*n* = 6)	4.24 ± 0.59	28.86 ± 3.84[b]	2.16 ± 0.44	3.52 ± 0.28	1.6 ± 0.23	38.83 ± 8.32	0.19 ± 0.03[a]	74.25 ± 14.25[b]	0.45 ± 0.04[b]
CUR (*n* = 6)	3.24 ± 0.73	23.17 ± 6.21[a]	2.08 ± 0.22	3.43 ± 0.33	1.24 ± 0.25	46.66 ± 7.36	0.23 ± 0.03[ac]	96.87 ± 12.38[b]	0.52 ± 0.07[b]

The values in the same column shown with different characters are statistically significant ($P < 0.05$).
AOS: Antioxidant status; GPx, glutathione peroxidase; IMA, ischemia-modified albumin; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; SOD, superoxide dismutase.

Table 3. Measurement results of biochemical and a oxidant/ntioxidant parameters in the kidney tissue
Tablo 3.Böbrek dokusunda biyokimya ve oxidant/antioksidant parametrelerin ölçüm sonuçları

Group	MPO (ng/ml)	MDA (nmol/mg protein)	NO (nmol/mg protein)	SOD (U/mg protein)	GPx [nmol/(min · mL)]	AOS (mmol/mg protein)
Control (*n* = 6)	1.06 ± 0.28[a]	2.62 ± 0.49[a]	6.88 ± 3.58[a]	0.81 ± 0.07[bc]	0.18 ± ±0.03	11.59 ± 1.53[bc]
Sham (*n* = 6)	1.19 ± 0.32[a]	2.89 ± 0.63[a]	6.07 ± 2.58[a]	0.88 ± 0.03[b]	0.18 ± 0.03	12.53 ± 1.64[b]
I/R (*n* = 6)	3.2 ± 0.71[b]	4.90 ± 0.84[b]	15.30 ± 5.41[b]	0.31 ± 0.04[a]	0.13 ± 0.03	7.20 ± 2.03[a]
CUR (*n* = 6)	2.73 ± 0.37[b]	4.02 ± 0.74[b]	4.8 ± 1.37[a]	0.52 ± 0.07[ac]	0.16 ± 0.03	8.61 ± 2.33[ac]

The values in the same column shown with different characters are statistically significant ($P < 0.05$).
AOS: Antioxidant status; GPx, glutathione peroxidase; IMA, ischemia-modified albumin; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitric oxide; SOD, superoxide dismutase.

Table 4. Measurement results of serum electrolyte parameters
Tablo 4. Serum elektrolit parametrelerinin ölçüm sonuçları

Group	K^+	Ca^{2+}	Na^+	Cl^-
Control	5.87 ± 0.52[a]	10.42 ± 0.66[a]	140.83 ± 4.57	105.5 ± 3.44
Sham	5.43 ± 0.71[c]	9.32 ± 0.4[b]	141,.33 ± 1.37[a]	103.83 ± 3.6
I/R	8.69 ± 0.84b**	9.95 ± 0.62*	136.57 ± 2.76[b]*	103 ± 4.61
CUR	6.93 ± 1.51[cd]	10.06 ± 0.82[bc]	139 ± 3.51	104 ± 3.78

The values in the same column shown with different characters are statistically significant ($P < 0.05$). *$P < 0.05$; **$P < 0.001$.

Table 5. Histopathological scoring results of the kidney tissues in each groups
Tablo 5. Gruplarda böbrek dokusunun histopatolojik skorları.

Group/Score	0	1	2	3	4	5
Control	100%	0%	0%	0%	0%	0%
Sham	100%	0%	0%	0%	0%	0%
I/R	0%	0%	0%	50%	33,3%	16.7%
CUR	0%	0%	0%	83.3%*****	0%*****	16.7%

Pearson chi-square test: *$P = 0.001$.

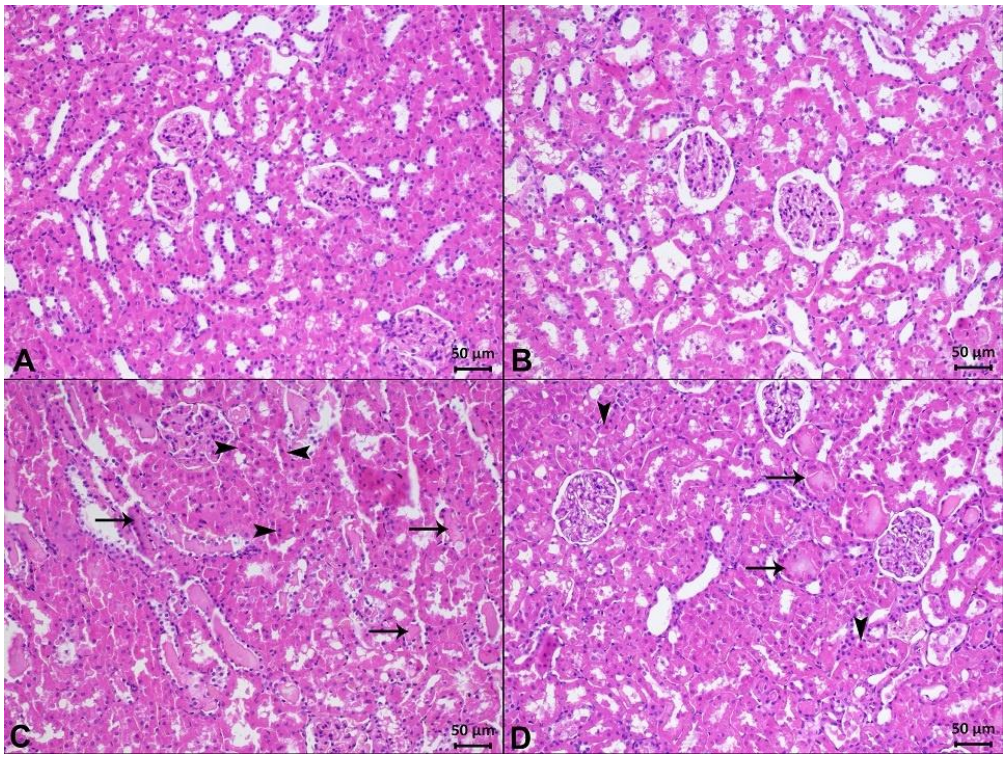

Figure 1. Histopathological view of experimental groups. **A. Control group:** histological structures were normal **B. Sham group:** histological structures are normal. **C. I/R group:** swelling in tubules in large field and severe necrosis (arrows), picnosis (arrowheads) in tubul epithelium and basal membrane separation in some epithelium. **D. CUR group:** less severe than the IR group; swelling and necrosis in the tubules (arrows), picnosis in the tubul epithelium (arrowheads) and basal membrane separation in some epithelium (HE.20x).
Şekil 1. Deney gruplarının histopatolojik görüntüsü. **A. Kontrol grubu:** histolojik yapılar normal görünümde **B. Sham grubu:** histolojik yapılar normal görünümde. **C. I/R grubu:** geniş sahalar halinde tubullerde şişme ve şiddetli nekroz (oklar), tubul epitellerinde piknoz (ok başları) ve bazı epitellerde bazal membrandan ayrılma. **D. CUR grubu:** IR grubuna göre daha az şiddette; tubullerde şişme ve nekroz (oklar), tubul epitellerinde piknoz (ok başları) ve bazı epitellerde bazal membrandan ayrılma (HE. 20x).

AKF is a clinical syndrome characterized by a rapid disruption of normal functions of the kidney for various reasons. This syndrome is characterized by the accumulation of nitrogenous waste materials and deterioration of fluid and electrolyte balance as a result of a rapid decline in GFR over hours or day by day (Adewusi and Afolayan, 2009; Atthe et al., 2009; He et al., 2011; Tanrıverdi and Karadağ, 2010; Uslu et al., 2009)

Serum urea and creatinine levels significantly increased in the I/R and CUR groups compared with those in the control group, but they remained within the normal reference levels. On the contrary, the serum K^+ level was significantly higher in the I/R group than in the CUR group, whereas the serum Ca^{2+} level was higher in the CUR group compared with that in the I/R group. No significant difference was seen in Na^+ and Cl^- levels between different groups.

Renal tubular epithelial cells may exhibit different structural and functional recovery, apoptosis, and necrosis depending on the duration and severity of ischemia (Dagher, 2004). Histopathological examination of renal tissue in the present study revealed nuclear shrinkage and severe vacuolization (moderate kidney damage) in 83.3% and complete tubular necrosis in 16.7% of the rats in the I/R group. Nuclear shrinkage and severe vacuolization (moderate kidney damage) was 50% and complete necrosis in the tubules 16.7% in the CUR group.
This study showed that the intraperitoneal application of CURat a dose of 500 mg/kg during 1-h ischemia had beneficial effects in correcting renal IRH in the rat renal ischemia/reperfusion model, considering biochemical measurements and oxidative markers. These results were also supported by histopathological results. Hence, it is believed that CURprotects the kidney against ischemia/reperfusion damage in the rat renal ischemia/reperfusion model.

Conflict of interest
Authors declerated no Conflict of interest.

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RESEARCH ARTICLE

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Determining The Yields and Percentages of Retail Cuts From Holstein Bull Carcasses Marketed in South Marmara Region of Turkey

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ABSTRACT

The objective of this study was to determine further-processing characteristics and share of valuable cuts in Holstein carcasses marketed in South Marmara Region of Turkey. The data-set collected from a commercial slaughterhouse included observations of 311 purebred Holstein bulls. The mean values were determined 510.27±5.11 kg for pre-slaughter weight, 273.07±2.75 kg for hot carcass weight and 53.57±0.15 % for hot carcass dressing. Mean cold-carcass weight was 266.34±2.67 kg and mean carcass bone content was 19.05±0.09 %. In addition, the means for valuable cuts including rib, roast, sirloin, cutlet and strip loin were 17.17±0.19 kg, 3.35±0.05, 2.74±0.04 kg, 8.74±0.11 kg and 4.65±0.06 kg, respectively. Total yield of valuable retail cuts were 36.66±0.42 kg with the percentage of 13.74±0.06 % and the processing loss was 8.19±0.11 kg with the percentage of 3.11±0.04 %. Statistical analysis revealed that highly significant differences were observed between slaughter age groups. Moreover, significant correlations were found between pre-slaughter / carcass weights and all carcass traits analyzed. The present results may be useful for an effective evaluation of carcass characteristics in beef market of Turkey.

Keywords: Beef Production, Carcass Characteristics, Retail Cut Yield, Valuable Cuts, Holstein

Türkiye'nin Güney Marmara Bölgesinde Piyasaya Sunulan Holstein Irkı Erkek Sığır Karkaslarında Perakende Parça Ağırlık ve Oranlarının Belirlenmesi

ÖZ

Bu çalışmanın amacı, Türkiye'nin Güney Marmara bölgesinde piyasaya sunulan Holstein karkaslarında ileri-işlem özellikleri ve değerli et oranlarının belirlenmesidir. Veri seti özel bir mezbahadan elde edilen 311 baş saf Holstein erkek sığıra ait verileri kapsamaktadır. Ortalama kesim öncesi ağırlığı 510.27±5.11 kg; sıcak karkas ağırlığı 273.07±2.75 kg; sıcak karkas randımanı ise % 53.57±0.15 olarak belirlenmiştir. Ortalama soğuk karkas ağırlığı 266.34±2.67 kg ve ortalama karkas kemik içeriği oranı ise % 19.05±0.09'dur. Bununla birlikte, biftek, rosto, bonfile, pirzola ve kontrfileden oluşan değerli et ortalamaları sırasıyla 17.17±0.19 kg, 3.35±0.05, 2.74±0.04 kg, 8.74±0.11 kg ve 4.65±0.06 kg'dır. Toplam değerli et verimi 36.66±0.42 kg, değerli et oranı % 13.74±0.06, ileri-işlem firesi 8.19±0.11 kg ve ileri işlem fire oranı % 3.11±0.04'tür. İstatistiksel analizler, kesim yaşı grupları arasında önemli farklılıkların bulunduğunu göstermiştir. Ayrıca, kesim öncesi ve karkas ağırlıkları ile incelenen tüm özellikler arasında anlamlı korelasyonlar bulunmuştur. Bu çalışmadan elde verilerin Türkiye sığır eti sektöründe karkas değerlendirmesinin etkili bir biçimde yapılabilmesi konusunda yararlı olacağı düşünülmektedir.

Anahtar Kelimeler: Sığır Eti Üretimi, Karkas Özellikleri, Perakende Parça Verimi, Değerli Et, Holstein

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INTRODUCTION

Total red meat production in Turkey has risen to approximately 1.2 million tonnes and approximately 91% of this production (1,059,196 tonnes), was composed of beef (Anonymous, 2018). However, inadequacy in beef production has resulted in high prices of red meat and importation of live animals and/or carcasses. Beef self-sufficiency in Turkey could better be achieved through research aimed to increase the maximum yield and quality gathered by individual cattle. Evaluating the ways to enhance the present situation in beef production and improvements in processing efficiency may presumably deliver gains to producers in terms of higher profitability and sustainability, while, gains to consumers in terms of lower beef retail prices and increased beef consumption (Hadi et al., 2002).

Slaughter and carcass trait evaluation of beef production among breeds representing a wide range of biological types of cattle provides a basis for characterization in respect to yield of salable product for gross measures of productivity (Jenkins et al., 1981). The Holstein-Friesian breed is predominant among dairy cattle in general and is accepted as a significant premier dairy breed with a high potential for milk production (Jurie et al., 2007) but, not negligibly, this breed is also suitable for the production of good quality beef (Węglarz, 2010; Nogalski et al., 2016). Holstein beef have been not so popular and applied limitedly in countries which can be evaluated as self-sufficient for beef production, such as USA, Korea and Canada, because not only they have inferior palatability characteristics as compared to beef-specific breeds, but also their poor eating quality does not make it a highly-preferred choice correspond to retail for consumers (Yim et al., 2015). However, evaluation of the dual capacity of the Holstein breed may be considered in several countries where both milk and meat are in short supply, and moreover, Holstein bulls pedigree selected for superiority in milk production have the capacity to produce beef and a potential for improvement in beef production according to their genetic variability for beef traits (Calo et al., 1973). In Turkey, cattle population has risen to 14.7 million (Anonymous, 2018). Of this population, Holstein breed has a significant impact on Turkish animal husbandry, with 5.5 million purebreds and 856 thousand crossbreds (Anonymous, 2016). Hence, Holstein's potential for improvement in beef production should be paid sufficient attention when evaluating Turkish meat industry.

Assessment of carcass characteristics and evaluation of valuable retail cuts of beef should be considered as important constituents in profitable meat production. In Turkey, there is still a strong need for studies regarding ways to improve red meat production. Population growth rate and economic dynamics have resulted in dramatic increases in beef prices. In this sense, encouraging the studies on the determination of retail cut yields and carcass quality traits may play a key role for improvement in Turkey's meat industry. Therefore, the aim of this study was to assess carcass traits and valuable retail cut yield from Holstein bull carcasses marketed in South Marmara Region of Turkey and to evaluate the outcomes with respect to retrospective perspectives.

MATERIALS and METHODS

The study was carried out from January 1, 2016 to December 31, 2016. Data from a total of 311 carcasses cut into primal weights from Holstein bulls slaughtered in a commercial slaughterhouse (slaughter age: 17.59±0.084 months) located in South Marmara region of Turkey was used in the current study. Only data with relevant records of slaughter weight and carcass traits including retail cuts were used in subsequent analyses. Hence inaccurate and/or deficient records in a way that would not describe a complete picture of slaughter history for a bull were excluded from the analysis. Hot carcass weight was measured without removing the subcutaneous fat and keeping the kidney and pelvic fat and was taken approximately 1 h postmortem (Journaux, 2007). Each carcass was divided down the back-bone to give two sides. After chilling at 4 °C for 24 h in a ventilated room, carcasses were again weighed to determine the cold-carcass weight.

All cuttings and bones were weighed and their yields were expressed as percentages of the cold carcass weight. Carcasses were evaluated for weight means (kg) of valuable cuts including rib, roast, strip loin, sirloin, and cutlet. In addition, the proportions (%) of bone, valuable cuts, and total meat (valuable cuts + chunk and mince) were determined on the basis of cold carcass weight. In this context, sides were quartered between the 12th and 13th ribs. Bodies of the thoracic vertebrae were removed by sawing to the point where they joined the spinous processes and ribs, but leaving the spinous processes and ribs attached to the rib roast. The sirloin tip was removed by cutting across the anterior end of the muscle in a line with the anterior edge of the aitch bone (parallel to the sacral vertebrae) and the strip loin was separated from the round on a line between the aitch bone and the posterior end of the 5th sacral vertebra (Koch and Dikeman, 1977). Apart from the valuable cuts, the mean weights of mince and chunk yield were determined. Processing loss was

defined as the percentage of meat weight loss in consequence of carcass processing.

All the statistical analyses were performed using Minitab software (MINITAB®, USA, v17.1.0). Descriptive statistics were determined belonging to all variables and the data were expressed as means, their corresponding standard deviations, coefficient of variation, and minimum-maximum. Phenotypic correlation coefficients were generated using the Pearson's correlation coefficient (PCC) option of correlation procedures. In this study, phenotypic correlations were classified into three groups according to levels of PCC ranges: low correlation if PCC is < 0.30, intermediate correlation if PCC is between 0.30 – 0.70 and high correlation if PCC is >0.70 (Buyukozturk, 2002). In order to determine differences in age groups, a one-way analysis of variance (ANOVA) was performed and when significant differences were identified, the mean values for group were contrasted using Tukey's test.

RESULTS

A summary of the descriptive statistics expressing means, standard deviations, the coefficients of variation and minimum-maximum values is given in Table 1 for carcass processing traits and wholesale cut components from Holstein bulls marketed in South Marmara Region of Turkey. The mean pre-slaughter weight of the animals was 510.27±5.11 kg and the mean hot carcass weight was 273.07±2.75 kg with dressing percentage of 53.57±0.15 %. The values of Holsteins were determined 266.34±2.67 kg for cold carcass weight and 50.29±0.44 kg for bone content. In addition, chilling loss was between 0.03 % and 0.15 % with a mean of 0.07±0.01 % in the carcasses analyzed. The yields of mince and chunk were 134.26±1.76 and 20.08±0.21 kg respectively; while the yields of valuable cuts were 17.17±0.19 kg, 3.35±0.05, 2.74±0.04 kg, 8.74±0.11 kg and 4.65±0.06 kg for rib, roast, sirloin, cutlet and strip loin, respectively. Results revealed that, the mean value for processing loss was 8.19±0.11 kg in Holstein carcasses analyzed.

Table 1. Descriptive statistics of slaughter weight, carcass traits and further-processing characteristics in Holstein bulls (n=311).
Tablo 1. Holstein erkek sığırlarda kesim ağırlığı, karkas ve ileri-işlem özelliklerine ait tanımlayıcı istatistikler (n=311).

Traits Analyzed	Mean	Standard Deviation	Coefficient of Variation	Minimum	Maximum
Slaughter age (month)	17.59	1.46	8.28	14	20
Slaughter weight (kg)	510.27	75.61	14.77	298.00	720.00
Hot-carcass weight (kg)	273.07	41.00	14.96	163.00	368.00
Hot-carcass dressing (%)	53.57	2.58	4.81	40.39	59.66
Cold-carcass weight (kg)	266.34	39.86	14.93	157.00	359.00
Cold-carcass dressing (%)	52.26	2.80	5.36	39.60	58.74
Chilling loss (%)	0.07	0.02	23.34	0.03	0.15
Bone content (kg)	50.29	6.37	12.66	31.00	67.00
Total meat yield (kg)	207.99	32.97	15.76	118.00	281.00
Mince (kg)	134.26	28.58	21.15	13.50	203.00
Chunk (kg)	20.08	3.31	16.38	10.70	30.00
Mince + chunk yield (kg)	171.36	27.56	15.99	96.60	234.50
Rib (kg)	17.17	2.90	16.84	3.50	24.50
Roast (kg)	3.35	0.76	22.60	1.50	6.00
Sirloin (kg)	2.74	0.64	23.12	1.00	5.00
Cutlet (kg)	8.74	1.62	18.50	5.00	13.00
Striploin (kg)	4.65	0.96	20.57	2.50	8.00
Total valuable cuts yield (kg)*	36.66	6.37	17.30	21.00	54.00
Processing loss (kg)	8.19	1.76	21.71	3.00	15.00

* Valuable cuts yield included rib, roast, sirloin, cutlet, striploin.

Table 2 shows the proportions of total meat, bone, valuable cuts, and processing loss. Total meat yield was 207.99±2.24 kg with the percentage of 77.94±0.12 % in Holstein bull carcasses analyzed. In addition, the mean values for carcass bone content and the trimmed meat percentage (mince + chunk) were determined 19.05±0.09 % and 64.19±0.13 %, respectively. Results indicated that, total yield of valuable retail cuts were 36.66±0.42 kg with the percentage of 13.74±0.06 %. Processing loss percentage was 3.11±0.04 % on the basis of cold carcass weight.

Pearson's correlation coefficients, shown in Table 3, indicated that all three groups of correlations, including low, intermediate and high, existed in the

present study. Results revealed that pre-slaughter weight highly correlated with, as expected, carcass and bone weights (0.95 and 0.81, respectively), mince + chunk (0.95), valuable retail cuts yield (0.81) and total meat yield (0.95) but did not significantly correlate with valuable cuts percentage (P>0.05). Besides, total meat yield correlated with all the traits analyzed, except valuable cuts percentage in different levels of significance. According to the present results, the correlation between valuable cuts percentage and carcass dressing indicated a low correlation (0.15); whereas the correlation between valuable cuts percentage and both mince + chunk percentage (-0.49) and valuable cuts yield (0.52) exhibited an intermediate correlation. The means of slaughter weight, carcass traits and further-processing characteristics for different age groups are presented in Table 4. Results indicated that the mentioned traits were highly influenced by the slaughter age (P<0.001), except for hot and cold carcass dressings and the percentage of valuable cuts.

Table 2. Descriptive statistics for proportions of retail cuts obtained from Holstein bull carcasses in further-processing (n=311).
Tablo 2. Holstein erkek sığırların ileri-işlemdeki karkaslarından elde edilen perakende parça ağırlık oranlarına ait tanımlayıcı istatistikler (n=311).

Traits Analyzed	Mean	Standard Deviation	Coefficient of Variation	Minimum	Maximum
Meat percentage	77.94	1.76	2.25	71.83	81.72
Bone content percentage	19.05	1.50	7.92	16.28	26.19
Mince + chunk percentage	64.19	2.02	3.14	57.03	70.20
Percentage of valuable cuts*	13.74	1.09	7.98	8.48	16.72
Processing loss percentage	3.11	0.64	20.78	1.13	7.74

* Valuable cuts included rib, roast, sirloin, cutlet, striploin.

DISCUSSION

Assessment of ways to improve profitability and price evaluation have an important role in the economic comparison of breeding strategies on the basis of beef production. Thus, determination of carcass processing traits based on the valuable retail cuts yield may provide a worthy contribution to meat industry. Recently, there is a clear discrepancy between the demand and supply because of the decreasing of domestic beef supply, and hence, the carcass assessment should be maintained to achieve maximum revenue and to evaluate the present situation and future needs in beef industry.

In many countries, beef production may be interpreted as two major categories based on the company structure: companies that produce a combination of dairy or meat products and companies that provide production from specific beef herds (Ardicli et al., 2017). In this context, Turkey's cattle farms generally comprise dairy cattle, dual-purpose breeds or their crossbreds, and the number of specific beef breeds is limited (Anonymous, 2016). Generally, proportions of hind quarter and/or lean meat are higher for beef crosses compared to purebred Holsteins. Thus, crossbreds produces more valuable carcasses (Huuskonen et al., 2013). A number of studies have confirmed a higher share of the most valuable cuts in the carcasses of beef or dual purpose breeds compared to Holstein bulls (Kempster et al., 1982; Barton et al., 2006; Kamieniecki et al., 2009; Pesonen et al., 2013). In the study performed by Pesonen et al. (2013), the means for tenderloin and loin yield were determined 2.2 kg (with the percentage of 1.1 %) and 5.1 kg-5.7 kg (with the percentage of 2.6-3.0 %) in Holstein bulls. These researchers also reported lower carcass bone content (35.4-36.8 kg) and bone percentage (17.3-18.5 %). Kempster et al. (1982) and Manninen et al. (2011) reported a similar share of valuable cuts in Holsteins. In the present paper, evaluation of valuable cuts suggested higher means for sirloin (2.74±0.04 kg) but lower means for striploin (4.65±0.06 kg). By contrast, lower means for loin (3.7–4.3 %), tender loin (1.4-1.6 %) and roast (1.7-2.0 %) were determined by Huuskonen et al. (2013) in purebred Holstein and Holstein x beef breed crossbred bulls including Aberdeen angus, Blonde d'Aquitaine, Charolais, Hereford, Limousin and Simmental. Keane and Allen (1998) and Pabiou et al. (2009) reported higher means for loin and rib proportions in Charolais x Holstein and Irish beef cattle, respectively.

Table 3. Pearson correlations among some values of carcass traits.
Tablo 3. Bazı karkas özelliklerine ait değerler arasındaki Pearson korrelasyonları.

Variables	SW	HCW	HCD	CCW	CCD	CL	BCW	BCP	PL	PLP	MCY	MCP	VCY[a]	VCP[a]	TMY
HCW	0.95***														
HCD	-0.11**	0.21***													
CCW	0.95***	0.99***	0.20***												
CCD	-0.15*	0.14*	0.90***	0.14*											
CL	0.63***	0.70***	0.28***	0.68***	0.20***										
BCW	0.81***	0.88***	0.26***	0.87***	0.23***	0.74***									
BCP	-0.53***	-0.52***	0.01	-0.53***	-0.02	-0.25***	-0.07								
PL	0.48***	0.51***	0.22***	0.50***	0.19**	0.54***	0.65***	0.09							
PLP	-0.24***	-0.22***	0.05	-0.23***	0.03	0.03	0.03	0.56***	0.70***						
MCY	0.95***	0.98***	0.10	0.98***	0.09	0.72***	0.81***	-0.62***	0.47***	-0.26***					
MCP	0.44***	0.36***	-0.27***	0.37***	-0.21***	0.14*	0.01	-0.81***	-0.22***	-0.60***	0.53***				
VCY	0.81***	0.87***	0.21***	0.87***	0.19**	0.65***	0.77***	-0.46***	0.48***	-0.16**	0.82***	0.09			
VCP	0.01	0.06	0.15**	0.06	0.14*	-0.01	0.03	-0.05	-0.01	-0.04	-0.04	-0,49***	0.52***		
TMY	0.95***	0.99***	0.13*	0.99***	0.12*	0.73***	0.83***	-0.61***	0.49***	-0.25***	0.99***	0.46***	0.88***	0.06	
TMP	0.51***	0.45***	-0.22***	0.46***	-0.16**	-0.16**	0.03	-0.96***	-0.26***	-0.72***	0.58***	0.84***	0.43***	0.05	0.56***

Slaughter weight (SW); Hot carcass weight (HCW); Hot carcass dressing (HCD); Cold carcass weight (CCW); Cold carcass dressing (CCD); Chilling loss (CL); Bone content weight (BCW); Bone content percentage (BCP); Processing loss (PL); Processing loss percentage (PLP); Mince + chunk yield (MCY); Mince + chunk percentage (MCP); Valuable cuts yield (VCY); Valuable cuts yield percentage (VCP); Total meat yield (TMY); Total meat percentage (TMP).
* $P<0.05$; ** $P<0.01$; *** $P<0.001$
[a]Valuable cuts yield included rib, roast, sirloin, cutlet, striploin.

Table 4. Comparison of slaughter weight, carcass traits and further-processing characteristics for different age groups in Holstein bulls (n=311).
Tablo 4. Holstein erkek sığırlarda kesim ağırlığı, karkas ve ileri-işlem özelliklerinin farklı yaş gruplarında karşılaştırılması (n=311).

Traits Analyzed	Age Groups			Significance
	14-16 months	17-18 months	19-20 months	
Slaughter weight (kg)	426.62±6.31^{c}	508.54±4.20^{b}	584.51±5.56^{a}	*P*<0.001
Hot-carcass weight (kg)	228.41±3.54^{c}	272.64±2.35^{b}	311.58±3.12^{a}	*P*<0.001
Hot-carcass dressing (%)	53.54±0.32	53.65±0.21	53.35±0.28	NS
Cold-carcass weight (kg)	222.46±3.43^{c}	265.79±2.29^{b}	303.45±3.03^{a}	*P*<0.001
Cold-carcass dressing (%)	52.46±0.35	52.31±0.23	51.96±0.30	NS
Chilling loss (%)	0.06±0.01^{c}	0.07±0.01^{b}	0.08±0.02^{a}	*P*<0.001
Bone content (kg)	44.65±0.64^{c}	50.05±0.43^{b}	55.34±0.56^{a}	*P*<0.001
Bone content percentage (%)	20.03±0.17^{a}	18.93±0.11^{b}	18.24±0.15^{c}	*P*<0.001
Total meat yield (kg)	172.14±2.88^{c}	208.43±1.93^{b}	238.94±2.53^{a}	*P*<0.001
Meat percentage (%)	76.73± 0.19^{c}	78.19±0.13^{b}	78.80±0.18^{a}	*P*<0.001
Mince (kg)	111.24±2.93^{c}	133.35±1.96^{b}	156.84±2.59^{a}	*P*<0.001
Chunk (kg)	17.04±0.33^{c}	20.15±0.22^{b}	22.68±0.29^{a}	*P*<0.001
Mince + chunk yield (kg)	141.58±2.42^{c}	171.66±1.62^{b}	197.27±2.12^{a}	*P*<0.001
Mince + chunk percentage (%)	63.09±0.24^{c}	64.41±0.16^{b}	65.06±0.21^{a}	*P*<0.001
Rib (kg)	14.63±0.30^{c}	17.29±0.20^{b}	19.15±0.27^{a}	*P*<0.001
Roast (kg)	2.66±0.08^{c}	3.36±0.06^{b}	3.94±0.07^{a}	*P*<0.001
Sirloin (kg)	2.28±0.07^{c}	2.73±0.05^{b}	3.16±0.06^{a}	*P*<0.001
Cutlet (kg)	7.23±0.16^{c}	8.69±0.11^{b}	10.09±0.14^{a}	*P*<0.001
Striploin (kg)	3.75±0.09^{c}	4.66±0.07^{b}	5.37±0.08^{a}	*P*<0.001
Total valuable cuts yield (kg)*	30.56±0.63^{c}	36.77±0.42^{b}	41.71±0.55^{a}	*P*<0.001
Percentage of valuable cuts*	13.64±0.14	13.79±0.09	13.74±0.12	NS
Processing loss (kg)	7.60±0.21^{b}	7.86±0.14^{b}	9.02±0.18^{a}	*P*<0.001
Processing loss percentage (%)	3.41±0.08^{a}	2.97±0.05^{b}	2.97±0.07^{b}	*P*<0.001

^{a,b,c} Different superscripts within a row indicate significant difference.
Not significant (NS) *P*>0.05
* Valuable cuts yield included rib, roast, sirloin, cutlet, striploin.

Pre-slaughter weight and carcass characteristics of beef cattle and correlations among them may vary according to the age, genetic background and sex of the animal, nutritional properties and environmental effects (Dannenberger et al., 2006). In this study, as expected, statistically significant differences in carcass characteristics were observed for different slaughter age groups. In this context, age group 19-20 months had higher means for all traits, except for hot and cold carcass dressings, the percentages of bone content, valuable cuts, and processing loss compared to other age groups (14-16 and 17-18 months). Slaughter weight was highly or intermediately correlated with all variables, except valuable retail cuts percentage. The same trend in correlation was also found in hot and cold carcass weights. The results for correlation structure were in accordance with results reported by Choy et al. (2010). It has been assumed that, carcass weight is one of the most important predictors to evaluate the yields and percentages of retail cuts of the carcasses (Chen et al., 2007; Choy et al., 2010; Cicek et al., 2016). According to the present results, there was a consistency in terms of correlation with the mentioned traits and valuable retail cuts yield. In this context, valuable retail cuts percentage significantly correlated with dressing percentages, even if the coefficients were indicated a low correlation (0.15 and 0.14 for hot and cold carcass dressing, respectively). However, there was no significant correlation between valuable retail cuts percentage and slaughter weight (*P*>0.05). Moreover, this same situation existed for the correlation with carcass weights (for both hot and cold carcass). Similarly, Chen et al. (2007) reported that the percentages of prime retail cuts (divided by chilled whole carcass weight) did not correlate with hot carcass weight. One possible explanation about this lack of a correlation may be associated with fat content of the carcasses. Choy et al. (2010) reported that retail cut percentage was highly correlated with body fat reserves and back fat thickness. On the other hand, Cicek et al. (2016) suggested that, a negative and intermediate correlation (-0.51) was found between hot carcass weight and the percentage of tenderloin, sirloin, rib roast, rump, knuckle, round eye and topside-outside flat (which was expressed as first degree retail cuts: FRC) in Holstein bull carcasses. An appropriate approach to optimum pre-slaughter and carcass weights may reveal beneficial outcomes in profitable beef production, especially considered in the case of dairy-type bulls. Thus, if beef output is to be maintained; carcass weights must increase

(Huuskonen et al., 2013). However, increasing carcass weight would not be desirable when beef carcasses are already adequately fat or over fat at existing-carcass weights (Herva et al., 2009; Herva et al., 2011). Taken together, profitability dynamics are needed to be contemplated in beef production, especially for the countries where the production cost is high, such as Turkey.

Valuable retail cut yield determined in this study was lower than the study performed by Cicek et al. (2016). Furthermore, sirloin weight found in the present study for Holsteins were higher than some earlier reports performed by Alpan (1972) and Akbulut and Tüzemen (1994) but lower than Baspinar et al. (1999), Koc and Akman (2003) and Kizil and Aydogan (2014). In the literature, there are several studies about the evaluation of carcass processing traits and valuable retail cuts in various cattle breeds, raised in Turkey or imported, such as mentioned above. However, the number of experimental animals is often limited when carcass traits of different breed groups are compared (usually not more than dozens of cattle per breed group). There is an apprehension about the representativeness of the mentioned animals compared with remaining animals from the same breed with respect to whether they delineate the whole variation and phenotypic spectrum in their respective populations. In addition, breed comparisons are mainly conducted based on environmental factors, for instance, their specific production conditions or genotypic structure contributions. Hence, further experiments based on large datasets collected from Turkish slaughterhouses are needed to evaluate and criticize the present situation in Tukey's beef sector and to study the potential of Holstein bulls for carcass assessment.

In the present study, evaluation of a relatively large dataset consisted of beef production and carcass traits regarding descriptive aspects were performed within the scope of retail cut yields collected from Holstein carcasses. The carcasses of this cattle, however, are characterized by poorer slaughter and quality parameters compared to beef-specific breeds (Węglarz, 2010); beef derived from Holstein breed is one of the significant sources of Turkish beef supply and bulls / cull cows make up a considerable proportion of the beef market. One possible approach for current situation could be commercial crossing of dairy cows with beef-breed bulls as suggested by many researchers (Grodzki et al., 2006; Węglarz, 2010; Huuskonen et al., 2013). On the other hand, more effective selection programs, especially based on genomic analyses, should be formed. In view of the cattle breed structure in Turkey, dual capacity of Holstein breed may be taken into account to cover the shortage of beef demand.

CONCLUSIONS

The large dataset collected in this study describes well the further processing characteristics and share of valuable cuts of Holstein carcasses marketed in South Marmara Region of Turkey. The present results confirm that admissible results were obtained for Holstein bulls. To improve beef production and to reach a potential of self-sufficient country, Turkey should perform a more effective process of carcass assessment. In addition the potential of Holstein breed in Turkey's beef production should be considered. Thus, results of the current study may be useful and indicative for evaluating the present situation in Turkish beef industry and for future studies on meat production traits in livestock.

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RESEARCH ARTICLE

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The Treatment of Infectious Bovine Keratoconjunctivitis Under Field Conditions: Intrapalpebral Injection Versus Subconjunctival Injection of Oxytetracycline

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ABSTRACT

The purpose of this study was to determine the efficacy of oxytetracycline when applied intrapalpebral (IPa) injection to the eye, compared with subconjunctival (SCo) injection, in the treatment of infectious bovine keratoconjunctivitis (IBK). Twenty eyes with severe clinical signs of IBK, of 15 Holstein Friesian female cattle aged 2–4 years were evaluated in this study. The affected animals had unilateral (n=10; 66.7%) and bilateral (n=5; 33.3%) symptoms. *Moraxella bovis* were identified in all ocular swabs samples on day 0 and all isolates were susceptible to oxytetracycline (100%). Oxytetracycline (100 mg/ml) was injected IPa (n=10) and SCo (n=10) at a total dose of 200 mg once daily on days 0, 3 and 6. After injections, the animals were re-examined for resolution of lesions associated with IBK weekly until the corneal ulcer healed. Microbiologic examination was repeated 3 times at intervals of 1 week. There is no effect of injection type on healing time of the lesion. Size and side of the lesion have a significant effect on healing time ($p<0.001$). It was found that small lesions had earlier clean in terms of the microbiologic evaluation. Lesion size has a significant effect on microbial growth ($p<0.001$). In conclusion, the same therapeutic effect was achieved in both applications. However, oxytetracycline given by IPa injection was comparatively easy, more comfortable and less invasive especially for painful eyes against IBK than SCo injection under the conditions of this study. In addition, it was enough for cattle with IBK at the dosage (200 mg, once daily on days 0, 3 and 6) of used in the study.
Keywords: cattle, IBK, intrapalpebral, oxytetracycline

Saha Koşullarında Enfeksiyöz Bovine Keratokonjuktivitis'in Sağaltımı: Subkonjuktival Oksitetrasiklin Enjeksiyonuna Karşı İntrapalpebral Enjeksiyon

ÖZ

Bu çalışmanın amacı, infeksiyöz bovine keratokonjuktivitis (IBK)'in sağaltımında, göze intrapalpebral (IPa) enjeksiyon ile uygulanılan oksitetrasiklin'in, subkonjuktival (SCo) enjeksiyon ile karşılaştırıldığındaki etkinliğini belirlemekti. Çalışmada, IBK'nın şiddetli klinik bulguları bulunan 2-4 yaşındaki, dişi, 15 Holstein Friesian sığıra ait 20 göz değerlendirildi. Etkilenen hayvanlar unilateral (n=10; % 66,7) ve bilateral (n=5; % 33,3) semptomlara sahipti. 0. günde tüm oküler swab örneklerinden *Morexella bovis* izole edildi ve oksitetrasikline duyarlı idi (% 100). 0, 3 ve 6. günlerde günde bir kez toplam 200 mg dozda oksitetrasiklin (100 mg/ml) IPa (n=10) ve SCo (n=10) olarak enjekte edildi. Enjeksiyonlardan sonra, korneal ülser iyileşmesi şekilleninceye kadar haftalık olarak IBK ile ilişkili lezyonlar yönünden hayvanlar tekrar muayene edildi. Mikrobiyolojik muayene birer hafta ara ile 3 kez tekrarlandı. Lezyonun iyileşme zamanı üzerine enjeksiyon tipinin etkisi yoktu. Lezyonun büyüklüğü ve bulunduğu tarafın iyileşme zamanı üzerine belirgin etkisi vardı ($p<0.001$). Küçük lezyonlar mikrobiyolojik değerlendirmede daha erken dönemde temiz bulundu. Lezyon büyüklüğü mikrobiyal gelişimi belirgin olarak etkiledi ($p<0.001$). Sonuç olarak, her iki uygulama ile aynı terapötik etki sağlandı. Bununla birlikte, bu çalışmada saha koşullarında özellikle ağrılı gözlerde IPa enjeksiyon yolu ile verilen oksitetrasiklin, SCo enjeksiyondan nispeten daha kolay, daha rahat ve daha az invazivdi. İlave olarak, bu çalışmada kullanılan doz (0, 3 ve 6. günlerde günde bir kez 200 mg) IBK'lı sığırlar için yeterli idi.
Anahtar kelimeler: IBK, intrapalpebral, oksitetrasiklin, sığır

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INTRODUCTION

Infectious bovine keratoconjunctivitis (IBK) is a highly contagious ocular disease and spreads rapidly among cattle in a herd (Alexander 2010, Angelos et al. 2001). *Moraxella bovis* (*M. bovis*) is considered the primary causal organism associated with IBK (Bedford 2004). However, the recently characterized Moraxella bovoculi (*Mor bovoculi*) has been isolated from eyes of calves and cattle affected with IBK. *Moraxella bovis* and *Mor bovoculi* are generally considered to be susceptible to a variety of different antibiotics (Angelos 2015). IBK treatment trials had been published in the ensuing decade that would enable better understanding of comparative efficacy of antibiotic treatment options for IBK (Cullen 2016). Numerous antibiotic drugs including penicillin, gentamicin, neomycin–bacitracin–polymyxin B, sulfonamides, oxytetracyclines, furazolidone, clindamycin, chloramphenicol, florfenicol and ceftiofur have been used in the treatment of IBK with systemic and topical applications, with variable results (Burns and O'Connor 2008). Long-acting intramuscular formulation of oxytetracycline is commonly used in cattle to treat infected with IBK. The main disadvantage of using the intramuscular route for treatments is the large amount of drug that has to be administered (Zielinski et al. 2002).
To achieve therapeutic drug concentration, topical antibiotic administration as solutions, powders, or ointments are required several times per day (Brown et al. 1998, McConnel et al. 2007). But the major disadvantage of topical treatment is the short duration of action of the active ingredient in ocular tissues due to the limited lacrimal fluid and low levels of drug achieved in the ocular tissue (Zielinski et al. 2002). Moreover, the daily multi-dose therapy is usually not practical for most farmers (Brown et al. 1998, McConnel et al. 2007).
Subconjuctival injections (SCo) was also used for the topically administration of antibiotics. Subconjunctival treatment requires only a small amount of drug and achieves high antibiotic levels in lacrimal fluid and ocular tissue, however, application is very difficult in fractious and painful animals and can cause ocular tissue damage (Zielinski et al. 2002). Different applications are required for cattle which have similar therapeutic efficacy with SCo but less invasive and easier to administer in field condition. In the literature, the application of tilmicosin by intrapalpebral injection (IPa) was also found effective but there is insufficient data to support this route of administration (Zielinski et al. 2002).

The purpose of this study was to determine the clinical efficacy of IPa oxytetracycline against naturally occurring cases of IBK and to compare this efficacy with a SCo dose of oxytetracycline under field conditions.

MATERIALS and METHODS

The study was conducted during the October and November at a dairy herd in Bursa, Turkey. Twenty eyes (n=20) with severe clinical signs of IBK, of 15 Holstein Friesian female cattle aged 2–4 years were evaluated in this study. The affected animals were separated from the herd. The ocular conjunctival swabs were obtained from the each animal's conjunctival sac of eyes using sterile cotton-tipped swabs for isolation of *M. bovis*. These samples were placed in screw-capped tubes containing sterile Stuart transport medium (Oxoid) and transported to the laboratory. Swabs were streaked on 10% sheep blood agar plates which were then incubated at 37 °C for 48 to72 h under aerobic conditions. *M. bovis* was isolated and identified by using standard microbiologic techniques (George et al. 1984). Antibiotic sensitivity testing was performed using the disk diffusion method (George et al. 1985).

Oxytetracycline (100 mg/ml; Primamycin®, Pfizer) was injected intrapalpebrally (IPa, n=10) and subconjunctivally (SCo, n=10) at a total dose of 200 mg (2 ml), once daily on days 0, 3 and 6. During the IPa injections, the animal's head simply hold and the upper eyelid skin was lifted to provide an acute angle with respect to the eye's surface, and a 21 gauge needle was inserted into the base of the fold created and antibiotic injection was made (Figure 1A). During the SCo applications, the animal's head was well restrained, the upper eyelid was rolled back, and a 25 gauge needle was inserted into the dorsal palpebral conjunctiva and antibiotic was injected (Figure 1B). After injections, the animals were re-examined for resolution of lesions associated with IBK weekly until the corneal ulcer healed. Corneal ulcers were photographed. Photographic images were recorded by a Kodak EasyShare DX4530 camera. Before photos were taken, a ruler with clear millimeter divisions was placed near the lower eyelid. Care was taken to ensure that the camera was angled perpendicular to the cornea.

The animals were assessed for 28 days after treatment for 8 clinical signs of infection and presented in Table 1. Microbiologic examination was repeated 3 times at intervals of 1 week (on days 7, 14 and 21).

Statistical analyze

To test the effects of injection type, size and side (unilateral or bilateral) of the lesion on healing time, three-way anova (analysis of variance) was conducted using GLM procedures. P values are set ≤.05 to be considered significant. The results are shown as means ± standard errors of the mean (SEM) (Table 2).

RESULTS

M. bovis was detected in all samples on day 0. All isolates were sensitive to oxytetracycline (100%), enrofloxacin, gentamicin, clindamycin and penicillin G, respectively. In animals, bacteriologic cultures were negative at the end of treatment (Table 1).

Table 1. Clinical and laboratory evaluation on day 0 and post treatment period (on days 7, 14, 21 and 28)
Tablo 1. 0. gün ve sağaltım sonrası dönemde klinik ve laboratuar değerlendirmeleri (7, 14, 21 ve 28. günler)

Oxytetracycline applications	Days	M.bovis isolation*	Swelling lids	Blepharospasm	Epiphora	Photophobia	Keratitis	Corneal ulcer**	Corneal stroma***	Partial loss of view
Subconjunctival injection group (SCo, n=10)	***0***	10	+	+	+	+	+	+	+	+
	7	8	↓	-	-	-	↓	↔	↓	↔
	14	5	-	-	-	-	↓	↓	↓	↓
	21	0	-	-	-	-	↓	↓	-	-
	28	-	-	-	-	-	-	-****	-	-
Intrapalpebral Injection group (IPa, n=10)	***0***	10	+	+	+	+	+	+	+	+
	7	8	↑	↓	-	-	↓	↔	↓	↔
	14	6	↓	-	-	-	↓	↓	↓	↓
	21	0	-	-	-	-	↓	↓	-	-
	28	-	-	-	-	-	-	-****	-	-

*: The number of cattle *M. bovis* isolated from ocular secretions, **: Ø ≥ 0.5 cm, ***: Common white to deep yellow opacity in the Corneal stroma, ****: Four animals recovered with permanent slight scarred cornea, +: yes, -: no, ↑: increased, ↓: decreased, ↔: stable

Table 2. The effects of injection type, size and side of the lesion on healing time (HT) and microbial growth (MG)
Tablo 2. İyileşme zamanı ve mikrobiyal gelişme (MG) üzerine lezyon büyüklüğünün, bulunduğu tarafın ve enjeksiyon tipinin etkileri

	Injection type (IT)			Lesion size (LSi)			Lesion side (LSd)		
	SCo (n= 10)	IPa (n=10)	P value	Small (n=4)	Large (n=16)	P value	Unilateral (n=10)	Bilateral n=10	P value
HT (day)	20.2±0.78	21.0±0.72	NS	14.0±1.08	23.9±0.58	**<0.001**	17.5±0.71	26.8±0.70	**<0.001**
MG (week)	2.01±0.16	2.17±0.15	NS	1.00±0.23	2.67±0.12	**<0.001**	1.75±0.15	2.83±0.15	NS
P Interactions of HT									
IT x LSi					NS				
IT x LSd					NS				
LSi x LSd					NS				
IT x LSi x LSd					NS				
P Interactions of MG									
IT x LSi					NS				
IT x LSd					NS				
LSi x LSd					NS				
IT x LSi x LSd					NS				

NS: Not significant

The affected animals had unilateral (n=10; 66.7%) and bilateral (n=5; 33.3%) symptoms. Prior to treatment, clinical signs of IBK generally included swelling of the lids, ocular discharge, blepharospasm, neovascularization and corneal ulcer diameter greater than 0.5 cm, a common white to deep yellow opacity in the corneal stroma (Figure 2A-D).

Corneal ulcer diameter were measured (cm) on picture and the values were found 1.35 ± 0.36 in SCo and 1.41 ± 0.48 in IPa group (Mean ± SD). Corneal opacity was clearly reduced in all cases which was noticed during the second injection. A painful swelling of the upper eyelid was occurred in all animals of IPa group after the second injection of oxytetracycline. This swelling disappeared by 72 hours after injection.

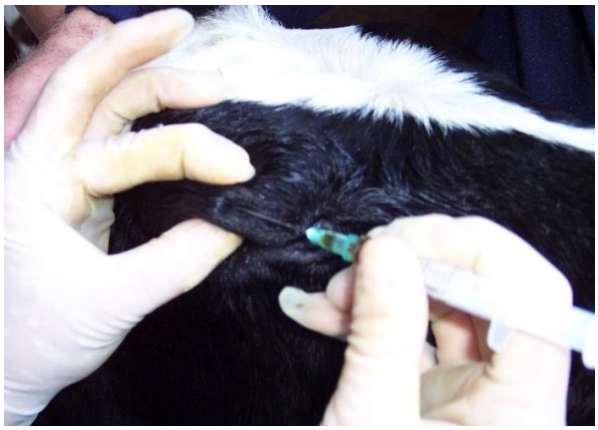

Figure 1A

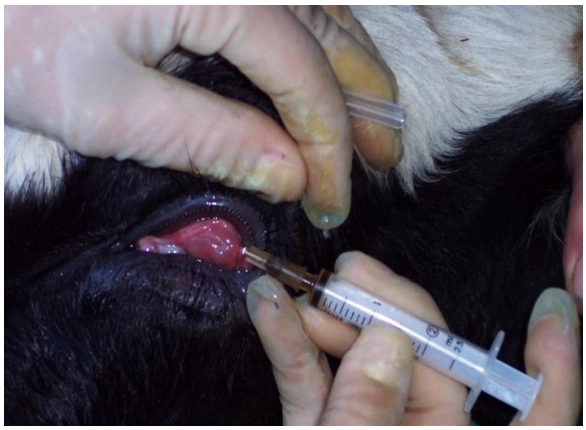

Figure 1B

Figure 1. Application of different oxytetracycline injection techniques in IBK cases. A. Intrapalpebral (IPa) injection, B. Subconjuktival (SCo) injection.

Şekil 1. IBK vakalarında farklı enjeksiyon tekniklerinin uygulanması. A. İntrapalpebral (IPa) enjeksiyon, B. Subkonjuktival (SCo) enjeksiyon.

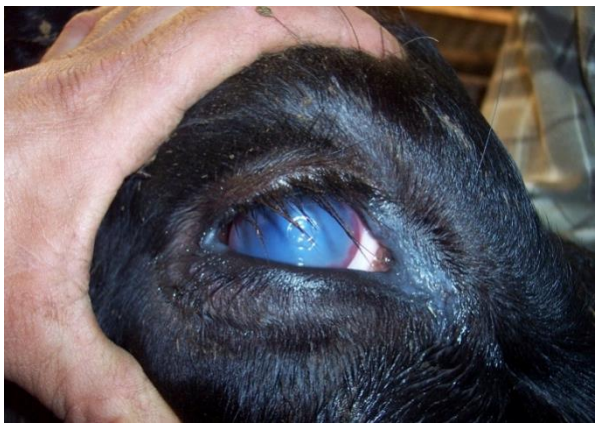

Figure 2A

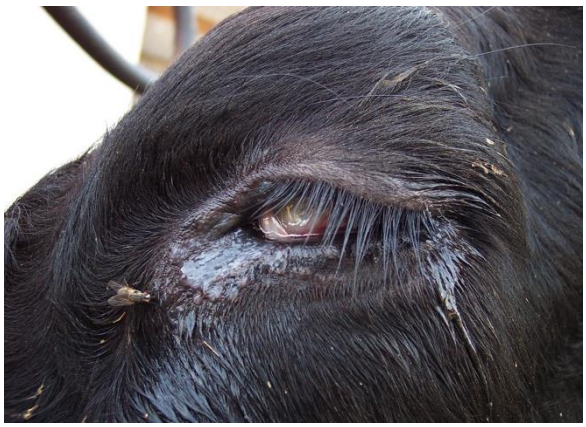

Figure 2B

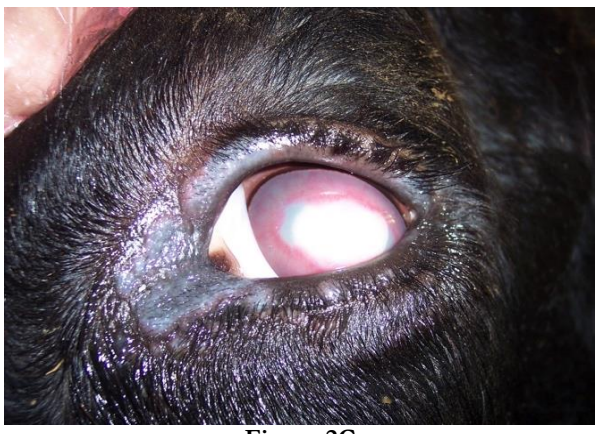

Figure 2C

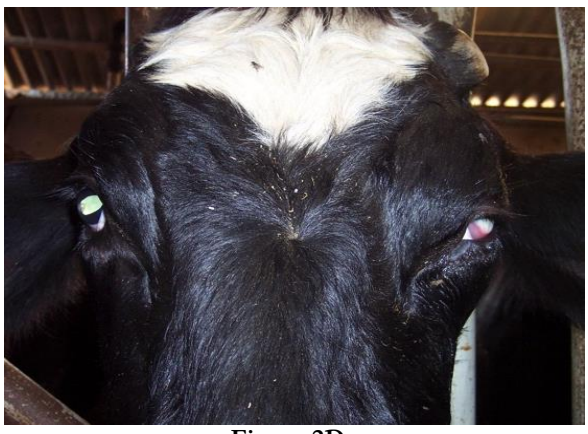

Figure 2D

Figure 2. A bluish-white opacity of the surface of the Cornea (A), deep corneal ulceration (B), a common white to deep yellow opacity in the corneal stroma and neovascularization (C) and swelling of the lids (D).

Şekil 2. Kornea üzerinde mavimsi beyaz opasite görünümü (A), derin korneal ülserasyon (B), korneal stromada sarımsı-beyaz yaygın opasite ve neovaskülarizasyon (C) ve göz kapaklarında şişlik (D).

Corneal ulcers recovered from day 14 (n=4), day 21 (n=8) and day 28 (n=8). In both groups 2 animals (2 eyes in 2 animals from group 1, and 2 eyes in 2 animals from group 2) were recovered with a permanent slight scarred cornea. There is no effect of injection type on healing time of the lesion ($p=0.408$). Size and side of the lesion have a

significant effect on healing time ($p<0.001$). The cattle with small ocular lesion had faster healing time (in 14 days) than that of the cattle with large ocular lesion (between 21 and 28 days). The lesions occurring one side led to faster healing time. In the microbiologic evaluation, small lesions were earlier clean (pathogen free) than large lesions. Lesion size has a significant effect on microbial growth ($p<0.001$). There were no interactions between injection type, lesion size and lesion side ($p>0.05$).

DISCUSSION

Subconjunctivally administered medications usually maintain higher corneal drug concentrations for longer periods of time than topical or parenteral applications (Senturk et al. 2007, Alexander 2010). In this application, the antibiotic must be placed beneath the dorsal palpebral or bulbar conjunctiva but it can be difficult to achieve in fractious cattle and requires good restraint (Quinn et al., 1994). It is very important that the animals should be properly restrained and fixation of the animal's head during SCo injection. This causes time and labour loss. On the other hand, palpebra is very mobile and pliable compared with skin elsewhere; therefore, the drugs will be injected more easily intrapalpebrally. During the literature search could not be reached adequate information about the advantages or disadvantages of IPa injection for treatment of IBK. There is no comparative study of SCo and IPa injection techniques. Researchers usually studied either the effects of different drugs or the effects of different doses of the same drug in cattle with IBK (George et al. 1985, Gokce et al., 2002, Zielinski et al. 2002, Senturk et al. 2007). In the present study, two injection techniques were compared. The IPa injection technique allowed easy application even in fractious animals and the drug was also injected more easily. Clinically the healing time was found similar in both groups. It was definitely a more comfortable application than SCo injection in field conditions and provided the same therapeutic effect.

It has been demonstrated that *M. bovis* is susceptibility to a variety of antibiotics (Prieto et al. 2013) and appropriate antimicrobial selection for the treatment of cattle infected with *M. bovis* requires knowledge of the minimum inhibitory concentration (MIC) for the bacterium, as well as an understanding of antibiotic distribution into ocular tissues and tears following administration (Shryock et al. 1998). The oxytetracycline is usually the first choice for antimicrobial treatment of IBK (Pickett 1999, Gokce et al., 2002, Senturk et al. 2007). Researchers have reported that the concentrations of the oxytetracycline-LA formulation might be administered for treatment of bacterial diseases in 2 days intervals but systemic administration of the drug may not ensure an effective therapy in eye disease (Zielinski et al. 2000, Zielinski et al. 2002). Maintaining consistent therapeutic drug concentration in tear film is difficult because of practical consideration. It has been reported to achieve therapeutic drug concentration, topical antibiotic administration is required several times per day; however, daily multidose therapy is not practical for most producers (Pickett 1999). Subcutaneous, intramuscular, and intravenous antibiotics are commonly used but very high dosages of an antibiotic are required to ensure adequate levels of the drug reach the eyes and tear glands (Brown et al. 1998, McConnel et al. 2007). Researchers have reported that the SCo administration of oxytetracyclineis reduce total dosages of drug than administration of systemic and daily multidose topical therapies (McConnel et al. 2007). High concentrations of antibiotic may be achieved in tear film (for 72 h) by SCo administration, although local irritation may occur (Brown et al. 1998). SCo injection of tetracyclines is effective but may cause necrosis at the injection site (Brown et al. 1998, Pickett 1999). Additionally, oxytetracycline-LA should not be recommended as a subconjunctivally, due to the it's severely irritation (Pickett 1999, Alexander 2010). The present study, all isolates were susceptible to oxytetracycline (100%) and conventional formulation of oxytetracycline preferred instead of oxytetracycline-LA for treating IBK by SCo and IPa routes. Only a painful swelling of the upper eyelid was occured in all animal of IPa injection group after the second injection of oxytetracycline, but there was no finding of necrosis in injection site at the end of treatment.

In the present study, all active symptoms such as blepharospasm, epiphora, photophobia were almost resolved at the end of 7 days in both groups, except of corneal ulcers (28 days). According to the findings and observations during this study, IPa injections ensure an effective therapy of the corneal ulcers caused by IBK in cattle. Additionally, it could be considered that, clinical findings of IBK rapidly and similarly resolved as in SCo injections. Based on literature knowledge (Brown et al. 1998, Zielinski et al. 2002)

in the present study these clinical results may be interpreted as the lacrimal fluid and ocular tissues contain sufficient quantity of oxytetracycline for a long time in both treatment group. In addition, it is safe for cattle with IBK at the dosage and the frequency of application of oxytetracycline used in this study. Since it was beyond of the scope of this study, the concentration of the oxytetracycline in the lacrimal fluid was not evaluated. The search of the amount of oxytetracycline in the lacrimal fluid after both local injections (SCo and IPa) during IBK treatment would be evaluated in the further studies.

CONCLUSION

In conclusion, 3 days intervals (days 0, 3 and 6), 3 IPa application of conventional formulation of oxytetracycline appears to be an effective method for the treatment of IBK with severe clinical symptoms such as corneal ulcers in this study, and IPa injection was recommended due to the easier application in field conditions. In addition, it was enough for cattle with IBK at the dosage (200 mg, once daily) used in the study.

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RESEARCH ARTICLE

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The Role of Pestiviruses (BDV and BVDV) in Ruminant Abortion Cases in the Afyonkarahisar Province

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ABSTRACT

Pestiviruses are important viral agents that can cause abortion in ruminants. In this study, roles of Border Disease Virus (BDV) and Bovine Viral Diarrhoea Virus (BVDV) were investigated in ruminant abortion cases. Aborted foetal tissue samples were collected from 101 animals (74 sheep foetuses and 27 bovine foetuses), each from epidemiologically different farms, during the months of January 2016 and December 2017 in the Afyonkarahisar Province. One step real-time duplex RT-PCR was used for the detection of BDV and BVDV RNA. Genetic characterization of the field isolates of pestiviruses was conducted by sequencing 5' untranslated region (5' UTR). BDV RNA was detected in 9 (12.16%) of the 74 aborted sheep foetuses, whereas BVDV RNA was detected in 6 (22.2%) of the 27 bovine foetuses. Phylogenetic analysis based on the 5' UTR region indicated that BDV isolates in the present study belong to BDV-7 genotype whereas BVDV isolates belong to BVDV-1 genotype. The results of this study showed that pestivirus infections play important role in ruminant abortion cases in Afyonkarahisar province.
Keywords: Border disease virus, bovine viral diarrhoea virus, abortion, sheep, cattle

Afyonkarahisar İlinde Ruminant Abort Vakalarında Pestivirusların (BDV ve BVDV) Rolleri

ÖZ

Pestiviruslar ruminantlarda abortlara neden olan önemli viral ajanlardır. Bu çalışmada, ruminant abort vakalarında Border Disease Virus (BDV) ve Bovine Viral Diarrhoea Virus (BVDV)'un rolleri araştırılmıştır. Abort olmuş fötus doku örnekleri 101 hayvandan (74'ü koyun fötusu, 27'si sığır fötus), her biri epidemiyolojik olarak farklı çiftliklerden, Ocak 2016 ve Aralık 2017 ayları arasında Afyonkarahisar ilinden toplanmıştır. BDV ve BVDV RNA'sının tespiti için tek adımlı real-time dubleks RT-PCR yöntemi kullanılmıştır. Sahadan izole edilen pestivirus'ların genetik karakterizasyonu 5' translate olmayan bölge sonunun (5' UTR) sekansı ile gerçekleştirilmiştir. BDV RNA'sı, 74 aborte koyun fötusunun 9 (%12.16)'unda, BVDV RNA'sı ise 27 sığır fötusunun 6 (%22.2)'sında tespit edilmiştir. 5' UTR bölgesinin filogenetik analizi bu çalışmada izole edilen BDV izolatlarının BDV-7 genotipine, BVDV izolatlarının ise BVDV-1 genotipine ait olduğunu göstermiştir. Bu çalışmanın sonuçları, pestivirus enfeksiyonlarının, Afyonkarahisar ilindeki ruminant abort vakalarında önemli rol oynadığını göstermektedir.
Anahtar Kelimler: Border disease virus, bovine viral diarrhoea virus, abort, koyun, sığır

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INTRODUCTION

Border Disease Virus (BDV), Bovine Viral Diarrhoea Virus 1 (BVDV-1) and Bovine Viral Diarrhoea Virus 2 (BVDV-2) belong to the *Pestivirus* genus of the *Flaviviridae* family, together with Classical Swine Fever Virus (CSFV). Pestiviruses are enveloped, single-stranded, positive-sense RNA viruses genome of 12.5 kb in length. Based on the genetic analysis, BDV isolates have been segregated into seven clusters (BDV-1 to BDV-7) whereas BVDV has two genotypes: BVDV-1 and BVDV-2 (Simmonds et al. 2012). Pestivirus infections have been associated with abortions, mummified foetuses, infertility, diarrhoea, respiratory disease and persistent infection (PI) of the offspring (Nettleton et al. 1998; Munoz-Zanzi et al. 2004).

It has been reported that pestiviruses are not host specific. Both BDV and BVDV can infect sheep, goat, cattle and swine (Nettleton et al. 1998; Passler and Walz 2010). Main route of transmission of pestiviruses is horizontal via transiently infected and PI animals. Furthermore, vertical transmission occurs in all host species (Van Campen and Frolich 2001).

Pestivirus infection has a worldwide distribution. Previous studies of abortion cases in ruminants in different regions of Turkey identified pestiviruses as the cause of abortion (Hasircioglu et al. 2009; Azkur et al. 2011; Avci et al. 2013; Berber and Sozdutmaz 2013; Tuncer-Goktuna et al. 2016; Ural and Erol 2017; Bulut et al. 2018). Small ruminants and cattle are important livestock in Afyonkarahisar province. Abortion in ewes and heifers causes serious economic losses in the livestock industry. Therefore, the aim of the present study was to investigate the role of BDV and BVDV in abortion cases of ruminants in the Afyonkarahisar Province.

MATERIAL and METHOD

Sample collection

During January 2016 and December 2017, foetal tissue samples (lung, liver, spleen, kidney and brain) were collected from 74 aborted sheep foetuses and 27 aborted bovine foetuses from flocks and herds where abortion cases occurred in the Afyonkarahisar province. Details of the sampled flocks and herds given in Table 1. Farmers reported that animals were not vaccinated against pestivirus infection in sampled flocks and herds.

RNA extraction and one step real-time duplex RT-PCR

Foetal tissue samples were homogenised in PBS using the TissueRuptor (Qiagen, Hilden, Germany). Viral RNA extraction was carried out from tissue homogenates using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. One step real-time duplex RT-PCR was performed with primers and probes that targeting 92 bp and 103 bp conserved regions of the 5'-UTR of BDV and BVDV, respectively (Table 2). The protocol described by La Rocca and Sandvik (2009) was used for detection of pestiviruses. One step real-time duplex RT-PCR reaction was carried out with one step RT-PCR kit (Cat No. 210212, Qiagen, Hilden, Germany) in a final volume of 25 µl reaction mix which contained 5 µl 5 x RT-PCR buffer, 200 µM of each dNTP, 1 µL enzyme mix, 0.4 µM of forward primer, 0.6 µM of reverse primers, 0.5 µM of each probes and 2.5 µL of sample RNA. Amplification was performed using LightCycler 2.0 real time PCR machine (Roche Applied Science, Indianapolis, IN, USA) with the following conditions: reverse transcription step of 10 min at 50 °C and 5 min at 95 °C, followed by 45 cycles at 95 °C for 15 s and 60 °C for 30 s. The samples that had a Ct value <35 were considered positive.

One-step RT-PCR amplification and sequencing of 5' UTR region

Samples that were positive by real-time duplex RT-PCR were subjected to one-step RT-PCR amplification using primers 324 and 326 which amplify a 288 bp region of the 5' UTR region (Vilcek et al. 1994). The protocol described by Vilcek et al. (1994) was used for detection of pestiviruses. RT-PCR reaction was carried out with one step RT-PCR kit (Cat No. 210212, Qiagen, Hilden, Germany) in a final volume of 25 µl reaction mix which contained 5 µl 5 x RT-PCR buffer, 400 µM of each dNTP, 1 µL enzyme mix, 1 µM each primer, and 2.5 µL of sample RNA. Amplification was performed using MJ Research thermal cycler with the following conditions: reverse transcription step of 30 min at 50 °C and 15 min at 95 °C, followed by 40 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s and final extension step in 72ºC for 5 min. The PCR products were analysed on 1.5% agarose gel stained with Gelred (Biotium, USA) after electrophoresis at 90 V for 60 min (Fig. 2). Amplified PCR products were sequenced both the forward and reverse directions on the ABI 3500XL DNA Analyser (Applied Biosystems, USA) with the

BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA) by Intron Saglik Urunleri (İzmir, Turkey). Primers 324 and 326 were used in sequence analysis. Phylogenetic tree was constructed, via the neighbour-joining method using MEGA software version 6, for the 5' UTR region of pestiviruses with additional sequences from GenBank. Kimura two-parameter model was used to describe the evolutionary distances between sequences.

Nucleotide sequence accession numbers

The 5' UTR region sequences reported in this paper are available in the GenBank under accession numbers MH395751 to MH395754.

Statistical analysis

The difference in the detected rate of BDV and BVDV was compared with Fisher's exact test. $P<0.05$ was considered to be statistically significant.

RESULTS

Detection of BDV and BVDV RNA by one step real-time duplex RT-PCR

BDV RNA was detected in 9 of the 74 aborted sheep foetuses whereas BVDV RNA was detected in 6 of the 27 aborted bovine foetuses (Table 1). Positive samples had Ct values between 20.17 and 34.06 (Fig. 1). There was no significant difference between the detected rate of BDV and BVDV ($P = 0.2193$). Furthermore, no significant differences were found between the districts where pestiviruses were detected ($P = 0.5294$).

Table 1. Districts where samples were collected
Tablo 1. Örneklerin toplandığı ilçeler

Districts	No. of examined flocks	No. of positive flocks	No. of examined herds	No. of positive herds
City Center	8	1	3	1
Çay	7	2	2	1
Çobanlar	5	-	3	1
Dazkırı	8	1	4	-
Dinar	7	-	3	1
Emirdağ	12	2	6	1
Hocalar	6	-	2	-
İhsaniye	7	-	1	-
Sinanpaşa	4	1	2	-
Sultandağı	10	2	1	1
Total	**74**	**9**	**27**	**6**

Table 2. Details of the primers and probes used for detecting pestiviruses by one step real-time duplex RT-PCR.
Tablo 2. Pestivirusların one step real-time dubleks RT-PCR ile saptanmasında kullanılan primerler ve problar

Primers and Probes	Sequence (5' - 3')	Target pestiviruses	Reference
106-F	CCATRCCCDTAGTAGGACTAGC	BDV-BVDV	La Rocca and Sandvik (2009)
190-R	GYGTCGAACCAYTGACGACT	BVDV	
179-R	GYGTYGAACTACTGACGACT	BDV	
Probe-162	FAM-TGGATGGCYKAABCCCTGAGTACAG-EDQ	BVDV	
Probe-128	YY-ACTAGCYDTCGTGGTGAGATCCCTG-EDQ	BDV	

Sequence and phylogenetic analyses of the 5' UTR region

Nucleotide sequences were obtained for two BDV and two BVDV field isolates. The analysis of the 5' UTR region sequences revealed that the homology between two BDV field isolates was 82.7% whereas the similarity with previously characterized BDV isolates ranged from 60.5% to 87%. The highest nucleotide homology was observed with

previous Turkish isolate (BDV-Aydin-04). The analysis of the 5' UTR region sequences revealed that the homology between two BVDV field isolates was 88.8% whereas the similarity with previously characterized BVDV isolates ranged from 70.2% to 96.5%. The highest nucleotide homology was observed with previous Germany isolate (BVDV CP7 strain).

The phylogenetic tree based on 5' UTR region sequences revealed that BDV field isolates in this study belonged to BDV-7 cluster whereas BVDV field isolates were typed as BVDV-1 (Fig. 3).

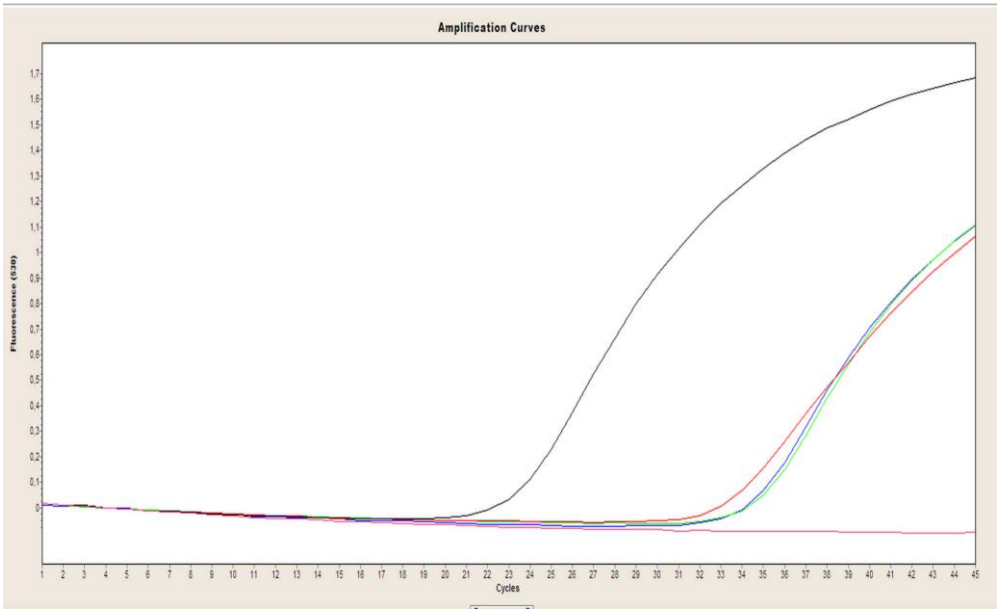

Figure 1. One step real-time duplex RT-PCR based on 5' UTR region of pestiviruses. Black line: positive control, pink line: negative control, other colourful amplification curves: positive pestivirus samples.

Şekil 1. Pestivirusların 5' UTR bölgesine dayalı one step real-time duplex RT-PCR. Siyah çizgi: pozitif kontrol, pembe çizgi: negatif kontrol, diğer renkli amplifikasyon eğirileri: pozitif pestivirus örnekleridir.

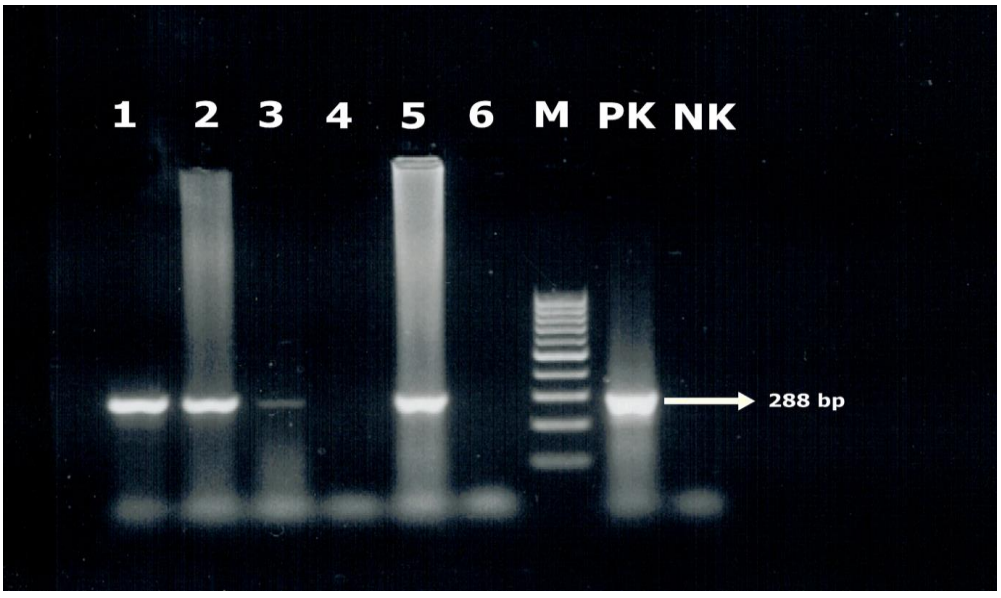

Figure 2. Agarose gel electrophoresis of RT-PCR product based on 5' UTR region of pestiviruses, M: Molecular marker of 100 bp, Lane 1-6: Samples, Lane PK: Positive control, Lane NK: Negative control.

Şekil 2. Pestivirusların 5' UTR bölgesine dayalı RT-PCR ürünlerinin agaroz jel elektroforezi, M: 100 bp moleküler marker, 1-6: Örnekler, PK: Pozitif kontrol, NK: Negatif kontrol.

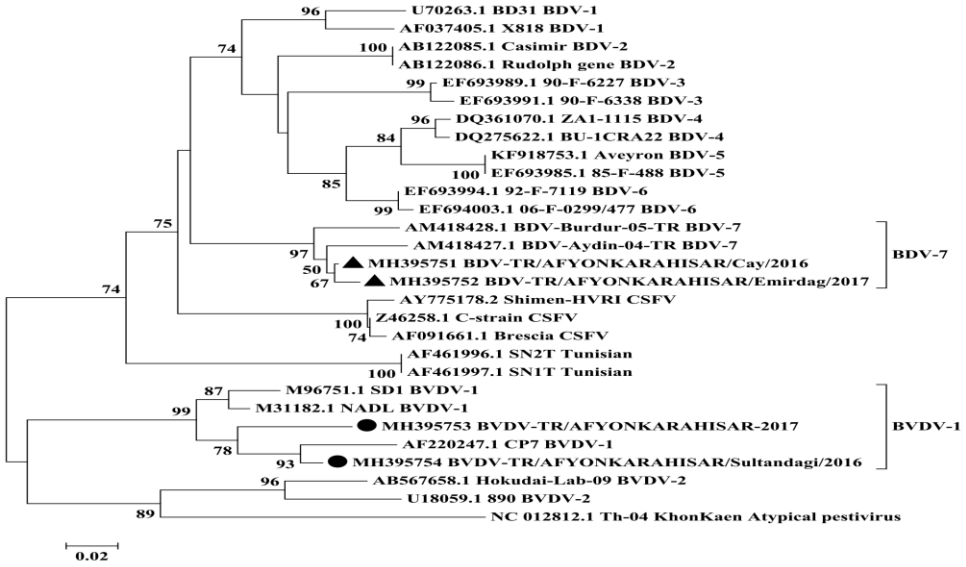

Figure 3. Phylogenetic tree constructed based on the 5' UTR region sequences using the Kimura two-parameter model. The BDV sequences obtained in this study are marked with black triangle (▲), and BVDV sequences are marked with round black spot (●).
Şekil 3. Kimura 2 parametre yöntemi kullanılarak oluşturulan 5' UTR bölgesi sekanslarının filogenetik ağacı. Bu çalışmada elde edilen BDV sekansları siyah üçgen ile (▲), BVDV sekansları ise siyah yuvarlak spotla (●) işaretlenmiştir.

DISCUSSION

Pestiviruses are distributed worldwide, and cause significant economic losses due to their impact on health and reproduction (Nettleton et al. 1998; Munoz-Zanzi et al. 2004). Pestiviruses are not highly host-specific (Nettleton et al. 1998; Passler and Walz 2010). Numerous studies have shown that both BDV and BVDV strains infect sheep, goat, cattle, swine and deer (Paton et al. 1995; Strong et al. 2010). However, in the study BDV RNA was only detected from aborted sheep foetuses, and BVDV RNA was from aborted bovine foetuses. Bulut et al. (2018) reported that prevalence of BVDV in sheep abortion cases in the Marmara and Eastern Anatolia regions in Turkey was 10.10% (40/396), and they suggested that the cause of BVDV infection in sheep may be pasture which contaminated with nasal drifts and saliva of persistently infected cattle. Furthermore, a previous study reported that close contact between small ruminants and cattle increases the risk of pestivirus transmission (Braun et al. 2013). In this study, BDV positive aborted sheep foetuses were from flocks which had only sheep for breeding, and according to farmers' report sheep and cattle were not use same pastures. Therefore there was no contact between sheep and cattle in BDV positive flocks. This could explain why BVDV RNA was not detected from aborted sheep foetuses.

The rate of pestiviruses in ruminant abortion cases in this study was 14.9% (15/101). This finding is in agreement with previous reports. Reported rates of pestiviruses in ruminant abortion cases in different regions of Turkey were between 0.93% and 66.6% (Cokcaliskan 2002; Hasircioglu et al. 2009; Albayrak et al. 2012; Avci et al. 2013; Tuncer-Goktuna et al. 2016; Bulut et al. 2018).

In this study, BDV RNA was found in 9 (12.16%) of the 74 aborted sheep foetuses. This result in agreement with previous report (Hasircioglu et al. 2009), but was lower than previous field studies that reported rates of the presence of pestiviruses in aborted sheep foetuses were 24.7%, 47.3% and 66.6% in the Marmara region, west part of Marmara region and Northern region of Turkey, respectively (Albayrak et al. 2012; Tuncer-Goktuna et al. 2016; Bulut et al. 2018). Possible explanations for this result may be the detection method, number of sampled animals and farm management. In this study, BVDV RNA was found in 6 (22.2%) of the 27 aborted bovine foetuses. This result in agreement with previous report (Albayrak et al. 2012), but was higher than previous study that detected BVDV antigen in 2.2% (2/92) of the aborted calves (Ozturk et al. 2012). Furthermore, Tuncer-Goktuna et al. (2016) detected pestivirus antigen in 31 (51.6%) of the 60 aborted calves in west part of Marmara region of Turkey. Possible explanations for these discrepancies may be the

number of sampled animals and number of sampled farms, and detection methods.
Serological and virological studies have been performed in the Afyonkarahisar province for pestiviruses (Gur 2009; Gur et al. 2009). However, molecular detection and genetic characterisation of pestiviruses in ruminant abortion cases in the Afyonkarahisar province has not been previously reported.

In previous studies, pestivirus isolates obtained from small ruminants in Turkey were classified into BDV-3, BDV-7 and BVDV-2 (Oguzoglu et al. 2009; Toplu et al. 2012; Yesilbag et al. 2014). Phylogenetic analysis of partial 5' UTR revealed that BDV field isolates in this study were of the BDV-7 genotype with the previous Turkish isolates (BDV-Burdur-05-TR and BDV-Aydin-04-TR). This result indicates that BDV-7 genotype is in circulation in the sheep population in Turkey.

The 5' UTR genetic analysis using sequences for pestiviruses revealed that BVDV field isolates in this study belonged to the BVDV-1 genotype (Fig. 3). The circulation of BVD-1 genotype in Turkey was also reported in previous studies (Yesilbag et al. 2008; Aslan et al. 2015). Furthermore, BVDV-2 genotype was detected from cattle in Turkey (Oguzoglu et al. 2010; Sarikaya et al. 2012; Yilmaz et al. 2012). It seems that both BVDV-1 and BVDV-2 are in circulation in cattle in Turkey.

In conclusion, a control programme for pestiviruses has not been applied in Turkey. Therefore, pestivirus infections are still animal welfare problem. Infection with pestiviruses causes serious economic losses in the livestock industry due to abortion problems, death and reduced reproductive performance. The results of this study showed that pestivirus infection play important role in ruminant abortion cases in Afyonkarahisar Province. A control programme for pestivirus infection will be beneficial to prevent economic losses.

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RESEARCH ARTICLE

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Türkiye' den Trichodinid Protozooan *Trichodina heterodentata* ve *T. pediculus* (Ciliophora: Trichodinidae) İçin Yeni Konak Kaydı[#]

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ÖZ

Bu çalışmada, Cichlidae familyasına ait sarı prenses (*Labidochromis caeruleus*), mavi prenses (Pseudotropheus socolofi) ve ahli çiklit (*Sciaenochromis fryeri*) balıkları ile Poecilidae familyasına ait velifera (*Poecilia velifera*) ve beyaz moli (*Poecilia sphenops*) balıklarının ektoparazitolojik yönden incelenmesi amaçlanmıştır. Aralık 2013- Kasım 2014 periyodunda ticari bir çiftlikten 600 balık örneği alınmıştır. Deri ve solungaç lamellerinden hazırlanan sürtme preparatların mikroskobik incelemesinde *Trichodina* sp.(Ehrenberg 1831) siliyatları tespit edilmiştir. Trichodinidler görüldüğünde Klein'in kuru gümüş boyama metodu kullanılarak yapışkan disk belirginleştirilmiştir. Sonuçlar *Trichodina pediculus*' ün radial iğne sayısı ve dentikül mesafesinin *T. heterodentata*' dan farklı olduğunu göstermiştir. *T. pediculus*' ün vücut çapı 55-70 (57,6 ± 1,03) µm ve *T. heterodentata*' nın vücut çapı 50-60 (54,72 ± 0,52) µm olarak ölçülmüştür. *T. pediculus*' ün yapışkan disk çapı 38-49 (44,78 ± 0,47) µm ve *T. heterodentata*' nın 34-49 (44,85 ± 0,51) µm olarak belirlenmiştir. Etkilenen balıklar anormal davranışlar veya klinik bulgu göstermemiştir. Bu çalışmada *Trichodina pediculus* ve *T. heterodentata* (Ciliophora: Trichodinidae) için yeni konak kayıtları bildirilmiştir.

Anahtar kelimeler: Akvaryum, protozoan, parazit, yeni konak

New Host Records For Trichodinid Protozoans, *Trichodina heterodentata* and *T. pediculus* (Ciliophora: Trichodinidae) from Turkey

ABSTRACT

In this study, we aimed, certain ornamental fish which the members of Cichlidae including the *electric yellow (Labidochromis caeruleus), powder blue cichlid (Pseudotropheus socolofi), electric blue hap (Sciaenochromis fryeri) and the members of Poecilidae including yucaten molly (Poecilia velifera), white molly (Poecilia sphenops) were examined for ectoparasitological.* Six hundred fish samples collected from a commercial farm December 2013 through November 2014. Microscopical examination of the smear preparations prepared skins and gill lamellae of them showed *Trichodina* sp. (Ehrenberg 1831) ciliates. When trichodinids observing, Klein's dry silver method was used to confirm the adhesive disc. Results showed numbers of radial pins and size of denticle span of *Trichodina pediculus* were different from those of *T. heterodentata*. The body diameter of *T. pediculus* was 55-70 (57,6 ± 1,03) µm and the body diameter of *T. heterodentata* was 50-60 (54,72 ± 0,52) µm. Width of the adhesive disc diameter was 38-49 (44,78 ± 0,47) µm for *T. pediculus* and was 34-49 (44,85 ± 0,51) µm for *T. heterodentata*. The affected fish did not show up unusual behavior or external clinical findings. *Trichodina pediculus* and *T. heterodentata* (Ciliophora: Trichodinidae) are informed for new hosts at first time in this study.

Keywords: aquarium, protozoan, parasite, new host

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GİRİŞ

Dünya genelinde akvaryum balığı endüstrisi Brezilya, Hindistan, Singapur ve Sri Lanka dahil birçok ülkede evcil hayvan endüstrisinin büyük bir kısmını oluşturmaktadır (Ghosh ve ark., 2003, Ling ve Lim 2005, Mandal ve ark., 2012, Martins ve ark., 2012). Günümüzde akvaryum balığı endüstrisi milyonlarca dolarlık bir sektör haline gelmiştir (Thilakaratne ve ark., 2003, Singh ve Sreedharan 2009). Evcil hayvan pazarlarındaki süs balıklarının büyük çoğunluğunun orijini tatlı su yetiştiriciliği oluşturmaktadır (Iqbal ve Haroon 2014). Diskus (*Symphysodona equifaciatus*), lepistes (*Poecilia reticulate*), kılıçkuyruk (*Xiphophorus helleri*), molly (*Poecilia sphenops*, *P. latipinna*) ve japon (*Carassius auratus*) yetiştiriciliği yapılan akvaryum balıkları içerisinde popüler balık türlerdir (Velasco-Santamaría ve Corredor-Santamaría 2011).

Parazitik enfeksiyonlar kültürü yapılan balıklar için en önemli sorunlar arasında yer alır. Uygun olmayan su kalitesi, su sıcaklığındaki dalgalanmalar dahil çevresel parametrelerdeki değişiklikler ile balıklara elleme ve yoğun olarak stoklama, farmasötik tedavi uygulamaları gibi işletme faaliyetleri yoğun kültür şartlarında balıklar üzerine etki ederek, balıkların homeostatik mekanizmalarına baskı yapar. Bu durum, balıkların ortamda bulunan parazitlere karşı savunmasız kalmalarına yol açar (Thilakaratne ve ark., 2003). Tatlı su balıklarında görülen parazitik türlere protozoan, myxozoan, helmint ve krustasean türleri dahildir. *Trichodina* sp. dahil protozoan ektoparazitler diğer parazitik türler arasında en tehlikeli gruptur. Trichodinidler tatlı su akvaryum balıklarının deri ve solungaçlarına yerleşerek balıkların büyüme performansları üzerine olumsuz etki yapar (Athanassopoulou ve ark., 2009, Iqbal ve Haroon 2014).

Trichodinidler aboral yüzey üzerindeki kompleks yapıları ve sahip oldukları yapışkan diskleriyle Trichodinidae familyası üyeleri arasında yer alır. Bu disk, parazitin hızlı ve dairesel şekilde hareket etmesine imkan verir. Yoğun kültür koşulları altında trichodinid sayısının hızla artması sonucu parazitin dairesel hareketleri konak vücut yüzeyinde aşındırıcı lezyonlara neden olabilir. Vücut yüzeyinde meydana gelen lezyonlar sonucu gelişen sekonder bakteriyel enfeksiyonlar balık kayıplarını arttırabilir (Iqbal ve Haroon 2014, Valladãa ve ark.,., 2015). Akvaryum balıklarında trichodinid enfeksiyonları Brezilya, Pakistan ve Sri Lanka dahil dünya genelinde bildirilmiştir (Thilakaratne ve ark., 2003, Martins ve ark., 2012, Iqbal ve Hassain 2013). Akvaryum balıklarından bildirilen trichodinid türler arasında *Trichodina nigra*, *T. acuta*, *T. reticulate*, *T. luzhoues* ve *T. mutabilis* gibi trichodinidler yer almaktadır (Valladão ve ark., 2015).

Trichodina pediculus ilk kez 1786' da *Cyclidium pediculus* olarak tanımlanarak 1838' de tür ismi *T. pediculus* şeklinde değiştirilmiştir. *T. pediculus* hidra, balık ve amfibien gibi geniş konak dağılımı gösterir (Gaze ve Wotten 1998). Bu trichodinid parazitin konak seçiciliğinin düşük olmasına karşın konak dağılımı Cypriniformes ve Perciformes olmak üzere iki takım ve Cyprinidae, Cichlidae, Centrarchidae, Odontobutidae ve Nonidae familyaları dahil beş familyayı içerir (Drobiniak ve ark., 2014). Kazubski (1991) *T. pediculus* Polonya' da japon balığından bildirmiştir. Bashe ve Abdullah (2010) bu türü Irak' ta dikenli yılan balığı (*Mastacembelus mastacembelus*)'nın derisinde tespit etmiştir. Ülkemizde ise Çapar Dinçer (2016) İç Anadolu Bölgesi' nin farklı göllerindeki siliyat faunasını araştırmış ve Ankara Mogan Gölü' nden *T. pediculus*'u bildirmiştir.

Trichodina heterodentata kozmopolitan bir tür olup ilk kez 1977' de Duncan tarafından rapor edilmiştir. Dove (2000) *T. heterodentata*' yı kılıç kuyruk (*X. helleri*) ve kırmızı plati (*X. maculatus*) balıklarından izole ederken, Dove ve O'Donoghue (2005) ise *T. heterodentata*' yı doğu sivrisinek balığı (*Gambusia holbrooki*) ve lepistes (*P. reticulate*)'ten bildirmiştir.
Türkiye' de yetiştiriciliği yapılan başlıca akvaryum balığı türleri arasında lepistes (Poecillia reticulata), melek balığı (Pterophylum scalarae), japon (Carassius auratus), diskus (Symphysodon aequifasciatus), ve moli (Poecilia sphenops) gelmektedir. Bu çalışma, Antalya civarında akvaryum balığı üretimi yapan ticari bir işletmeden temin edilen farklı süs balığı türlerinden *Trichodina heterodentata* ve *T. pediculus* trichodinidleri için yeni konak kayıtlarını bildirmektedir.

MATERYAL ve METOD

Çalışma Akdeniz Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından 2013.07.03 protokol numarasıyla Etik Kurul onayı alınarak gerçekleştirilmiştir. Çalışma süresince Antalya civarında faaliyet gösteren ticari bir akvaryum balığı işletmesinden Cichlidae familyasına dahil sarı prenses (*Labidochromis caeruleus*, Fryer 1956), mavi prenses (*Pseudotropheus socolofi*, Johnson 1974), ahli çiklit (*Sciaenochromis fryeri*, Konings 1993) ve velifera (*Poecilia velifera* Regan, 1914) ile Poecilidae familyası üyelerinden beyaz moli (*P. sphenops*, Valenciennes 1846)'nin dahil olduğu toplamda altıyüz balık örneği ile çalışılmıştır. Örnekleme çalışmalarına Aralık 2013 de başlanılıp çalışma, Kasım 2014'e kadar devam etmiştir. Örnekleme zamanlarında

akvaryumlarda bulunan suyun sıcaklığı, çözünmüş oksijeni ve pH'ı ölçülerek kaydedilmiştir.
Balık örnekleri işletmeden laboratuvara içerisinde havalandırılmış su içeren polietilen torbalar kullanılarak nakledildi. Laboratuvar koşullarında balıkların vücut ağırlıkları ölçülüp kaydedildi. Balıklardaki mevcut parazitleri incelemek için standart parazitolojik yöntemler kullanıldı (Roberts 1989, Lom ve Dykovà 1992). Balıkların deri ve solungaçlarından sürtme preparatlar hazırlanarak mikroskop altında incelendi. Balıkların mide barsak, karaciğer, dalak ve böbreklerinden örnekler alınarak incelendi. Sürtme preparatlarda trichodinid parazitler gözlendiğinde, bu preparatlar havada kurutalarak yapışkan diski ortaya çıkarabilmek için Klein'in kuru gümüş boyama yöntemi ile boyandı (Klein 1958). Tricodinid türlerinin belirlenmesinde, parazitlerin vücut çapları, yapışkan disk çapları, radyal iğneler, diş halka çapları, sınır membranı, bıçak uzunluğu, ışın uzunluğu ve dentikül mesafesi ölçümleri mikrometrik oküler kullanılarak yapıldı (Resim 1). Parazit fotoğrafları, mikroskoba takılı olan Nikon kamera ile çekildi.

Tüm ölçümlerin aritmetik ortalamaları hesaplandı. Prevalans, ortalama yoğunluk ve ortalama bolluk değişimleri için varyans analizi ve Dukan testi uygulandı. Parazitler arasında ortalama bolluk ve su sıcaklığı arasındaki ilişki korelasyon analizi ile test edildi. İstatistiksel olarak anlamlı olan $p \leq 0.05$ seçildi.

BULGULAR

On iki ay boyunca her ay işletmeye gidilerek suyun çözünmüş oksijen, pH ve sıcaklık değerleri ölçüldü. Elde edilen verilere göre, en yüksek su sıcaklık değeri 25,8 °C ile Haziran, Temmuz ve Ağustos aylarında, en düşük su sıcaklık değerinin 23,1 °C ile Kasım ayında olduğu belirlendi. En yüksek çözünmüş oksijen miktarı 6,9 mg/l ile Aralık ayında, en düşük çözünmüş oksijen miktarı 4,7 mg/l ile Mayıs, Haziran, Temmuz ve Ağustos aylarında tespit edildi. En yüksek pH değeri 8,40 değeri ile Ocak ayında, en düşük pH değeri ise 7,10 ile Haziran ve Temmuz aylarında kaydedildi.

Çalışma süresince ortalama 1,5-2,5 gram ağırlığında ve 3,5-4,5 santimetre uzunluğunda sarı prenses, mavi prenses, ahli çiklit, velifera ve beyaz moli türleri ile çalışılarak toplam altıyüz balık örneği incelendi. Bu balıklardan %13,6' sının iki farklı trichodinid türü ile enfeste olduğu bulundu. Çalışmada *T. pediculus*' un sarı prensesin (*L. caerulus*) %6,6 sını, mavi prensesin (*P. socolofi*) %8,3 ünü, veliferanın (*P. velifera*) %9,1 ini, beyaz molinin (*P. sphenops*) ise %10,8 ini enfeste ettiği, *T. heterodentata*' nın ise ahli çiklitin (*S. fryeri*) %25,8 ini ve beyaz molinin (*P. sphenops*) %7,5 ini enfeste ettiği tespit edilmiştir. Enfeste balık örneklerinin sadece derilerinden hazırlanan sürüntülerde trichodinid türleri gözlenmiştir. Parazitten etkilenen balıklarda normal olmayan davranışlar ve/veya klinik bulgular tespit edilmemiştir. Altıyüz balık için trichodina türlerinin prevalansı %13,7 olup, en yaygın görülen tür ise %51,2 ile *T. pediculus* olmuştur. *T. heterodentata*'nın baskınlığı ise %3.3 olarak bulunmuştur. Çalışmada, tespit edilen *T. pediculus* ile *T. heterodentata*'nın (Familya Trichodinidae Claus 1874, Cins *Trichodina* Ehrenberg 1838) tanımları, şekilleri, prevalansları ile ortalama yoğunlukları Tablo 1 de verilmiştir. Balık örneklerinin endoparazit yönünden incelenmesi sonuçlarına göre, balıklarda herhangi bir endoparazit türüne rastlanılmamıştır.

Trichodina pediculus dentüküllerinde orak şeklinde bıçakları, hafif kavisli ve çok uzun ışınlara sahiptir. Bıçaklar kenarına doğru sivrileşir (Resim1). Vücut 55-70 µm (57.6 ± 1.03), yapışkan disk 38.0-49.0 µm (44.78 ± 0.47), dentikül halkasının çapı 21.0-35.0 µm (28.92 ± 0.63), merkezi halka 11.0-20.0 µm (14.61 ± 0.49) çapındadır. Dentükül halkası 23-24 dentükül içerir ve dentikül başına 9-12 radyal iğne bulunur. Merkezi bölgenin eni 2-3 µm, dentikül mesafesi 4 µm' dir. Makronukleus at nalı şeklindedir ve vücudun merkezinde ışınların son noktasına yerleşmiştir. Mikronukleus küçük çubuk şekillidir ve makronukleusun son ucuna yerleşmiş haldedir. Morfometrik veriler Tablo 2' de verilmiştir. Havada kurutulmuş *T. pediculus* preparatları gümüş boyama yöntemiyle boyanmış ancak örnek boyayı almamıştır.

Trichodina heterodentata dolgun bıçaklara sahiptir. Bu trikodinid parazitin dentikülleri geniş bıçak, güçlü çubuk şekilli olmaları ile karakterize edilir bunlar merkezdeki sivri uçlar ile bağlantılı olan bıçakların apofizi ile sağlanır. Merkezi cisimcikler yoktur, bıçağın antreriör kenarı keskin biçimde aşağı doğru kıvrımlıdır. Bıçaklar y ve y^{-1} eksenleri arasındaki boşluğu doldurur ve bıçağın tepe kısmı yuvarlaktır y^{+1} eksenine değer. Bıçak bağıntıları kalındır. Işın uzun sağlam ve y ile y+1 eksenleri arasında konumlanmıştır (Resim2 a ve b). *T. heterodentata* ya ait morfometrik veriler Tablo 3 de verilmiştir. Morfometrik verilere ilişkin değerlendirmeler Roberts (1989), Lom ve Dykovà (1992) ve Özer ve Öztürk (2015) isimli araştırmacıların çalışmalarından yararlanılarak yapılmıştır.

Tablo 1. Enfeste balıkların trikodinid parazitlerinin prevalansı ve yerleşim yerleri.
Table 1. The prevalance and localities of trichodinid parasites of the infested fish.

Konak	Örnek Sayısı	Parazit Türü	Yerleşim Yeri	Enfeste Balık	Prevalans
L. caeruleus	120	*Trichodina pediculus*	Deri	3	% 2,5
P. socolofi	120	*Trichodina pediculus*	Deri	4	% 3,3
S. fryeri	120	*Trichodina heterodentata*	Deri	3	% 2,5
P. velifera	120	*Trichodina pediculus*	Deri	4	% 3,3
P. sphenops	120	*Trichodina pediculus*	Deri	3	% 2,5
		Trichodina heterodentata	Deri	1	% 0,8

Trichodina pediculus (n=14) (Resim1)

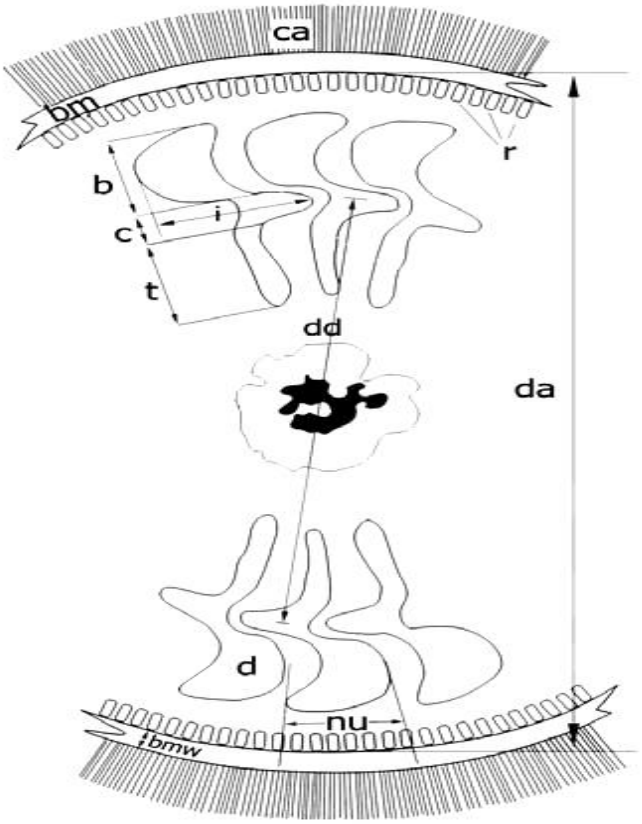
Tablo 2. *Trichodina pediculus*' un morfometrik verileri (parantez içinde aritmetik ortalama ve standart hata µm olarak verilmiştir).
Table 2. Morphometrical data of *Trichodina pediculus* (with arithmetic mean and standard error in parentheses, all measurements in µm).

Araştırmacı	Bu Çalışma	Kazubski (1991)	Gaze veWootten (1998)	Bashe ve Abdullah (2010)
Konak	*L. caeruleus, P. socolofi, P. velifera, P. sphenops*	*Carassius carassius*	*Gasterosteus aculeatus*	*Mastacembelus mastacembelus*
Yerleşim	Deri	-	Deri	Deri
Yer	Antalya, Türkiye	Kortowo, Polonya	Airthrey Loch, Central Region	Greater Zab Nehri, Irak
Vücut Çapı	55-70 (57.6±1.03)	-	-	55-70
Yapışkan disk çapı	38-49 (44.78±0.47)	54.96±4.52	46.0-57.2 (50.1±3.6)	43-66
Dentiküler halka çapı	21-35 (28.92±0.63)	35.70±2.83	29.3-34.0 (32.0±1.6)	28.3-44.6
Merkezi halka çapı	11-20 (14.61±0.49)	-	-	11-24
Dentikül sayısı	23-24	28-29	26-29 (27)	25-30
Radial iğne/dentikül	9-12	-	6-8 (7.5±0.8)	9-9.6
Sınır membran	3-5 (3.42±0.11)	3.9	3.4-5.0 (4.4±0.4)	-
Bıçak uzunluğu	5-7 (5.76±0.13)	-	4.9-6.2 (5.6±0.3)	-
Işın uzunluğu	7-9 (7.19±0.16)	-	9.2-13.6 (11.6±1.3)	-
Merkezi bölge eni	2-3 (2.30±0.07)	-	1.3-2.5 (1.9±0.3)	-
Dentikül mesafesi	4	19.12±2.38	16.6-21.8 (18.9±1.6)	-

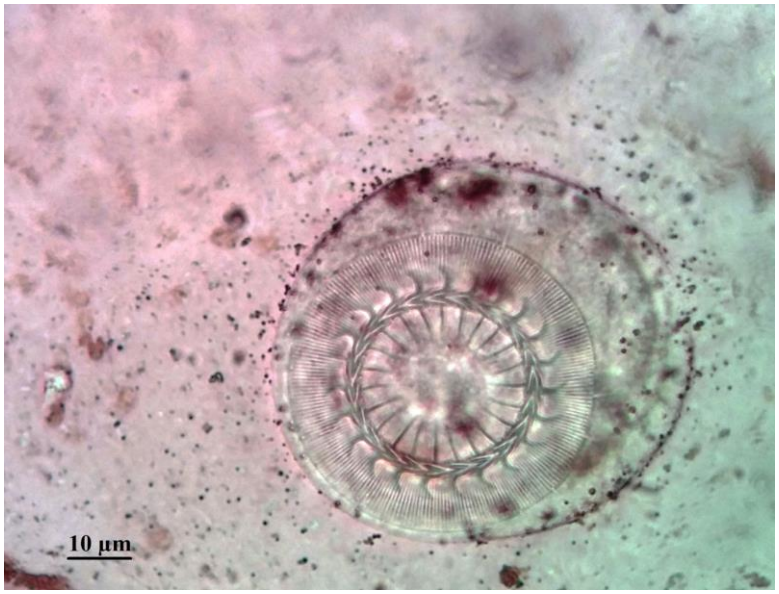
Trichodina heterodentata (n=4) (Resim2)

Tablo 3. *Trichodina heterodentata* nın morfometrik verileri (parantez içinde aritmetik ortalama ve standart hata, tüm ölçümler µm cinsinden).
Table 3. Morphometrical data of *Trichodina heterodentata* (with arithmetic mean and standard error in parentheses, all measurements in µm).

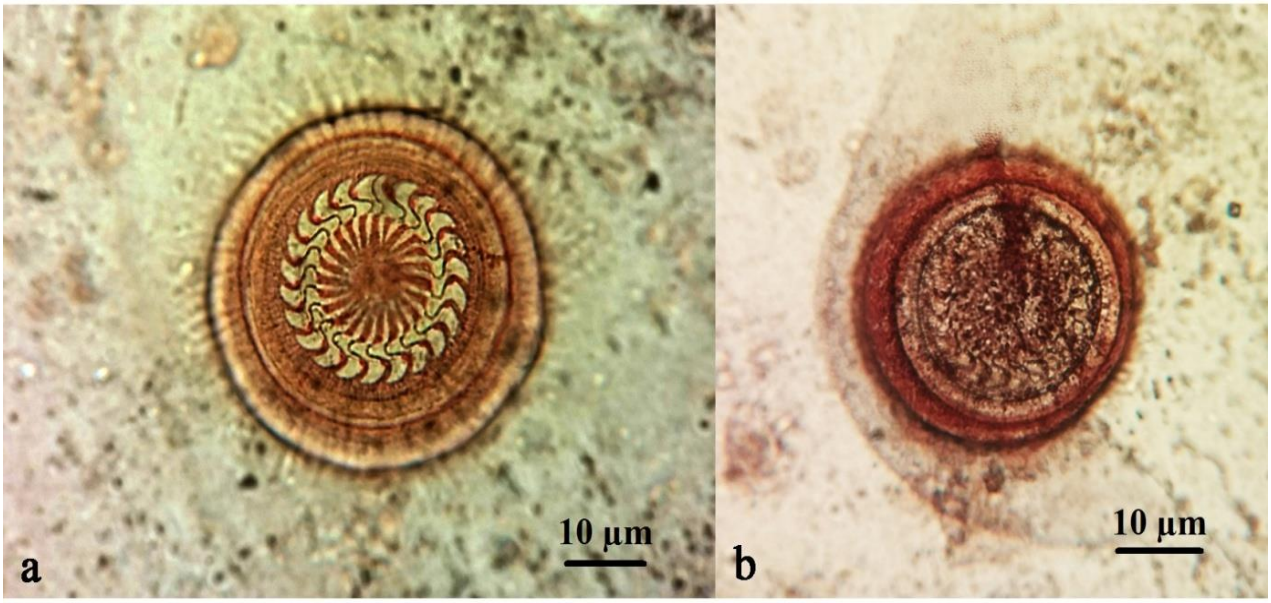
Araştırmacı	**Bu çalışma**	**Asmat (2004)**	**Martins ve ark., (2010)**	**Öztürk ve Çam (2013)**
Konak	*S. fryeri* *P. sphenops*	*Anabas testudineus* *Puntius gelinus*	*Ictalurus punctatus*	*Neogobius fluviatilis* *Proterorhinus marmoratus* *Pomatoschistus marmoratus*
Yerleşim Yeri	Deri	Solungaçlar	Deri ve solungaçlar	Deri solungaç ve yüzgeçler
Yer	Antalya, Türkiye	West Bengal, India	Porto Uniao ve Santa Catarina, Brezilya	Kızılırmak Delta, Samsun, Türkiye
Vücut çapı	50-62 (54.72±0.52)	46.1-61.2 (54.6±3.3)	27.0-77.0 (59.4±8.5)	45-64 (51.17±3.09)
Yapışkan disk çapı	34-49 (44.85±0.51)	41.8-52.0 (45.6±2.8)	40.0-72.0 (60.2±6.7)	37-55 (43.42±2.60)
Dentüküler halka çapı	23-33 (25.4±0.41)	26.0-33.6 (30.4±1.7)	27.0-47.0 (38.5±4.5)	24-39 (27.16±1.70)
Merkezi halka çapı	9-13 (11.25±0.12)	9.2-17.3 (13.5±2.1)	-	-
Dentükül sayısı	23-24	21-26 (23.1±1.2)	23-28 (24.4±1.6)	20-26
Radyal iğne/dentikül	6-9	9-13 (10.8±1.2)	5.0-15.0 (11.8±2.1)	7-8
Membran sınır	2.0-4.0 (2.65±0.10)	3.1-5.6 (4.5±0.6)	3.0-7.0 (5.1±1.7)	4-5 (4.75±0.15)
Bıçak uzunluğu	6.0-7.0 (6,30±0,07)	4.0-8.0 (6.2±0.8)	4-6 (4.75±0.22)	4.1-7.1 (5.3±0.6)
Işın uzunluğu	6.0	5.9-8.2 (6.9±0.7)	3.0-12.0 (8.5±1.7)	5-8 (5.58±0.34)
Merkezi bölge genişliği	3.0	2.0-3.1 (2.8±0.4)	2.0-6.0 (3.8±0.7)	1-3 (2.17±0.21)
Dentikül mesafesi	8.0	13.7-17.9 (15.0±1.0)	7.0-13.0 (10.3±1.2	11-17 (12.88±0.65)



Resim 1. Trichodinid türlerinin tayini için ölçümü yapılan bölgeler: bıçak uzunluğu (b), membran sınırı (bm), membran sınır genişliği (bmw), merkezi bölge eni (c), adoral zon silleri (ca), dentikül (de), yapışkan disk çapı (da), dentikül çapı (dd), dentikül sayısı (dn), dentikül mesafesi (i), radyal iğne sayısı (nu), radyal iğne (r), ışın uzunluğu (t) (Öğüt ve Altuntaş 2011).
Figure 1. Measuring organelles in trichodinid species; blade lenght (b), border membrane (bm), border membran width (bmw), central part witdh (c), cilia of adoral zone (ca), denticle (de), adhesive disc diameter (da), denticle diameter (dd), denticle number (dn), denticle span (i), radial pin number (nu), radial pin (r), ray lenght (t) (Öğüt and Altuntaş 2011).



Resim 2. Işık mikroskobu altında görüntülenen *Trichodina pediculus*
Figure 2. *Trichodina pediculus* under the light microscopy.



Resim 3 a; *S. fryeri* den izole edilen ve gümüş-nitrat solüsyonu ile boyanan *Trichodina heterodentata* nın ventral görüntüsü, **b;** *T. heterodentata* nın dorsal görüntüsü.
Figure 3 a; Ventral view of *Trichodina heterodentata* from *S. fryeri* stained with silver-nitrate solution, **b;** dorsal view of *T. heterodentata*

TARTIŞMA

Trichodinidler sucul ekosistemin en sık görülen parazitlerinden olup konaklarını tatlı su ve deniz balıkları oluşturur. Bu parazitler akvaryum balıklarında ciddi kayıplara neden olmakla birlikte, akvaryum balıkları arasında beta (*Betta splendens*), neon tetra (*Paracheirodon innesi*) ve lepistes (*P. reticulata*) türlerinin bu parazitlere daha duyarlı olduğu bildirilmiştir (Dobberstein ve Palm 2000, Koyuncu 2009).

Ülkemizde 1998 yılına kadar trichodinid türler hakkında bir rapor mevcut olmayıp, ilk kez 1998 de *Trichodina acuta*, *T. mutablis* ve *T. nigra* trichodinidleri doğal ve kültür balıklarından bildirilmiştir (Özer ve Öztürk 2015). Daha sonra, Öztürk ve Çam (2013) ile Özer ve Öztürk (2015) *T. heterodentata*'yı gobi (*Neogobius fluviatilis*, *Proterorhinus marmaratus* ve *Pomatoschistus marmaratus*), dişli sazancık (*Aphanius danfordii*) ve sudak (*Sander lucioperca*)'dan tespit etmiştir. Cins seviyesinde ise *Trichodina* sp. japon (*C. auratus*), lepistes (*P. reticulata*), altın moli (*P. latipinna*), kılıçkuyruk (*X. helleri*) ve kırmızı plati (*X. maculatus*) balıklarından bildirilmiştir (Koyuncu and Cengizler 2002, Bulguroğlu ve Korun 2013).

Trichodina gibi protozoan parazitler, balıklarda az sayıda bulunduklarında klinik bir bulguya neden olmazlar. Bu nedenle, trichodina enfestasyonlarında tanı sadece mikroskobik gözlem ile yapılır. Parazitle enfeste balıklarda durgunluk, vücut ağırlığında azalma ve solungaçlarda şişkinlik görülür (Durborow 2003). Tang ve Zhao (2007) *T. heterodentata* ile enfeste japon balıklarının solungaçlarında epitelyal dökülme, yangı ve yapısal bozukluk bildirirken, Vallado ve ark., (2015) ise bu trichidinid türün tespit edildiği balıklarda ciddi proliferatif lezyonları bildirmiştir. Mevcut çalışmada, 600 balık örneğinin 82'sinin trichodinid türler ile enfeste olduğu bulunmuştur. Bu türlerin 42'si *Trichodina pediculus*, 40'ı ise *T. heterodentata* olarak tanımlanmıştır. Çalışmada *T. pediculus* ve *T. heterodentata* ile enfeste balıklarda, parazit sayısının düşük olması nedeniyle Durborow (2003), Tang ve Zhao (2007) ve Vallado ve ark., (2015) tarafından bildirildiği gibi durgunluk, vücut ağırlığında azalma ve solungaç filamentlerinde hasar nedeni ile solungaç doku yapısında değişiklik ve bozukluk tespit edilmemiştir.

Trichodinid türlerinin çoğu balıkların solungaçlarında bulunurken, az bir kısmı ise balıkların sadece vücut yüzeyinde bulunur. *T. pediculus* ve *T. acuta* türlerinin enfeste balıkların derisinde gözlenmesine karşın solungaçlarda bildirilmemiştir. Bununla birlikte, *T. heterodentata* gibi sık rastlanılan türlerin ise balıkların solungaç ve derilerinde görüldüğü rapor edilmiştir (Basson ve Van As 2006, Abowei ve ark., 2011). Çalışmada enfeste sarı prenses, mavi prenses, velifera, beyaz moli ve ahli çiklit balıklarının derisinden hazırlanan sürtme preparatlarda *T. pediculus* ve *T. heterodentata* türleri tespit edilirken, solungaçlardan hazırlanan preparatlarda ise *T. heterodentata* tespit edilmemiştir. *T. pediculus*' ün ayırt edici karakteristik özelliği dentikül sırasıdır. Bu sıralar uzun ve gittikçe sivrilen yapıdadır. Yapışkan diskin merkezinde, merkezi kısım belirgin olmaksızın granüler görünür (Gaze ve Wootten 1998). Bashe ve Abdullah (2010) Irak' ta *Mastacembelus mastacembelus*' un derisinden *T. pediculus*'u izole etmiştir. Araştırmacılar, parazitin vücüt çapını 55-70 µm, yapışkan disk çapını 43-66 µm, dentiküler halka çapını 28.3-46.6 µm, dentikül

sayısını 25-30, ışın uzunluğunu 9-9.6 µm, bıçak uzunluğunu 5-6 µm olarak bildirmiştir. Bu ölçümler çalışmamızdaki *T. pediculus* ölçümleri ile benzerlik gösterdiği anlaşılmıştır. Valladao ve ark., (2014), *Prochilodus lineatus* larvasının ağız, solungaç, yüzgeçler ve vücut yüzeyinden *T. heterodentata*'yı izole etmiştir. Araştırıcılar, parazitin vücut çapını 48.4-65.9 (56.9±3.6) µm, yapışkan disk çapını 39.4-55.3 (47.7±3.6) µm, dentiküler halka çapını 23.0-37.6 (29.4±2.6) µm, dentikül sayısını 20-26, membrane sınır genişliğini 2.8-5.7 (4.5±0.4) µm, dentikül uzunluğunu 5.8-9.3 (7.8±0.7) µm, bıçak uzunluğunu 3.8-5.7 (4.6±0.4) µm, ışın uzunluğunu 6-9.9 (7.7±0.8) µm, dentikül mesafesini 13.0-17.6 (15.4±1.0) µm ve dentikül başına düşen radial iğne sayısını 6-12 olarak belirlemiştir. Bu ölçümler, çalışmamızda elde edilen ölçümler ile benzerlik gösterdiği bulunmuştur.

Piazza ve ark., (2006) Brezilya' da akvaryum balığı türlerinin parazitik faunasını araştırmışlardır. Araştırmacılar japon, plati, kılıçkuyruk ve moli dahil 189 balığı incelemiştir. Çalışma sonuçlarına göre incelenen balıkların *T. acuta* ile enfeste oldukları ve bu trichodinid parazitin prevalansının %4.7 olduğu tespit edilmiştir. Çalışmamızda *Trichodina* türlerinin prevalansı 600 balıkta % 14.9 olarak bulunmuştur. En sık görülen trichodinid türü *T. pediculus* (11.6%) olup, *T. heterodentata*' nın baskınlığı % 3.3 olmuştur. Durborow' un (2003) bildirdiği gibi çalışmada enfeste balıklarda parazit sayısının düşük olması nedeniyle, bu balıklarda ciddi kayıplara neden olmadığı anlaşılmıştır.

Çoğu trikodinid tür, çok az konak spesifikliği sergilerken diğerleri konağa spesifiktir. Konağa spesifik trichodinidler, sadece bir kaç istisnai durumda solungaç parazitidir. *T. centrostrigata*, Cichlidae familyası ile ilişkilidir. Japon balıklarında *T. reticulate* bildirilirken, *T. kazubski* Güney Afrika' da *Barbus* spp.' de bulunmuştur, *T. nobilis* ve *T. kupermani* ise asya sazanında tespit edilmiştir (Basson ve Van As 2006, Abowei ve ark., 2011).
T. heterodentata çiklit türlerini tercih eden tür olarak tanımlanır ancak parazit farklı balık türlerini de etkileyebilir. Şu ana kadar, dünya genelinde 50'den fazla balık türünde tanımlanmıştır. En çok etkilenen balık türleri çiklitler ve cyprinidler olmasına rağmen, Eleotridae ve Poecilidae familyalarına dahil olan balıkların çoğunun bu türle enfeste olduğu bildirilmiştir (Miranda ve ark., 2012). Çalışmamızda, *T.* heterodentata' nın ahli çiklit (*S. fryeri*) ve beyaz moli (*P. sphenops*)' yi enfeste ettiği tespit edilmiştir. Miranda ve ark., (2012), diğer araştırmacıların bu parazitin konak balık tercihinin olmadığı hipotezine dikkat çekerek, bu soruyu cevaplamak için daha fazla sayıda familyaya ait balıklarla yapılmış çalışmaya ihtiyaç duyulduğunu öne sürmüştür. Bununla birlikte, mavi prenses ve beyaz moli türleri, Cichlidae ve Poecilidae familyalarının üyeleri olup, *T. heterodentata*' nın konak tercihi üzerine verilen yukarıdaki bilgiler, bu çalışmanın verileriyle uyumlu olduğu anlaşılmaktadır.

SONUÇ

Trichodinid parazitler balıkta düşük sayıda bulunduklarında klinik bir bulguya yol açmayabilir. Sonuç olarak, çalışmamızda incelenen balık türlerinde trichodinid parazitlerin klinik bir bulguya neden olmadığı tespit edilmiştir. *Trichodina heterodentata* ülkemizde ilk kez aşağı Kızılırmak Delta'sında mevcut balık türlerinden bildirilirken (Özer ve Öztürk 2015), çalışmamızda ise akvaryum balığı türlerinde tespit edilmiştir. *T. pediculus* ise Çapar Dinçer (2016) tarafından Mogan Gölü'nde tespit edilirken, çalışmamızda ise ciklit ve molilerden izole edilmiştir. Bu türün konak seçiciliğinin az, ancak konak dağılımının *Cyprinidae*, *Cichlidae*, *Centrarchidae*, *Odontobutidae* ve *Nonidae* familyaları ile sınırlı olduğunun rapor edilmesine (Drobinia ve ark., 2014) karşın, çalışmamızda *Poecilidae* familyasına ait moliden tespit edilmiştir.

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Detection of Ochratoxin A In Bulk Tank Milk

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ABSTRACT

Ochratoxin A (OTA) produced by several *Aspergillus* and *Penicillium* species is a mycotoxin that contaminates different foods and feedstuffs, including cereals, coffee beans, nuts, cocoa, pulses, beer, wine, spices, dried vine fruits, meat, milk. In humans and animals, OTA has been observed to be particularly nephrotoxic, hepatotoxic, immunotoxic, neurotoxic, embryotoxic, carcinogenic and teratogenic. Ochratoxin A is a stable molecule and can remain unchanged even after the processes applied. In this study, it was aimed to determine the presence of ochratoxin A in milk samples (n:40) collected from bulk tank milks in Burdur province of Turkey. The presence of OTA in the samples was analyzed by using ELISA. The analyzes were performed according to the manufacturer's instructions. As a result, Ochratoxin A was found in 40 cow's milk samples (range 2-270 ng/l) collected from bulk milk tanks. The results of this study show that cow's milk should be considered as a potential OTA source in the human diet. It is proposed to examine the presence of OTA more intensively in dairy products and to determine their maximum limit values by conducting necessary studies.

Keywords: Burdur, Ochratoxin A, Milk, Mycotoxin

Süt Toplama Tanklarında Okratoksin A Varlığının Belirlenmesi

ÖZ

Okratoksin A (OTA), *Aspergillus* ve *Penicillium* türü mantarlar tarafından sentezlenen ve tahıl, kahve çekirdeği, fındık, kakao, bakliyat, bira, şarap, baharat ve kuru üzümde bulunabilen bir mikotoksindir. İnsanlarda ve hayvanlarda, OTA özellikle nefrotoksik, hepatotoksik, nörotoksik, embriyotoksik, immunotoksik, teratojenik ve karsinojenik etkiler gösterir. Okratoksin A kısmen kararlı bir moleküldür ve gıdalara uygulanan işlemlerden sonra bile değişmeden kalabilir. Bu çalışmada; Burdur bölgesinde bulunan süt toplama tanklarında (n:40) OTA varlığının belirlenmesi amaçlandı. Örneklerde OTA varlığı ELISA kullanılarak analiz edildi. Analizler üreticinin talimatlarına göre yapıldı. Süt toplama tanklarından alınan 40 inek süt örneğinde (2-270 ng/l aralığında) OTA bulundu. Bu çalışmanın sonuçları, inek sütünün insan beslenmesinde potansiyel bir OTA kaynağı olarak görülmesi gerektiğini göstermektedir. OTA varlığını süt ürünlerinde daha yoğun bir şekilde incelenmesi ve gerekli mevzuat çalışmaları yapılarak maksimum limit değerlerinin belirlenmesi önerilmektedir.

Anahtar kelimeler: Burdur, Okratoksin A, Mikotoksin, Süt

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INTRODUCTION

Mycotoxins produced by *Fusarium, Aspergillus* and *Penicillium* cause toxic effects on humans and animals by consumption of mycotoxin-contaminated foods such as cereals, corn, fruits, milk, egg and feeds such as grain (Capriotti et al., 2012; Binder, 2007; García-Moraleja et al., 2015). Milk is one of the most important sources of animal protein in human nutrition. Especially for the children who are in the age of growth should consume milk for adequate and balanced nutrition. Therefore, it is important that milk shouldn't contain harmful toxic components for human health especially for children who are more sensitive for toxins rather than older people (Flores-Flores et al., 2015). Although many studies have demonstrated that aflatoxin M_1 is the most common mycotoxin in milk, some researchers have also detected other mycotoxins such as ochratoxin A (OTA), fumonisin, aflatoxin G_1 (Herzallah, 2009; Gazzotti et al., 2009; Huang et al., 2014).

Ochratoxin A is a mycotoxin that produced by *Aspergillus* and *Penicillium* species. It can be found in spices, raisins, cereal, coffee beans, nuts, cocoa, pulses, beer and wine (Varga et al., 2006). Ochratoxin A has immunotoxic, nephrotoxic, embryotoxic, teratogenic, neurotoxic, hepatotoxic, genotoxic, and carcinogenic effects (Weidenbach et al., 2004; Malir et al., 2013). As OTA is a stable compound, high temperatures (above 250 °C) are required to decrease the toxin levels. It is not destroyed by common food preparation process (Boudra et al., 1995). It was classified by Agency for Research on Cancer as group 2B (possibly carcinogenic to humans) (IARC, 1993). Therefore, OTA's Provisional Tolerable Weekly Intake (PTWI) is 120 ng/kg of body weight (bw) according to the European Commission. Contamination of OTA is receiving increasing attention worldwide, owing to possible harmful effects on human and animal health (Keyvan and Yurdakul, 2015). Animal origin food products contaminated by OTA can create a risk to human health. For this reason, animal origin food products like meat and milk should be analyzed in order to detect OTA contamination (Duarte et al., 2012). The aim of this work was to detect the potential presence of ochratoxin A in bulk tank milks collected from Burdur province of Turkey.

MATERIAL and METHODS

Milk samples

A total of 40 bulk tank milk samples were obtained from Bulk milk tanks in Burdur province, located in the southern side of Turkey from July to October 2017. The milk samples were stored at -20 °C until they were used.

Determination of OTA in the milk

After the samples reached room temperature, 750 µl of methanol was added to 250 µl of the milk sample. The mixture was stirred at room temperature for 5 min. Subsequently, centrifugation was performed and the supernatant was used for analysis. For analysis of OTA, Ochratoxin A Serum/Milk ELISA test kit (Helica Biosystem Inc; 9410CH01M-96) was used and analyzes were performed according to the manufacturer's instructions.

Statistic

The standard curve was prepared according to the manufacturer's instructions.

RESULTS

In the current study, the contamination of OTA in bulk milk tank milk collected from bulk tank milk in Burdur province of Turkey was detected by ELISA. The standard curve was linear with a determination coefficient (R^2) of 0.969 for OTA (Figure 1). Ochratoxin A was found in 40 cow's milk samples (range 2-270 ng/l) (Figure 2).

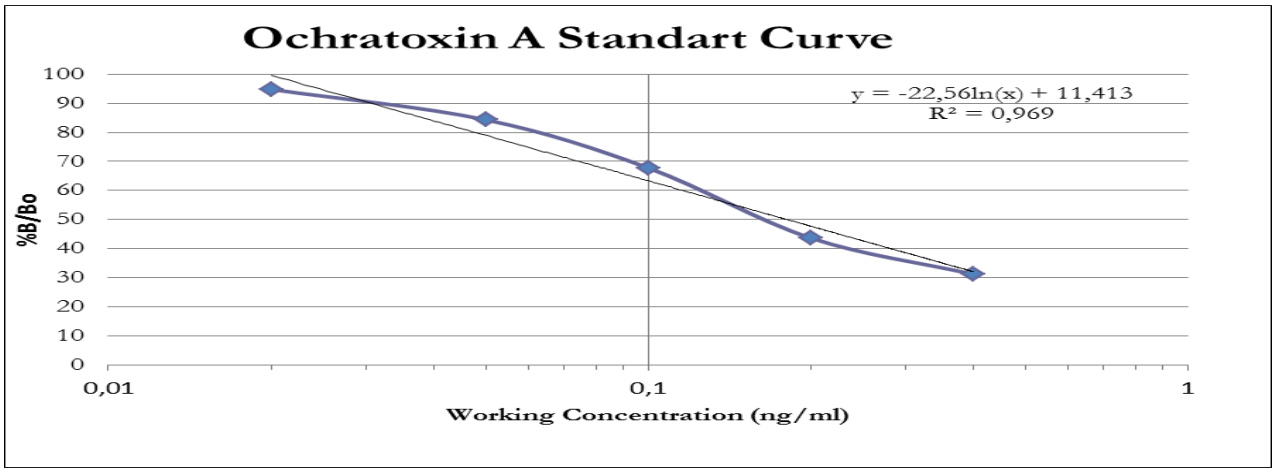

Figure 1. Ochratoxin A standard curve and equation.

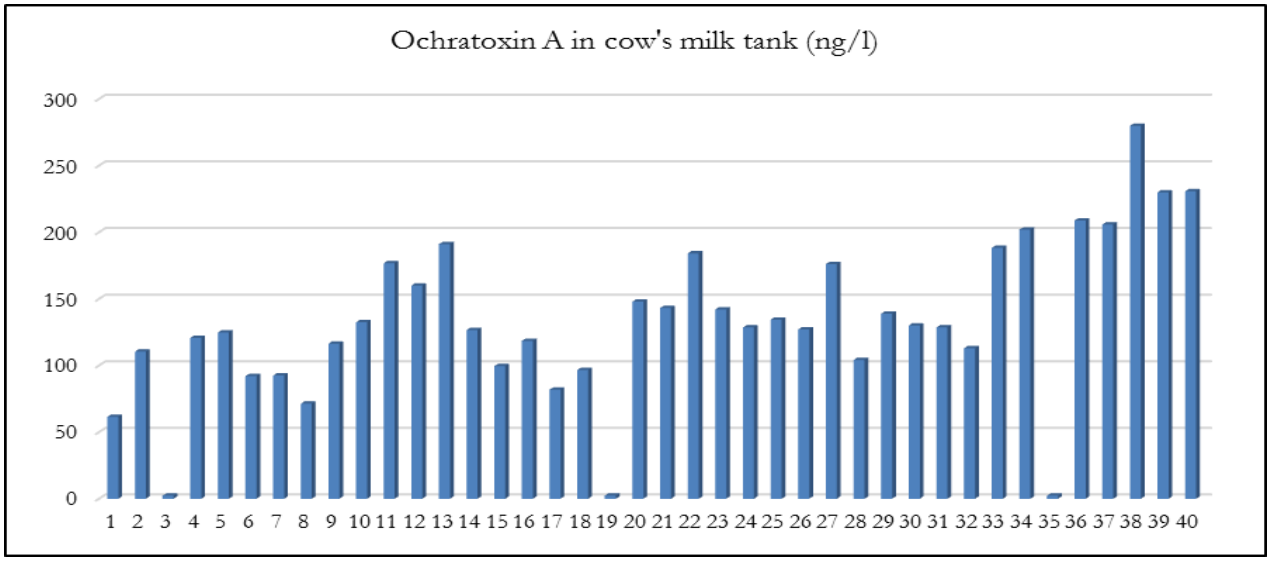

Figure 2. Levels of Ochratoxin A in cow's milk sample collected from bulk milk tanks in Burdur Province.

DISCUSSION

Ochratoxins have been found in wide range of foods and feedstuffs. Ochratoxin A (OTA) is the most important toxin in groups of Ochratoxin which can be also found animal-derived products, such as meat and milk (Alshannaq and Yu, 2017). In a study, OTA was detected in 6 out of 40 conventional cow's milk samples (range 11-58 ng/l), and in 5 out of 47 organic milk samples (range 15-28 ng/l) in Norvey (Skaug, 1999). Ochratoxin A was found in 5 out of 36 cow's milk samples (range 10-40 ng/mL) collected from Sweden (Breitholtz-Emanuelsson et al. 1993). However, no OTA was detected in the samples in samples (n=121) of cow's milk obtained from a northern region of Germany (Valenta and Goll, 1996). In all three studies, the detection limit was set at 10 ng/l. In this study, OTA was detected in 37 out of 40 cow's milk samples (range 10-270 ng/l) collected from milk collection tanks in Burdur province of Turkey. Differences observed in the results of the studies can be attributed to the fact that the weather conditions during growth, harvesting and storage of crop have a great influence on OTA levels (Jørgensen et al., 1996). Presumably, the amount of OTA in the feed, which is dependent on these factors, changes the OTA level in the milk. Differences in climatic and husbandry procedures may explain the changes in OTA contamination between countries and different farms.

According to Regulations (EC) No. 1881/2006 and 105/2010, maximum levels (µg/kg) for OTA in foodstuff have been established to minimize exposure of the public in European Union. Some of European Union (EU) members constrict the limits or set limits in commodities not specified by

the European Union harmonized guidelines in some cases. Slovakia set a limit of 5 µg/kg for milk although there are no regulations in other countries with EU for OTA in milk (Duerta et al. 2010). The OTA levels in this study were well below the limit values of Slovakia which set the OTA limit. However, when it is evaluated in the aspect of tolerable daily intake (TDI) or tolerable weekly intake (TWI), OTA levels found in this study was

higher than the maximum limit of OTA calculated. A TWI of OTA has been reported by the European Food Safety Authority as 120 ng/kg bw in 2006 (EFSA, 2006). The Nordic Working Group (1991) has suggested a TDI of OTA in humans of 5 ng/kg bw. Assuming that a child (4-years-old) is 15 kg and consumes about 400 ml milk every day, no more than 200 ng/l OTA must be present in the milk according to the Nordic Working Group. However, in this study, 6 (15%) out of 40 positive samples showed more than the maximum limit of 200 ng/l OTA. The OTA levels in cow's milk (15%) found in this study are adequate to lead to a higher intake of OTA than the proposed TDI of 5 ng/kg bw in children consuming large amounts of milk.

Risk assessments made do not differentiate between risk group, especially children, and adult groups (Skaugh 1999). Children represent a particularly sensitive population group in which a specific TDI should be assessed, particularly considering the inappropriate dose/body weight ratio. The presence of contaminants in cow's milk is likely to have greater impacts on infants and children than adults who can be fed on a more diverse diet. The results of this study and other studies demonstrate that cow's milk should be considered as a potential OTA source in the human diet. It is proposed to examine the presence of OTA more intensively in dairy products and to determine their maximum limit values by conducting necessary studies.

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Bacterial and Fungal Species Isolated From Dogs With Otitis Externa[#]

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ABSTRACT

This study was conducted to detect the distribution of bacterial and mycotic agents and the antimicrobial susceptibility of bacterial isolates from dogs with infective otitis externa for an 11-year period. Samples, collected from the external ear canal of 475 dogs, were analysed by conventional bacteriological and mycological methods between the years of 2005 and 2016. Antimicrobial susceptibility of the isolates was determined by Kirby-Bauer disc diffusion method. Bacterial growth was observed in 328 of 475 swab samples collected from the dogs. Of 434 isolated bacteria, 281 isolates (64.7%) were Gram-positive cocci, 151 isolates (34.8%) were Gram-negative rods and 2 isolates (0.5%) were Gram-positive rods. The most frequently isolated microorganisms was *Staphylococcus intermedius* (18.7 %), followed by *Pseudomonas aeruginosa* (12.9%), *Escherichia coli* (7.1%) *Proteus mirabilis* (6.7 %) *Micrococcus* spp (4.1%) and *Streptococcus canis (*2.5 %). Mycological growth was also observed from 213 of 475 matching swabs. The results showed that the need for bacterial culture and antimicrobial susceptibility tests for appropriate antimicrobial therapy. Mycological culture should also be performed in infectious otitis externa cases of dogs.

Keywords: Infectious otitis externa, Dog, Culture, Microorganism, Antimicrobial susceptibility

Otitis Eksternalı Köpeklerden İzole Edilen Bakteri ve Maya Türleri

ÖZ

Bu çalışma, 11 yıllık bir süre boyunca infektif otit eksternaları olan köpeklerden bakteriyel ve mikotik ajanların dağılımını ve bakteriyel izolatların antimikrobiyal duyarlılıklarını saptamak amacıyla yapıldı. 2005-2016 yılları arasında, 475 köpeğin dış kulak kanalından toplanan numuneler, geleneksel bakteriyolojik ve mikolojik yöntemlerle incelendi. İzolatların antimikrobiyal duyarlılıkları Kirby-Bauer disk difüzyon yöntemi ile belirlendi. Köpeklerden toplanan 475 sürüntü örneğinin 328'inde bakteriyolojik üreme gözlendi. İzole edilen 434 bakteriden 281'i (% 64.7) Gram pozitif kok, 151'i (% 34.8) Gram negatif çomak ve 2 izolat (%0.5) Gram pozitif basil olarak belirlendi. En sık izole edilen mikroorganizma *Staphylococcus intermedius*'tu (% 18.7), bunu *Pseudomonas aeruginosa* (% 12.9), *Escherichia coli* (% 7.1), *Proteus mirabilis* (% 6.7), *Micrococcus* spp (% 4.1) ve *Streptococcus canis* (% 2.5) izledi. Aynı zamanda, 475 swabın 213'ünde mikolojik üreme de görüldü. Sonuçlar, uygun antimikrobiyal tedavi için bakteri kültürü ve antimikrobiyal duyarlılık testlerine ihtiyaç duyulduğunu göstermektedir. Bunun yanısıra, köpeklerin enfeksiyöz otitis eksterna olgularında mikolojik kültür de yapılmalıdır.

Anahtar Kelimeler: İnfeksiyöz otitis eksterna, Köpek, Kültür, Mikroorganizma, Antimikrobiyal Direnç

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INTRODUCTION

Otitis externa (OE) which is the inflammation of the external auditory meatus, is the most common ear disease of the canine and feline (Guedeja-Marron et al.1998, Rosser 2004). The prevalence of the OE is estimated between 5 and 20% (Rougier et al. 2005).

Otitis has many predisposing factors which can be classified as primary, predisposing and perpetuating. The primary causes such as parasites, foreign bodies, hypersensitivity and allergic diseases, keratinization disorders, autoimmune diseases initiate otitis externa in otherwise normal ears. The predisposing factors such as anatomic and conformational factors, excessive moisture, iatrogenic factors, and obstructive ear disease make the ear more susceptible to the development of OE but do not cause it alone. Bacteria, yeast, otitis media, progressive pathologic changes are considered as perpetuating factors and they are responsible for aggravation of the process and therefore avoid spontaneous resolution (Rosser 2004, Lyskova et al. 2007).

Regardless of the primary ear lesion, acute and suppurate otitis of canine are predominantly caused by the microbial contamination (Guedeja-Marron et al.1998, Bernardo et al. 1998). The microorganisms the most commonly isolated from canine otitis externa are *Staphylococcus intermedius* and *Malassezia pachydermatis* (Kiss et al. 1997).

This study was conducted to detect the distribution of bacterial and mycotic agents and the antimicrobial susceptibility of bacterial isolates from dogs with infective otitis externa for an 11-year period.

MATERIALS and METHODS

Collection of samples

Canine cases clinically suspected of otitis externa and presented at the Department of surgery were included in the study. Diagnosis of the disease was based on historical data, clinical signs or findings on physical examination. At eleven year period, between 2005 and 2016, the samples were obtained from 475 dogs. In each case, two sterile bacteriological swabs were used to collect cerumen from the external ear canal. Swabs were processed within 2 hours.

The animals belonged to both sexes, with ages ranging from 2 months to 19 years old. The dog breeds were Golden Retreiver, Cocker spaniels, Terrier, German shepherd dogs and the other breeds (mix, Rottweiler, Anatolian Shepherd, Pekingese, Bulldog, Siberian Husky, Setter, Chow Chow, Boxer, Pointer, Beagle, Collie, Labrador Retreiver, Akbash, Miniature Pincher, Chihuahua, King Charles, Yorkshire Terrier, Dalmatian, Dogo Argentina, Pug, Kopay, , Saint Bernard, Mastiff).

Microbiological analysis

In each case, one of the swabs was inoculated in Nurient Agar containing 7% sheep blood and Nutrient Broth containing horse serum and incubated microaerobically at 37°C for 24-48 hours (Quinn et al. 2002). Gram staining was performed from the cultures and identification conducted by biochemical identification kits API Staph, API 20 Strep API 20 E, API 20 NE (BioMérieux; Marcy-L'Etoile, France). The other swab set was inoculated onto Sabouraud Dextrose Agar (SDA) and the plates were incubated at 37°C for 1 week. After the incubation, Gram staining was performed from the cultures and standard methods were used for the identification of the yeast (Quinn et al. 2002).

Antibiotic susceptibility test

The *in vitro* susceptibility of isolated strains was investigated by using Kirby-Bauer agar disk diffusion method compliant with the Clinical and Laboratory Standards Institute (CLSI 2006). For this purpose, gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), amoxicillin–clavulanic acid combination – AMC (20 μg), ampicillin (10 μg), penicillin G (10 unit), ampicillin/sulbactam (10 μg), cefoperazone (75 μg), erythromycin (15 μg) and tetracycline (30 μg) were tested.

RESULTS

Isolation and identification findings

In this study, the most commonly represented breeds were: Golden Retreivers (103/475), Cocker spaniels (89/475), Terriers (47/475), German shepherd dogs (32/475) and the other breeds (204/475) (mix (85), Rottweilers (17), Anatolian Shepherds (16), Pekingeses (13), Bulldogs (9), Siberian Huskies (8), Setters (8), Chow Chows (6), Boxers (6), Pointers (5), Beagles (5), Collies (4), Labrador Retreivers (4), Akbashs (3), Miniature Pinchers (3), Chihuahuas (2), King Charles (2), Yorkshire Terriers (2), Dalmatian (1), Dogo Argentina (1), Pug (1), Kopay (1) , Saint Bernard (1) and Mastiff (1)).

Bacterial growth was observed in 328 of 475 swab samples collected from the dogs. In 233 of the cases bacteriological culture revealed single species. In 84 cases, two species were cultured from the

sample. Three or more species isolated from 11 samples. Of 434 isolated bacteria, 281 isolates (64.7%) were Gram-positive cocci, 151 isolates (34.8%) were Gram-negative rods and 2 isolates (0.5%) were Gram-positive rods. The most frequently isolated microorganisms were *Staphylococcus intermedius* (81, 18.7 %) and *Pseudomonas aeruginosa* (56, 12.9%), followed by *Escherichia coli* (31, 7.1%), *Proteus mirabilis* (29, 6.7%), *Micrococcus* spp (18, 4.1%) and *Streptococcus canis* (11, 2.5 %). The dispersions of the isolates are summarized in the table 1.

Mycological growth was also observed from 213 of 475 (45.05%) matching swabs. 149 isolates (70%) were *Malassezia* spp, and 64 isolates (30%) were *Candida* spp. When isolated microorganisms evaluated according to dog breeds, *S. intermedius* was the most frequently bacteria in all breeds (except cocker), whereas in Cocker spaniels, *P. aeruginosa* was the most frequently isolated bacteria. The distribution of the isolates according the dog breeds are summarized in the table 2.

Antibiotic susceptibility test findings

In all strains, the most active susceptibility occurred to ciprofloxacin (72%), enrofloxacin (66.3%), amikacin (66.2%) and cephoperazone (65.3%). All *Pseudomonas aeruginosa* strains were resistant to eritromisin (100%), and most all to penicillin (97.5%), and tetracycline (96.4%). The rates of resistance of the most frequently isolated bacteria are summarized in the table 3.

Table 1. Distribution of the isolates

Isolates		Number of isolates	Percentage of results (%)
Bacteria	***S. intermedius***	81	18.7
	P. aeruginosa	56	12.9
	E. coli	31	7.1
	P. mirabilis	29	6.7
	***Micrococcus* spp.**	18	4.1
	S. canis	11	2,5
	Other Gram negative rods *	35	8.1
	Other Gram positive bacteria**	173	39.9
Total		434	100
Yeasts	***Malassezia* spp.**	149	70
	***Candida* spp.**	64	30
Total		213	100

* Other Gram negative rods : Members of the *Enterobactericeae* family

** Other Gram positive bacteria: Members of the *Staphylococcaceae* family, *Streptococcaceae* family, *Micrococcaceae* family and *Enterococcaceae* family

Table 2. Distribution of the isolates according the dog breeds

	Isolates	Breeds					Total
		Golden Retreiver No (%)	Cocker spaniels No (%)	Terrier No (%)	German shepherd No (%)	Mix breeds No (%)	
Bacteria	***S. intermedius***	11 (13.6)	17 (21)	8 (9.9)	10 (12.3)	35 (43.2)	81
	P. aeruginosa	9 (16.1)	19 (33.9)	5 (8.9)	3 (5.4)	20 (35.7)	56
	E. coli	6 (19.3)	6 (19.3)	2 (6.5)	7 (22.6)	10 (32.3)	31
	P. mirabilis	2 (6.9)	12 (41.4)	3 (10.3)	-	12 (41.4)	29
	***Micrococcus* spp.**	2 (11.1)	6 (33.3)	-	1 (5.6)	9 (50)	18
	S. canis	1 (9)	5 (45.5)	-	-	5 (45.5)	11
	Other Gram negative rods *	6 (17.1)	7 (20)	3 (8.6)	2 (5.7)	17 (48.6)	35
	Other Gram positive bacteria**	31 (17.9)	28 (16.2)	24 (13.9)	12 (6.9)	78 (45.1)	173
Yeasts	***Malassezia* spp.**	41 (27.5)	25 (16.8)	15 (10.1)	8 (5.4)	60 (40.2)	149
	***Candida* spp.**	12 (18.8)	10 (15.6)	5 (7.8)	7 (10.9)	30 (46,9)	64

Table 3. Percentages of *in vitro* resistance to antimicrobial agents

Resistance rate (%)	Antibiotic										
	GN	**AM**	**CIP**	**ENR**	**AMC**	**AMP**	**PEN**	**SAM**	**CPZ**	**E**	**TE**
S. intermedius	38,9	25,7	18,1	30,9	22,2	62,5	73,9	30	24,7	75	71,8
P. aeruginosa	34,9	22,2	9,3	40	96,2	91,3	97,5	93,3	30,2	100	96,4
E. coli	43,3	36,4	58,6	25	85,7	0	53,8	86,9	64	25,9	79,2
P. mirabilis	25	33,3	48,1	33,3	78,6	28,6	92,9	88,2	30,4	20	95,7
***Micrococcus* spp**	69,2	37,5	33,3	31,3	73,3	50	69,2	92,3	41,2	38,9	43,8
S. canis	100	88,9	60	44,4	75	100	100	66,7	60	44,4	100
Total	38,6	33,8	28	33,7	59,7	64,2	78,4	63,6	34,7	53,7	81,3

GN: Gentamicin AM: Amikacin CIP: Ciprofloxacin ENR: Enrofloxacin AMC: Amoxicillin/Clavulanic acid AMP: Ampicillin PEN: Penicillin SAM: Ampicillin/Sulbactam CPZ: Cefoperazon E: Erythromycin TE: Tetracycline

DISCUSSION

Otitis externa may occur in any dog. Although a predisposition has been recognized in Cocker Spaniels, Poodles, Pyrenean shepherds and Labrador retrievers. Saridomichelakis et al. (2007) indicated that this breed predisposition is more important in cocker spaniels, in which a combination of conformational factors including the long, pendulous and hairy ear pinnae and the increased density of compound hair follicules and ceruminous glands in the ear canal may contribute to the higher frequency of OE. In this study, similar to the other studies the most commonly represented breeds were Golden Retreivers, Cocker spaniels, Terriers and German shepherd dogs (Kiss et al. 1997, Bernardo et al. 1998, Cafarchia et al. 2005, Saridomichelakis et al. 2007).

In this study, most frequently isolated microorganism was *S. intermedius* (18.7%). Oliveira et al. (2008) reported that many studies have described the presence of *S. intermedius* as components of the normal microbiota of the canine ear and pointed their association with canine OE. Other researchers have isolated most frequently *S. intermedius* in canine otitis externa (Kiss et al.1997, Morris et al.2006). The results of some researchers are disagreeing with these findings. Sarıerler et al. (2004) have reported that 11.53% *S. aureus* and 5.12% coagulase-negative Staphylococci were isolated and *S. aureus* was the most frequent bacteria for canine otitis externa. *P. aeruginosa* was the next most common, followed by *P. mirabilis* and *E. coli*. Kuyucuoğlu and Sarıtaş (2010) indicated that the most frequently isolated microorganism from dog ears was *S.aureus* (31.5%), followed by *Streptococcus spp* (16.4%) and *Bacillus spp.* (12.3%). Similar to the results, Martin Barrasa et al. (2000) reported the incidence of Gram-negative bacteria isolated in their study corresponds with that reported previously: a high incidence of *Pseudomonas*, followed by *P. mirabilis* and *E. coli*. *S. canis* isolation rate was 2.5 % in dogs. Similar to this results, by Hariharan et al. (2006), *S. canis* rate was reported as 9.9% of otitic ears of dogs. On the contrary, Lyskova et al. (2007) reported were isolated 29.9% *S. canis* in dogs. The geographical location and previous drug use might be cause this argumentative results.

There are many bacteria in healthy ears as well as a small number of *Staphylococcus* genus which are the most common pathogens in otitis externa. Gram negative microorganisms are not routinely identified from the healthy ear canal. for this reason *P. aeruginosa, P. mirabilis, K. pneumoniae* and *E. coli* are important Gram-negative bacteria causing otitis externa (Penna et al. 2010). Also, *P. aeruginosa* is commonly isolated in otitis externa and often shows resistance to multiple antimicrobial agents, including fluoroquinolones (Colombini et al. 2000). In this study, *P.aeruginosa* isolates were resistant to enrofloxacin (40%) as the report but contrary susceptible to ciprofloxacin (90.7%). However, it has been well known fact that previous misusage of fluoroquinolones (ciprofloxacin, enrofloxacin or marbofloxacin) lead to the development of resistant to others (Gebru et al. 2011). The history of the individuals was investigated; excessive or inaccurate use of fluoroquinolones was not verified (unpublished data).

Sarıerler et al. (2004) indicated that the yeasts may be isolated from normal ear canals but if enviromental conditions are suitable, the otitis externa can be created by yeasts. *Malassezia pachydermitis* is the most common yeast isolated from otitis externa case. *Candida sp.* may also be found in canine otitis externa . In this study,

mycological growth was also observed from 213 of 475 (44.8%) matching swabs. 149 isolates (70%) were *Malassezia* spp, and 64 isolates (30%) were *Candida* spp. These results are consistent with the findings of the other studies (Bernardo et al. 1998, Sarıerler et al. 2004, Cafarchia et al. 2005, Lyskova et al. 2007, Saridomichelakis et al. 2007).

Sfaciotte et al. (2015) reported that the major bacterial pathogens were *Staphylococcus* spp. (65.85%), *Pseudomonas* spp. (12.19%) and *Enterobacteria* species (19.51%) in 36 dogs with clinical otitis and they emphasized that the antimicrobial agents against this pathogens considered most resistant were penicillin (75%) and tetracycline (50%). In the current study, in a similar vein, tetracycline and penicillin resistance rates were found relatively high as 81.3% and 78.4% respectively. The lowest resistance rates were found to ciprofloxacin (28 %), enrofloxacin (33.7 %), amikacin (33.8 %) and cephoperazone (34.7 %). All of *P. aeruginosa* strains were resistant to eritromycin and % 97.5 to penicillin. In addition, all of *S.canis* strains were resistant to gentamicin, tetracycline, penicillin and ampicillin.

Aminoglycosides, such as amikacin and gentamicin, have been suggested for topical application in otitis externa caused by Gram-negative bacteria (Hariharan et al. 2006). In this study, amikacin (70%) and gentamicin (65.3%) were sensitive against to Gram-negative bacteria.

The patient material comprised 26 dog breeds, of which the Golden Retreiver (103, 21,7%) and the Cocker spaniels (90, 18,9%) were the most frequently affected. The cocker spaniel is said to be predisposed to the disease by its long, pendulous ears, its liking for water and the frequent entry of grass awns into the ear canal(Kiss 97) while, the Golden Retreiver dog may be predisposed by the hyperactivity of its cerumen producing glands.

CONCLUSION

Treatment of OE is generally challenging for the small animal practitioner due to multi-factorial structure of the disease, probability of long-term antimicrobial therapy usage. Consequently, bacterial culture and susceptibility test are very important factors for treatment success. Mycological culture should also be performed in infectious otitis externa cases of dogs.

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RESEARCH ARTICLE

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Çeşitli Bitki Ekstraktlarının Çiğ köfte Üzerindeki Antimikrobiyal Etkisi

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ÖZ

Çiğ köfte günlük olarak hazırlanıp, birkaç saat içerisinde tüketilmesi gereken, raf ömrü kısa ve mikrobiyal açıdan riskli bir gıdadır. Bu araştırmada; çiğ köfte numunelerine belirli oranlarda değişik baharat ekstrakları ilave edilmesinin, çiğ köftelerin raf ömrü üzerine etkisi etkileri incelenmiştir. Çalışmada kullanılacak çiğ köfte hazırlandıktan sonra, %0,5 ve %1 oranında bitki ekstraktları (yeşil çay, hibiskus, tarhun, ceviz kabuğu, limon kabuğu yağı, portakal kabuğu yağı) ilave edilerek +4°C de 21 gün süreyle depolanmıştır. Depolamanın 0, 7, 14 ve 21. günlerinde, *Lactobacillus* spp., *Lactococcus* spp., maya ve küf, *Pseudomonas* spp., *Escherichia coli,* , *Staphylococcus aureus*, toplam koliform, *Listeria* spp., *Salmonella* spp., toplam aerobik mezofilik ve toplam aerobik psikrofilik mikroorganizma varlığı araştırılmış; ayrıca depolama süresince, baharatların çiğ köftenin rengine etkisi de incelenmiştir. Depolama süresi boyunca numunelerin sırasıyla parlaklık-koyuluk, kırmızı-yeşil ve sarı-mavi renk göstergesi olan; *L*, a** ve *b* değerlerinde artış gözlenmiştir. Yapılan mikrobiyolojik analiz sonuçlarına göre ise numunelerin hepsinde maya-küf sayısı, *Pseudomonas* cinsi bakteri sayısı, laktik asit bakteri sayısı ve *Lactococcus* cinsi bakteri sayılarında azalma görülürken; toplam aerobik mezofilik bakteri sayısında kullanılan ekstrakta göre farklılık gözlemlenmiş, patojen mikroorganizma ve *Staphylococcus aureus* cinsi bakterilerin üreme göstermediği tespit edilmiştir.
Anahtar Kelimeler: Çiğ köfte, antimikrobiyal etki, baharat, ekstrakt, raf ömrü

Antimicrobial Effect of Various Plant Extracts on Raw Meatballs

ABSTRACT

Raw meatballs is a food which should be prepared daily and consumed within a few hours, shelf life is short and microbial in terms of risk. This research was carried out with the aim of determining the effect of various spices and modification of raw meatballs on shelf life. The effects of the spice extracts on the shelf life were investigated by adding the spice extracts to the prepared raw meat ball samples at certain ratios. After preparing the raw meatballs to be used in the study, plant extracts (green tea, hibiscus, tarragon, walnut shell, lemon peel oil, orange peel oil) were added at the rates of 0,5% and 1% and stored at + 4 ° C for 21 days. On days 0, 7, 14 and 21 of storage; total aerobic mesophilic bacteria, yeast and mold, total coliform bacteria, *Lactobacillus* spp., *Lactococcus* spp., *Pseudomonas* spp., *Listeria* spp., *Salmonella* spp. *Escherichia coli and Staphylococcus aureus* counts has been researched. In addition, the effect of the spice on the color of raw meatballs during storage was also examined. During the storage period; the samples were of brightness-darkness, red-green and yellow-blue color indicator, respectively; L *, a * and b * values were increased. According to the microbiological analysis results; yeast-mold count, *Pseudomonas* spp., lactic acid bacteria and *Lactococcus* bacteria counts decreased in all samples. In contrast, the total aerobic mesophilic bacterial counts differed according to the extract used. In addition, *Listeria* spp, *Salmonella* spp, *Escherischia coli* and *Staphylococcus aureus* were found not to reproduce.
Keywords: Raw meatball, antimicrobial effect, spice, extract, self life.

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GİRİŞ

Çiğ Köfte, Türkiye'de hazırlanan önemli geleneksel gıdalardan birisi olup, aslen ülkenin doğu bölgelerine özgü bir üründür. Ancak son zamanlarda batısında da yaygın olarak tüketilmektedir (Gezgin 2005).
Yapımı ve içeriği bölgesel olarak farklılıklar göstermesine karşın genel hammaddesi bulgur, tercihe bağlı olarak yağsız sığır kıyması, soğan, sarımsak, salça, maydanoz, tuz ve çeşitli baharatlardır. Çiğ köftenin yapımında kullanılacak malzemeler yoğrulup el ile şekil verilerek satışa sunulur. Tercihen küçük bir parça marul ve lavaşa sarılır, üzerine limon sıkılarak tüketilir. (Dağlıoğlu ve ark. 2005).

Ürünün üretiminde kullanılan hammaddeleri çoğunluğu kıyma ve bulgurdan gelen mikroorganizmalara ek olarak, üretim aşamasında ilave edilen katkı maddeleri, su, çiğ köfteyi hazırlayan personel ve üretim yapılan tesisin atmosferi dahil her türlü araç- gereç, bir çok riskli mikroorganizmayı içinde barındırmaktadır (Arslan ve ark. 1992, Başoğlu 1982, Çetin ve ark. 1985, Erol ve ark.. 1993, Sağun ve ark. 1997a, Sağun ve ark. 1997b).

Çiğ köftenin tüketim süresi, depolama koşullarına göre 1-2 gün arasındadır. Fakat formülasyonunda bulunan bileşenlerden kaynaklanan yüksek risk nedeniyle hazırlanışının ardından kısa süre içerisinde tüketilmesi tavsiye edilir (Öcal 1997). Ayrıca içerdiği hammaddelerin mikrobiyal yükü ve herhangi bir ısıl işleme tabi tutulmaması nedeniyle de çiğ köfte, sağlık açısından da risk taşıyan bir gıdadır (Gezgin 2005).

Çiğ köftelerin mikrobiyal güvenliği ile ilgili yapılmış çok sayıda çalışma mevcuttur. Pek çok araştırmacı tarafından çiğ köfte üretiminde kullanılan kıyma ve baharatlarda patojen bakteri suşları kontaminasyonu (*Staphylococcus aureus,* koliform bakteri*, Escherichia coli, Enterococci* and *Bacillus cereus*) olduğu rapor edilmiştir (Erol ve ark. 1993, Tekinşen ve ark. 1980).

Konu ile ilgili olarak Tunçel ve Tiryaki (2001) çalışmalarında, piyasadaki çiğ köftelerde patojenik mikroorganizma varlığı araştırılmıştır. Elde edilen sonuçlara göre, numunelerin %14' ünden *Salmonella* izole edilirken aynı zamanda çok sayıda koliform grubu bakteri de tespit edilmiştir.
Çiğ köftedeki bu koliform ve patojen bakterilerin çiğ etten, formülasyonda kullanılan diğer katkı maddelerinden veya personelden kaynaklanmış olabileceği vurgulanmıştır.

Erol et al. (1993) yapmış olduğu çalışmada, çiğ köfteye katılan baharatların antimikrobiyal etkisinin olduğu düşünülmüş fakat bu etkinin *Salmonella* ya da diğer patojen mikroorganizmaları tamamen ortadan kaldırabilecek kadar etkili olmadığı kanısına varılmıştır. Gıdalarda bulunabilecek bu mikroorganizmaları inhibe etmek, gelişimlerini yavaşlatmak ve gıdaların raf ömrünü daha uzun hale getirebilmek için çeşitli kimyasal maddeler kullanılmaktadır. Ancak son dönemde, sentetik antimikrobiyal maddelerin güvenirliğine ilişkin endişeler nedeni ile bitkisel ekstraktlar gibi doğal yolla elde edilen bileşiklerin gıdalarda antimikrobiyal, antioksidan ve diğer çeşitli amaçlarla kullanımında bir artış söz konusudur. (Koca ve Bostancı 2013).

Baharatların antioksidan ve antimikrobiyal özelliklerinin araştırılması 1880'lerde başlayıp 20. yüzyıla kadar süregelmiştir. Yapılan araştırmalar baharat ekstraktları ve çeşitli esansiyel yağları da kapsayacak şekilde günümüzde de devam etmektedir. (Coggins 2001). Günümüzde baharatların esansiyel yağları ya da aktif bileşenleri, gıdalarda mikrobiyal gelişimin ve oksidatif bozulmanın kontrolünde, kimyasallara karşı tercih edilen önemli bileşikler haline gelmiştir (Arın 2009). Günümüzde çeşitli çalışmalara konu olan yeşil çay yapraklarının antimikrobiyal etkilerinin varlığı, yapılan araştırmalar sonucunda ortaya konulmuştur. Yeşil çayın başlıca fenolik bileşiklerinden olan kateşinlerin, kuvvetli antioksidan özellik göstermelerinin yanı sıra, gerek gıdalarda bozulma yapan mikroorganizmalara, gerekse insanlarda hastalık etmeni olan patojen mikroorganizmalara karşı kuvvetli bir antimikrobiyal etki gösterdikleri de belirtilmiştir (Keskin ve ark. 2017). Amarowicz ve Shahidi (1996) tarafından yapılan bir çalışmada, yeşil çay polifenollerinin *Escherichia Coli* K12 suşuna karşı antibakteriyel etkisinin olduğu rapor edilmiştir.

Araştırmacılar tarafından antimikrobiyal etkisi ortaya konulan diğer bir bitki ise *Hibiscus sabdiriffa*' dır. *Malvaceae* familyasına dahil, tek yıllık otsu bir bitki olan hibiskus bitkisi, hayvan yemi ve lif üretimi gibi çeşitli amaçlar ile yetiştirilmesinin yanı sıra yapraklarının çaya benzer şekilde demlenmesi ile içecek olarak da tüketilmektedir. (Plotto ve ark. 2004). Bunun dışında hibiskus bitkisi kimyasal zehirlenmeler ve mantar zehirlenmelerinde antidot olarak da kullanılmaktadır (Şen 2011). Ayrıca hibiskus bitkisinin antimikrobiyal etkisinin olduğu, çeşitli çalışmalar sonucu ortaya konulmuştur. Fullerton ve ark. (2011) tarafından yapılan bir çalışmada, antibiyotiğe karşı dirençli olan *Campylobacter* suşları ile kontamine olmuş et sularında, agar yüzeyine hibiskus ekstraktı

uygulamasıyla suşların gelişiminin etkili bir şekilde engellendiği rapor edilmiştir.

Tarhun (*Artemisia dracunculus L.*) tıbbi ve aromatik amaçlı yaygın olarak kullanılan ve çeşitli çalışmalar ile antimikrobiyal özelliği ile bilinen bitkilerden birisidir (Azırak 2007). *Artemisia dracunculus L.* bitkisinin antimikrobiyal özelliklerinin de incelendiği bir çalışmada, bu bitkinin uçucu yağlarının antimikrobiyal aktiviteye sahip olduğu yapılan analizler sonucunda ortaya konulmuştur. (Tüylü ve ark. 2009).

Esansiyel yağlar da Gram (-) ve Gram (+) bakteriler dahil, çeşitli mikroorganizma üzerinde antibakteriyel etkiye sahiptir (Bayaz 2014). Araştırmalar sonucunda limon ve portakal kabuğu yağlarının da içerisinde bulunduğu çok çeşitli bitkisel uçucu yağların antimikrobiyal etkiye sahip olduğu ortaya konulmuştur (Karanki 2013).

Ceviz yeşil kabuk ve yaprak kısımları da geleneksel tedavi yöntemlerinde halk arasında kanama durdurucu, damar kuvvetlendirici, , antihelmintik, antidiaretik, hipoglisemik, hipotansiv, sedativ buna ek olarak antifungal özellikleri ile bilinmekte ve kullanılmaktadır. Değişik çalışmalarda cevizin özellikle ağaç kabuğu, yaprak, yeşil meyve kabuğu ve juglon maddesinin antimikrobiyal aktivitesi olduğu belirlenmiştir (Yiğit ve ark. 2009).
Bu çalışmada çeşitli mikroorganizmalar üzerinde inhibe edici özellik gösterebileceği ön görülen bitki ekstraktları, çiğ köfteye değişik oranlarda ilave edilmiş ve mikroorganizma türlerinin raf ömrü boyunca gelişimi gözlenmiştir.

MATERYAL ve METOT

Çiğ köfte, Öcal (1997)'ın belirttiği formülasyon modifiye edilerek Tablo.1 de belirtildiği oranlarda karıştırılarak etsiz olarak hazırlanmıştır. Çiğ köftenin yapımında kullanılan hammaddelerin temini Afyonkarahisar İl Merkezi'ndeki bir satış noktasından temin edilmiştir. Bitkilerden ekstrakt eldesi amacıyla Karakoç ve Gökçe (2013) tarafından belirtilen yöntem referans alınmıştır. Bu yönteme göre her bitki materyalinden 50 gr tartılıp 1000 ml' lik erlenmayerlerin içerisine konulmuştur. Sonrasında bitkilerin üzerine 500 ml %80' lik etanol eklenmiştir. Bu karışımın üzeri alüminyum folyo ile ile kapatılarak erlenmayerler, 24 saat boyunca shaker (WiseShake ®SHO-2D) kullanılarak 120 rpm' de karıştırılmıştır. Bu süre sonunda karışımlar süzgeç kağıdından (Whatman ® Grade 40) süzülerek, süzüntü içerisinde bulunan alkol, rotary evaporatör (Heidolph Hei- VAP value) kullanılarak 120 rpm'de 60°C de sıcaklıkla ayrılmıştır (Karakoç ve Gökçe 2013). Hazırlanan çiğ köfte 250 şer gram olmak üzere toplam 13 adet steril numune kaplarına alınmıştır. 1 adet kontrol olmak şartıyla, her bir ekstrakttan %0,5 ve %1 oranlarında numunelere ilave edilmiştir. Kontrol numunesi ve ekstrakt ilave edilen numuneler 21 gün süre ile +4°C' de muhafaza edilmiştir. Muhafaza süresince 0, 7, 14 ve 21. günlerde mikrobiyolojik ve fizikokimyasal analizleri yapılmıştır.

Baharat ekstraktları ilave edilmiş çiğ köfte numunelerinden, steril numune kaplarına 250 gram alınarak, analizi yapılıncaya kadar 4°C de muhafaza edilmiştir. Numunelerden steril numune kaşığı yardımı ile 10 gram alınarak steril numune poşetlerine aktarılmış ve üzerinde 90 ml steril ringer çözeltisi ilave edilmiştir. Numunenin homojen hale gelmesi için stomacher cihazında (BagMixer® 400 P-080921247) iyice karışması sağlanmıştır. Hazırlanmış olan bu 10^{-1}'lik dilüsyondan steril pipet ile 1 ml alınarak tüm numunelerin dilüsyonları hazırlanmıştır.

Ardından, hazırlanmış olan 10^{-1}'lik dilusyondan steril otomatik pipet yardımı ile 1 ml alınarak, içerisine önceden 9 ml steril ringer çözeltisi konulan ağzı kapalı steril deney tüplerine aktarılmış ve 10^{-2} lik diüsyon hazırlanmıştır. İşlemlere aynı şekilde devam edilerek, 10^{-3} ve 10^{-4} 'lük dilusyonlar elde edilmiştir (Anonim 1998, Seçkin ve Karagözlü 2004, Anonim, 2011). Dilüsyonlardan steril pipet ile 1 ml alınıp, belirtilen (Tablo.3, Tablo.4) mikroorganizmalar için, gerekli ise ön zenginleştirme yapılıp, ilgili besiyerlerine yayma plak yöntemi ile ekim yapılarak petri kapları inkübasyona bırakılmıştır. Çiğ köfte numunelerinin renk analizleri, 0, 7, 14 ve 21. günlerinde kolorimetre cihazı ile (Minolta Chroma Meter CR-400, Osaka, Japan) analiz edilmiş, bu analizlerde L*, a*, b* değerlerinin ölçümü yapılmıştır (Metzger ve ark. 2008, Voss 1992).
Sonuçların istatistiksel değerlendirmesi Duncan çoklu karşılaştırma testi, IBM SPSS ver. 23.0 (2015) paket programı kullanılarak yapılmıştır (SPSS 2015).

Tablo 1. Çiğ Köftenin Formülasyonu
Table 1. Formulation of raw meatball

Hammadde	Miktar
Bulgur	1000 g
Kuru soğan	600 g
Yeşil soğan	160 g
İsot	240 g
Salça	200
Tuz	20 g
Karabiber	6 g
İçme suyu	1200 ml

Tablo 2. Ekstraktlar ve oranları
Table 2. Extracts and ratios

Numune	Baharatlar ve seyreltme oranı
K1	Kontrol numunesi
C1	%1'lik yeşil çay etanol ekstraktı
C2	%0,5' lik yeşil çay etanol ekstraktı
D1	%1'lik hibiskus etanol ekstraktı
D2	%0,5'lik hibiskus etanol ekstraktı
G1	%1'lik tarhun etanol ekstraktı
G2	%0,5'lik tarhun etanol ekstraktı
L1	%1'lik ceviz kabuğu etanol ekstraktı
L2	%0,5'lik ceviz kabuğu etanol ekstraktı
M1	%1'lik limon kabuğu yağı etanol ekstraktı
M2	%0,5'lik limon kabuğu yağı etanol ekstraktı
T1	%1'lik portakal kabuğu yağı etanol ekstraktı
T2	%0,5'lik portakal kabuğu yağı etanol ekstraktı

Tablo 3. Analizlerde kullanılan besiyerleri, inkubasyon koşulları ve kullanılan metodlar
Table 3. The Mediums Used in the Analysis, the Incubation Conditions and the Methods Used.

Mikroorganizma	Besiyeri	İnkübasyon Koşulları	Kullanılan Kaynak
Toplam Aerobik Mezofilik Bakteri	Plate Count Agar (Merck 1.05463)	30°C – 48/72 saat- aerobik	ISO 4833 (Anonymous 2003)
Toplam Aerobik Psikrofilik bakteri	Plate Count Agar (Merck 1.05463)	4°C – 5/7 gün-aerobik	FAO
Lactobacillus spp.	MRS (Man Rogasa) Agar (Merck 1.10661)	30°C – 24/48 saat-anaerobik	ISO 15214 (Anonymous 1998)
Lactococcus spp.	M17 Agar (Merck 1.15108)	30°C – 24/48 saat-anaerobik	Corroler et al(Corroler et al 1998)
Küf / Maya	Potato Dextose Agar (Merck 1.10130)	22°C – 4/5 gün –aerobik	Pichhardt 1993
Toplam Koliform Grubu	Violet Red Bile Agar (Merck 1.01406)	30°C – 24/48 saat/ aerobik	ISO 4832 (Anonymous 1991a)
Staphylococcus aureus	Baird Parker Agar (Merck 1.05406)	37°C –24/48 saat/ aerobik	ISO 6888-1(Anonymous 1991b)
Pseudomonas spp.	Pseudomonas Selective Agar Base (PSA) (Merck 1.07620)	37°C – 24/48 saat- aerobik	ISO 13720 (Anonymous 2010)
Esherichia coli	Chromocult TBX Agar (Merck 1.16122)	44 °C'-24/48 saat	ISO-16649-1 (Anonymous 2001a) ISO-16649-2 (Anonymous 2001b) ISO-16649-3 (Anonymous 2015

TAMB: Toplam aerobik mezofilik bakteri. TAPB: Toplam aerobik psikrofilik bakteri
TAMB: Total Aerobic Mesophilic Bacteria, TAPB: Total Aerobic Psychrophilic Bacteria

Tablo 4. Analizlerde kullanılan ön zenginleştirme broth'ları, inkubasyon koşulları ve kullanılan metodlar
Table 4. Pre-Enrichment Broths Used in The Analyzes, Incubation Conditions and Methods Used.

Mikroorganizmalar	Besiyeri	Supplament	İnkübasyon koşulları	Kullanılan kaynak
Listeria spp.	Oxford (Merck 1.07004)		37°C – 24/48 saat- aerobik	ISO 11290-1:2017
	Palcam (Merck 1.11755)	PLSS (Merck 1.12122)		(Anonymous 2017b) ISO 11290-2:2017 (Anonymous 2017c)
Salmonella spp .	BPLS (Merck 1.07232)		37° C -24/48 saat- aerobik	ISO 6579-1:2017
	XLD (Merck 1.05287)	----		(Anonymous 2017a)

PLSS: Palcam Listeria Selective Supplament acc. to van Netten et al. BPLS: Brillant-Green Phenol-red Lacoste Sucrose.
XLD: Xylose Lysine Deoxycholate.

BULGULAR

Toplam Aerobik Mezofilik Bakteri Sayısı (TAMB) Depolama süresince örneklerde Toplam aerobik mezofilik bakteri sayısındaki değişim Tablo 5'de gösterilmiştir.

Maya – küf sayısı
Depolama süresince örneklerde maya – küf sayısındaki değişim Tablo 6'da gösterilmiştir.

***Pseudomonas* sayısı**
Depolama süresince örneklerde *Pseudomonas* sayısı değişimi Tablo 7'de gösterilmiştir.

Laktik asit bakterileri sayısı
Depolama süresince örneklerde laktik asit bakterileri sayısındaki değişimi Tablo 8'de gösterilmiştir.

***Lactococcus* cinsi bakteri sayısı**
Depolama süresince örneklerde *Lactococcus* cinsi bakteri sayısındaki değişimi Tablo 9'da gösterilmiştir.

Diğer mikroorganizmalar
Depolama süresince numunelerin tümünde *Salmonella* spp., *Listeria* spp., *Escherichia coli, Staphylococcus aureus,* toplam koliform grubu bakteri ve toplam aerobik psikrofilik cinsi bakterilerde üreme tespit edilememiştir.

Tablo.5. Toplam aerobik mezofilik bakteri sayısının depolama süresince değişimi (log kob/g)
Table.5. Throughout the storage period number of the Total aerobic mesophilic bacteria (log cfu/g)

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	5,83Ba	5,90Aba	6,37Aba	6,78Aa
C1	5,65Aa	5,00ABab	4,81Bcd	4,64Bc
C2	5,72Aa	5,20ABab	4,89Bc	4,56Bc
D1	5,68Aa	4,92ABb	4,81Bcd	4,65Bc
D2	5,79Aa	5,15ABab	4,76Bcd	4,46Bc
G1	5,77Aa	4,68Bc	4,63Bd	4,60Bc
G2	5,83Aa	5,11ABab	4,85Bcd	4,61Bc
L1	5,80Aa	4,89Bb	4,86Bcd	4,78Bc
L2	5,90Aa	5,04ABab	4,92Bc	4,87Bc
M1	5,72Aa	5,23ABab	4,85Bcd	4,60Cc
M2	5,87Aa	5,34ABab	4,94Bc	4,75Cc
T1	5,78Aa	5,89Aa	5,90Ab	5,91Ab
T2	5,89Aa	5,90Aa	5,92Ab	5,96Ab

A, B, (→) : Aynı satırda büyük farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.
a, b, c, d (↓): Aynı sütunda farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.

Tablo.6. Maya-küf sayısının depolama süresince değişimi (log kob/g)
Table.6. Throughout the storage period number of the yeast / mold (log cfu/g)

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	6,39Aa	6,55Aa	6,83Aa	6,91Aa
C1	6,30Aa	4,26Bb	3,73Cc	2,49Dc
C2	6,37Aa	4,40Bb	3,80Cc	2,95Dc
D1	6,11Aa	5,60Bab	4,60Cb	4,58Cb
D2	6,15Aa	5,60Bab	4,52Cb	4,45Cb
G1	6,32Aa	6,37Aa	3,67Bc	2,18Cc
G2	6,37Aa	4,20Bb	3,77Cc	2,79Dc
L1	6,00Aa	4,45Bb	3,68Cc	2,80Dc
L2	6,15Aa	4,56Bb	3,78Cc	2,87Dc
M1	6,37Aa	5,37Bab	4,45Cb	4,54Cb
M2	6,39Aa	5,78Bab	4,78Cb	4,80Cb
T1	6,11Aa	4,58Bb	3,81Cbc	2,90Dc
T2	6,26Aa	4,89Bb	3,95Cbc	2,99Dc

A, B, C, D (→) : Aynı satırda büyük farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.
a, b, c, (↓): Aynı sütunda farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.

Tablo 7. *Pseudomonas* bakteri sayısının depolama süresi boyunca değişimi (log kob/g)
Table 7. Throughout the storage period number of the *Pseudomonas* spp. (log cfu/g)

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	5,81Aa	5,83Aa	5,86Aa	5,86Aa
C1	5,76Aa	5,11Aa	4,93ABab	4,83Bb
C2	5,80Aa	5,18Aa	4,94ABab	4,88Bab
D1	5,80Aa	4,93ABb	4,87Bb	4,72Bb
D2	5,78Aa	4,93ABb	4,90ABab	4,81Bb
G1	5,80Aa	5,78Aa	4,97Bab	4,94Bab
G2	5,81Aa	5,80Aa	4,98Bab	4,96Bab
L1	5,73Aa	5,00Aa	4,91ABab	4,81Bb
L2	5,76Aa	5,08Aa	4,94ABab	4,86Bb
M1	5,56Aa	4,86Bb	4,79Bb	4,70Bb
M2	5,68Aa	4,93ABb	4,90ABab	4,86Bb
T1	5,72Aa	4,99Ba	4,93Bab	4,90Bab
T2	5,80Aa	5,20Aa	4,95ABab	4,85Bb

A, B, (→) : Aynı satırda büyük farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.
a, b, (↓) : Aynı sütunda farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.

Tablo 8. Laktik asit bakteri sayısının depolama süresi boyunca değişimi (log kob/g)
Table 8. Throughout the storage period number of the Lactic acid bacteria (log cfu/g)

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	5,67Ba	5,78Aba	5,94Aa	6,11Aa
C1	5,61Aa	5,50Aa	4,83Bb	4,66Bb
C2	5,67Aa	5,66Aa	4,87Bb	4,78Bb
D1	5,11Aa	4,63Bb	4,45Bb	4,18Bb
D2	5,51Aa	4,76Bb	4,51Bb	4,39Bb
G1	5,58Aa	5,53Aa	4,93Bb	4,61Bb
G2	5,64Aa	5,60Aa	4,97Bb	4,72Bb
L1	5,66Aa	5,61Aa	4,83Bb	4,30Bb
L2	5,68Aa	5,65Aa	4,91Bb	4,69Bb
M1	5,51Aa	4,83Bb	4,74Bb	4,18Bb
M2	5,58Aa	4,93Bab	4,78Bb	4,51Bb
T1	5,41Aa	4,90Bab	4,83Bb	4,71Bb
T2	5,53Aa	4,93Bab	4,87Bb	4,82Bb

A, B, (→) : Aynı satırda büyük farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.
a, b, (↓): Aynı sütunda farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.

Tablo 9. *Lactococcus* spp. cinsi bakteri sayısı depolama süresi boyunca değişimi (log kob/g)
Table 9. Throughout the storage period number of the *Lactococcus* spp. (log cfu/g)

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	3,83Ba	3,99ABa	4,52Aa	4,96Aa
C1	3,80Aa	3,78Ab	3,75Ab	3,08Bb
C2	3,83Aa	3,82Ab	3,77Ab	3,20Bab
D1	3,74Aa	3,70Ab	3,34ABb	3,04Bb
D2	3,77Aa	3,72Ab	3,41ABb	3,28Bab
G1	3,82Aa	3,74Ab	3,69Ab	3,52Aab
G2	3,80Aa	3,78Ab	3,70Ab	3,57Aab
L1	3,83Aa	3,70Ab	3,62Ab	3,48Aab
L2	3,78Aa	3,78Ab	3,72Ab	3,63Aab
M1	3,77Aa	3,64Ab	3,50Ab	3,41Aab
M2	3,81Aa	3,76Ab	3,63Ab	3,50Aab
T1	3,80Aa	3,71Ab	3,50Ab	3,20Bab
T2	3,81Aa	3,80Ab	3,62Ab	3,34Aab

A, B, (→) : Aynı satırda büyük farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.
a, b, (↓): Aynı sütunda farklı harflerle gösterilen değerler birbirinden $p<0,05$ düzeyinde farklıdır.

Renk Analizleri

L* Değerindeki değişmeler
Çiğ köfte numunelerinde depolama süresince L* değerleri Tablo.10' da gösterilmiştir.

Tablo 10. Depolama süresi boyunca L* değerindeki değişimler
Table 10. Changes in L * value over the storage time

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	37,35	40,49	38,91	40,32
C1	36,94	37,85	38,65	39,74
C2	36,53	38,88	39,91	39,14
D1	36,20	39,95	39,06	39,24
D2	35,41	38,09	38,10	39,77
G1	38,04	39,90	39,78	38,96
G2	37,66	39,50	39,40	39,03
L1	36,94	37,93	39,23	40,60
L2	36,63	37,81	40,12	40,27
M1	33,99	39,35	38,46	41,06
M2	36,49	39,26	39,10	40,05
T1	35,83	38,29	39,16	39,59
T2	37,04	39,82	39,39	40,36

a* Değerindeki değişmeler
Çiğ köfte numunelerinde depolama süresince a* değerleri Tablo.11'de gösterilmiştir.

Tablo 11. Depolama süresi boyunca a* değerindeki değişimler
Table 11. Changes in a* value over the storage time

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	37,35	40,49	38,91	40,32
C1	36,94	37,85	38,65	39,74
C2	36,53	38,88	39,91	39,14
D1	36,20	39,95	39,06	39,24
D2	35,41	38,09	38,10	39,77
G1	38,04	39,90	39,78	38,96
G2	37,66	39,50	39,40	39,03
L1	36,94	37,93	39,23	40,60
L2	36,63	37,81	40,12	40,27
M1	33,99	39,35	38,46	41,06
M2	36,49	39,26	39,10	40,05
T1	35,83	38,29	39,16	39,59
T2	37,04	39,82	39,39	40,36

b Değerindeki değişmeler
Çiğ köfte numunelerinde depolama süresince b* değerleri Tablo.12' de gösterilmiştir.

Tablo 12. Depolama Süresi Boyunca b* değerindeki değişimler
Table 12. Changes in b* value over the storage time

Numune	0.Gün	7.Gün	14.Gün	21.Gün
K1	22,40	27,95	25,25	27,08
C1	24,44	24,14	24,79	27,94
C2	21,77	25,91	25,54	25,94
D1	19,10	27,17	25,36	27,13
D2	20,72	23,80	23,35	27,29
G1	22,13	26,97	24,67	26,31
G2	20,97	23,90	25,68	27,49
L1	21,21	22,78	24,92	26,80
L2	21,22	23,53	26,29	32,14
M1	23,13	25,25	25,15	28,88
M2	20,01	24,41	24,61	28,07
T1	20,33	24,23	25,50	28,73
T2	19,56	25,02	23,68	29,48

TARTIŞMA

Yapılan analizler sonucunda %1 oranında yeşil çay etanol ekstraktının, *Lactococcus spp.* cinsi bakteriler üzerinde en etkili ekstrakt olduğu; %0,5 oranında ilavesinin ise, çalışmada kullanılan diğer ekstraktlara göre, toplam aerobik mezofilik bakteri sayısında en etkili bitki ekstraktı olduğu tespit edilmiştir.

Çalışmamızda elde ettiğimiz sonuçlara benzer şekilde, Velioğlu (2007), yeşil çayın antimikrobiyal etkisini araştırdığı bir çalışmasında, antimikrobiyal etkinin kullanılan ekstrakt konsantrasyonu ile ilgili olduğunu vurgulamış ve fermente olmamış çayların antimikrobiyal aktivitesinin; fermente olan çaylara göre daha yüksek olduğunu belirtmiştir. Ayrıca bu antimikrobiyal etkinin, çay yapraklarında bulunan kateşinlerden kaynaklandığını ortaya koymuştur.
Amarowicz ve ark. (1996) tarafından yapılan başka bir çalışmada ise yeşil çay polifenollerinin *Escherichia Coli* K12 suşuna karşı antibakteriyel etkisinin olduğu raporlanmıştır.

Kullanılan diğer bir ekstrakt olan hibiskus etanol ekstraktının %1 oranında ilavesinin *Pseudomonas, Lactobacillus* spp. ve *Lactococcus* spp. cinsi bakteri sayısında; %0,5 oranında ilavesinin ise toplam aerobik mezofilik bakteri sayısında azalmaya neden olduğu saptanmıştır.

Çömlekçioğlu ve ark. (2014) bazı bitki ekstraktlarının antimikrobiyal aktivitelerinin belirlenmesi üzerine yapmış oldukları çalışmanın sonuçları, analizlerimizi doğrular nitelikte olup, hibiskus bitkisinden elde olunan ekstraktların antimikrobiyal yönünün kuvvetli etki gösterdiği bulgularını ortaya koymuştur. Depolama süresi boyunca çiğ köfte numunelerinin maya/ küf sayısının azalmasında en etkili ekstrakt %1 oranında ilave edilen tarhun ekstraktı olmuştur.

Tarhun bitkisinin antimikrobiyal özelliklerinin de incelendiği bir çalışmada, bu bitkinin uçucu yağlarının antimikrobiyal aktiviteye sahip olduğu tespit edilmiştir. (Tüylü ve ark. 2009).

Çiğ köfte numunelerinin, depolama süresi boyunca tümünde *Pseudomonas* cinsi bakteri sayılarında bir azalma görülmüş olmasına karşın; %1 oranında ilave edilen limon kabuğu yağı, kullanılan diğer ekstraktlara göre bu bakteri cinsinde daha yüksek antimikrobiyal etki göstermiştir. Limon kabuğu yağının etki gösterdiği diğer bir mikroorganizma ise *Lactobacillus lactis* olarak tespit edilmiştir.

Çoksever (2009) tarafından yapılan bir araştırmada limon ekstraktının antimikrobiyal etkileri incelenmiş ve sadece *Lactobacillus lactis* üzerine etki gösterdiği tespit edilmiştir. Bu sonuç, çalışmamızın doğruluğunu kanıtlar nitelik taşımaktadır. Konu ile ilgili yapılan diğer bir çalışmada ise limon meyvesinin yağ asidi ekstraktlarının, *K.pneumoniae* ve *Staphylococcus aureus* dışında, tüm bakteri (*Escherichia Coli)*, maya (*Candida albicans*) ve fungusların (*Epidermophyton* spp.) gelişimlerini değişen

oranlarda önlediği ortaya konmuştur. (Erecevit ve Kırbağ 2017).

Çalışmada kullanılan portakal kabuğu yağı etanol ekstraktının ise her iki oranda (%1 ve %0,5) kullanımı, toplam aerobik mezofilik bakteri sayısının düşürülmesinde etkili olmamıştır. Kullanılan diğer bir ekstrakt olan, ceviz kabuğu etanol ekstraktı, tüm mikroorganizma grupları üzerinde belirli oranlarda antimikrobiyal etki göstermiştir.

Yiğit ve ark. (2009) yapmış oldukları çalışmada ceviz (*Juglans regia L.*) bitkisinin çeşitli aksamlarının su ve metanol ekstraktlarının *Staphylococcus aureus, Staphylococcus epidermidis* ve *Pseudomonas aeruginosa* üzerinde antimikrobiyal aktiviteye sahip olduğunu belirlemişlerdir.

Oliveira ve ark (2008) tarafından yapılan bir araştırmada yaprak aksamının su ile ekstraksiyonunun *Candida albicans* ve *Cryptococcus neoformans* üzerinde antifungal etki gösterdiği tespit edilmiştir.

Mehrabian ve ark. (2000) ise, Ceviz (*Juglans regia L.*) yeşil kabuk ve yaprak kısımlarından elde ettikleri ekstrelerinin antimikrobiyal özelliğini inceledikleri çalışmada aksamların metanol ekstrelerinin, etanol ekstrelerine göre daha etkili antimikrobiyal özelliğe sahip olduğunu belirtmişlerdir.

Çoksever (2009) tarafından yapılan bir çalışmada portakal kabuğundaki fenolik maddeler, metanol ile ekstrakte edilerek kullanıldığında antimikrobiyal aktivitenin yanı sıra, portakal kabuğu ekstraktının, yüksek antioksidan etkiye sahip olduğu ortaya konmuştur. Başka bir çalışmada ise portakal ekstraktının *Lactococcus lactis* ile *Listeria innocua* üzerine etkili olduğu tespit edilmiştir. (Frazier 1980).

Çalışmamızda elde edilen bulguların Çoksever (2009) ve Frazier (1980)' in sonuçlarından farklı olmasının nedeni, ekstraksiyon sırasında kullanılan çözücü maddeden ve kullanılan konsantrasyon değerlerinden kaynaklanabileceği düşünülmektedir.
Analiz süresi boyunca her 0, 7, 14 ve 21. günlerde çiğ köfte numunelerinin renk analizleri yapılmış, bu analizler sonucunda kontrolün L^*, a^* ve b değerinin zamana bağlı olarak artış gösterdiği gözlemlenmiştir. L^* değeri; ürünün parlaklık ve koyuluğunu ifade etmektedir. (Voss 1992, Anonim 2012). Başlangıçta en yüksek L^* değeri 38,04 (P>0,05) ile G1 numunesine ait olup, 21 gün sonunda en düşük L^* değeri; 39,59 (P>0,05) ile T1 numunesinde görülmüştür. Baharat ekstraktı ilavesinin; G1 ve G2 numuneleri hariç, diğer tüm çiğ köfte numunelerinde L^* değerinin önce düşmesine ve daha sonra artmasına neden olmuştur. Depolama süresi sonunda en fazla değişim gösteren ve en yüksek L^* değerine sahip numune, 41,06 (P>0,05) değeri ile %1 oranında limon kabuğu yağı ekstraktı içeren M1 numunesi olmuştur. Kırmızı etin depolanması ve muhafaza ömrü ile ilgili yapılan bir araştırmada limon ekstraktı kullanılmış ve L^* değerinin düştüğü tespit edilmiştir. Bu durum limondaki su tutan bileşenin suyu absorbe etmesi ve buna bağlı olarak L^* değerinin düşmesi ile açıklanmıştır. (Çoksever 2009).

Çoksever (2009)' in limon kabuğu ekstraktı ile yapmış olduğu çalışma sonucu, çalışmamız ile kıyaslandığında farklı sonuçlar elde edilmesinin nedeni olarak ürün çeşidi ve kullanılan konsantrasyon miktarından kaynaklı olduğu düşünülmektedir.

Çalışmada kullanılan numunelere bitki ekstraktları ilavesinin öncesinde ve hemen sonrasında renk analizi yapılmış, ilk sonuçlara kıyasla D2, M1 ve C1 numuneleri dışında diğer tüm ekstraktların L^* değerinde önce bir düşüş yaşanmış, ardından zamana bağlı olarak artış gözlemlenmiştir. Diğer bir renk parametresi olan a^* değeri; kırmızı ve yeşil renkleri ifade etmektedir. (Voss 1992; Anonim 2012).

Ekstrakt ilavesinin hemen ardından ölçüm yapılmış, a^* değerinin M1, D2 ve C1 numunelerinde, kontrol numunesine kıyasla bir artış gözlenmiştir. Diğer numunelerde ise başlangıçta en düşük a^* değeri 19,42 (P>0,05) ile M1; depolama süresi sonunda en yüksek a^* değerine sahip ise 21,84 (P>0,05) değeri ile D2 numunesi olmuştur. Analiz süresi boyunca, numunelerin a* değerinde genel olarak bir artış gözlemlenmiştir.

Çoksever (2009), antioksidan aktivitesi düşük olan turunçgil ekstraktlarının bulunduğu örneklerin en düşük a^* değerine sahip olacağı kanısına karşın Higgins ve ark. (1998) yapmış olduğu analizlerde; turunçgillerde mevcut olan karotenoidlerin oksidasyonu azalttığı ve a^* değerini yükselttiğini ortaya koymuştur. Bu durumun çalışmamız ile olan farklılığının kullanılan hammaddeden kaynaklanabileceği öngörülmektedir. Sarı ve mavi renkteki değişimler ifade eden b* değerinin depolama süresi boyunca tüm çiğ köfte numunelerinde arttığı tespit edilmiştir. Başlangıçta en düşük b değeri 19,10 (P>0,05) ile D1 numunesi iken; analiz süresi sonunda en yüksek b değerine sahip numune 32,14 (P< / >0,05) değeri ile L2 numunesi olmuştur.

SONUÇ

Son yıllarda tüketimi sıkça artan geleneksel gıdalarımızdan biri olan çiğ köfte, raf ömrü oldukça kısa ve mikrobiyal riski yüksek olan bir gıda maddesidir. Araştırmacılar tarafından, gıdaların raf ömrü üzerine yapılan birçok çalışmada, antimikrobiyal etkisi bilinen çeşitli bitkiler veya bu bitkilerin ekstraktları kullanılmış ve oldukça önemli sonuçlar elde edilmiştir. Bu çalışmada bazı bitki ekstraktlarının, çiğ köfte üzerindeki antimikrobiyal etkisi araştırılmış, çalışma esnasında farklı oranlarda bitki ekstraktları kullanılmıştır. Bu çalışmadan elde edilen sonuçlar, çiğ köfte üretimi sırasında, hijyen ve sanitasyon koşullarına dikkat edildiği taktirde, kullanılan bitki ekstraktlarının mikroorganizmalar üzerinde belirli oranlarda antimikrobiyal etki gösterdiğini ortaya koymuştur. Çiğ köfte üretiminde bu bitki ekstraktlarının kullanımı ile birlikte, ürünün raf ömründe artışla beraber mikrobiyal risk azalacaktır.

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Yeni Doğan Buzağılarda Karşılaşılan Femur Kırığı Olgularının Lokalizasyonu, Şekli ve Sağaltım Seçeneklerinin Değerlendirilmesi

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ÖZ

Bu çalışmada 2017 yılında Kafkas Üniversitesi Veteriner Fakültesi Hayvan Sağlığı Eğitim, Araştırma ve Uygulama Hastanesine femur kırığı şikâyeti ile getirilen buzağılarda kırık oluşum nedeni, kırığın lokalizasyonu ve sağaltım yöntemleri ile sonuçlarının değerlendirilmesi amaçlandı. Hayvan materyalini farklı cins ve ırklara ait 13 adet yeni doğan buzağı oluşturdu. Kırık etiyolojileri belirlendikten sonra kırık lokalizasyonu ve tipi saptanarak yapılacak sağaltım yöntemi planlandı. Operasyonlar subaraknoid anestezi altında gerçekleştirildi. Kırık fiksasyonu için 9 olguda retrograd pin, 1 olguda plaka uygulaması gerçekleştirildi. Yeni doğan buzağılarda kırığın en çok (%53.84) güç doğuma bağlı bilinçsiz yaklaşım veya kriko kullanımına bağlı geliştiği tespit edildi. Suprakondüler ve orta diafizer oblik kırıkların eşit oranda ve diğer kırık tiplerine göre daha yaygın (%30.76) görüldüğü belirlendi. Buzağıların postoperatif dönemde kısa süre (2.3 gün) içerisinde ilgili ekstremitelerini kullandıkları görüldü. Yeni doğan buzağılarda karşılaşılan femur kırıklarının tedavisinde intramedullar pinleme ile %88.88 oranında başarı elde edilebileceği tespit edildi.

Anahtar Kelimeler: Buzağı, Femur kırıkları, Sağaltım

Assessment of Localization, Shape and Treatment Options of Femur Fracture Cases in Newborn Calves

ABSTRACT

In this study, it was aimed to evaluate the results of fracture formation, fracture localization and treatment methods and results of the fractures brought to the Hospital of Kafkas University Faculty of Veterinary Medicine Research and Practice Hospital between 2017 by complaint of femur fracture. The animal material consisted of 13 newborn calves belonging to different sex and breeds. After the etiology of the fractures, the localization of the fracture and type was determined, treatment were planned. Operations were performed under subarachnoid anesthesia. For fracture fixation, ıt was conducted in 9 cases retrograde pin and 1 case of plate application. It was found that the most developed (53.84%) of the fractures in newborn calves were due to forced extraction during dystocia. Supracondylar and mid diafizer oblique fractures were found equally and more common (30.76%) in comparison with other types of fractures. It was observed that the calves used the relevant limbs within a short period of time (2.3 days) in the postoperative period. In the treatment of femur fractures encountered in newborn calves, intramedullary pinning was found to be a success rate of 88.88%.

Key Words: Calf, femur fractures, treatment.

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GİRİŞ

Buzağılar yeni doğan dönemlerinde enfeksiyöz ve non efeksiyöz olarak birçok hastalığa maruz kalırlar. Travma kemiklerde kırık oluşumunda rol oynayan en önemli non enfeksiyöz faktörlerden biridir (Akin 2014, Nichols ve ark. 2010). Yeni doğan buzağılarda güç doğuma bağlı olarak yapılan hatalı girişimler (Aksoy ve ark. 2009, Arıcan ve ark. 2013, Ferguson ve ark. 1990, Ferguson 1994, Nichols ve ark. 2010) ile doğum sonrasında diğer hayvanlar tarafından tekmelenme, annenin yavrunun ekstremitelerine basması, kayma ve düşme (Akin 2014, Arıcan ve ark. 2013, Bilgili ve ark. 1999, Durmuş ve ark. 2009, Yanmaz ve ark. 2014), transport ve trafik kazaları (Akin 2014, Yanmaz ve ark. 2014) gibi nedenler kırık oluşumuna yol açmaktadır. Buzağılarda kırıklar en çok metakarpusta bunu izleyerek de femurda şekillenmektedir (Akin 2014, Durmuş ve ark. 2009, Ferguson ve ark. 1990, Hoederman ve ark. 2012).
Yeni doğan buzağılarda karşılaşılan femur kırıkları çoğunlukla proksimal epifiz ve distal metafizde şekillenmekte olup genellikle transversal ve oblik kırıklar biçimindedir. Fragmentlerin tamamen yer değiştirmesi, periostun tamamen sıyrılması ve komşu dokulardaki yaralanmalar tipik olarak görülen bozukluklardır (Hoederman ve ark. 2012).
Cerrahi tekniklerin gelişmesi, veteriner cerrahi alanında sığırlara yapılan ortopedik girişimleri daha tercih edilebilir hale getirmiştir (Ferguson ve ark. 1990) ancak kırık tedavisi yapılmadan önce hayvanın genetik potansiyelinin, ekonomik değerinin ve kırık tipi ile lokalizasyonunun değerlendirilmesi gereklidir (Görgül ve ark. 2004). Kırk iyileşmesi, kırık hattındaki hareketin derecesi, bakteriyal kontaminasyonun varlığı, yumuşak doku yaralanmalarının şiddeti, kırık tipi ve lokalizasyonu, genel sağlık durumu ile hastanın yaşı gibi faktörler tarafından etkilenmektedir (Aithal ve ark. 2004, Gangl ve ark. 2006).

Femurda konservatif tedavi ile başarılı sonuçlar alınma olasılığı oldukça düşüktür. Thomas splint ya da intra-medullar pin uygulamaları sağaltım amacıyla kullanıldığında distal metafizdeki kırıklara göre diafizer kırıklarda olumlu sonuçlar alma şansı daha yüksektir. Distal metafizyal kırıkların fiksasyonu için kullanılan steinman pinler migrasyon ve instabilite ile sonuçlanmaktadır. Plaka osteosentezi ile yapılan sağaltım kırık lokasyonu tarafından etkilenmez. Yeni doğan buzağılarda femoral kemiğin yumuşak olması plaka osteosentezindeki en büyük olumsuzluklardandır. Çünkü yumuşak neonatal kemik, vida gevşemesine predispozisyon yaratabilir ve sonrasında fiksasyonda instabilite ile karşılaşılabilir (Hoederman ve ark. 2012). Yeni doğan buzağıların femur kırıklarında sağaltım amacıyla kullanılan intermedullar interloking pinleme tekniğinin prognozu kırık lokasyonu ne olursa olsun iyi olduğu bildirilmiştir (Bellon ve Mulon 2011, Hoederman ve ark. 2012, Junior ve ark. 2010).
Bu çalışmanın amacı yeni doğan buzağılarda karşılaşılan femur kırıklarının etiyolojileri ile lokalizasyonlarının belirlenmesi ve sağaltım sonuçlarının aktarılmasıdır.

MATERYAL ve METOT

Çalışma materyalini 2017 yılında Kafkas Üniversitesi Veteriner Fakültesi Hayvan Hastanesi Cerrahi Kliniği'ne arka ekstremite topallığı şikâyeti ile getirilen ve femur kırığı tespit edilen farklı ırk, yaş ve cinsiyetten 18 adet buzağıdan kaput ve kollum femoris kırığı olan 5 adet buzağı hariç 13 adet buzağı oluşturdu. 13 buzağıdan 10 tanesine tedavi uygulanırken, 3 tanesine hayvan sahiplerinin tedaviyi kabul etmemesinden dolayı sağaltım uygulanmadı. Kırık lokalizasyonları ve tiplerinin de araştırıldığı bu çalışmada tedavi edilmeyen bu 3 olguya da yer verildi.

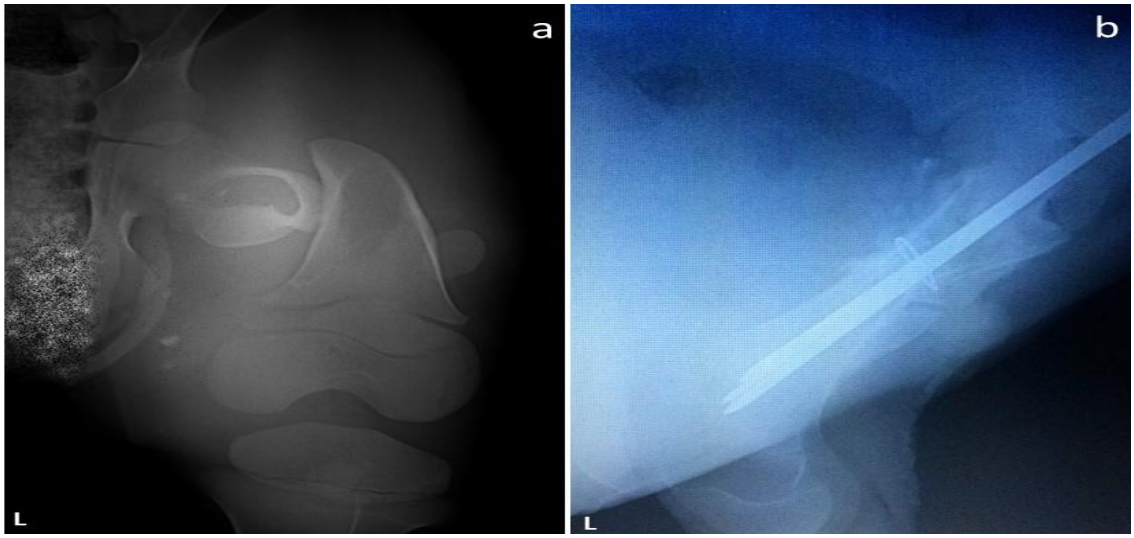
Hasta sahiplerinden alınan anamnez bilgileriyle kırık etiyolojileri belirlendi. Elde edilen verilerin dağılımı yüzde olarak ifade edildi. Klinik ve radyografik muayenelerden sonra kırık lokalizasyonu ve tipi saptanarak yapılacak sağaltım yöntemi planlandı. Hastalarda preoperatif hazırlıklar tamamlandıktan sonra ilgili bölgenin tıraş ve dezenfeksiyonu yapıldı. Anestezi yöntemi olarak subaraknoid anestezi tercih edildi. Operasyon masasına 30 derecelik eğim verildi. Hastanın kranial kısmı yüksekte ve kırık ekstremite yukarıda kalacak biçimde lateral pozisyonda yatırılan hasta operasyon masasına sabitlendi. Lumbosakral bölge enjeksiyon amacıyla hazırlandıktan sonra deri ve deri altı dokuların infiltrasyon yöntemi ile lokal anestezisi sağlandı ve 25 gouge (Egemen® anestezi iğnesi) spinal iğne ile lumbosakral aralığa girildi. BOS sıvısı gelişi ile subaraknoid bölgeye ulaşıldığı doğrulandıktan sonra 0.02 mg/kg dozunda ropivacaine HCl (Naropin® 10 mL amp., AstraZeneca, Germany) 0.5 mL/dak hızında enjekte edildi. Ekstremitelere yapılan derin ağrı duyusu testi ve pink prik uygulamalarıyla ağrı duyusunun ortadan kalktığı belirlendi ve anestezik ajan uygulaması sonlandırıldı. Kırık hattına kraniyolateral olarak yaklaşıldı. Kırık bacağın lateral yüzeyi proksimalde trochanter majörden distalde femurun lateral epikondilüsüne kadar ensize edildi. Muskulus tensor fasiya lata künt diseksiyon ile ayrıldı. Femura ulaşmak için muskulus vastus lateralis ile biseps femoris kasları sınır boyunca ayırt edildi. Biseps femoris kası kaudal, vastus lateralis kası ise kraniyal yönde ekarte edildi. Kırık bölgesine ulaşıldıktan sonra kırık hematomu aspire edildi ve kırık bölgesi 0.9% NaCl ile yıkandı. Kırık fragmentlerinin uçları kemik tutma pensleri yardımıyla kırık bölgesinden dışarı alınarak 7 olguda

retrograd pin, 2 olguda ise çapraz pin uygulaması gerçekleştirildi. Bu olgularda 4 mm'lik ve 2 mm'lik pinler kombine olarak kullanıldı. Bir olguda ise geçici stabilizasyon kemik tutma pensleri ile sağlandıktan sonra 4 mm kalınlığında plaka ve 3 adet 3 mm' lik ile 3 adet de 4.5 mm' lik vida kullanılarak kalıcı fiksasyon sağlandı. Operasyon bölgesi rutin olarak kapatıldı. Operasyonlardan sonra kontrol grafileri alındı. Hastalar aynı gün taburcu edildi ve postoperatif profilaksi amacıyla sefazolin sodyum (1 g/12 saat, IM 5 gün) ve analjezi amacıyla meloxicam (2mg/kg, IM, 3 gün) önerildi. İlk hafta her gün sonrasında 15' er gün arayla olmak üzere hasta sahipleri telefonla aranarak buzağıların durumu öğrenildi. Klinik ve radyografik kontroller için hasta sahiplerinin tamamı 15' er günlük periyotlarda hastaneye çağrılmasına rağmen yalnızca 4 tanesi kontrol için hastasını getirdi. Diğer olguların durumları telefonla bilgi alınarak öğrenildi. Osteosentez sonrası buzağılara bandaj uygulanmadı. Hayvan sahiplerine buzağıları dar bir alanda bakması ve hareket kısıtlaması önerildi. Buzağıların ilgili ekstremite üzerine yük bindirdikleri gün ekstremiteyi kullandıkları gün olarak kabul edildi.

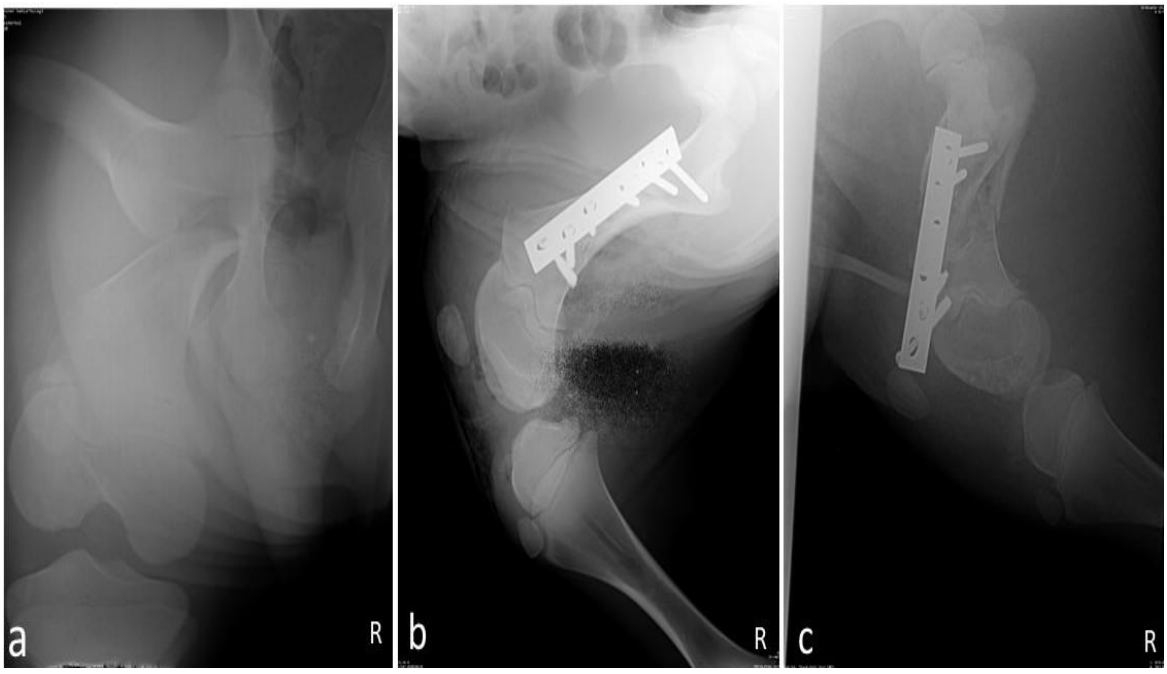
BULGULAR

Olguların ırk, yaş cinsiyet, kırık lokalizasyonu, sağaltım seçenekleri ve sonuçları Tablo 1'de veriliştir. Hayvanların ırklara göre dağılımı Simental 7, Esmer 3, Esmer-Simental melezi 2 ve Esmer-Doğu Anadolu Kırmızısı melezi 1 olarak belirlenirken, olguların 9'u dişi 4'ü erkekti. Hastaların ortalama yaşları 26.69 (min: 1, max: 180) gün olarak saptandı. Olgularda karşılaşılan kırık sebepleri 7 tanesinde güç doğum (%53.84), 3 tanesinde düşme (%23,87), 1 tanesinde mazgal arasına sıkışma (%7,69), 1 tanesinde tekmelenme (%7,69) ve birinde de (%7,69) annenin yavrunun ekstremitesine basması olarak belirlendi. Kırık lokalizasyonu ve şekli incelendiğinde ise olguların 4' ünde suprakondüler kırık (%30,76), 4' ünde orta diafizer oblik kırık (%30,76), 2'sinde orta parçalı diafizer kırık (%15,38), 2' sinde distal diafizer oblik kırık(15,38), 1' inde ise orta diafizer transversal kırık (%7,69) saptandı (Tablo 1).

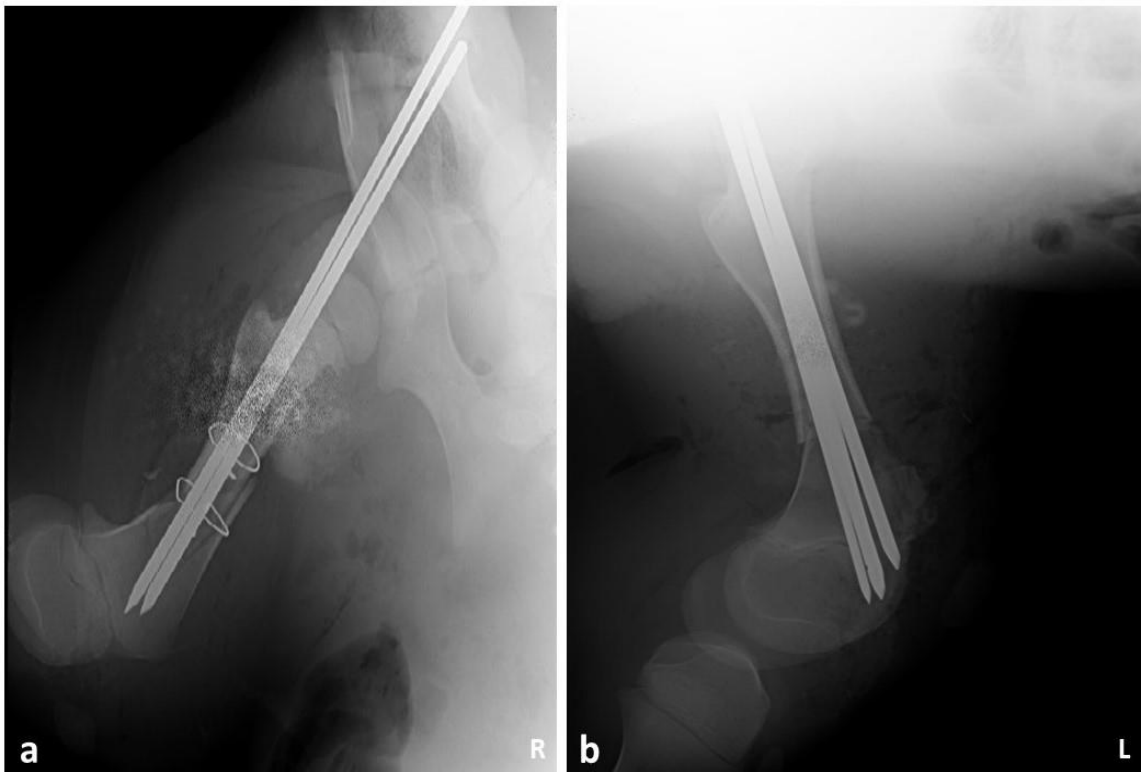
Olgulardan 3'üne hasta sahipleri istemediği için operasyon yapılmadı, kalan 10 olgudan 7'sinde intra medullar pinleme (Resim 1), 2'sinde çapraz pinleme yapılırken, 1 olguda plak uygulaması gerçekleştirildi. Operasyon sonrası hastaların ekstremitelerini kullandıkları ortalama gün 2.3 (min: 1 gün, max: 4) gün olarak belirlendi. Post-operatif dönemde 1 hasta sahibine ulaşılamazken, diğer hasta sahiplerinden telefonla bilgi alındı. Bazı hasta sahipleri ise hastalarını getirerek pinleri çıkartıldı. Olguların post operatif en kısa 2 ay süre ve en uzun 11 aylık sürede kontrolleri yapıldı. Bu süreler zarfında 1, 3, 4, 7, 9, 10, 11, 12 numaralı olgular tamamen iyileşirken, 2 numaralı olgunun 15. günde yapılan muayenesinde saptanan enfeksiyon nedeniyle ötenazi kararı verildi. Hastalardan 13 nolu olguda post operatif 15. günde vidaların gevşediği ve distal kırığın iyileşmediği saptandı (Resim 2). Bu olguda hasta sahibi yeniden operasyonu kabul etmedi ve hasta kendi haline bırakıldı. Post-operatif 35. günde tekrar kontrolü yapılan hastada kaynamanın anormal olduğu ve topallığın devam ettiği belirlenerek plaka çıkartıldı. Plaka osteosentezi yapılan olgu, intramedullar pinleme tekniği ile karşılaştırma amacından ziyade hem kırık lokalizasyonunun hem de kırık etiyolojisinin belirlenmesinde veri oluşturması sebebiyle çalışmaya dahil edildi. Hasta sahipleri 5, 6 ve 8 nolu olgularda sağaltımı kabul etmedi.



Resim 1. 7 Numaralı olguya ait radyografik görüntüler. (a. Operasyon öncesi, b. Operasyon sonrası 15. gün)
Figure 1. Radigrahic view of case 7 (a. Preoperatively, b. Postoperative 15. day)



Resim 2. 13 Numaralı olguya ait radyografik görüntüler. (a. Operasyon öncesi, b. Operasyon sonrası c. 35. gün)
Figure 2. Radigraphic view of case 13. (a. Preoperatively, b. Postoperatively, c. Postoperative 35. day)



Resim 3. 4 ve 10 numaralı olgulara ait post-operatif kontrol grafileri. (a. 4 numaralı olgu, b. 10 numaralı olgu)
Figure 3. Post-operative control radiograms for cases 4 and 10. (a. Case number 4, b. Case number 10)

Tablo 1. Olguların ırk, yaş cinsiyet, kırık lokalizasyonu, sağaltım seçenekleri.
Table 1. Age, gender, fracture localization and treatment options of cases.

Olgu	Irk	Yaş	Cinsiyet	Kırık Sebebi	Kırık Şekli ve Bölgesi	Sağaltım	Ekstremiteyi kullandığı gün	Sonuç
1	S	2 A	D	Düşme	Suprakondüler kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	3	Ulaşılamadı
2	S	15 G	E	Güç doğum	Orta diafizer oblik kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	2	-
3	E	5 G	D	Güç doğum	Suprakondüler kırık	2 adet 4 mm steinman pin	3	+
4	E	1 G	E	Güç doğum	Orta diafizer oblik kırık	2 adet 3 mm steinman pin	1	+
5	S	10 G	E	Mazgal arasına sıkışma	Orta diafizer oblik kırık	Hasta sahibi operasyonu kabul etmedi	-	/
6	S	10 G	D	Tekmelenme	Suprakondüler kırık	Hasta sahibi operasyonu kabul etmedi	-	/
7	S	3 G	E	Güç doğum	Orta diafizer oblik kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	2	+
8	S	12 G	E	Düşme	Orta diafizer parçalı kırık	Hasta sahibi operasyonu kabul etmedi	-	/
9	ES	20 G	E	Güç doğum	Suprakondüler kırık	2 Adet 3 mm steinman pin	3	+
10	ED	2 G	E	Güç doğum	Orta diafizer transversal kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	2	+
11	E	7 G	E	Annesinin yavruyu ezmesi	Distal diafizer oblik kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	2	+
12	ES	6 A	D	Düşme	Orta diafizer parçalı kırık	2 adet 4 mm, 1 adet 2 mm steinman pin	4	+
13	S	2 G	E	Güç doğum	Distal diafizer oblik kırık	2 mm plak, 3 mm 3 adet ve 4.5 mm 3 adet vida	1	+

E: Esmer, S: Simental, ES: Esmer-Simental Melezi, ED: Esmer-Doğu Anadolu Kırmızısı Melezi, +: İyileşme var, -: İyileşme yok, /: Tedavi denenmedi.
E: Brown Swiss, S: Simmental, ES: Brown Swiss x Simmental crossbred , ED: Brown Swiss x Eastern Anadolian Red crossbred , +: Positive result, -: Negative result, /: No treatment.

TARTIŞMA ve SONUÇ

Yeni doğan buzağılarda en önemi kırık nedenlerinden biri güç doğum sırasında yapılan hatalı müdahaleler olarak bildirilmiştir (Aksoy ve ark. 2009, Arıcan ve ark. 2013, Durmuş ve ark. 2009, Nichols ve ark. 2010, Ferguson ve ark. 1990, Ferguson 1994, Yanmaz ve ark. 2014). Bunun yanı sıra doğum sonrasında diğer hayvanlar tarafından tekmelenme, annenin yavrunun ekstremitelerine basması, kayma ve düşme (Akin 2014, Arıcan 2013, Bilgili ve ark. 1999, Durmuş ve ark. 2009, Yanmaz ve ark. 2014) transport ve trafik kazaları (Akin 2014, Yanmaz ve ark. 2014) gibi nedenler kırık oluşumuna yol açmaktadır. Olgularımızdan 7'sinde kırık oluşma sebebi güç doğum sırasındaki kriko kullanımı gibi hatalı müdahaleler olarak belirlenmiş olup diğer olgularda başka sebepler etiyolojide rol oynamakta idi. Bu bulgu ile gerek hasta sahiplerinin gerekse veteriner hekimlerin doğuma müdahale sırasında çok dikkatli olmaları gerektiği ve usulünce girişimlerde bulunmaları gerektiği sonucuna varılmıştır. Doğum sırasında yapılan hatalı manipülasyonların ülke ekonomisine oldukça yüksek oranda zarar verdiği açıkça görülmektedir.

Yeni doğan buzağılardaki femur kırıklarında femoral arter lasere olabileceğinden yaşamı tehdit edici kanamalarla karşılaşabilir (Anderson 2015).

Olgularımızdan hiçbirinde şiddetli kanama ile karşılaşılmamış olması femoral arter laserasyonunun olgularımızda oluşmadığı görüşünü uyandırmıştır. Bir çalışmada yeni doğan buzağılarda karşılaşılan femur kırıkları çoğunlukla proksimal epifiz ve distal metafizde şekillendiği ve genellikle transversal ve oblik kırıklar biçiminde görüldüğü bildirilirken (Hoederman ve ark. 2012) Bellon (2011) ise femur diafizindeki kırıkların oblik ve spiral oluştuğunu bildirmiştir. Çalışmamızdaki kırıkların lokalizasyonları incelendiğinde orta diafizer tip ile 7 olguda karşılaşıldığı ve bunlardan 4'ünün oblik kırık, 2'sinin parçalı kırık birinin ise transversal kırık şeklinde olduğu görülmüştür. Güç doğuma bağlı şekillenen kırık olgularında orta diafizer kırık fazlalığı dikkatimizi çekmiştir. Kırık tiplerinin lokalizasyonları Hoederman ile uyumlu değil iken (Hoederman ve ark. 2012) oblik kırıkların çok sayıda olması daha önceki çalışmalar (Bellon ve Mulon 2011, Fırat 2007) ile örtüşür nitelikte bulunmuştur. Yenidoğan buzağılarda femur kırıklarının sağaltımında birçok farklı yöntem önerilmektedir (Bellon ve Mulon 2011, Nichols ve ark. 2010). Yaş, vücut ağırlığı, kırık konfigürasyonu ve ekonomik değer gibi etkenler sağaltım yönteminin seçilmesinde önemli rol oynar (Anderson ve Miesner 2015, Nicholas ve ark. 2010). Konservatif sağaltım genellikle başarılı olmamaktadır (Hoederman ve ark. 2012). Kliniğimize getirilen olgular incelendiğinde ve ekonomik değerleri göz önüne alındığında femur kırıklarının bandaj ile iyileşme şansının oldukça düşük olduğu da dikkate alınarak olgularımızın 10'u operatif olarak sağaltılmış olup 3 olguda hasta sahipleri müdahaleyi kabul etmemişlerdir. Post-operatif dönemde bir hasta sahibine ulaşılamazken diğer olguların 8'inde sağaltımdan başarılı sonuç alınmış, bir olgudan post-operatif bilgi alınamamış ve 1 olgu da ise tatminkâr sonuç elde edilememiştir. Yeni doğan buzağıların femur kırıklarında operasyon ile başarılı sonuçlar alındığı ve bu yöntemin mutlaka değerlendirilmesi gerektiği düşünülmektedir.

İntramedullar pin uygulamasının maliyeti düşük, uygulanması ve çıkarılması kolay aynı zamanda kısa sürede gerçekleştirilmesi ile epifizer plağa en az düzeyde zarar vermesi gibi avantajları vardır. Rotasyonel stabilizasyonun zayıf olması, endosteal kallus oluşumuna olumsuz etki yapması (Bellon ve Mulon 2011, Durmuş ve ark. 2009) ile pin migrasyonu dezavantajlarıdır. Buzağılarda distal diafizer kırıkların sağaltımında İM pinleme yapmış ve oldukça başarılı sonuçlar almıştır (Bellon ve Mulon 2011, Nicholas ve ark. 2010). Olgularımızdan 9'unda steinman demet pinleme yöntemi kullanılmıştır. Olguların 6'sında 2 adet 4 mm ve bir adet 2 mm steinman pin kullanılmıştır. Demet pinleme gerçekleştirdiğimiz olgularımızdan hiç birinde belirtilen dezavantajlar ile karşılaşılmamış olup düşük maliyet ve uygulama kolaylığı açısından son derece kullanışlı bulunmuştur. Operasyon sırasında genellikle buzağıların 10 mm çapında medullar kanala sahip oldukları görülmüş olup operasyon planlaması yapılırken bu verinin dikkate alınması gerektiği sonucuna ulaşılmıştır.

Plaka uygulamasının diğer yöntemlere göre daha fazla doku hasarı oluşturduğu ve periosteal dolaşıma zarar verdiği bildirilmektedir (Durmuş ve ark. 2009). Yeni doğan buzağılarda femoral kemiğin yumuşak olması plaka osteosentezindeki en büyük endişelerden bir tanesidir. Çünkü yumuşak neonatal kemik vida gevşemesine predispozisyon yaratabilir ve sonrasında fiksasyonda instabilite ile karşılaşılabilir (Hoederman ve ark. 2012). Femurun distaline lokalize olmuş kırıklarda, kırık hattının ekleme yakın olması, distal fragmentin kemiğin corpus'una oranla oldukça geniş olması, distal fragmentin spongioz kemik dokudan oluşması gibi nedenler ile stabilizasyon problemi sık yaşanır; ve bu nedenlerle de buzağılarda distal femur kırıklarının prognozu genellikle olumsuz olarak kabul edilmektedir (Akın ve ark. 2014, Bellon ve Mulon 2011, Ferguson 1994, Ferguson ark. 1990). Distal diafizer kırıklı bir olgumuzda uyguladığımız plaka osteosentezinde distal vidalarda gevşeme ile karşılaşılmış olup olgunun sınırlı hareketlerine izin verilmiş ve kallus oluşumuna kadar plaka çıkarılmamış sonrasında çıkarma işlemi gerçekleştirilerek olgunun ayağını kullanabildiği görülmüştür. Bu vida gevşemesinin literatürlerde belirtilen nedenlerden kaynaklandığı düşünülmektedir.

Sonuç olarak, yeni doğan buzağılarda oldukça yüksek insidansta karşılaşılan femur kırıklarının lokalizasyonu ve şekli belirlenmiş olup sağaltımları gerçekleştirilmiştir. Özellikle diafizer femur kırıklarının sağaltımında kullandığımız demet pinleme tekniğinden başarılı sonuçlar elde edilmiştir. Bunun yanı sıra kilitli intramedullar pin tekniği gibi yöntemleri deneme şansımız olmamış, plaka osteosentezi ise çok yetersiz sayıda kalmıştır. Demet pinleme tekniği ile diafizer femur kırıklarının başarılı şekilde sağaltılmasının yanı sıra diğer kırık tiplerinde farklı yöntemlerin ilerideki yapılacak çalışmalar ile netlik kazandırılması görüşüne varılmıştır. Ayrıca buzağılardaki femur kırıklarının %53.84 oranında güç doğumlarda bilinçsizce yaklaşıma bağlı olarak insan kaynaklı geliştiği tespit edilmiş ve yetiştiricilerin bu konuda bilgilendirilmesi gerektiği kanaatine varılmıştır.

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Köpeklerde Göz Kapağı Tümörleri: 47 Olguda Retrospektif Çalışma (2006-2017)[#]

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ÖZ

Çalışmada 2006-2017 yılları arasında İstanbul Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne göz kapağında kitle şikayeti ile getirilen ve sağaltımları yapılan toplam 47 köpeğe ait göz kapağı tümörleri olguları retrospektif olarak değerlendirildi. Olguların ayrıntılı göz muayenesi yapıldı. Kitlelerin uzaklaştırılmasında V şeklinde eksizyon yeğlenerek, tümör tam katlı olarak bölgeden uzaklaştırıldı. Yapılan histopatolojik inceleme sonucu, meibomian bezlerden köken alan neoplazmaların çoğunlukta olduğu belirlendi. Çalışma sonunda elde edilen veriler ile göz kapağı tümörü oluşumunda ırk predispozisyonu, yaşın ve cinsiyetin etkisi ile en yaygın görülen göz kapağı tümörü belirlenip, kullanılan cerrahi yöntemin rekonstruktif blefaroplastiye gerek kalmadan, kitlelerin uzaklaştırılmasında yeterli olduğu görüldü.

Anahtar Kelimeler: Köpek, göz kapağı tümörleri, V eksizyon, retrospektif, okuler.

Eyelid Tumors in Dogs: 47 Cases Retrospective Study (2006-2017)

ABSTRACT

In this study, the eyelid tumors and the treatment results of 47 dogs referred to Istanbul University Faculty of Veterinary Medicine Department of Surgery with a complaint of eyelid masses were evaluated retrospectively. Cases underwent detailed ophtalmic examination. A V-shaped excision was performed and the tumor was removed in full-thickness. Histopathological examination revealed that most of the neoplasms originating from the meibomian glands were observed. The most common eyelid tumors and formation of the eyelid tumors with respect to the race predisposition, and effects of age and sex were defined. The surgical method used were sufficient for removal of the masses without the necessity of a reconstructive blepharoplasty.

Keywords: Dog, eyelid tumors, V-shaped excision, retrospective, ocular.

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GİRİŞ

Köpeklerde göz kapağının neoplastik oluşumlarına oldukça sık rastlanır. Kedilerdeki görülme oranı ise köpeklerden çok daha azdır (Hedlund CS 2007, Aquino MS 2008, Slatter DH 2003, Maggs DJ ve ark 2012, Gelatt KN ve ark 2012, Finn M ve ark 2008). Göz kapağı tümörleri Beagle, Siberian Husky ve English Setter gibi köpek ırklarında daha yaygın görülür. Buna karşın melez köpek ırklarında oluşum riski daha azdır (Hedlund CS 2007, Roberts SM ve ark 1986).

Papilloma ve histiyositoma dışındaki göz kapağı tümörleri, genellikle 10 yaşından büyük yaşlı köpeklerde ortaya çıkar. Yapılan retrospektif çalışmalarda, bu neoplazmaların çoğunlukla bening karakterde olduğu bildirilmektedir (Aquino MS 2008, Krehbiel JD and Langham RF 1975, Roberts SM ve ark 1986, Romkes G 2014). Buna karşın, yapılan histopatolojik incelemeler sonrasında, malign karakterde olduğu tespit edilen göz kapağı tümörlerinin bile, olumlu bir prognoza sahip oldukları ve sağaltıma cevap verdikleri belirtilmektedir (Hedlund CS 2007, Gelatt KN ve ark 2012, Slatter DH 2003).

Köpeklerin göz kapağı tümörlerinin yaklaşık %40-77'sini meibomian bez neoplazmaları oluşturur. Bu tümörler adenomalar, epiteliomalar ve karsinomalar olarak sınıflandırılır (Labelle AL and Labelle P 2013, Black LJ ve ark 2018, Werner J ve ark 2017). Meibomian bez neoplazmlarını sırasıyla melanomalar ve papillomalar izler (Aquino MS 2008, Gelatt KN ve ark 2012, Willis AM and Wilkie DA 2001). İyi huylu göz kapağı tümörlerini sebasöz adenoma, bening melanoma, histiyositoma ve papillomalar oluşturur. Bu tümörlerden sebasöz adenoma en yaygın görülen göz kapağı tümörüdür. Göz kapağında görülen maling tümörler ise melanoma, skuamöz hücre karsinoması, adenokarsinoma, bazal hücre karsinoması, mast hücre tümörü, hemanjiyosarkoma ve fibrosarkoma şeklinde sıralanabilir (Willis AM ve Wilkie DA 2001, Hedlund CS 2007, Aquino MS 2008, Maggs DJ ve ark 2012, Gelatt, KN ve ark 2012, Roberts SM ve ark 1986).

Göz kapağı tümörlerinin klinik bulgusunu çoğunlukla kitlenin kendisi oluşturur. Kitlenin korneada oluşturduğu irkiltiye bağlı olarak, epifora ve blefarospazm ortaya çıkar. Tümörün göz kapaklarının fonksiyonunu bozacak derecede büyümesi sonucu ise ülseratif keratitis gibi daha ciddi oküler bulgular görülebilir (Hedlund CS 2007, Finn M ve ark 2008).

Göz kapağı tümörlerinin tanısı için ince iğne aspirasyon biyopsisi yapılır. Ayırıcı tanı için ise kitlenin tamamen uzaklaştırılmasının ardından mutlaka histopatolojik değerlendirmenin yapılması önemlidir (Willis AM and Wilkie DA 2001, Hedlund CS 2007).

Sağaltımı cerrahi yöntemler ile yapılır. Bu amaçla elektroşirurji, kriyoablasyon, şirurjikal eksizyon, karbondioksit laser tedavisi, immunoterapi, radyasyon tedavisi, kemoterapi gibi tekniklerden yararlanılır (Willis AM ve Wilkie DA 2001, Romkes G ve ark 2014, Gelatt KN ve ark 2012, Şaroğlu M 2013). Sağaltım sırasında öncelikle göz kapağının yapı ve fonksiyonlarının korunması son derece önemlidir (Aquino MS 2008).

Küçük tümörlerin uzaklaştırılmasında çoğunlukla şirurjikal kama eksizyon yöntemi yeğlenir. Bu uygulama yönteminde farklı teknikler kullanılır. Tümörün gözkapağı kenar uzunluğunun üçte birinden daha küçük olması halinde, V şeklinde eksizyon yapılarak, tümör tam katlı olarak bölgeden uzaklaştırılır. Böylelikle yaranın kapatılması için ilave bir onarıcı sağaltıma gerek duyulmaz. Diğer bir yöntem 4 kenarlı kama tam kalınlık eksizyondur. Her iki teknikte de iki katlı apozisyon sağlanması önemlidir (Romkes G ve ark 2014, Finn M ve ark 2008).

Çalışmada 2006-2017 yılları arasında İstanbul Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne göz kapağında kitle oluşumu şikayetiyle getirilen toplam 47 köpek retrospektif olarak değerlendirildi. Çalışma sonunda elde edilen bulguların, bilimsel literatüre ve meslek pratiğine aktarılması amaçlandı.

MATERYAL ve METOD

Çalışma materyalini 2006-2017 yılları arasında İstanbul Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalı Kliniği'ne göz kapağında kitle oluşumu şikayetiyle getirilen değişik yaş, ırk ve cinsiyetteki toplam 47 köpek oluşturdu. Olguların ayrıntılı anamnezlerinin ardından fiziksel ve oftalmolojik muayeneleri yapıldı. Göz kapağındaki kitlenin operatif olarak uzaklaştırılmasına karar verilen hastaların, hemogram (Eritrosit-RBC, hemoglobin-Hgb, hematokrit-HCT, lökosit-WBC) ve bazı biyokimyasal parametrelerine (Aspartataminotransferaz-AST,b alanin aminotransferaz-ALT, Glikoz, Üre, kreatinin, total protein) bakılarak genel sağlık durumları belirlendi. Anesteziye alınmalarında sakınca olmayan hastalara genel anestezi uygulandı. Bu amaçla premedikasyonda; ksilazin HCl (Rompun, %2, Bayer®, Almanya) 2mg/kg dozda kasiçi (IM) yolla

verildi. Anestezi indüksiyonu ketamin HCl (Alfamine, %10, Eczacıbaşı®, Türkiye)'in 10mg/kg dozda IM yapılmasıyla sağlandı. Hastaların uygun büyüklükteki entübasyon tüpleri (Rüsh-Almanya) ile endotrakeal entübasyonlarının yapılmasının ardından, genel anestezi başlangıçta %4, devamında %2 konsantrasyonda isofluran (Forane®,100ml, Abbott, İsviçre) ile devam ettirildi.

Hastalar sağaltım uygulanacak göz üstte kalacak şekilde operasyon masasına yatırılarak, uygun pozisyon verildi. Bölgenin tıraş ve dezenfeksiyonu yapıldı. Olguların tamamında neoplazmalar şirurjikal eksizyonla uzaklaştırıldı. Bu amaçla V şeklinde eksizyon yapıldı. Bu uygulama, tümörlerin sorunsuz şekilde uzaklaştırılması için yeterli oldu. Tümörün uzaklaştırılmasını takiben göz konjunktivası 4/0-6/0 emilebilir iplik poliglikolik asit P.G.A. (Çetin Kimya Sağlık Ara., Türkiye) ile basit sürekli dikiş yöntemiyle kapatıldı. Deri ise 3/0-4/0 emilmeyen monoflament Propilen (Medeks, Türkiye) dikiş materyali ile basit ayrı dikiş tekniği kullanılarak kapatıldı (Şekil -1 ve 2).

Operasyon sonrası hastaların gözüne lokal antibiyotik olarak günde 4 kez, 1-2 damla olacak şekilde ofloksasin (Exocin®%0.3 oftalmik damla, Alergan, Türkiye) ve aynı zamanda günde 2 kez fusidik asit (Fucilthalmic® oftalmik pomad, Abdi İbrahim, Türkiye) damlatıldı. Bu uygulama 2 hafta süreyle devam ettirildi. Operasyon bölgesini korumak için hastalara Elizabeth yakalığı takıldı.

Post operatif dönemde 7 gün boyunca sistemik antibiyotik de uygulandı. Bu amaçla seftriakson (Novosef® 0.5g, Zentiva, Türkiye), 25 mg/kg dozda IM olarak yapıldı. Ayrıca postoperatif analjezi için 3 gün boyunca meloksikam (Metacam® 1.5mg/ml, Boehringer İngelheim, Türkiye) 0.2 mg/kg dozda peros verildi.

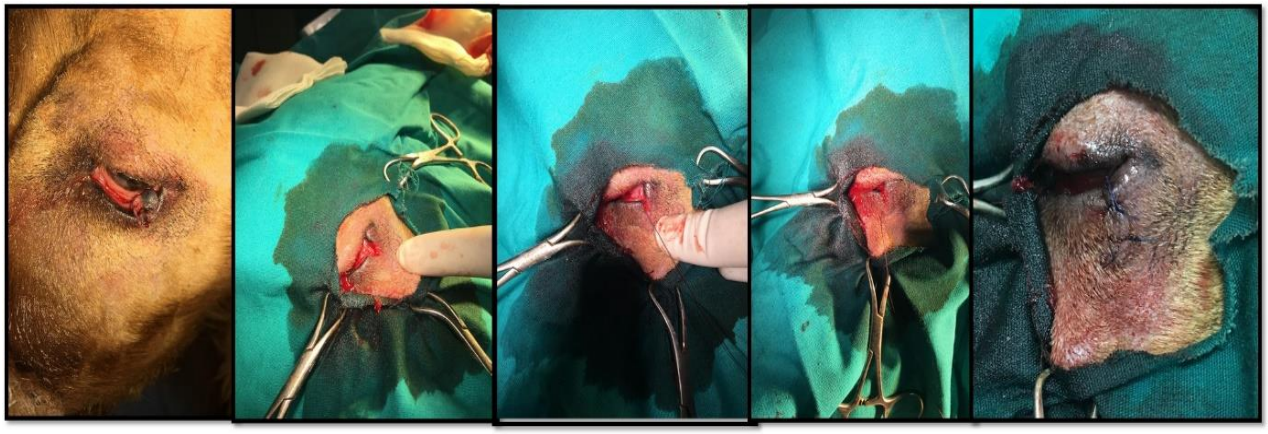
Tüm hastaların göz kapaklarından uzaklaştırılan kitlelerin histopatolojik incelemesi İstabbul Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'nda yapıldı. Nötral tamponlu %10 'luk formalin ile fikse edilen doku örnekleri, rutin işlemlerden geçirilerek parafine gömüldü. Parafin bloklardan 4-5 mikrometre kalınlığındaki kesitler alınarak, hematoksilen eozin ile boyandı. Hazırlanan preparatlar, ışık mikroskobunda değerlendirildi.

BULGULAR

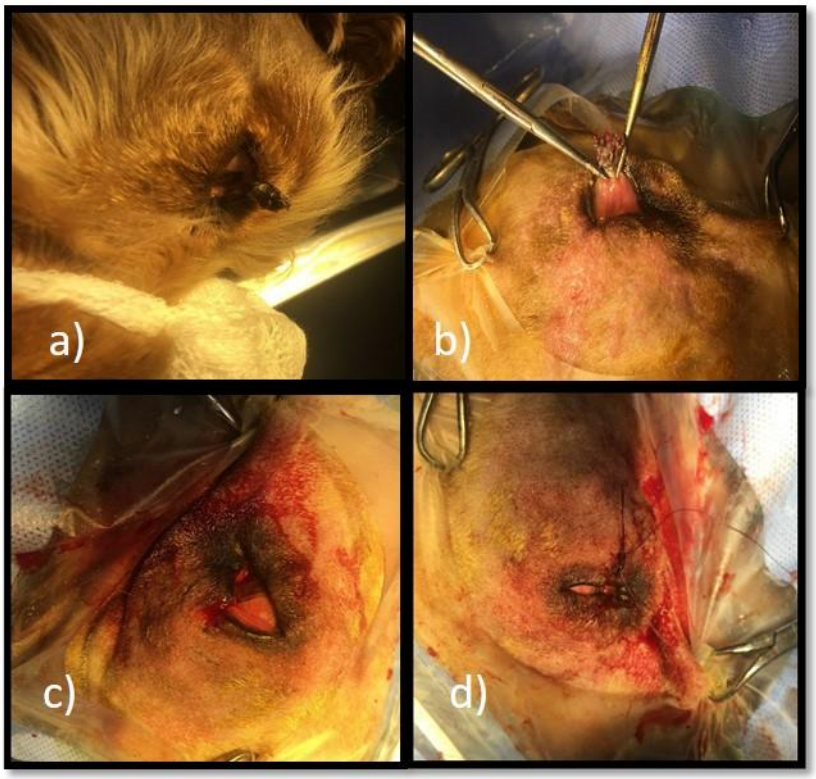
Hastaların yapılan klinik muayeneleri sonrasında 7 olguda (Olgu No: 1, 4 10, 17, 21, 24, 34) (şekil 2 ve 3) göz kapağında kitle oluşumunun yanı sıra, tümörden kaynaklanan değişen derecelerde epifora, blefarospazm, konjunktivitis, korneal ülserasyon ve vaskülarizasyon gibi bulgulara da rastlandı. Bu olguların operasyon gününe kadar medikal sağaltımları yapıldı.

Operasyonla uzaklaştırılan kitlelerin boyut, hacim, renk ve görünüşlerinin, kitlenin tipi ve var oluş süresine göre değişiklik gösterdiği belirlendi.

Olgulara ait ırk dağılımları tablo-1, yaş aralığı tablo-2 ve cinsiyet dağılımı tablo-3'de gösterilmiştir. Histopatolojik muayene sonrasında elde edilen bulgular tablo-4 ve tümörlerin göz kapaklarına göre dağılımları tablo 5'de sunulmuştur. Histopatolojik değerlendirme sonucuna göre; meibomian epitelioma (%36,17) ve meibomian adenoma (%27,65) görülme sıklığının diğer göz kapağı tümörlerine kıyasla belirgin şekilde yüksek olduğu belirlenmiştir.



Şekil 1: 10 no'lu olgunun alt göz kapağında meibomian epitelioma'nın V şeklinde ensizyonla uzaklaştırılmasının intraoperatif görüntüleri.
Figure 1: Case number 10, intraoperatively view of the removal of the "meibomian epithelioma" at lower eyelid.



Şekil 2: 21 no'lu olguya ait a)üst göz kapağında meibomian adenoma'nın intraoperatif uzaklaştırma, b) V şeklinde ensizyonla, c)uzaklaştırılmış ve d) bölgenin iki katlı apozisyonu ile kapatılmış görüntüsü
Figure 2: Case number 21, intraoperatively views of the removal of a a)meibomian adenoma at upper eyelid with b) V-shape excision, c) after removal d) two-layer closure for an eyelid defect.

Şekil 3: 34 no'lu olgunun alt göz kapağındaki sebasöz epitelioma'ya bağlı şekillenmiş kornea ülseri görüntüsü.
Figure 3: Case number 34, view of a corneal ulcer due to sebaceous epithelioma at lower eylid.

Şekil 4: 10 numaralı olgunun alt göz kapağında oluşan meibomian epitelioma'ya bağlı epifora görüntüsü.
Figure 4: case number 10, epiphora due to meibomain epithelioma at lower eyelid.

Tablo 1. Olgulara ilişkin ırk dağılımı.
Table 1. Breed distribution of the cases.

Irk	Olgu Sayısı
Terrier	6
Cocker	9
Golden Retriever	5
Melez	5
Husky	4
Boxer	3
Collie	2
Kangal	2
Akita	1
Dachshund	1
French Bulldog	1
Pincher	1
Labrador	2
Pekingese	1
Alman Çoban Köpeği	1
Doberman	1
Chow Chow	1
Cavailer King Charles	1

Tablo 2. Olgulara ait yaş dağılımı.
Table 2. Age distribution of the cases.

Yaş aralığı	Olgu sayısı
0-3 Yaş	4
3-6 Yaş	5
6-9 Yaş	14
9-11 Yaş	15
11-14 Yaş	9

Tablo 3. Olgulara ait cinsiyet dağılımı
Table 3. Sex distribution of the cases.

Cinsiyet Dağılımı	Olgu Sayısı	Dağılımı (%)
Dişi	20	42,55
Erkek	27	57,45

Tablo 4. Olguların histopatolojik sonuçlarına ilişkin bulgular.
Table 4. Histopathological results of the cases.

Histopatolojik Sonuçlar	Olgu sayısı	Görülme Oranı (%)
Meibomian epitelioma	17	36,17
Meibomian adenoma	13	27,65
Meibomian karsinoma	1	2,12
Papilloma	1	2,12
Sebasöz adenoma	1	2,12
Sebasöz epitelioma	4	8,51
Sebasöz karsinom	1	2,12
Mast hücre tümörü	1	2,12
Malign trikoepiteliom	1	2,12
Melanom	2	4,25

Tablo 5. Tümörlerin göz kapaklarına göre dağılımı.
Table 5. Localization of the eyelid neoplasms of the cases.

Tümörün Lokalizasyon	Olgu Sayısı	Dağılımı (%)
Üst göz kapağı	28	59,57
Alt göz kapağı	19	40,43

TARTIŞMA ve SONUÇ

Göz kapakları göz küresi ve korneanın korunmasını sağlayan anatomik yapılardır. Bunun yanı sıra göze giren ışının kontrolünde, nazolakrimal drenaj sisteminde, gözyaşı ve meibomian bezlerden salınan film tabakasının preoküler dağılımında da görev alır (Gelatt KN and Plummer CE 2017).

Göz kapağı tümörleri genellikle orta ve ileri yaş köpeklerde daha yaygın görülür (Aquino MS 2007, Maggs DJ ve ark 2012). Sunulan bu retrospektif çalışmada yaklaşık 11 yıllık bir değerlendirme yapılmış olup, göz kapağı tümörü görülme yaşının kaynaklarda (Aquino MS 2007, Maggs DJ ve ark 2012, Gellat KN and Janice P 2011) bildirildiği gibi, orta yaş ve üzeri köpeklerde daha yaygın görüldüğü belirlenmiştir.

Göz kapağı tümörlerinin ortaya çıkmasında ırk predispozisyonunun etkisinin yanı sıra, ülke ya da bölgedeki popüler ırklara göre de oranlar değişebilmektedir (Şaroğlu M 2013). Çoğunlukla Beagle, Siberian Husky ve English Setter ırkı köpeklerde daha yaygın görülmektedir (Hedlund CS 2007, Roberts SM ve ark 1986). Çalışmada göz

kapağı tümörlerinin ırklara göre dağılımı incelendiğinde, Cocker Spaniel (%19,56), Terrier (%12,76) ve Golden Retriever (% 10,63) gibi ırkların ilk sıralarda yer aldığı belirlenmiştir. Bu ırk köpeklerin bölgedeki popüler köpek ırkları olmalarından ötürü ilk sıralarda yer almış olabilecekleri (Şaroğlu M 2013) değerlendirilmiştir. Göz kapağı tümörlerinin melez ırklarda nadir görüldüğünü ifade eden araştırmanın (Roberts SM ve ark 1986) aksine, çalışmada 5 melez ırk köpekte de göz kapağı tümörüne rastlanmıştır.

Göz kapağı tümörlerinin oluşumunda cinsiyetin önemli olup olmadığına ilişkin herhangi bir istatistiki veri bulunmamaktadır (Gelatt KN ve ark 2012). Sunulan çalışmada, 27 olgunun erkek (%57,45) ve 20 olgunun dişi (%42,55) köpek olduğu görülmüştür. Buna göre göz kapağı tümörlerinin erkeklerde daha yaygın görüldüğü ifade edilebilir. Göz kapağı tümörlerinin büyük çoğunluğunu meibomian bezlerden köken alan neoplazmalar oluşturur (Maggs DJ ve ark 2012). Sunulan bu çalışmada da meibomian epitelioma (%36,17) ve meibomian adenoma (%27,65)ilk sıralarda yer almış olup, bulgunun kaynaklarla (Gelatt KN ve ark 2012, Maggs DJ ve ark 2012) uyumlu olduğu görülmüştür.

Göz kapağı tümörleri çok nadir metastaz yaparlar. Bu tümörlerin malignitesi, genellikle lokal yayılım yapmalarıyla ortaya çıkar (Şaroğlu M 2013, Aquino MS 2007). Köpeklerde gerek kriyoşirurji gerekse cerrahi sağaltım uygulanmış göz kapağı tümörlerinin nüks etme oranlarında önemli bir fark bulunmamaktadır (Roberts SM ve ark 1986). Nüks oluşum süresi kriyoşirurjiyi takiben 7.4 ay iken cerrahi eksizyon sonrası bu süre 28.3 ay olarak bildirilmektedir (Roberts SM ve ark 1986). Sunulan çalışmada tümörlerin tümüne cerrahi eksizyon uygulanmış olup, olguların postoperatif kontrolleri dikiş materyallerinin uzaklaştırılma süreci ile sınırlı kalmıştır. Devam eden süreçte hasta sahipleri herhangi bir nüks şikayeti bildirmemiş ve sağaltımı yapılan olgular yeniden nüks bulgusuyla kliniğe getirilmemişlerdir.

Sonuç olarak; bu çalışmada göz kapağı tümörlerinin cerrahi eksizyon ile sağaltımlarının ardından, alınan kitlenin histopatolojik incelemesi yapılmıştır. Elde edilen sonuçlar ile göz kapağı tümörü oluşumunda ırk predispozisyonu, yaşın ve cinsiyetin etkisi ile en yaygın görülen göz kapağı tümörü belirlenmiştir. Ayrıca çalışmada tercih edilen cerrahi yöntem ile rekonstruktif blefaroplastiye gerek kalmadan eksizyon hattının rahatlıkla kapatılabileceği görülmüş olup, bulguların bilimsel literatüre ve meslek pratiğine katkı sağlaması amaçlanmıştır.

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RESEARCH ARTICLE

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Protective Effect of N-Acetylcysteine on Testicular Oxidative Damage, Spermatological Parameters and DNA Damage in Glyphosate-Based Herbicide-Exposed Rats

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ABSTRACT

The aim of this study was to examine the protective effect of N-acetylcysteine (NAC) on testicular oxidative damage, spermatological parameters and DNA damage caused by Glyphosate (GLF) in rats. In total, twenty-eight Wistar male rats were evaluated by being separated into four groups in an equal way. Rats in group I, which represented the control group, were fed normal diet without GLF or NAC, group II received normal feed containing 160 mg/kg/daily NAC, group III received normal feed containing 375 mg/kg/daily GLF, and group IV received normal feed containing 160 mg/kg/daily NAC + 375 mg/kg/daily GLF. GLF administration decreased sperm motility, abnormal sperm rate, sperm plasma membrane integrity, glutathione level and superoxide dismutase in the rats' testicular tissue. On the other hand, high malondialdehyde level and DNA damage were detected in the group administered with GLF. Besides, in histopathological terms, a decrease in sperm concentration and degeneration of sertoli cells were determined in the testicular tissue. NAC and NAC+GLF administration reversed lipid peroxidation and DNA damage induced by GLF, the activity of antioxidant enzymes and cell integrity in rats' testis. The above-mentioned findings indicate that NAC reduces lipid peroxidation caused by GLF, improves the antioxidant defense mechanism and regenerates tissue damage in rats' testis.

Keywords: DNA damage, Glyphosate, N-acetylcysteine, Rat sperm

Glifosat Bazlı Herbisite Maruz Kalan Sıçanlarda N-Asetilsisteinin Testis Oksidatif Hasarı, Spermatolojik Parametreler ve DNA Hasarı Üzerindeki Koruyucu Etkisi

ÖZ

Bu çalışmanın amacı, N-asetilsisteinin (NAC) sıçanlarda Glifosat (GLF) 'nin neden olduğu testiküler oksidatif hasar, spermatolojik parametreler ve DNA hasarı üzerindeki koruyucu etkisini incelemektir. Toplam yirmi sekiz Wistar erkek sıçan, eşit bir şekilde dört gruba ayrılarak değerlendirildi. Kontrol grubunu olan grup I'deki sıçanlar, GLF veya NAC olmaksızın normal diyetle beslendiler, grup II'deki hayvanlara 160 mg / kg / günlük NAC içeren normal rat diyeti uygulandı, grup III'deki hayvanlara 375 mg / kg / günlük GLF içeren normal rat diyeti verildi ve grup IV'deki hayvanlara 160 mg / kg / günlük NAC + 375 mg / kg / günlük GLF içeren normal rat diyeti uygulandı. GLF uygulaması sıçanların testis dokusunda spermatozoon motilitesini, anormal spermatozoon oranını, spermatozoon plazma membran bütünlüğünü, glutatyon ve süperoksit dismutaz düzeyini azaltmıştır. Diğer taraftan GLF uygulanan grupta yüksek malondialdehid düzeyi ve DNA hasarı saptandı. Ayrıca histopatolojik olarak testis dokusunda spermatozoon konsantrasyonunda azalma ve sertoli hücrelerin dejenerasyonu belirlendi. NAC ve NAC + GLF uygulaması, lipid peroksidasyonunu ve GLF tarafından indüklenen DNA hasarını, sıçanların testislerinde antioksidan enzimlerin aktivitesini ve hücre bütünlüğünü tersine çevirdi. Yukarıda belirtilen bulgular, NAC'nin GLF'nin neden olduğu lipit peroksidasyonunu azalttığını, antioksidan savunma mekanizmasını geliştirdiğini ve sıçanların testislerinde doku hasarını rejenere ettiğini göstermektedir.

Anahtar Kelimeler: DNA hasarı, Glifosat, N-Asetilsistein, Rat spermatozoon

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INTRODUCTION

Herbicides are only one of many pesticide types. These are chemical substances that farmers use to weed. When people are exposed to pesticides in food and plastic, industrial pollutants and synthetic chemicals, these substances are thought to affect the endogenous reproductive hormone function adversely. This can lead to a variety of reproductive anomalies (Dallegrave et al., 2007). In the studies conducted, it has been demonstrated that the above-mentioned compounds and their metabolites represent the main pollutants of surface waters and they usually continue to exist in agricultural products, which constitutes a significant threat to the health of humans and animals (Feron et al., 2002; Bohm et al., 2008; Clair et al., 2012). Glyphosate (GLF)-based herbicides are the first herbicides started to be utilized around the world. It is expected that GLF is specific on plant metabolism, and it has been claimed to have adverse impacts on animals and humans (Takahashi et al., 2001). GLF is a broad-spectrum herbicide commonly utilized for killing undesired plants in lands used for agricultural as well as nonagricultural purposes. It has been determined that GLF alters the cellular antioxidant status considerably, which induces glutathione depletion, enzymatic disorders and an increase in lipid peroxidation in keratinocytes (Gehin et al., 2006). GLF may have an endocrine disruptor effect or affect the reproductive system of males since it may cause changes in aromatase activity and expression, genes regulating estrogen and testosterone levels (Richard et al., 2005; Romano etl., 2010; Clair et al., 2012).

N-acetylcysteine (NAC), which was developed in the 1960s, represents the N-acetyl derivative of the amino acid L-cysteine that occurs naturally (Aitken et al., 1993). It is thiol-based antioxidant, takes a significant part both in protecting the components of cells against oxidative damage and in detoxifying numerous electrophiles (Martha et al., 1998). NAC has a wide area of use in the clinic as a mucolytic agent and an antidote for acetaminophen overdose. Moreover, NAC can play a role in regulating gene expression related to oxidative stress, therefore, it has an antagonistic impact on oxidative damage (Meister, 1991). NAC is almost nontoxic, and it is commonly utilized for decreasing the elasticity and viscosity of mucus due to its capacity to reduce disulfide bonds. NAC can enter into direct interaction with oxidants and different thiols, including glutathione, in addition to being a perfect scavenger of hydroxyl radicals. Along with the scavenger function of NAC, numerous pieces of evidence have demonstrated that it stimulates cellular glutathione production as well. Hence, it is possible that oxidant-mediated damage in cell cultures or animals is decreased or inhibited by NAC (Ciftci et al., 2009).

Thus, the aim of this study was to examine the protective effect of NAC on testicular oxidative damage, spermatological parameters and DNA damage caused by GLF in rats.

MATERIALS and METHODS

Chemicals

Knockdown 48 SL which was a commercial preparation used as a GLF source, (Hektaş, Kocaeli, Turkey) and N-acetylcysteine (600 mg/20 tablet) was used (Basel, İstanbul, Turkey). Ketalar (Ketamin HCl 50 mg/ml; Pfizer, İstanbul, Turkey) and xylazine (20 mg/ml; Bayer, İstanbul, Turkey) were used for anesthesia purpose and euthanasia.

Animals and Experimental Design

In this study, 28 male Wistar Albino rats aged 12 weeks on average and weighed 290-350 g were used at Afyon Kocatepe University Experimental Animal Research Center. Approval for conducting experiments was obtained from the Animal Care and Use Committee (2017-49533702 / 26) at Afyon Kocatepe University, while the National Institutes of Health performed the care and use of laboratory animals. The animals were randomized into four groups (n = 7) and housed in a controlled environment (22 ° C, 12 h light-dark cycle), and free access to food and water was ensured. Group I (the control group) was fed a normal diet without GLF or NAC, group II received normal feed with 160 mg / kg / day NAC, group III received normal feed with 375 mg/kg/daily GLF, and group IV received normal feed containing 160 mg/kg/daily NAC + 375 mg/kg/daily GLF. The 8-week period of administration is required to identify the impact of these substances on the production of sperm since the exact spermatogenic cycle in rats, consisting of spermatocytogenesis, meiosis and spermiogenesis, requires 40-50 days.

Epididymal Sperm Assessment

The percentage of progressive sperm motility was evaluated in accordance with the study of Sönmez et al. (2005) using a phase contrast microscope with a heated stage. Briefly, a heated slide was put on a phase contrast microscope, which was heated to the temperature of 37 ° C, and afterwards a few drops of Tris buffer solution [0.3m Tris (hydroxymethyl) aminomethane, 0.027m glucose, 0.1m citric acid], a very small drop of liquid obtained from the epididymis of the left cauda using a pipette was put into the Tris buffer

solution, and its mixture with a cover-slip was ensured. The visual assessment of the ratio of forward progressive sperm motility was performed at 200 x and 400 x magnification. The estimation of motility was carried out in three various areas in every specimen. Three different forecast averages were utilized as the final motility score.

The Hypo-osmotic Eosin stain test (HE-test) was used in the semen samples in which the ratio of dead-live spermatozoa and the hypo-osmotic swelling test were applied together (Ducci et al., 2002; Fukui et al., 2004; Mansour, 2009).

The estimation of sperm cells abnormal in morphological terms was performed on a wet mount slide by using 2 - 3 semen drops thinned in Hancock's solution (Hancock, 1952) under a phase contrast microscope (Olympus CX 31, Olympus Optical Co., Ltd., Japan), and spermatozoa ratios were recorded.

Homogenate preparation

Ice cold 0.9% NaCl was used to wash testicular tissues obtained from the rats. Foreign materials were flushed out of the tissues. Cold 0.15 M Tris-HCl buffer (pH 7.4) was used to rinse them, and the homogenization of the tissues in buffer was performed for the purpose of obtaining 10% (w/v) homogenate. Afterwards, they were subjected to centrifugation for 10 minutes at 2100 g and kept in a deep freeze prior to the use (Kucukkurt et al., 2008).

MDA, GSH, SOD and CAT measurement in tissue homogenates

The technique described by Ohkawa et al. in 1979 was employed for the determination of malondialdehyde (MDA), and the technique described by Beutler et al. (1963) was used in order to measure GSH concentration in the tissue homogenates. The methods described by Sun et al. (1988) and Aebi (1984) were employed in order to measure SOD and CAT antioxidant enzyme activity, respectively, in the tissue samples. The colourimetric method described by Lowry et al. (1951) was employed for the measurement of the protein concentration in the tissue. A Shimadzu 1601 UV–VIS spectrophotometer (Tokyo, Japan) was utilized in the spectrophotometric measurements.

DNA Integrity

Sperm DNA damage was investigated using the single cell gel electrophoresis (comet) assay, which is generally performed under high alkaline conditions (pH ≥ 13). The evaluation was performed by the visual scoring method. DNAs with no damage were scored as 0, and the damaged DNAs were scored from 1 to 4 according to the degree of damage. The results were evaluated as arbitrary unit (AU) (Gündoğan et al., 2010).

Histologic Examination

The collection of testicular tissues from all animals and their fixation in Bouin's solution were performed, following which they were embedded in paraffin wax and cut into sections 5 μm in thickness. Mayer's hematoxylin and eosin (H&E) were utilized for staining. The assessment of the tissues was performed using a light microscope (Olympus Bx51 model) having a camera (Olympus DP20).

Statistical Analysis

The data were presented as mean ± standard error of means (SEM). The value of $P<0.05$ was accepted to be the level of significance. The differences between the groups in relation to all the sperm properties, histological results and biochemical parameters were detected by employing the one-way analysis of variance (ANOVA) and post hoc Duncan test. The SPSS/PC (Version 10.0; SPSS, Chicago, IL) package program was utilized for performing all the analyses.

RESULTS

Table 1 contains information on the epididymal sperm motility and abnormal sperm rate, while Table 2 contains information on plasma membrane integrity. A significant increase in the sperm motility ($P < 0.05$) was observed in the NAC administration group. The abnormal sperm rate was significantly decreased in both NAC and NAC+GLF administration groups in comparison with the control and GLF groups. A significant increase in plasma membrane integrity (HE test) ($P < 0.05$) was detected in the NAC administration group in comparison with the remaining groups. A considerable increase in the testis MDA levels was determined in the GLF group when compared to the control group ($P < 0.05$). Furthermore, the GSH level decreased in the GLF group in comparison with the control group. As can be seen from Table 3, the activities of antioxidant enzymes, CAT and SOD were detected in the rats' testis tissue. The lower SOD activity was detected in the testis tissues ($P < 0.05$) in the GLF group when compared to the control group. At the same time, the GLF-induced change in MDA, GSH and SOD levels was reversed as a result of NAC administration. As can be seen from Fig. 1, damage to DNA was identified in the rats' sperm cells. The

high levels of DNA damage (94.8±3.79 AU) were detected in the GLF group in comparison with the control group (38.8±2.04 AU) ($P<0.05$). Moreover, DNA damage was determined to be at 25.8±2.34 AU in the NAC group. The above-mentioned findings indicated that administering NAC inhibited the change in DNA damage caused by GLF in sperm cells ($P<0.05$). The detailed description of histopathological alterations in the testis of the experimental group is presented in Fig. 2. In the GLF group, a decrease in sperm concentration and degeneration of Sertoli cells in the testis were detected (Fig. 2 A3). In the NAC groups, insignificant histopathological alterations were observed in the rats' testis tissues (Fig. 2 A2). Beside, no considerable histopathological alterations were detected in the rats' testis tissues in the control group (Fig. 2 A1).

Table 1. Mean (± SEM) spermatological parameters in epididymal rat semen.

GROUPS	Motility %	Head %	Mid-Piece %	Tail %	Total %
CONTROL	72.8±2.86^{b}	2.3±0.14^{b}	0.6±0.13^{ab}	7.8±0.51^{b}	10.7±0.53^{b}
NAC	85.7±2.02^{a}	1.9±0.17^{b}	0.1±0.09^{c}	3.3±0.24^{d}	5.3±0.40^{d}
GLF	35.7±2.02^{c}	6.2±0.70^{a}	0.9±0.07^{a}	11.2±0.42^{a}	18.3±0.92^{a}
NAC +GLF	72.8±2.75^{b}	2.2±0.21^{b}	0.3±0.17^{bc}	5.5±0.43^{c}	8.1±0.50^{c}

Values (Mean ± S.E.M.) with different superscripts (a and c) within the same column showed significant differences ($P<0.05$).

Table 2. Mean (± SEM) HE test parameters in epididymal rat semen.

GROUPS	H+/E- %	H-/E- %	H+/E+ %	H-/E+ %
CONTROL	63.7±1.53^{b}	29.2±1.35^{a}	3.4±0.57^{c}	3.5±0.81^{b}
NAC	70.0±0.88^{a}	23.1±2.38^{b}	3.2±1.12^{c}	3.57±0.99^{b}
GLF	18.4±1.63^{c}	7.8±0.96^{c}	44.1±1.33^{a}	29.5±2.69^{a}
NAC +GLF	62.3±2.36^{b}	22.0±1.48^{b}	7.5±1.68^{b}	8.1±0.73^{b}

Values (Mean ± S.E.M.) with different superscripts (a and c) within the same column showed significant differences ($P<0.05$).

Table 3. Mean (± SEM) oxidative stress parameters in epididymal rat semen.

GROUPS	MDA (nmol/ml)	GSH (nmol/g doku)	SOD (U/µg protein)	CAT (k/µg protein)
CONTROL	3.6±0.09^{b}	11.3±0.23^{a}	2.8±0.06^{b}	0.02±0.03
NAC	3.5±0.05^{b}	11.7±0.32^{a}	3.2±0.04^{a}	0.01±0.02
GLF	5.3±0.56^{a}	9.5±0.36^{b}	2.6±0.06^{c}	0.02±0.04
NAC +GLF	3.7±0.13^{b}	10.9±0.33^{a}	2.8±0.04^{bc}	0.02±0.02

Values (Mean ± S.E.M.) with different superscripts (a and c) within the same column showed significant differences ($P<0.05$).

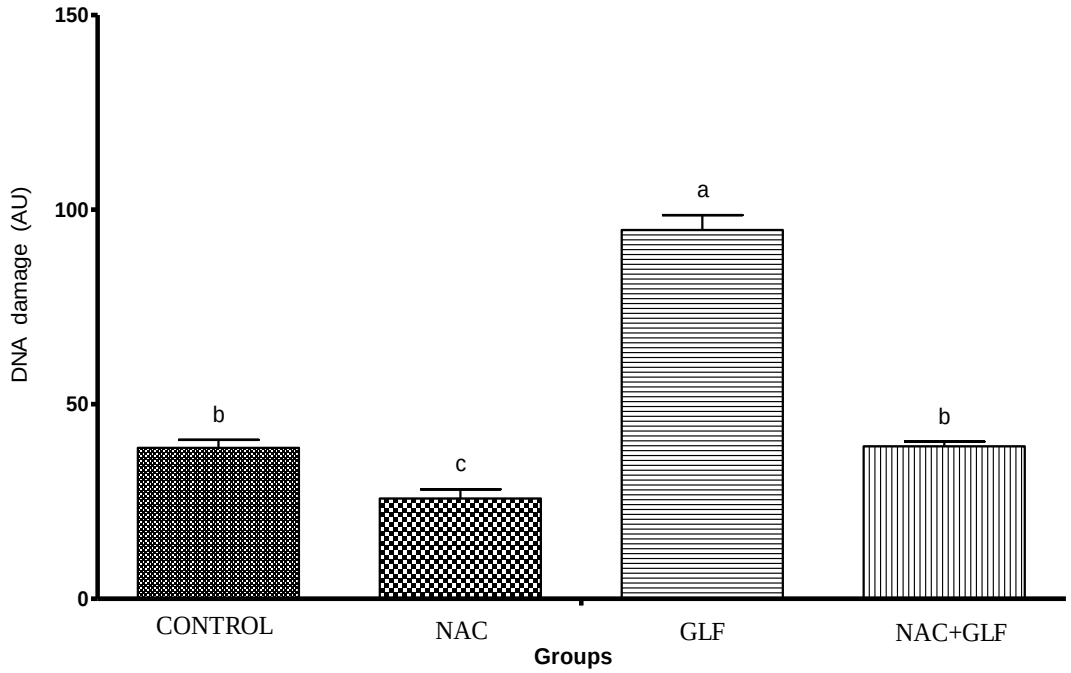

Fig. 1 The effect of GLF alone and treated with NAC on DNA damage in rats. Results are expressed as mean + SEM of seven rats. a,b,c,: different letters show statistically significant differences ($p<0.05$).

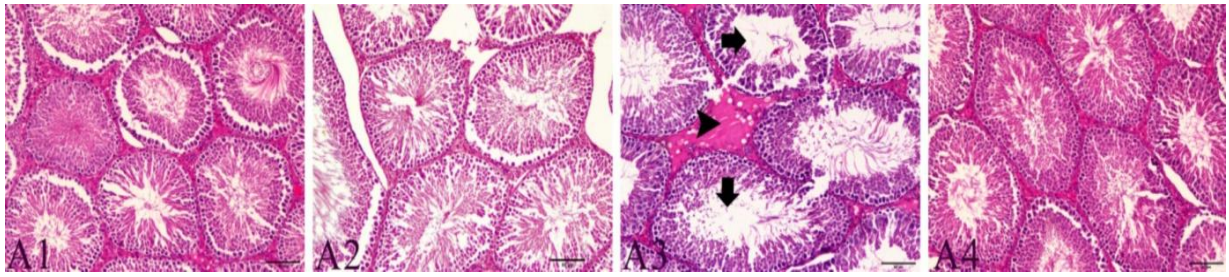

Fig 2. The effect of N-acetylcysteine (NAC) on GLF-induced damage in testis of rats. Representative figures were stained with H&E. Arrows and arrow heads indicate decreases sperm concentration and degeneration of Sertoli cells (Fig. 2 A3). Control group (A1), animals treated with NAC (A2) animals treated with GLF (A3), animals treated with GLF+ NAC (A4).

DISCUSSION

GLF has influenced the rats' reproductive system and has caused a decrease in the epididymal sperm motility and morphology. In the present study, GLF caused a reduction in motility, plasma membrane integrity of spermatozoa and an increase in abnormal sperm rate. This finding is in agreement with the findings of many studies conducted on different animal species. Reduced serum testosterone levels, decreased daily sperm count, increased ratio of abnormal sperm in adult male rats exposed to glyphosate-Roundup were indicated in the study of Dallegrave et al. (2007). Lopes et al. (2014) studied zebrafish (Danio rerio) and they reported that glyphosate could have negative impacts on reproductive parameters, including a reduction in sperm motility and the motility period and the alteration in question would decrease the fertility rate of the above-mentioned animals. In contrast, the study performed by Bhide (1988) reported no negative impact on the reproductive system of rats that were exposed to glyphosate in the maximum dose of 15 mg/kg/day. NAC effect has been shown to increase the body's major antioxidant glutathione levels. Glutathione takes a significant part in the removal of toxic substances and free radicals, therefore, it has a protective effect on the cells. In this study, GLF-associated sperm morphology was prevented by NAC and it caused an increase in the epididymal sperm motility and improved the capacity of sperm plasma membrane integrity. Similar results are also reported in some studies conducted on different kinds of animals. Michael et al. (2009) reported that the addition of tris-based diluent 1.5 mM NAC improved the sperm motility of frozen-thawed canine sperm. Partyka et al. reported that NAC (15 mM) improved the parameters of chicken sperm during storing liquid at the temperature of 5 ^{o}C for 24 and 48 h. Oeda et al. (1997) reported that the semen specimens of humans containing and not

containing NAC (1.0 mg/mL) were incubated at room temperature and the researchers determined that NAC enhanced the total sperm motility and considerably decreased ROS, indicating that the function of impaired sperm could be enhanced by NAC. Çiftçi et al. (2009) reported that the study group consisting of 60 males was given NAC in the dose of 600 mg/d orally for three months and the control group also consisting of 60 males was given a placebo. NAC considerably improved the motility, volume, and viscosity of semen. The assessment of ROS formation and sperm function prior to and following the administration of NAC was performed in the study of Akiyama (1999) and the researcher observed no improvement in sperm density and sperm motility, however, there was a tendency for sperm function to get better, and a significant decrease in the ROS level in human sperm was determined following the administration of NAC. A possible toxic impact of 5 and 10 mM NAC doses on ram semen in the process of cryopreservation was reported in the study of Yıldız et al. (2015). Bisedes same researchers identified the harmful impacts of higher NAC doses on sperm motility. Contrary to the results of the above-mentioned studies, an improvement in sperm motility following the administration of NAC was determined in this study.

In this study, the MDA content of the testis considerably increased as a result of treatment with GLF. Increased MDA can demonstrate an increase in ROS production, which may damage sperm and other cytoplasmic organelle membrane structures as a result of lipid, protein and nucleotide peroxidation, therefore, leading to a change in sperm motility. A physiological impact of glutathione (GSH) is known in the repair of cellular oxidative damage through the formation of disulfide via the action of glutathione peroxidase (Raina et al., 2009a). In this study, a reduction in GSH was observed in GLF exposure. Superoxide is the enzyme that catalyzes the dismutation of superoxide, H_2O_2, the first step of advocating the dismutase antioxidant defense. A reduction in the testicular SOD activity was determined in GLF exposed group when compared to the control group. The oxidative stress induced by GLF exposure may have depleted the cellular SOD level (Raina et al., 2009b). Oxidative stress and multiple stress-response pathways were caused by GLF, which induced a death of Sertoli cells in the testis of prepubertal rats. A Ca^{2+} overload and a cell signaling misregulation were stimulated by glyphosate. The cellular stress response and/or the decreased antioxidant defenses might have an impact on the disruption of Sertoli cells. Therefore, they might affect spermatogenesis and the fertility of males (de Liz Oliveira Cavalli et al., 2013). Jasper et al. (2012) observed that glyphosate caused ROS generation in the exposed rats' testes, therefore, leading to polyunsaturated fatty acid peroxidation in the membrane of the testes that caused MDA, which represents one of the by-products of lipid peroxidation, to form. Upon conducting in vitro studies, a noticeable scavenging impact of NAC against ROS was confirmed. The structure and chemical reaction of NAC, which bears a similarity to that of glutathione, explain the above-mentioned antioxidant features. It is considered to function primarily as a pre-indicator of intracellular cysteine and glutathione and as a stimulator of cytosolic enzymes taking part in glutathione metabolism. Furthermore, NAC can function by entering into direct chemical reaction with radical species and/or ROS-dependent by-products (Cocco et al., 2005). Reddy et al. (2011) reported that the administration of intraperitoneal NAC in the dose of 75 mg/kg/day to mice induced with 4 ppm sodium arsenide for drinking water for 35 days reduced the oxidative damage of NAC. Farombi et al. (2008) reported that NAC considerably alleviated the toxic impact of tetracycline on the parameters of sperm, the negative histopathologic alterations caused by antibiotic were not enhanced by the antioxidants, and NAC considerably decreased the toxic impacts of tetracycline on the antioxidant and testicular marker enzymes along with oxidative stress markers. The contribution of oxidative stress to defective spermatogenesis as a result of decreasing the antioxidant level in the testes and therefore causing the infertility of males was reported in the study of Nithya and Elango (2015). Baker et al. (1996) indicated that the protection of spermatozoa against the damaging impact of leucocyte derived ROS on sperm movement in humans was ensured by the high concentrations (10 mM) of antioxidants, including glutathione and NAC. Arı et al. (2016) reported that NAC in medium doses, for example, 0.5 mM and below, added in skim milk based extenders may be utilized for the protection of ram sperm cells against oxidative stress. This study showed that NAC inhibited ROS-related oxidative stress (MDA, GSH and SOD) in animals exposed to GLF as a result of its antioxidant activity. The findings in question are consistent with the studies reported above.

Cells might possess higher sensitivity to chemical agents, which are capable of binding DNA, leading to damage, as a result of which single-strand DNA breaks may occur (Kumaravel and Jha, 2006). The adverse impacts of glyphosate on reproductive parameters in zebrafish Danio rerio, including damage to sperm DNA, decreasing the mitochondrial membrane integrity and functionality, were reported in the study of Lopes et al. (2014). In the present study, GLF damaged sperm DNA and caused a more significant DNA damage in comparison with the control and other

groups but NAC alleviated DNA damage, and the reason for this may be the pharmacological impacts of NAC particularly on antioxidant activities. Erkkıla et al. (1998) reported that NAC considerably prevented apoptosis in the testicular germ cells of humans in vitro, therefore demonstrating the important role of antioxidative mechanisms for the survival of germ cells in the seminiferous tubules in spermatogenesis. Li Ji et al. (2013) reported the protective effect of NAC from germ cell apoptosis caused by cadmium as a result of its preventing endoplasmic reticulum stress in the CD-1 mice testes. Whitker et al. (2012) showed that adding of 1.0 mM NAC enhanced the usability of frozen-thawed boar sperm in IVF since it causes a decrease in the DNA fragmentation and lipid peroxidation of the sperm.

The degenerative changes observed in the seminiferous tubule and interstitial cells of the testes of rats signify that GLF is toxic to the reproductive system of male rats. Romano et al. (2010) detected a decrease in epithelium depth and bigger diameter from the lumen in the seminiferous tubule of rats to which GLF was administered. This result is consistent with the present study, GLF caused a decrease in sperm concentration in the lumen and degeneration of Sertoli cells in the testis. It is possible to inhibit the GLF toxicity and preserve the normality of the testicular architecture as a result of treatment with NAC.

As a result of the current study, it has also been determined that administering NAC decreases oxidative stress and DNA damage caused by GLF through reducing lipid peroxidation and causing antioxidant enzyme activation in the testis. Therefore, it improves suppressed reproduction in rats which has been induced by GLF.

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RESEARCH ARTICLE

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Effect of Immunocastration Vaccine Administration At Different Doses on Performance of Feedlot Holstein Bulls

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ABSTRACT

The aim of the study is to determine the effect of immunocastration vaccine administration at different doses on fattening performance of feedlot Holstein bulls. In this research, 94 Holstein male calves assigned to the 4 treatments. Control group; 1 mL of 0.9% saline solution was subcutaneously injected to intact bulls on 1st and 60th days of the feedlot as placebo. On the same days of the feedlot, Immunocastration vaccine (Bopriva®) at two doses of 1 mL and 1 mL for Trial-1 group, 1.5 mL and 1.5 mL for Trial-2 group, 1.5 mL and 1 mL for Trial-3 group were subcutaneously injected to bulls. The feedlot lasted 180 days. Immunocastration vaccine administration at different doses did not affect the live weights (LWs) and cold carcass yields of feedlot Holstein bulls ($P>0.05$). However, it reduced fattening performance between 61-120 days ($P<0.05$) and 1-180 days ($P<0.01$). As a result, it was decreased the fattening performance that administration of Bopriva® at different doses as a GnRH vaccine in Holstein male bulls; whereas it was determined that numerically increase in average daily live weight gain was found in the Trial-2 group than the other groups to which the immunocastration vaccine was applied.

Keywords: GnRH, fattening, immunocastration

Farklı Dozlarda İmmunokostrasyon Aşı Uygulamasının Entansif Koşullarda Yetiştirilen Holstein Erkek Danalarının Besi Performansı Üzerine Etkisi

ÖZ

Bu çalışmanın amacı, farklı dozlarda GnRH aşısının Holştayn erkek buzağılarında besi performansı ve karkas randımanı üzerine etkisinin belirlenmesidir. Araştırmada, 94 baş Holştayn ırkı erkek buzağı kullanılmış ve rastgele 4 gruba ayrılmıştır. Besinin 1. ve 60. gününde kontrol grubuna plasebo olarak 1 mL %0.9'luk tuzlu su çözeltisi derialtı yolla enjeksiyon yöntemi ile uygulanmıştır. Besinin aynı günlerinde, Deneme-1 grubundaki buzağılara 1 mL ve 1 mL, Deneme-2 grubundaki buzağılara 1.5 mL ve 1.5 mL ve Deneme-3 grubundaki buzağılara ise 1.5 mL and 1 mL olmak üzere iki doz immunokastrasyon aşısı (Bopriva®) derialtı yolla enjeksiyon yöntemi ile uygulanmıştır. Besi 180 gün sürüştür. Farklı dozlarda immunokastrasyon aşısı uygulamasının entansif koşullarda yetiştirilen Holştayn erkek danalarında, canlı ağırlık ve karkas randımanı üzerine etkisinin olmadığı belirlenmiştir ($P>0.05$). Buna karşın immunokastrasyon aşısı uygulamasının besinin 61-120 ($P<0.05$) ile 1-180. gün ($P<0.01$) arası dönemde besi performansını azalttığı belirlenmiştir. Sonuç olarak, Holştayn erkek danalarında GnRH aşısı olarak Bopriva®'nın farklı dozlarda uygulamasının; besi performansını düşürdüğü buna karşın immunokastrasyon aşısı uygulanan gruplar içinde rakamsal olarak en yüksek canlı ağırlık artışının Deneme-2 grubunda olduğu tespit edilmiştir.

Anahtar Kelimeler: GnRH, besi, immunokastrasyon

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INTRODUCTION

Castration of male animals is a widespread farming method reported in the literature for more than 50 years and is used world-wide in controlling fertility. Castration has been commonly conducted to enhance growth, metabolism, carcass, and meat quality through decreased pH in the carcasses. It has also been used to improve body fat deposition, reduce aggressive and sexual behaviour for handling the animals in an easier manner, to obtain less carcass damage and to improve animal welfare for animal producers, consumers and owners. Unless the animals are not castrated, they may become dangerous because of aggressive behaviours among themselves and to the people who handle them (Wierbicki et al. 1955, Field 1971, Lofthouse and Kemp 2002, Duff and McMurphy 2007, Freitas et al. 2008, Amatayakul-Chantler et al. 2012, Miesner and Anderson 2015).

Surgical castration is commonly applied, although different castration methods are applied in animals. However, surgical castration (i.e. gonadectomy) usually comes with complications (stress, pain, discomfort etc..) and consequent reductions in post-castration performance (decrease in feed efficiency and rate of growth, and elongated deterioration in productivity etc.) (Marti et al. 2015, Ison et al. 2016).

Vaccination for gonadotropin-releasing hormone (GnRH), which is also called as immunocastration, is considered to be an animal-friendly alternative for surgical castration has received particular attention in male and female mammals. Both for males and females, GnRH, a hypothalamic hormone, has an important role in the regulation of reproductive functions. For this reason, immunization for GnRH (GnRH vaccine) ends up in the neutralization of endogenous GnRH with the subsequence suppression of the gonadotropin-luteinizing hormone (LH) and follicle-stimulating hormone (FSH) expression by anterior pituitary. As a result of this, testicular testosterone and androsterone production is reduced (Bonneau and Enright 1995, Thompson 2000).

GnRH secretion have marked increases after 4 months of age (happening at the same time with the increase in the secretion of LH) in Bull calves, at which time prepubertal transition and testicular development begins (Rodriguez and Wise 1989, 1991). However, benefits on carcass enhancement and testicular growth resulted with one immunization in 4 - 12 months of age (Adams et al. 1996).

The potential to use GnRH vaccine has caused specific attention in major livestock including cattle (Robertson et al. 1979, Finnerty et al. 1998, Huxsoll et al. 1998), goats (Godfrey et al. 1996), pigs (Caraty and Bonneau 1986, Molenaar et al. 1993, Meloen et al. 1994) and sheep (Clarke et al. 1978, Brown et al. 1995, Clarke et al. 1998).

A cattle-specific GnRH vaccine (for immunocastration) (Bopriva®, Zoetis Australia Ltd., West Ryde, Australia) was approved to be used in heifers and bulls in New Zealand, Australia, Mexico, Brazil, Argentina, Turkey, and Peru (Balet et al. 2014). The immunocastration vaccine is applied in 2 doses. With the 1st dose, the bovine immune system is prepared; and the immune response is activated with the 2nd dose. The animal is deemed immunocastrated only when the second dosage (i.e. the booster) is applied (Hennessy 2008). Suppression of GnRH in the hypothalamic axis through antibody induction by GnRH vaccine, reduced the testosterone concentration released, and as a result, the function of the gonads (Sherwood et al. 1993).

It has been reported in several studies conducted before that immunological castration may be very effective to prevent aggressive and sexual behaviour in bulls (Jago et al. 1997a, Marti et al. 2015, Price et al. 2003), but, literature data show that there is no clear effect of immunocastration on performance. The growth of immunocastrated animals was reported to be equal to castrates and less in intact bulls (Cook et al. 2000; Ribeiro et al. 2004, Hernández et al. 2005), intermediate between those that are intact and castrates (Adams et al. 1996, Aïssat et al. 2002) or equal to bulls that are intact (Adams and Adams 1992, Finnerty et al. 1994, Adams et al. 1996, Huxsoll et al. 1998, D'Occhio et al. 2001, Amatayakul-Chantler et al. 2012, Pérez-Linares et al. 2017).

In order to cover the increasing red meat demands of the ever-increasing population of the world, different strategies have been developed and different husbandry methods are used as well as castration. Although those who deal with livestock for meat have used high meat yield cattle bred as Angus and Charolais, they thought of fattening Holstein bull calves as an option, provided that they yield certain advantages to cattle producers like obtaining high-quality carcass (Duff and McMurphy 2007).

When it is considered that the studies in which the effects of immunocastration on growth performance are investigated are limited in number, and the fact that Holstein male calves are

used to produce red meat by producers of livestock for meat are considered together, the purpose of the present study is to define the immunocastration dose that ensures the best breeding performance and to investigate the effects of immunization against gonadotropin-releasing hormone at different doses on feeding performance in Holstein male calves.

MATERIAL and METHODS

All animal-use protocols were carried out in accordance with Directive 2010/63/EU of the European Parliament and Council of 22 September 2010 on the protection of animals used for scientific purposes (EUD 2010). Research was conducted according to the institutional committee on animal use (protocol/file number 2016/16).

A total of 94 Holstein male calves (309.5 ± 2.58 kg LW and 267 days old) were distributed to one of the 4 treatment groups: intact bulls (Control), animals vaccinated with first and second (60 days after the first vaccination and starter of the feedlot) dose of with GnRH (vaccinated) which dose are 1.0 mL and 1.0 mL (Trial-1), 1.5 mL and 1.5 mL (Trial-2), 1.5 mL and 1.0 mL (Trial-3), respectively. The study was conducted in a private farm in Sirvan County of the Siirt province.

Between the arrival and the time when the trial started, the animals were handled in an equal manner. During the trial, animals were blocked based on BW. The animals were fed with the same feed (50.0% corn, 15.0% barley, 10.0% soybean meal, 12.2% sunflower meal, 1.75% limestone, 0.50% salt, 0.25% DCP, 0.3% premix; 16.1% CP, 5.2% ash, 11.2 Mcal MJ/kg; DM basis) and barley straw (4.1% CP, 6.3% ash; DM basis) ad libitum throughout the experiment. On day 0 and 60 of the feedlot, different dozes of GnRH vaccine (Bopriva®, Zoetis, Turkey) was given subcutaneously to animals in treatment group on neck's left side with a 12.5-mm 16-gauge needle in one dose with a safety vaccinator. On the same days of the feedlot, 1.0 mL of 0.9% saline solution was injected subcutaneously to control group as placebo.

In order to adopt the calves to the feed that will be used in breeding in 14 days, the feed was increased slowly before the study started. The animals were weighed with a scale in every 15-day period to determine their LWs. The feeding lasted for 180 days. With the help of the LWs taken initially, at the end of the feeding period, and in 15-day periods, the LW and average daily live weight gain (ADG) were determined in various periods. In addition, 12 animals were slaughtered from each group after the feeding period, and the hot and cold carcass yields were determined.

The statistical analysis for normal distribution data of the treatment groups was carried out with the general linear model procedure of SPSS software 20.0 (SPSS Inc., Chicago, IL, USA). The results are given as mean ± standard deviation. Duncan's multiple range test was employed for multiple comparisons in important groups. Data points with different letters were considered to be different at a significant level ($P \leq 0.05$).

The data were statistically analyzed using general linear model procedure adopted by SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) statistics software with One-way ANOVA. The results are given as mean ± standard deviation. Data points with different letters were considered to be different at a significant level ($P \leq 0.05$). Statistical significant effects were further analyzed and means were compared using Duncan's multiple range test.

RESULTS

In different periods of the feeding, it was determined that the immunocastration application at different doses did not have any effects on LW of the Holstein male calves ($P<0.05$). However, applying immunocastration at different doses reduced the ADG in the period between days 61 and 120 ($P<0.01$) and throughout the feeding period (days 1-180) ($P<0.05$) and also reduced the ADG. In other words, it was determined that the ADG of the calves in the Control Group were higher than the ADG of the calves throughout the feeding and between the days 61-120 when compared with the trial groups. In addition to this, it was determined that there were no statistically significant differences between the trial groups in terms of ADG ($P>0.05$) (Table 1).

It was also determined that applying immunocastration at different doses did not affect the hot carcass weight, hot carcass yield, cold carcass weight, and cold carcass yield of Holstein male calves ($P>0.05$) (Table 2).

Table 1. Effect of Immunocastration Vaccine Administration at Different Doses on live weight and daily live weight gain in various periods in Feedlot Holstein Bulls
Tablo 1. Holştayn Erkek Danalarında Farklı Dozlarda İmmunokastrasyonun çeşitli dönemlerdeki canlı ağırlık ve günlük canlı ağırlık artışı üzerine etkisi

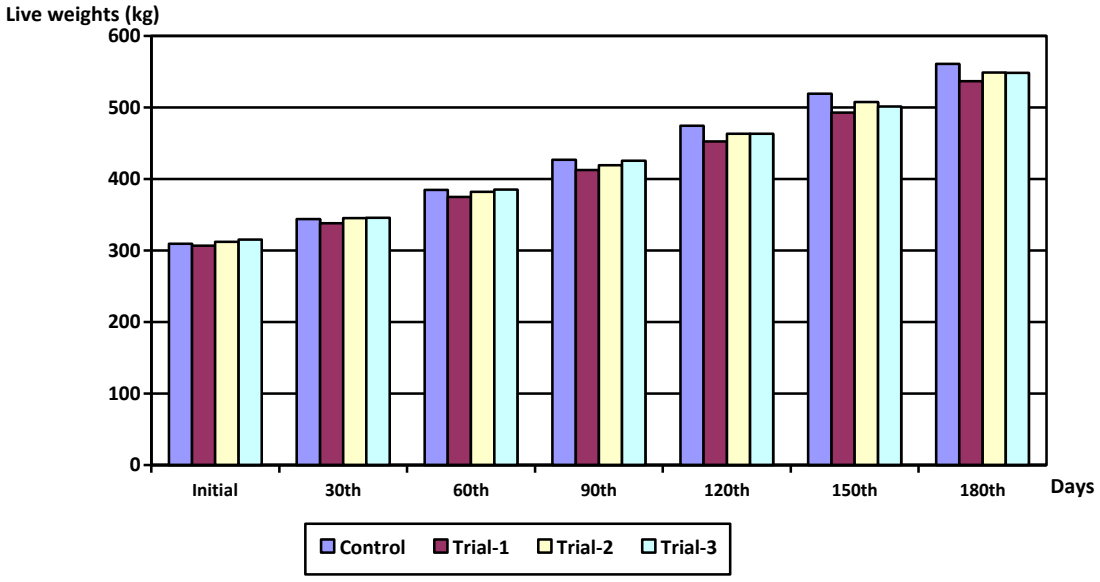
	Control	Trial-1	Trial-2	Trial-3	P-Value
			Live Weight		
Initial	309.21±5.49	306.62±4.22	312.11±5.45	315.39±5.23	0.652^{ns}
30th	343.72±5.29	338.30±4.28	345.41±6.39	345.70±7.06	0.693^{ns}
60th	384.72±5.46	374.95±4.40	381.96±6.77	385.05±8.59	0.501^{ns}
90th	426.93±6.28	412.39±4.70	419.13±7.74	425.44±10.21	0.325^{ns}
120th	474.32±6.48	452.53±4.83	463.18±7.81	462.95±10.7	0.137^{ns}
150th	518.98±6.68	492.62±4.94	507.31±7.25	501.19±11.43	0.053^{ns}
180th	560.88±8.27	536.67±5.35	548.56±8.36	548.25±11.95	0.144^{ns}
			Average Daily Live Weight Gain		
1-60th	1.26±0.05	1.14±0.02	1.16±0.06	1.16±0.07	0.210^{ns}
61-120th	1.49±0.05^{a}	1.29±0.02^{b}	1.35±0.05^{b}	1.30±0.05^{b}	0.001**
121-180th	1.44±0.08	1.40±0.03	1.42±0.06	1.42±0.04	0.938^{ns}
1-180th	1.40±0.04^{a}	1.28±0.02^{b}	1.31±0.03^{b}	1.29±0.04^{b}	0.016*

$^{a, b}$: Means with different superscripts in the same column differ significantly (P<0.05).
^{ns}: non-significant (P>0.05); *: P<0.05; **: P<0.01.

Table 2. Effect of immunocastration vaccine administration at different doses on carcass weight and percentage in feedlot Holstein bulls
Tablo 2. Holştayn Erkek Danalarında Farklı Dozlarda Bopriva ile İmmunokastrasyonun karkas ağırlığı ve oranı üzerine etkisi

	Control	Trial-1	Trial-2	Trial-3	P-Value
Hot carcass weight	291.86±10.55	279.21±4.94	282.96±5.01	289.93±6.28	0.451^{ns}
Hot carcass percentage	52.87±0.62	51.45±0.40	52.02±0.49	51.97±0.44	0.308^{ns}
Cold carcass weight	284.81±10.34	273.07±4.87	278.99±5.52	283.26±6.23	0.517^{ns}
Cold carcass percentage	51.59±0.61	50.32±0.40	50.85±0.46	50.77±0.45	0.398^{ns}

^{ns}: non-significant (P>0.05).



Graphic 1. The live weights at different periods in feedlot Holstein bulls
Grafik 1. Holştayn erkek danalarda besinin farklı dönemlerdeki canlı ağırlıklar

DISCUSSION

The LW, ADG, and carcass weight and yield are significant properties for farmers, livestock producers and industry. As well as providing heavier commercial cuts, heavier carcasses allow to diffuse costs by optimizing the industrial process. They are also payment to producers. In addition to these, it is significant to have a pattern as carcasses of different weights make it compulsory to have similar labour and process time; however, they have clear industrial profitability (Pazdiora et al. 2013).

It was determined that the GnRH vaccine at different doses did not have any effects on the LW of the animals in different periods of the trial in Holstein bulls vaccinated on days 0 and 60. These results were similar to those reported by Adams and Adams 1992, Freudenberger et al. 1993, Finnerty et al. 1994, Huxsoll et al. 1998, D'Occhio et al. 2001, Amatayakul-Chantler et al. 2012, Marti et al. 2013, Pérez-Linares et al. 2017, and different from those reported by Adams and Adams 1992 and Amatayakul-Chantler et al. 2013.

The results of previous studies show that the magnitude of the response in immunocastration has different effects for bulls. The heterogeneity in the results reported previously stems from the use of different vaccine formulation in previous studies, applying different vaccine programs (one, two or three booster doses, different duration of effect from booster-slaughter date), using different race, using implant or not, the difference in husbandry or management practice and from the different types of feed.

Steroid hormones stimulate hypertrophy of the neck, chest and rump muscles, and provide a more forequarter yield (Pazdiora et al. 2013). The impact of testosterone on intact males that develop muscles throughout life occur because of increased nitrogen retention (Galbraith et al. 1978). Prior et al. 1983 claimed that testosterone had an effect that inhibits lipogenic enzyme activities in adipose tissue and induces higher basal lipolytic rates. GnRH-vaccinated cattle, other factors, such as modified sexual or aggressive behaviour may assist in maintaining growth compensating for the decreased natural anabolic hormone testosterone concentrations (Jago et al. 1997b, Price et al. 2003, Amatayakul-Chantler et al. 2012).

In the present study, it was determined that the LW of the calves to which immunocastration was applied were lower than the calves that were included in the Control Group in terms of numbers. In addition, this situation may be referred to the fact that the LW of the calves to which immunocastration is applied may be lower than the calves included in the Control Group in terms of numbers depending on the longer duration for fat deposition in the calves to which immunocastration is applied when compared with the intact calves and with the foresight claiming that testosterone has a lipogenic inhibitory effect on the enzymatic activity of the fat tissue that increases the basal level of the lipolytic activity because of the anabolic effect of testosterone explained above (Coutinho et al. 2006, De Freitas et al. 2015, Andreo et al. 2016).

In the present study, the result showing that applying immunocastration at different doses on days 61-120 ($P<0.01$) and throughout the feeding period (days 1-180) ($P<0.05$) reduces ADG was similar to the result reported by Marti et al. 2017 and Moreira et al. 2017; and different from the result reported by Adams et al. 1993, Huxsoll et al. 1998, Cook et al. 2000, Amatayakul-Chantler et al. 2012, Pérez-Linares et al. 2017.

It was determined that applying immunocastration at different doses did not affect the carcass weight and yield in Holstein male calves; however, this application reduced the carcass weight and yield in terms of numbers when compared with the Control Group. It was verified in previous studies that there are no differences in carcass dressing % between intact bulls and the animals that were vaccinated (Adams and Adams 1992, Adams et al. 1993, Freudenberger et al. 1993, Ribeiro et al. 2004, Amatayakul-Chantler et al. 2012, Marti et al. 2013. On the other hand, unlike our study, some previous studies reported that carcass percentage of bulls was higher compared to that of vaccinated animals (Huxsoll et al. 1998, Cook et al. 2000). The reduced carcass percentage in vaccinated animals compared to bulls may be explained by the taking away of the excessive fat around the kidneys and heart, and from the pelvis of the carcasses.

CONCLUSIONS

Physical castration causes stres and reduces performance in animals, on the other hand active immunization against GnRH maintains (or with slight reduce) performance by maximizing welfare in bulls, and controls unwanted sexual and aggressive behavior. Considering these facts about physical- and immunocastration, it was decreased the fattening performance that administration of Bopriva® at different doses as a GnRH vaccine in Holstein male bulls; whereas it was determined that numerically increase in average daily live weight gain was found in the Trial-2 group than the other

groups to which the immunocastration vaccine was applied.

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RESEARCH ARTICLE

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Bozdoğanda (*Falco columbarius*) Pecten Oculi'nin Morfolojisi ve Stereolojik Metot ile Hacminin Hesaplanması[#]

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ÖZ

Corpus vitreum içerisinde yer alan pecten oculi yüksek damarlaşma ve pigmentasyona sahiptir. Retinanın beslenmesinde ve hareket eden objelerin iyi görülmesi için yardımcı bir aygıt olduğundan gündüz avlanan kuşlar için büyük önem taşır. Araştırmada 5 adet ergin erkek bozdoğanın (*Falco columbarius*) pecten oculi'sinin morfolojik ve stereolojik incelemeleri yapılmıştır. Morfolojik incelemeler sonucu bozdoğan pecten oculi'sinin plikalı tipte ve 17-18 adet kıvrıma sahip olduğu, şeklinin çeşitkenar yamuğa benzediği saptandı. Bu yapıların ortalama uzunlukları ise 12.26 ± 0,21 mm (bazal), 7.97 ± 0,08 mm (apikal), 5.83 mm ± 0,12 (bazal-apikal arası) olarak tespit edildi. Stereolojik hacmi ise ortalama 17.28 ± 0,28 mm^3 olarak tespit edilmiştir. Bozdoğanda pecten oculi'nin morfolojik yapısı ortaya konarak stereolojik metotla hacmi hesaplanmış olup, tür ayrımında birçok çalışmaya kaynak olabileceği düşünüldü.

Anahtar Kelimeler: Bozdoğan, Falco columbarius, Morfoloji, Pecten oculi, Stereoloji

Morphology and Volume Measurement of Pecten Oculi by Stereology in Merlin (*Falco columbarius*)

ABSTRACT

Pecten oculi seeded in corpus vitreum poses high vascularisation and pigmentation. It very important for day-time hunter birds because it is involved in retinal nutrition and contributes for better vision to moving objects. In this study, morphologic and stereologic evaluation of pecten oculi in 5 Merlin (*Falco columbarius*) was carried out. It was observed that pecten oculi of merlin was plicated type with 17-18 convulations and resembled scalene trapezium in shape. Average lenght of these structures were 12.26 ± 0,21 (basal), 7.97 ± 0,08 mm (apical) and, 5.83 mm ± 0,12 (basal and apical intermission). The avarage stereological volume was 17.28 ± 0,28 mm^3. It was concluded that morphology and the volume of pecten oculi by stereological approach were determined and the data generated here may be useful for the differentiation of species.

Keywords: Falco columbarius, Merlin, Morphology, Pecten oculi, Stereology

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GİRİŞ

Bozdoğan Amerika, Avrupa ve Asya'nın Kuzey bölgelerinde, Ortadoğu ve Orta Asya'da yaşar (Gooders, 1995).Orta boyda bir doğan türü olup, yaklaşık mavi alakarga boyutunda bir kuştur. Uzun ve sivri kanatlara sahip olup hızlı kanat çırpmaları ile karakterizedirler. (Johnsgard, 1990). Erkeğin üst kısımları ve kuyruk üstü açık mavimsi gridir. Kuyruğun ucunda kalın siyah bir bant bulunur. Alt tarafı açık portakal renginde ve boyuna koyu çizgilidir. Dişilerin üst kısımları kahverengi, alt kısımlar boyuna çizgili olup bu çizgiler erkeğinkinden daha kalındır ve kuyruğunda enine bantlar bulunur (Johnson ve Coble, 1967; Jordan ve Shelton, 1982).

Pecten oculi kanatlılara özgü bir yapıdır. Bu yapı discus nervi optici'nin tabanında retina'dan orijin alıp corpus vitreum içerisine doğru uzanır (Brach, 1977; Dursun, 2002; Gültiken ve ark., 2012). Pecten oculi, humour vitreus içerisine kıvrımlı olarak girer, lense kadar ulaşmaz (Dursun, 2002, Nickel ve ark., 1977). Kuş türlerinde pecten oculi'nin anatomik yapısı farklılıklar gösterir. Kiwi türü kuşlarda (Yeni Zelanda'ya özgü bir kuş) koni (Meyer, 1977), devekuşunda pervane (Kiama ve ark., 2006) ve diğer kuş türlerinde ise plikalı olmak üzere üç tipten bahsedilmektedir (Baumel ve ark., 1993).

Pecten oculi, yüksek damarlaşma ve pigmentasyona sahiptir (McLelland, 1990; Micali ve ark., 2012). Kanatlılarda a. centralis retinae bulunmadığından pecten oculi, corpus vitreum'un besin ve sıvı dağılımına hizmet eder. Corpus vitreum içine uzanan pecten oculi'nin diffüzyon yoluyla retina'nın oksijen ve diğer gıdalarla beslenmesinde rol oynadığı, bunun yanında humor vitreus ile pecten oculi arasında meydana gelen bazı kimyasal değişikliklere de ev sahipliği yaptığı ileri sürülmektedir (Dursun, 2002; Nickel ve ark., 1977; Mclelland, 1990; Hazıroğlu ve Çakır, 2017).. Aynı zamanda soğuk havalarda ani hareket esnasında gözün ısınmasını sağlar (Doğuer ve Erençin, 1964; McLelland, 1990). Ayrıca hareket eden objelerin iyi görülmesi için yardımcı bir aygıttır. Pecten oculi'nin gölgesinin gözün arka planına düşmesi vasıtasıyla hareketin fark edilme derecesi yükselir. Bundan dolayı gündüz avlanan kuşların pecten oculi'si büyük, dane yiyen kuşlar ile gece kuşlarının pecten oculi'si ise küçüktür.

Yapılan bu araştırma ile bozdoğanlarda pecten oculi'nin morfolojik ve stereolojik olarak incelenmesi, diğer kanatlı türleri ile benzerlik ve farklılıklarının ortaya konulması amaçlanmıştır. Böylece bu konuda çalışma yapacak araştırmacılara ve literatür bilgisine katkı sağlayacağı düşünülmüştür.

MATERYAL ve METOT

Bu araştırmada 2003 ile 2009 yılları arasında Afyon Kocatepe Üniversitesi Veteriner Fakültesi kliniklerine getirilen, tedavi edilemeyecek durumda olan beş adet ergin erkek bozdoğan kullanıldı. Cerrahi Anabilim Dalı tarafından ketamin (60 mg/kg) ve xylazine (6 mg/kg) kombinasyonu ile ötenazi edilen kuşlar Anatomi Anabilim Dalı tarafından %10'luk formaldehit solüsyonu içerisinde tespit işlemine tabi tutuldu. Pecten oculi'yi elde etmek için kadavralar stereo mikroskop (Olympus optical Co. Ltd. Tokyo. Japonya) altında diseke edilerek bulgular alındı ve Mitotoyo marka dijital kımpas ile ölçümler yapıldı. Elde edilen bulgular dijital fotoğraf makinesi (Sony DSC F 717 Japon) ile fotoğraflandı. İsimlendirmelerde Nomina Anatomica Avium (Baumel ve ark., 1993) esas alındı.

Morfolojik inceleme sonrasında materyaller histolojik doku takibi ve parafin bloklara gömülme işlemlerine tabi tutuldu. Parafin bloklara gömülen örneklerden stereolojik inceleme amaçlı 40 µm kalınlığında seri kesitler alındı. Seri kesitlerin her dokuzuncu kesiti değerlendirmeye tabi tutularak örnekleme yapıldı. Elde ettiğimiz kesitler jel kaplı lamlara alınarak Hematoksilen-Eozin boyama prosedürü ile boyandı ve entellan vasıtasıyla lamların üzerine lameller kapatıldı. Elde ettiğimiz seri kesitlerden motorize tablaya (Lang MS 316) sahip Olympus MD2 ışık mikroskobuna entegre M-Shot MDX4 marka mikroskop kamerasında, M-Shot Digital Imaging System 9.3.3.1 ve Stereom I (Oğuz ve ark., 2007) yazılımları vasıtasıyla uzunluk ve hacim ölçümleri yapıldı.

Alan ölçümleri yaparken nokta uzaklığı 1 mm olan noktalı alan cetveli kullanıldı ve aşağıdaki formül kullanılarak pecten oculi hacmi hesaplandı;

$V = (t \cdot a/p \cdot \Sigma P)\ cm^3$

t : kesit kalınlığı (~ 1.7 mm),

a/p : noktalar arası alan (1 mm x 1 mm),

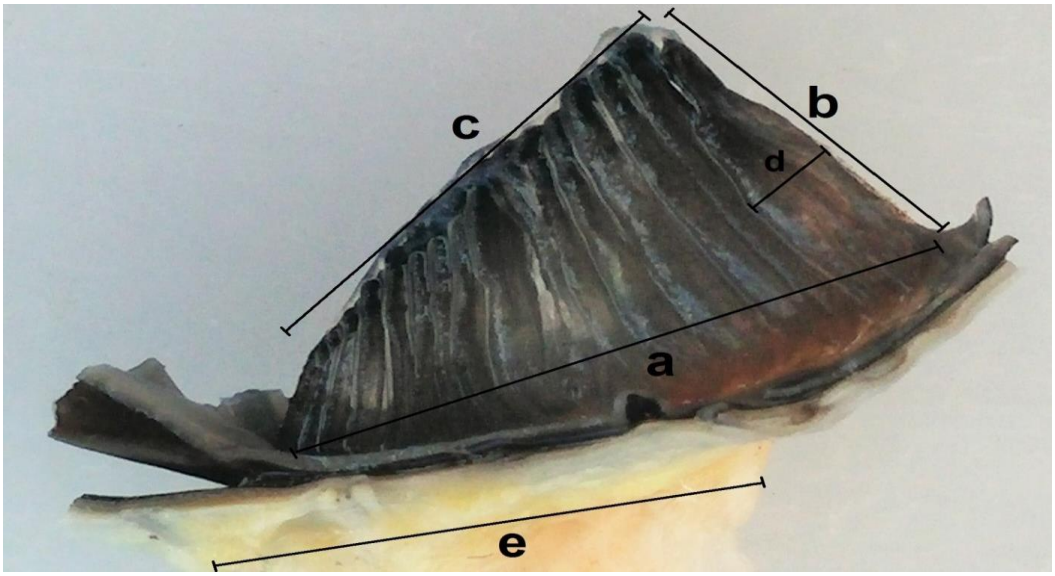
ΣP : Kesit yüzeyinde düğüme denk gelen noktaların sayısı

BULGULAR

Bozdoğanda yapılan araştırmada pecten oculi'nin discus nervi optici yakınında retina'ya bağlanan ve corpus vitreum içerisine uzanan kıvrım şeklindeki yapılardan oluştuğu belirlendi (Şekil 1).

Şekil 1. Bulbus oculi ile birlikte pecten oculi.
Figure 1. Pecten oculi with bulbus oculi.

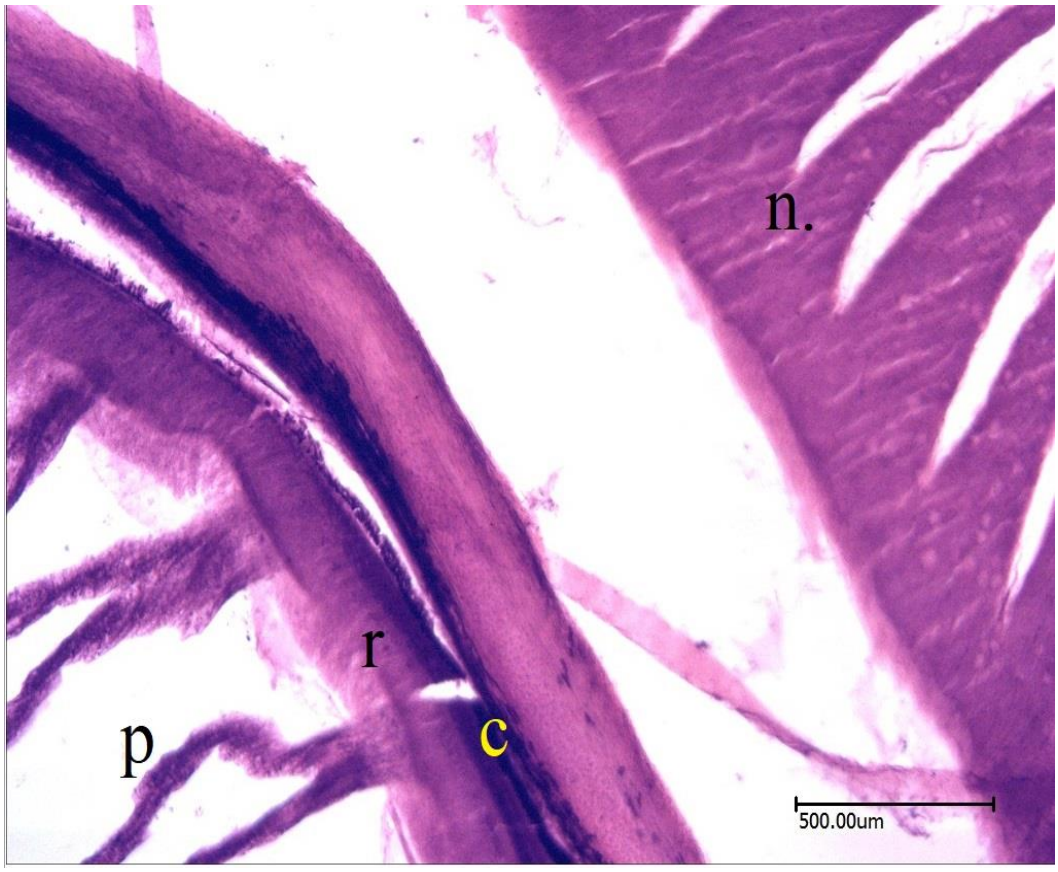
Bu yapının posterio-anterior yönde seyrettiği belirlendi. Bazal kısmı testere ağzı şeklinde olup buranın retina'ya bağlandığı gözlendi. Bazal uzunlukların ortalaması ile standart sapma değeri 12.26 ± 0,21 mm, apikal uzunlukların ortalaması ile standart sapma değeri 7.97 ± 0,08 mm, bazal ve apikal arasındaki uzunlukların ortalaması ile standart sapma değeri ise 5.83 mm ± 0,12 olarak tespit edildi. Pecten oculi'nin pigmentasyon yoğunluğu nedeniyle kahverengi siyahımsı bir renge sahip olduğu belirlendi. Şeklinin çeşitkenar yamuğa benzediği saptandı. Bu yapının ortalama 17-18 adet kıvrıma sahip olduğu görüldü (Şekil 2).



Şekil 2. Pecten oculi ve nervus opticus. (a: bazal uzunluk, b: bazal ile apikal arası uzunluk, c: apikal uzunluk, d: plikalar, e: n. opticus)
Figure 2. Pecten oculi and nervus opticus. (a: basal distance, b: basal to apical distance, c: apical distance, d: plicas, e: n. Opticus

İlk kıvrımın kendinden daha kısa sekonder bir kıvrımı ve bu kıvrımın da kendinden daha uzun tersiyer bir kıvrımı şekillendirdiği, sonrasında ise tek kıvrım şeklinde apikale doğru uzandığı belirlendi. Diğer kıvrımların apikale sekonder ve tersiyer kıvrım yapmadıkları tespit edildi. Bu kıvrımların apikalde ince bir bant ile birbirine bağlanarak serbest bir uç halinde corpus vitreum içinde lens'e doğru uzandığı görüldü.

Mikroskopik incelemelerimizde pecten oculi'nin nervus opticus'un retina'ya girdiği bölgede retina'ya bağlandığı (Şekil 3) ve apikal yöne kıvrımlar yaparak uzandığı belirlendi.

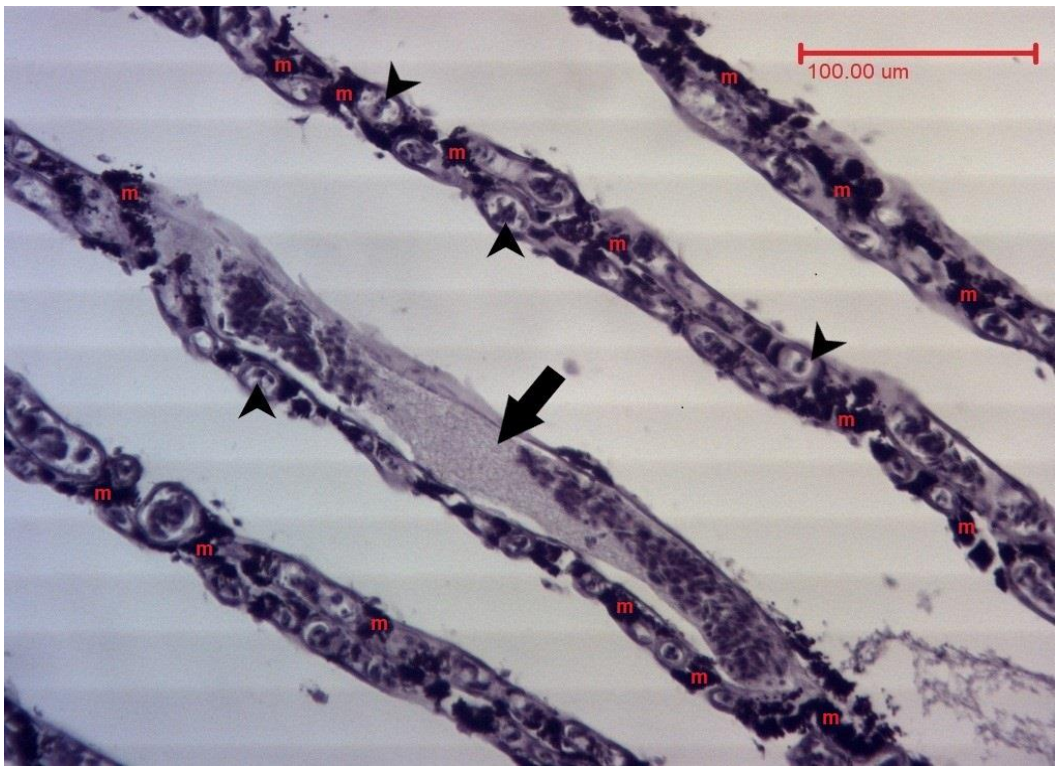


Şekil 3. Pecten oculi'nin bazali (4X büyütme). n: nervus opticus, c: choroidea, r: retina, p: pecten oculi

Figure 3. Pecten oculi baseline (4X magnification). n: nervus opticus, c: choroidea, r: retina, p: pecten oculi

Pecten oculi dokusunda iki tip damara rastlandı. Bunlardan büyük olanlar afferent ve efferent damarlar olup, bunların bazılarının lümenlerinde eritrositlerin varlığı tespit edildi. Daha küçük çapta fakat daha çok sayıda olan damarlar ise kapillar damarlardı (Şekil 4). Bunların dışında pecten dokusunun, bazalde daha seyrek olmakla birlikte apikale doğru artan miktarda ve kapillarlar arasında birikmiş bir şekilde melanin pigmenti içeren granüllere (melanosit) sahip olduğu da gözlendi (Şekil 4).

Son olarak elde ettiğimiz seri kesitler üzerinde noktalı alan cetveli ile yaptığımız ölçümler sonucunda (Şekil 5) ise pecten oculi'nin hacim ortalaması ve standart sapma değeri 17.28 ± 0,28 mm^3 olarak bulundu.



Şekil 4. Pecten oculi dokusundaki afferent-efferent damarlar (ok), kapillar damarlar (ok başı) ve melanositler (m). (20X büyütme)
Figure 4. Afferent-efferent vessels (arrow), capillar veins (arrowhead) and melanocytes (m) in the pecten oculi tissue (20X magnification)

Şekil 5. Stereom I programı vasıtasıyla pecten oculi hacminin hesaplanması
Figure 5. Volume estimation of the pecten oculi by Stereom I software

TARTIŞMA ve SONUÇ

Yapılan morfolojik araştırmalarda gözün yapısında bulunan pecten oculi'nin tipi, kıvrım sayısı, rengi ve uzunluğunun kuş türlerine göre farklılık gösterdiği belirtilmiştir (Baumel ve ark., 1993; Branch, 1977). Pecten oculi'nin devekuşunda (Baumel ve ark., 1993; Kiama ve ark., 2006) pervane, kiwi (Baumel ve ark., 1993) türünde ise koni tipinde olduğu bildirilmiştir. Bu yapının bozdoğanda; boynuzlu baykuş (Braekevelt, 1993), peçeli baykuş (Yılmaz ve ark., 2017), güvercin (Braekevelt, 1988; Dayan ve Özaydın, 2013; Korkmaz, 2017), ördek ve hindi (Dayan ve Özaydın, 2013), martı (Gezer-İnce ve ark., 2017), muhabbet kuşu (Micali ve ark., 2012) ve Japon bıldırcınında (Orhan ve ark., 2011; Pourlis, 2013) olduğu gibi plikalı tipte olduğu gözlendi. Pecten oculi'nin şekli de türlere göre farklılık göstermektedir. Devekuşunda (Kiama ve ark., 2006) koni, bıldırcında ikizkenar yamuk (Pourlis, 2013) ya da deniz kabuğu (Orhan ve ark., 2011), leylekte (Onuk ve ark., 2013) akordiyon, güvercinde (Braekevelt, 1988) fan, evcil kanatlılarda (Nickel ve ark., 1977) kama şeklinde iken bizim araştırmamızda pecten oculi'nin şeklinin çeşitkenar yamuğa benzediği saptandı. Rengi şahin (Gültiken ve ark., 2012), devekuşu, ördek (Dayan ve Özaydın, 2013), martı (Gezer-İnce ve ark., 2017) ve diğer pek çok kuş türünde (Baumel ve ark., 1993; Lord, 1956) olduğu gibi bizim araştırmamızda da kahverengi siyahımsı olarak tespit edildi. Pecten oculi'nin muhabbet kuşlarında (Micali ve ark., 2012) 10-12, devekuşunda (Kiama ve ark., 2006) 16-19, leylekte (Onuk ve ark., 2013) 15-17, güvercinde (Korkmaz, 2017) 14 ya da 15-17 (Braekevelt, 1988), Japon bıldırcınında (Pourlis, 2013) 18-22 ya da ortalama 19 (Orhan ve ark., 2011), şahinde (Gültiken ve ark., 2012) 17, serçede 20, çayırkuşunda 25, hindide 10, atmacada 15, kırmızı kuyruklu şahinde 16 (Lord, 1956), boynuzlu baykuşta (Braekevelt, 1993) 7-8, bizim araştırmamızda ise bozdoğanlarda 17-18 adet kıvrıma sahip olduğu belirlendi. Devekuşunda (Dayan ve Özaydın, 2013; Kiama ve ark., 2006) primer lamella'dan lateral'e daha ince sekonder bir lamella, bu lamellaların bazıları da tersiyer lamella verdiği bildirilirken, bizim araştırmamızda yalnızca primer kıvrımın kendinden daha kısa sekonder kıvrımı ve buda kendinden daha uzun tersiyer kıvrımı şekillendirerek tek kıvrım şeklinde apikale uzandığı belirlendi. Atmacada (Lord, 1956) basal uzunluk 4 mm, apikal uzunluk 2.75 mm, leylekte (Onuk ve ark., 2013) bazal uzunluk ortalama 10.05 mm, apikal uzunluk 6.07 mm, bıldırcında (Pourlis, 2013) bazal uzunluk 4-5 mm, apikal uzunluk 2.5 - 3 mm, şahinde (Gültiken ve ark., 2012) bazal uzunluk sağ gözde ortalama 11.13 mm, solda 10.9 mm, muhabbet kuşunda (Micali ve ark., 2012) bazal uzunluk 8-9 mm, boynuzlu baykuşta (Braekevelt, 1993) bazal uzunluk 5-6 mm, apikal uzunluk 3mm olduğu bildirilirken, bozdoğanda bazal uzunluk ortalama 12.26 mm, apikal uzunluk 7.97 mm olarak tespit edildi. Devekuşunda (Kiama ve ark., 2006) bazal ve apikal arasındaki uzunluğun 11 mm olduğu bildirilirken, bozdoğanda bu uzunluk 5.83 mm olarak ölçüldü.

Güvercin, muhabbet kuşu, martı, bıldırcın, leylek ve boynuzlu baykuş gibi türlerde gerek ışık mikroskobu ve gerek elektron mikroskobu ile yapılan çalışmalarda (Braekevelt, 1993; Orhan ve ark., 2011; Micali ve ark., 2012; Onuk ve ark., 2013; Gezer-Ince ve ark., 2017; Korkmaz, 2017) pecten oculi'nin pecten bazali vasıtasıyla nervus opticus'un retinaya girdiği bölgeye oturduğunu ve apikale doğru kıvrımlar yaparak devam ettiğini bildirilmiştir. Bu çalışmamızda bozdoğanda pecten oculi'nin benzer lokasyonda ve şekilde köken aldığı gözlemlendi. Korkmaz (2017) ile Dayan ve Özaydın (2013)'ın güvercinler üzerinde yaptıkları çalışmalarda pecten oculi'ye ait damarları büyüklüklerine göre primer, sekonder ve tersiyer damarlar olarak adlandırmışlardır. Diğer bazı çalışmalarda ise (Gezer-Ince ve ark., 2017; Micali ve ark., 2012; Onuk ve ark., 2013) damarlar afferent-efferent damarlar ile kapillar damarlar olmak üzere iki tipte incelenmiştir. Bizim araştırmamızda ise pecten oculi dokusunda daha büyük çaplı olan damarları afferent ve efferent damarlar, küçük çaplı ve daha yaygın olan damarları ise kapillar damarlar olarak nominaya göre adlandırıldı. Farklı kanatlı türlerine ait yapılan çalışmalarda pecten oculi'nin yapısında melanin pigmenti içeren granüllerden bahsedilmiştir (Korkmaz, 2017; Micali ve ark., 2012; Orhan ve ark., 2011; Braekevelt, 1993). Melanosit adı verilen bu granüllerin ise kıvrımların apikalinde daha yoğun olduğu bildirilmiştir (Gültiken ve ark., 2012; Gezer-Ince ve ark., 2017; Onuk ve ark., 2013). Bozdoğanda da diğer araştırmalara paralel olarak melanositlerin apikalde daha yoğun olduğu saptandı.

Pecten oculi hacminin hesaplanması ile ilgili yapılan tek çalışmada Kiama ve ark. (2006) deve kuşlarında pecten oculi hacmini 185 mm^3 olarak bulmuştur. Bu çalışmada ise seri kesitler üzerinde stereolojik olarak yapılan ölçümlerde pecten oculi hacmini 17.28 mm^3 olarak tespit edildi.

Sonuç olarak yapılan bu çalışma ile bozdoğanda pecten oculi'nin morfolojik yapısı ortaya konup stereolojik metotla hacmi hesaplanarak diğer kuş türleri ile arasındaki benzerlik ve farklılıkları tespit edilmiştir. Genel olarak yapı itibariyle şahin türlerini andırdığı saptanmıştır. Böylece bu makalenin tür farklarını ortaya koyabilecek birçok çalışmaya da kaynak olabileceği düşünülmektedir. Kanatlılarda

göz muayenesi ve cerrahi girişimlerde araştırmacılara ve veteriner hekimlere katkı sağlayacaktır.

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RESEARCH ARTICLE

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Köpeklerde Eozinofilik Gastroenteritis: 14 Köpeğin Retrospektif Analizi ve Endoskopik Biyopsilerde İki Diagnostik Yöntemin Karşılaştırılması

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ÖZ

Köpeklerde eozinofilik gastroenterit'in (EGE) klinik ve laboratuvar bulguları hastalık için spesifik değildir. Gastrointestinal kanalda histolojik olarak eozinofillerin belirlenmesi ise zordur. Bu retrospektif çalışmanın amacı EGE'nin klinik ve bazı laboratuvar bulgularının değerlendirilmesi ve dokularda eozinofillerin belirlenmesinde kullanılan iki tekniği karşılaştırmaktı. Bu amaçla EGE'li 14 köpeğin medikal raporları ve biyopsi sonuçları tekrar değerlendirildi. Eozinofil peroksidaz monoklonal antikoru (Epx mAb) ile immunohistokimyasal (IHC) ve hematoksilen eozin (H&E) ile boyanan EGE'li köpeklerin mide ve duodenal kesitlerinde eozinofiller sayıldı. Bütün köpeklerde en yaygın gastrointestinal semptomlar kusma ishal, kilo kaybı iken en yaygın laboratuvar bulgusu hipoalbuminemi ve eozinofili idi. EPX ile saptanan eozinofillerin sayısı H&E ile saptananlara oranla istatistiksel olarak yüksek bulundu ($p \leq 0.05$). Bu çalışma EGE'li köpeklerde kusma ishal, kilo kaybı, hipoalbünemi ve eozinofili görülmesine rağmen doğru tanının histopatolojik yöntemle konulması gerektiğini, Epx mAb ile eozinofillerin saptanmasıda H&E boyama yöntemine göre daha güvenilir olduğunu gösterdi.

Anahtar Kelimeler: Köpek, Eozinofil peroksidaz, Eozinofilik gastroenterit, Hipoalbuminemi, Eozinofili.

Canine Eosinophilic Gastroenteritis: Retrospective Analysis of Diagnosis in 14 Dogs and Comparison of Two Different Diagnostic Methods in Endoscopic Biopsies

ABSTRACT

Clinical manifestations and laboratory findings of eosinophilic gastroenteritis (EGE) in dogs are nonspecific. Identification of eosinophils in the GI tract of dogs with EGE, by histological evaluation is challenging. The aim of this study was to evaluate the clinic and laboratory findings and compare two different methods used to detect eosinophils in order to diagnose the disease. Medical records and biopsies from 14 dogs with EGE were retrospectively reviewed. Sections were immunolabeled with monoclonal antibodies (mAbs) against the eosinophil granule protein eosinophil peroxidase (Epx) and stained by H&E. The number of eosinophils were manually quantified. The most common observed gastrointestinal symptoms were vomiting, diarrhea and weight lost. The most common laboratory findings were hypoalbunemia and hypereosinophilia. The number of eosinophils detected in Epx mAb-labeled stomach and duodenal sections was significantly higher compared with that in H&E-stained sections ($p \leq 0.05$). The result of this study suggests that clinical findings may not be enough for the diagnosis of eosinophilic gastroenteritis in dogs. The diagnosis of canine EGE requires histopathological evaluation of GI biopsy. Immunohistochemical detection of Epx provides a more precise method to detect GI tract eosinophils compared to H&E staining and could be used as an alternative and reliable diagnostic tool for EGE.

Keywords: Dog, Eosinophil peroxidase, Eosinophlic gastroenteritis, Hypoalbuminemia, Eosinophlia.

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GİRİŞ

Eozinofilik gastroenterit ender olarak görülen ve gastrointestinal (GI) mukozada eozinofil infiltrasyonu ile karakterize bir hastalıktır (Samiullah ve ark. 2016, Collins ve ark. 2018). Eozinofilik infiltrasyon en çok mide ve ince bağırsağın proksimal kısmında gelişmesine rağmen gastrointestinal kanalın özofagustan rektuma kadar olan her bölümünde de görülebilir (German ve ark., 1999). Köpeklerde EGE insanlardan farklı olarak idiopatik inflamatory bowel disease (IBD) in bir formu olduğu kabul edilmektedir (German ve ark. 2001, Haas ve ark. 2015).

Eozinofilik gastroenterit etiyopatogenezi tam olarak bilinmemesine rağmen gelişiminde hipersensivite reaksiyonunun rol oynadığı düşünülmektedir (Ingle ve ark. 2011, Guilford 1996, Mehta ve Furuta 2015). İnsanlarda yapılan çalışmalarda EGE'li hastaların %50'sinde aynı zamanda astım, rinit, egzama ve ilaç alerjisi olduğu görülmüştür. Fakat köpeklerde bu konuda bir çalışma yapılmamıştır (Cerquetella ve ark. 2010). Eozinofillerin periferal dolaşımdan ilk göç alanları özofagusun dışındaki diğer GI organlardır ve normal fizyolojik koşullar altında eozinofiller mide ve intestinal mukozada bulunmaktadır (Rothenberg ve ark. 2001, Hogan 2009, Sattasathuchana ve Steiner 2014). Eozinofil, luminal patojenlere karşı konakçı savunmasında ve intestinal epithelial homeostasisin sağlanmasında çok önemli rol oynamaktadır. (Rothenberg ve ark. 2001, Powell ve ark. 2010, Hogan 2013). Major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil protein X (EPX) gibi sitotoksik eozinofil proteinleri doku hasarı ve disfonksiyonuna neden olarak, gastrointestinal permabilitede artış ve inflamasyona neden olduğu düşünülmektedir (Forbes ve ark. 2004, Carlson ve ark. 1999, Furuta ve ark. 2005, Hurell 2011, Horgan ve ark. 2013). İnsanlarda ve köpeklerde eozinofilin hastalıklarda ve normal durumlarda gastrointestinal sistemdeki rolü tam olarak açıklığa kavuşturulamamıştır. Bu nedenle eozinofilin gastrointestinal sistem hastalıklardaki rolü araştırıcıların ilgi odağı olmuştur (Furuta ve ark. 2005).

Köpeklerde ve insanlarda bu hastalığın tanısı genellikle zordur. Tanıda gastrointestinal sistem hastalıklarında yaygın görülen semptomların varlığı (kusma, ishal, kilo kaybı, melena, hematoemez), periferal eosinofilinin saptanması ve gastrointestinal organlardan alınan biyopsi örneklerinde eozinofil sayısındaki artış kriter alınır (Powell ve ark. 2010, Sattasathuchana and Steiner 2014). Özellikle endoskopik biyopsi örneklerinin histojik olarak değerlendirilmesi hastalığın tanısında çok önemlidir (Day ve ark. 2008, Washabau ve ark. 2010, Mehta ve Furuta 2015). Fakat patologlar arasındaki yorum farkı, alınan doku örneği sayısının az olması, boyama sırasında meydana gelen hatalar hastalığın tanısının doğrulanmasını zorlaştırmaktadır (Day 2008, Washabau ve ark. 2010). Ayrıca, eozinofilik infiltrasyonun belirlenmesinde, rutin olarak kullanılan hematoksilen eosin boyama yöntemi yetersiz kalmaktadır, bununla birlikte bu boyama yöntemi hastalığın patogenezinde çok önemli bir rol oynayan degranüle eozinofilleri belirleyememektedir (Bastan ve ark. 2017).

Bu çalışmada EGE'li köpeklerde hastalığın klinik, histopatolojik ve bazı laboratuvar bulgularına dikkat çekmek ve eozinofillerin belirlenmesinde EPX mAb kullanarak alternatif yeni bir tanı yöntemi geliştirilmek amaçlandı.

MATERYAL ve METOT

Hayvan Materyali

Bu retrospektif çalışmada 2012-2017 yılları arasında Ankara Üniversitesi Veteriner Fakültesi Hayvan Hastanesine kusma, ishal, iştahsızlık, kilo kaybı ve melena şikayetleri ile getirilen, mide ve duodenumdan alınan biyopsi örneklerinin histopatolojik muayenesi sonucunda eozinofilik gastroenterit tanısı konulmuş, yapılan klinik ve laboratuvar muayenelerinde paraziter, bakteriyel ve neoplazik hastalığı bunmayan 14 köpek ile gastrik ve duodenal biyopsi örneklerinin histopatalojik değerlendirmesinde herhangi bir abnormalite saptanmayan 10 köpek kullanıldı. Her bir hastanın histopatolojik, demografik, klinik, tam kan ve serum biyokimyasal (üre, kreatinin, total protein, albümin, globulin, alanin aminotransferaz, alkalen fosfataz, glukoz, sodyum, potasyum, kalsiyum, kolestrol, bilirubin, bazal kortizol, trypsin like immunoreactivity, folat, B12, canine pancreas-specific lipase) parametreleri değerlendirildi.

Histopatalojik ve immunohistokimyasal boyama

Biyopsi örneklerinden eozinofil sayılarını belirlemek için formalin ile fikse edilmiş parafin bloklardan 4-μm kalınlığında 3 seri kesit alındı. Birinci kesitler hematoksilen eozin, ikinci kesitler Bastan ve ark., (2017) tarafından tanımlanan immunohistokimyasal yönteme göre EPX mAb ile boyandı (Mayo Clinic, Scottsdale, AZ). Negatif kontrol için üçüncü kesitlerin boyanmasında normal mouse IgG (negatif kontrol, Sigma-Aldrich, St. Louis, MO) kullanıldı. EPX ve H&E ile boyanan her bir slayttan 5 en büyük biyopsi dokusu seçildi ve her bir doku kesintisiz olarak 40'lık büyütmede Olympus BX53 mikroskop kullanılarak sayıldı. Tüm biyopsi örnekleri histopatolojik olarak

WSAVA kriterleri (Washabau ve ark., 2010) kullanılarak morfolojik parametre (epitel hasarı, kriptlerde genişleme, lakteal dilatasyon ve mukozal fibrozis) ve inflamasyon kriterlerine (intraepitel lenfosit imitasyonu, lamina propriada lenfosit, eozinofil, plazma hücresi ve lenfosit infilrasyonu) göre değerlendirildi.

İstatistiksel Analiz

İstatistiksel analiz için R Statistical Software (version 3.4.3) kullanıldı. İki farklı boyama (H&E ve EPX) yöntemiyle elde edilen eozinofil sayıları paired Wilcoxon testi ile karşılaştırıldı ve $p \leq 0.05$ değeri istatistiksel olarak önemli kabul edildi.

BULGULAR

Eosinofilik gastroenterit tanısı konmuş ortalama yaşları 5.9 ± 2.4 yıl olan 14 köpeğin 6'sı dişi 8'i erkek idi. Bu köpeklerin 3'ü Labrador retriever, 3'ü melez, 2'si Rottweiler 1'i Alman çoban köpeği, 1'i Terrier ırkı idi. Klinik semptomlar olarak en yaygın kusma, ishal ve kilo kaybı belirlendi (Tablo-1). Tam kan sayımı sonucunda 14 EGE'li köpeğin 8'inde hipoalbünemi ((%57.1), 6'sında (% 42.8) eozinofili, 3'ünde (% 24.1) anemi, 1'inde trombositosis (%7.1) saptandı (Tablo-2), diğer kan parametreleri normal sınırlardaydı. Histopatolojik inceleme sonucunda sağlıklı köpeklerin mide ve duodenumunda herhangi bir abnormal selüler infiltrasyona rastlanmamasına rağmen (Şekil-1) 14 köpeğin mide ve duodenumun lamina propriasında eosinofilik infiltrasyon saptandı (Şekil-1).

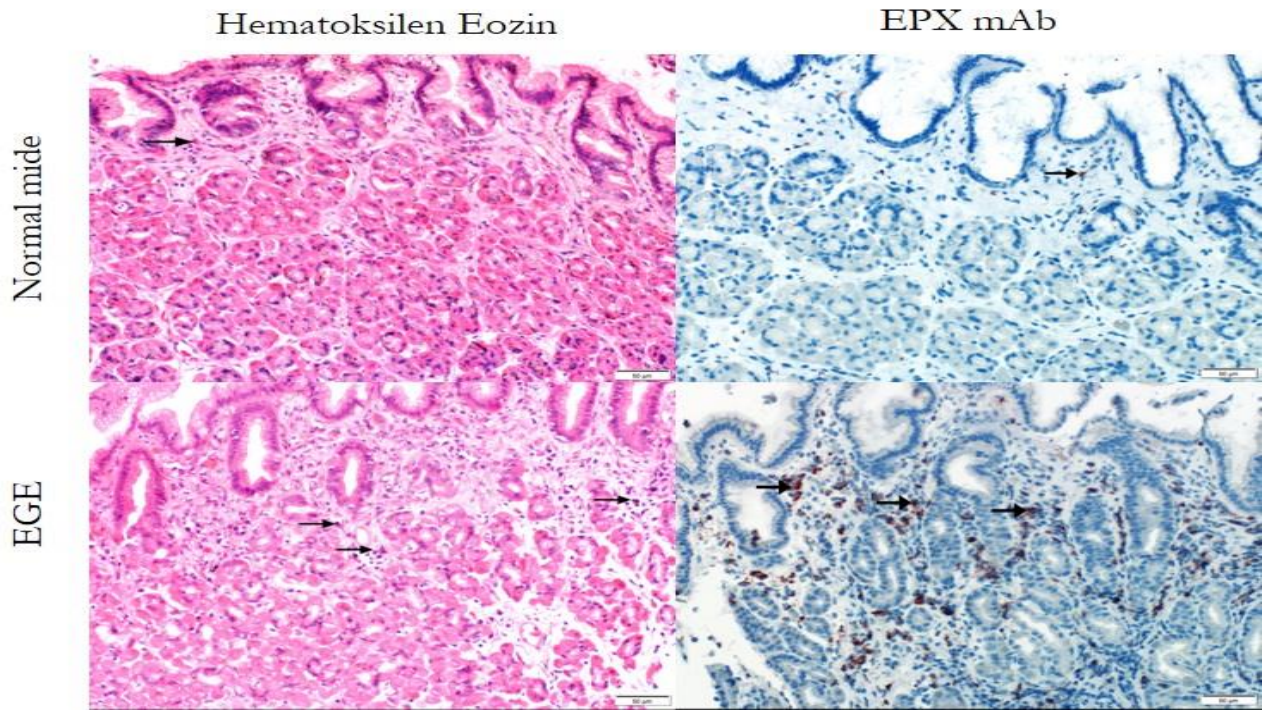
Midede ve duodenumda, EPX mAb ile belirlenen eozinofil sayısı sırasıyla 27,5±9.3, 38.3±9.3 iken her iki dokuda H&E tekniği ile belirlenen eozinofil sayısı sırasıyla 10,7±6,3, 25,7±7,6 idi. Her iki dokuda da EPX mAb ile belirlenen eozinofil sayısı, H&E tekniği ile belirlenen eosinophil sayısından yüksekti. Bu sonuç istatiksel olarak anlamlı bulundu ($p \leq 0.05$).

Tablo 1. Eozinofilik gastroenteritisli 14 köpekteki klinik bulgular
Table 1. Clinical sings of 14 dogs with eosinophilic gastroenteritis

Klinik bulgular	Köpek sayısı (yüzdesi)
Kusma	10 (%71,4)
İshal	9 (%64,2)
Kilo kaybı	8 (%57,1)
Hematoemez	2 (%14,2)
Melena	2 (%14,2)

Tablo 2. Eozinofilik gastroenteritisli 14 köpekteki serum biyokimyasal ve hematolojik bulgular
Table 2. Biochemical and hematological findings for 14 dogs with EGE

Parametre	Hayvan sayısı (%)	Ortalama Değer	Referans değer
Albümin (g/l)	8 (%57,1)	23,9±3,1	32-38
Eozinofil (10^9/l)	6 (%42,8)	2,4±0,4	0.2-1.4
PCV (%)	3 (%24,1)	29±0,7	35-50
Trombosit (10^9/l)	1 (%7,1)	662	170-500



Şekil 1. Normal ve EGE' li köpeklerin midelerinde H&E ve Epx boyamaları. Eozinofillerin midenin lamina propriyasındaki dağılımı. Sol tarafta H&E boyama, sağ taraf Epx IHC boyama. H&E ve Epx ile boyanan mide dokularında intact eozinofiller (siyah oklar). Bar = 50 µm.

Figure 1. H&E staining and Epx labeling in stomach of dogs with normal and EGE. Distribution of eosinophils within LP of stomach of normal and EGE of dogs. H&E stained sections are on the left and Epx IHC sections on the right. İntact eosinophils in stomach tissues by stained with H&E and Epx (Black arrows). Scale Bar = 50 µm.

TARTIŞMA

Eozinofilik gastroenterit gastrointestinal kanalda eozinofil infiltrasyonu ile karakterize ender görülen bir hastalık olup, etiyolojisi tam olarak bilinmemektedir. Bu hastalıkta kusma, hematemesis, iştahsızlık, kilo kaybı, abdominal ağrı ve melena gibi kronik GI hastalık bulguları görülür (Fonseca-Alves ve ark. 2012, Mehta ve Furuta 2015). Bu çalışmada da yukarıdaki araştırmacıların belirttiğine benzer klinik semptomların gözükmesi, hastalığın bu semptomlarla seyrettiğini doğrular nitelikteydi. Fakat EGE de gelişen semptomlar diğer gastrointestinal hastalıkların bulguları ile benzerlik gösterdiğinden hastalığın kesin tanısı için diğer diagnostik yöntemlere başvurulmalıdır (Sattasathuchana ve Steiner 2014).

Bu çalışma da Craven ve ark. (2004)'nın yaptığı çalışmaya benzer olarak köpeklerin %51.4'ünde hipoalbünemi saptandı. Eosinofilik gastroenteritte; iştah kaybı, hemoroji, intestinal permabilitenin artışı, villus atrofisi ve fibrozis nedeni ile intestinal yüzey kaybına bağlı malabsorbsiyon sonucu hipoalbünemi geliştiği düşünüldü. EGE olgularının tümünde periferal kanda eozinofili gözlenmezken olguların %20'sinde eozinofili görüldüğü bildirilmiştir (Talley 1990, Fonseca-Alves ve ark. 2012). Bu çalışmada köpeklerin %42'sinin periferal kanında eozinofili saptandı. Bunun nedenin kan alma zamanlarındaki farklılıklar olabileceği düşünüldü. Çünkü köpeklerde eozinofiller 1 saat gibi çok kısa sürede periferal dolaşımda kalırlar ve çok hızlı bir şekilde başta GI sistem dokuları olmak üzere diğer dokulara göç ederler (Young ve Meadows 2010).

Köpeklerde normal fizyolojik şartlarda özofagus dışındakı diğer GI organlarında eozinofil bulunmaktadır (German ve ark. 1999, Washabau ve ark. 2010, Rothenberg ve ark. 2001). Eozinofillerin parazitik hastalıklar, neoplaziler gibi hastalıklar dışında sindirim kanalı mukozasında birikmesi EGE' nin en karakteristik özelliğidir (Hogan ve ark. 2013, Day ve ark. 2008). H&E boyaması bu hastalığın tanısında rutin olarak kullanılmaktadır (Day ve ark. 2008, Washabau ve ark. 2010), IBD'li köpeklerde yapılan bir çalışmada postmortem olarak elde edilen köpeklerin jejunumunda, H&E boyamasının sadece yapısı bozulmamış (intact) eozinofillerin belirlendiği, degranüle olmuş eozinofilleri saptanamadığı fakat Epx mAb IHC boyamanın her iki tip eozinofili de saptadığı gösterilmiş ve immunohistokimyasal yöntem ile Epx mAb'nin hastalığın doğru tanısı için kullanılabilecek yeni bir yöntem olduğunu belirtilmiştir (Bastan ve ark. 2017). Eozinofilik özofagitli insanlarda yapılan bir çalışmada, Epx

mAb'nin, H&E boyamasına oranla 4 kat daha fazla eozinofili saptadığı ve ayrıca bu yöntemin eozinofilik özofagitis ve gastroözofageal refluks hastalığının ayırıcı tanısını kolaylaştırdığı bildirilmiştir (Protheroe ve ark. 2008). Akut akciğer hasarı olan insanlar üzerinde yapılan diğer bir çalışmada eozinofillerin saptanmasında Epx mAb'nin H&E boyamasına oranla 40 kat daha hassas olduğu belirlenmiştir (Willetts ve ark. 2011). Bu çalışmanın sonuçları da mide ve duodenum biyopsilerinde eozinofillerin saptanmasında Epx mAb'nin kullanabileceğini ve yukarıdaki çalışmalara benzer olarak Epx mAb'nin eozinofilleri belirlemede rutinde kullanılan H&E boyamasına oranla daha güvenilir bir yöntem olduğunu gösterdi. Bu bakımdan bu yöntem gelecekte eozinofilik hastalıkların tanısında umut vadeden bir tanı yöntemi olarak düşünülmektedir.

Hematoksilen eosin boyamasının bir diğer dezavantajı degranüle olmuş eozinofilleri saptayamamasıdır (Protheroe ve ark. 2008, Bastan ve ark. 2017). Gastrointestinal eozinofil infiltrasyonu artmış insanlarda yapılan çalışmalar; artan degranüle eozinofil sayısının hastalığın prognozunun kötüye gittiğini ve mukozal hasarın gelişmesinde önemli bir rol oynadığını göstermiştir (Bischoff ve ark. 1999, Kristjansson ve ark. 2004, Smyth ve ark. 2013). IBD'li köpeklerde yaptığımız bir önceki çalışmamızda tedavi edilmeyen köpeklerde, tedavi edilenlere ve sağlıklı olanlara oranla degranüle eozinofil sayısında artış saptandı (Bastan ve ark. 2017). Bu nedenle hem intact hem de degranüle olmuş eozinofillerin sayısının belirlenmesi hastalığın patogenezisinin anlaşılmasında önemli olabilir. Bu çalışmada amacımız 2 yöntemin karşılaştırılması olduğu için sadece intact eozinofiller sayıldı. Fakat gelecekteki çalışmalarda EPX mAb ile normal ve degranüle eozinofillerin GI mukozada meydana getirdiği hasarın belirlenmesi ile hastalığın patogenezisinin aydınlatmasında önemli rol oynayacağını düşünmekteyiz.

Sonuç olarak, bu çalışma EGE'li köpeklerde kusma ishal, kilo kaybı, hipoalbünemi ve eozinofili görülmesine rağmen tanının histopatolojik yöntemle yapılması gerektiğini, Epx mAb IHC ile eozinofillerin saptanmasında H&E'ni yöntemine göre daha güvenilir sonuçlar vereceğini ve bu hastalığın tanısında güvenilir bir yöntem olarak kullanılabileceğini gösterdi.

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REVIEW

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Anthelmintic Resistance in Farm Animals

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ABSTRACT

Anthelmintic resistance means that developing genetically transmitted lack of susceptibility to an anthelmintic which is previously known to be susceptible to a parasite population. Anthelmintic resistance has an increasing importance in recent years. The anthelmintic resistance which is developed especially due to use of unconscious anthelmintics also brings with it economic problems. Investigations have shown that resistance has developed to anthelmintics in a short period of time after launch to the market and even in some countries, several sheep and goat farms have been closed due to anthelmintic resistance. For this reason, especially in livestock breeding; The development of resistance should not be overlooked while planning of treatment and control programs and choosing anthelmintics. In this review, resistance mechanisms which is developed to anthelmentic drugs, resistance detection methods and anthelmentic resistance status of livestock in Turkey were evaluated.

Key words: Anthelmintics, Cattle, Goat, Resistance, Sheep

Çiftlik Hayvanlarında Antelmentik Direnç

ÖZ

Bir parazit populasyonunun daha önce duyarlı olduğu bir antelmentiğe karşı gelişen ve genetik yolla aktarılan duyarlılık kaybı olarak değerlendirilen antelmentik direnç, son yıllarda giderek artan bir öneme sahiptir. Özellikle bilinçsiz ilaç kullanımına bağlı olarak gelişen antelmentik direnç ekonomik problemleri de beraberinde getirmektedir. Yapılan araştırmalar bazı ilaçların piyasaya sürümünü takiben kısa süreler içinde ilaca karşı bir direnç geliştiğini hatta bazı ülkelerde sadece direnç gelişmine bağlı olarak çiftliklerin kapatıldığı göstermektedir. Bu nedenle özellikle çiftlik hayvanları yetişrtiriciliğinde; antelmentik kullanılırken veya helmint enfeksiyonlarının tedavi ve kontrol programları planlanırken direnç gelişimi göz ardı edilmemeilidir. Bu derlemede antelmentik ilaçlara gelişen direnç mekanizmaları ve direnç tespit yöntemleri ile Türkiye'de çiftlik hayvanlarında belirlenen antelmentik direnç hakkında özlü bilgi verilmiştir.

Anahtar kelimeler: Antelmentikler, Direnç, Keçi, Koyun, Sığır

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INTRODUCTION

Infections which is caused by parasites limit the welfare and yield of livestock around the world. The control of helminth infections is mostly based on the use of anthelmintic drugs (McKellar and Jackson, 2004). Anthelmintic resistance has developed in a short period of time after drug launched to the market, as a result of the intense and unconscious use of drugs. World Association for the Advancement of Veterinary Parasitology (WAAVP) published methods to detect anthelmintic resistance to draw attention to this issue in 1992 (Coles et al. 1992). Anthelmintic resistance has become a serious problem, especially in sheep nowadays. In some countries such as Australia, United Kingdom, New Zealand and South Africa, some sheep and goat farms have been closed due to multiple drug resistance (Kaplan 2004, Geary 2005).

Anthelmintics

Anthelmintics constitutes the cornerstone to control helminth infections. Until recently, there were three main broad-spectrum anthelmintic groups in the market. These are benzimidazoles (BZs); imidazothiazole and tetrahydropyrimidines (I/Ts) and macrocyclic lactones (MLs). They are also classified as white, yellow and clear drug groups. Monepantel which is a member of amino-acetonitrile derivatives (AAD) was found about 30 years later than ivermectin and classified as the fourth anthelmintic group. Finally, Derquantel which is from spiroindoles group was classified as a fifth anthelmintic group and launched to the market as a combination with abamectin. Fourth group and fifth groups are shown with orange and purple respectively (Abbott et al. 2012).

Use of Low Dose Anthelmintics

To ensure that the treatment is fully effective, the animals should be weighed and appropriate dose should be given by calculating. The use of low-dose drugs are caused to remain alive of more parasites and accelerates the development of anthelmintic resistance after treatment. Decreased bioavailability of the drug is related to drug administration routes and the animal species. Especially irregular topical (pour-on) applications are caused predisposition to the development of anthelmintic resistance. The pharmacokinetics of the anthelmintics are also effective with regards to the development of resistance. As a result of using long-acting or slow releasing anthelmintics, the host is exposed to low doses at the end of the elimination phase. Thus, short-acting anthelmintics are preferred (Wolstenholme et al. 2004, Sutherland and Leathwick, 2011).

Genetic And Biological Factors Contributing Anthelmintic Resistance

Resistance is the heritable ability of the worms to survive a dose of anthelmintic which would normally be effective. The resistance is inherited and passed to the next generation. If a drug resistance develops to one anthelmintic in a class, other drugs in the same class will be effected and it is called as side resistance. If a drug resistance develops to two or more different anthelmintic groups, it is described as a cross or multiple resistance. Several sheep and goat farms have been closed cause of multiple drug resistance in Australia, South Africa and New Zealand (Kaplan 2004, Geary 2005, Abbott et al. 2012).

Although the development of anthelmintic resistance seems to be slow at the beginning, the resistance, it is increasingly continued after each treatment and the susceptibility is eventually lost. Once resistance has developed in the parasite population, it is not possible to sensitize this population again until now (Sangster and Dobson, 2002).

It is thought that the parasite population carries a resistant allele even before the drug is administered (Wolstenholme et al. 2004). According to another hypothesis, it is thought that the resistance occurs as a result of spontaneous and repetitive mutations (Skuce et al. 2010). If an individual carries two alleles or copies of a gene, it is called as homozygous. If it carries different alleles or copies of a gene, it is called as heterozygous. Homozygous parasites could be sensitive or resistant. Although the genetics of resistance is not fully understood, participating in a single gene for resistance leads to develop resistance faster. In case of resistance genes are dominant, resistance will be developed faster compared to recessive genes. Moreover, some parasites have various biologic features such as direct (monoxen) development without an intermediate host, short life cycle and high fertility rate, which accelerates the development of resistance in a parasite population (Sangster et al. 1998, Coles 2005).

Detection of Anthelmintic Resistance

Anthelmintic resistance means that developing genetically transmitted lack of susceptibility to a drug which is previously known to be susceptible to a parasite population. Detection of anthelmintic resistance in a parasite population is very important when the frequency of alleles is low. Thus, development of anthelmintic resistance could be delayed and susceptibility of the drug could be preserved (Martin et al. 1989).

Currently, the detection of anthelmintic resistance is based on in vivo and in vitro tests. Using of these tests are limited because of taking a long time, expensive, labor-intensive and required test animals. Some of the tests which are used to detect anthelmintic resistance are only successful if the target parasite population is 25% or more resistant phenotypically (Coles et al. 1992).

In Vivo Methods for The Detection of Anthelmintic Resistance

The two most commonly used methods for detecting anthelmintic resistance are; fecal egg count reduction test (FECRT) and the controlled efficacy test (CET). Although the controlled-efficacy test is the most trustable method, the fecal egg count reduction test is the most widely used test as an in vivo method (De Graef 2013).

Fecal Egg Count Reduction Test (FECRT)

Fecal egg count reduction test is the most practical in vivo method for detecting anthelmintic resistance and has been recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP). This method is based on the calculation of the difference as a percentage after counting of nematode eggs in feces for pre-treatment and post-treatment (10-14 days later). It is considered to develop resistance if the number of eggs per gram (EPG) in the stool decreases by a percentage below 95% after treatment (Coles et al. 1992).

This test is available for all the anthelmintics. If parasite population has more than 25% resistance, it can be said that this technique is reliable. Ideally, ten animals which have higher than 150 EPG are selected for each group (Coles et al. 2006).

If there are less than 50 EPG in feces, the modified McMaster technique cannot be used and this situation limits the use of the technique. In case that, the number of eggs in the stool before treatment is less than 150, it is recommended to use a more sensitive method. Another disadvantage of this method is the lack of species specificity (Coles et al. 2006, Levecke et al. 2009).

Controlled Efficacy Test (CET)

The controlled efficacy test is seen as the best method to determine the effect of the anthelmintics (Martin et al. 1989, Cook et al. 2006). In this test, the animals are experimentally infected with known resistant and susceptible L3 and then treated with different concentrations of the anthelmintic. After a certain period of time, parasites are collected from the abomasum. If the decrease in the number of parasites is less than 90% or more than 1000 parasites remain alive after treatment, it is considered to be resistant. The disadvantages of this method are being expensive, time-consuming and labor-intensive. Also, using animals for testing possess some ethical problems (Coles et al. 1992, Taylor et al. 2002).

In Vitro Methods for The Detection of Anthelmintic Resistance

The advantages of in vitro tests are being low cost, not differ from host to host and not necessary to use testing animals. Many methods have been developed to detect anthelmintic resistance using the nematode larvae. The most of these tests are not widely used practically because of reliability, reproducibility, sensitivity, and easy interpretation of tests are not at the desired level. Only Egg Hatching Test (EHT) and Larval Developmental Test (LDT) are widely used (De Graef 2013). In addition, Larval Paralysis Test, Micro-Motility Measurement Test, Larval Migration Inhibition Test and Molecular-Based Tests are used (Coles 2005, Jabbar et al. 2006, Demeler et al. 2010).

Egg Hatching Test (EHT)

The egg hatching test is only used to detect benzimidazole and levamisole resistance, but can not be used in macrocyclic lactones due to not being ovicidal. Eggs are incubated at various concentrations with anthelmintics to calculate the percentage of egg hatching after the eggs are obtained from the feces (Taylor et al. 2002, Coles 2005). The optimal dose is calculated using sensitive isolates. In the tested samples, the percentage of egg hatching is also regarded as the percentage of resistance. An advantage of this method is that only once stool collection is sufficient (Coles et al. 2006). The results of the egg hatching test are generally interpreted using values of ED50 (50% inhibition value) or ED99 (99% inhibition value). If the ED50 is used as the threshold value, resistant parasites in the population must be at least 25% to detect benzimidazole resistance. The sensitivity of the test was increased with the use of ED99 value, and it has become possible to detect resistant parasites with a low percentage in the population (Várady et al. 2007).

Larval Developmental Test (LDT)

The larval development test was developed to measure the potential of the anthelmintic drug with regards to inhibiting egg development. Trichostrongylid type eggs are incubated with tested anthelmintics in the medium containing *Escherichia coli* for 6-8 days in this method and then the ratio of developed L3 is calculated. The use of freshly collected eggs is the most important factor for working efficiently of the test (Demeler et al. 2010). The larval development test is used to determine the resistance of many anthelmintics

including macrocyclic lactones, although it is more labor-intensive and time-consuming than the egg hatching test. Larval growth test is more sensitive than FECRT and egg hatching test (EHT); it is able to detect up to 10% of parasites carrying resistance in populations (Jabbar et al. 2006).

Larval Migration Inhibition Test

Motility and migration and tests are based on paralyzing muscles of the trichostrongylid nematodes by way of anthelmintics. The third stage larvae are incubated with anthelmintic serial dilutions for twenty-four hours and then transferred onto a mesh for another twenty-four hours. In spite of resistant L3s pass through the mesh, sensitive L3s remains on the mesh. Then the percentage of migrating larvae is calculated. Migration inhibition is determined by the curve resulting from different concentrations (Demeler et al. 2010).

Molecular-Based Tests

DNA-based tests have been developed to identify genetic-based qualitative or quantitative changes (differences in gene expression). Low benzimidazole resistance which can not be detected by in vitro methods can be determined with the development of molecular-based tests. Molecular-based tests are more sensitive and faster than in vivo and in vitro tests, in spite of expensive equipment and materials. These tests allow individual detection of parasites which is carrying resistance genes in a population (Von Samson-Himmelstjerna 2006). Theoretically, even if the frequency of resistance is low in the population, molecular-based tests can detect resistance alleles. Polymerase Chain Reaction (PCR) based tests detect benzimidazole resistance by using of single nucleotide polymorphisms (SNPs). Subsequent to PCR, DNA is separated on agar electrophoresis and the bands revealed. Then real-time PCR and pyrosequencing techniques began to be used. So far, most of the molecular research has been conducted on benzimidazoles to detect anthelmintic resistance. The resistance mechanism of other anthelmintics is not as well known as benzimidazoles yet, but studies are still being continued (Kwa et al. 1994, Jabbar et al. 2006, Von Samson-Himmelstjerna 2006).

Refugia

Parasites are called refugia, which have not been exposed to an anthelmintic drug in a parasite population. Refugia is the basis of the large majority of sustainable parasite control programs. Refugia constitute one of the sources of re-infection and prevent resistant parasites from becoming a majority of the population. Also, it consists of developmental stages of the parasite from the egg to L3 in nature, the cysted larvae in the abomasal glands and the untreated parasites in the host (Abbott et al. 2012). Reducing the proportion of resistant parasites in the population and delaying the development of resistance by increasing the proportion of susceptible parasites constitutes the principle of refugia. Short treatment intervals reduce the reproduction of susceptible parasites, while also reduce the number of unexposed parasites. In addition, some animals in the herd should not be treated to maintain the presence of sensitive parasites (Sangster and Dobson, 2002).

Mechanism of Anthelmintic Resistance

Benzimidazole Resistance

In genetic studies on benzimidazole-resistant gastrointestinal nematodes, several specific changes in the beta-tubulin-encoding sequence lead to point mutations, thus reducing drug susceptibility (Von Samson-Himmelstjerna et al. 2007, Dicker 2010). Genetic studies on *Teladorsagia circumcincta*, *T. colubriformis*, *H. concortus* and *Cooperia oncophora* have shown that tyrosine (resistant, TAC) is encoded instead of phenylalanine (sensitive, TTC) at codon 200 in beta-tubulin isotype 1 gene (Phe200Tyr or F200Y) which is caused point mutation (Kwa 1994, Von Samson-Himmelstjerna et al. 2007). The second, less common, benzimidazole resistance mechanism is the phenylalanine-tyrosine (Phe-Tyr) polymorphism at codon 167 which is seen especially in the nematodes of horses (Wolstenholme et al. 2004, Hodgkinson et al. 2008, Silvestre and Cabaret, 2002). Tyr (Tyrosine) was required at codon 200 for benzimidazole resistance in *Haemonchus concortus*; The homozygous phenylalanine-phenylalanine (Phe/Phe), the heterozygous phenylalanine-tyrosine (Phe / Tyr) or homozygous tyrosine-tyrosine (Tyr/Tyr) at codon 167 can cause the parasite to become resistant in *T. circumcincta*. Tyr (Tyrosine) is required at codon 200 for benzimidazole resistance in *Haemonchus concortus*. The homozygous phenylalanine-phenylalanine (Phe / Phe) at codon 200 and the heterozygous phenylalanine-tyrosine (Phe/Tyr) or homozygous tyrosine-tyrosine (Tyr/Tyr) at codon 167 in *T.circumcincta* can cause the parasite to become resistant (Silvestre and Cabaret, 2002, Von Samson-Himmelstjerna et al. 2007). Resulting from point mutation at codon 198, alanine (Ala) is encoded instead of glutamic acid (Glu) as an alternative mechanism of benzimidazole resistance which is found in *H. contortus* (Ghisi et al. 2007). Some of the studies have shown that P-glycoproteins are indirectly involved in benzimidazole resistance of nematodes. Another mechanism of resistance to benzimidazole is the deletion of β-tubulin isotype 2 in the *H.concortus* population. While heterozygous parasites are

advantageous compared with susceptible parasites in terms of benzimidazole resistance, although it is not completely resistant. Parasites are more likely to survive, especially after inadequate dosing of anthelmintics (Roos et al. 1995, Von Samson-Himmelstjerna 2006).

Levamisole Resistance

There are insufficient studies on the resistance mechanisms of tetrahydropyrimidines including levamisole, imidazothiazole and pyrantel (Kopp et al. 2009). Studies on *Caenorhabditis elegans* have shown; 5 genes that encode the subunits (L-AChRs) of ionotropic acetylcholine receptors which is sensitive to levamisole resistance. These are 3 α-subunit genes (*lev-8*, *unc-63*, *unc-38*) and 2 non-α subunit genes (*lev-1*, *unc 29*) (Fleming et al. 1997, Boulin et al. 2008). In addition, the L-AChR expression is lost in muscle cells due to mutations in *ric-3*, *unc-74* and *unc-50* and resulting in loss of sensitivity to levamisole. For the development of the levamisole resistance, glycine (Gly) must be encoded instead of glutamic acid (Glu) at codon 153 in the unc-38 gene of *C. elegans* (Rayes et al. 2004, Martin and Robertson, 2007). Lack of susceptibility to pyrantel receptors has occurred as a result of encoding glycine (Gly) instead of glutamine (Gln) at codon 57 of the *unc-63* gene of *C.elegans*. (Bartos et al. 2006). Nicotinic acetylcholine receptors consist of 5 glycoprotein subunits which are arranged around a central ion channel and each subunit gains different pharmacological properties to the nAChR (Fleming et al. 1997). Expression difference in HA17, which is a gene fragment, between levamisole-sensitive and levamisole resistant parasites was identified by cDNA-AFLP technique and aimed to be a potential marker for detection of levamisole resistance (Neveu et al. 2007). In Ancylostoma caninum, significant polymorphic differences were not observed in ARR-29, ARR-38 and ARR-63, which are subunits of the pyrantel, but it was proven that expression of these genes is significantly reduced in resistant parasites. These genes are ortholog with the *unc-29*, *unc-38* and *unc-69* genes which is found in *C.elegans* (Kopp et al. 2009).

Macrocyclic Lactone Resistance

The mechanism of macrocyclic lactone resistance has not been fully understood yet. Glutamate-gated chloride channels and acetylcholine receptors have a similar structure and the central ion channel are constituted by the combination of 5 subunits (α and β). The α subunits contain the glutamate binding site, while the β subunits contain the ivermectin binding site (Martin et al. 1997, Bartos et al. 2006). Some of the genes which are involved in ivermectin resistance include glutamate and GABA-gated chloride channels (Gilleard, 2006). Changes in allele frequencies of glutamate and GABA chlorine subunits were observed in different populations of *Haemonchus contortus*, but the changes in a single allele were not correlated with resistance (Blackhall et al. 1998, Blackhall et al. 2003). Macrocyclic lactone resistance is emerged by mutation of a few glutamate-gated chloride subunit genes in *C. elegans* (McCavera et al. 2007). In order to develop a high level of ivermectin resistance in *C. elegans*, simultaneous mutation is required in all three genes (*avr-14*, *avr-15* and *glc-1*) which is encoding α-subunit of the glutamate-gated chloride channel. *Avr-15* encodes GluCla2 which is expressed in the pharyngeal muscles of *C. elegans* and *Avr-14* encodes GluCla3 which is expressed in the extrapharyngeal nerve cell of *C. elegans*. One of the most important mechanisms of action of ivermectin is the inhibition of the pharyngeal pump which causes starvation of parasites (Dent et al. 2000, Cook et al. 2006). While parasitic nematodes have different GluCl subunit genes compared to *C. elegans*, there are also orthologs that reduce the sensitivity of ivermectin, such as *avr-14* in *C. oncophora* (McCavera et al. 2007). The genetic mechanism of the ivermectin resistance in Trichostrogylid parasites is not fully understood (Geary 2005, Prichard and Roulet, 2007).

Changes in the γ-amino butyric acid (GABA) receptor genes are thought to be responsible for the macrocyclic lactone resistance (Blackhall et al. 2003).

Detoxification process of P-glycoproteins (PGP) is thought to play a role in macrocyclic lactone resistance. P-glycoproteins are a member of the ATP binding cassette superfamily and provide active transport of endogenous and exogenous hydrophobic molecules across the membrane (Sangster and Dobson, 2002). P-glycoproteins are significantly localized in the digestive tract and it is expressed at high levels in the membranes of the intestinal and pharyngeal cells (Smith and Prichard, 2002). The main role of P-glycoproteins is to protect the organism by pumping toxic agents out of the cell. It has been reported that Tc-Pgp-9, which is a kind of PGP in the study on ivermectin resistant *T.circumcincta*, has increased expression at mRNA level, high level of polymorphism in sequence, and helminths may play an important role in resistance to ivermectin. It has been reported that increased expression at mRNA level and high level of polymorphism in the sequence are observed in Tc-Pgp-9 which is obtained from ivermectin resistant *T. circumcincta* of sheep and it has been determined that it may play an important role with regards to ivermectin resistance of helminths. Pgp-inhibited mice and Collie dogs with

PGP deficiency are highly susceptible to ivermectin and result in death as a result of extreme neurotoxicity (Lespine et al. 2008, Dicker et al. 2011).

Verapamil, as a calcium channel blocker, inhibits the binding site of Pgp, thereby increasing the efficiency of the anthelmintics. In vitro using of verapamil as a Pgp inhibitor has shown that macrocyclic lactone-resistant parasites become more sensitive (Demeler et al. 2013).

Amino-Acetonitrile Derivatives (AAD) Resistance

Monepantel was first used in small ruminants in 2009 with the commercial name Zolvix®, and the first resistance case was reported four years later after introduced to the market (Scott et al. 2013). Then there are different resistance reports from various parts of the world (Mederos et al. 2014, Love 2014, Cintra et al. 2016).

Monepantel which is a member of amino-acetonitrile derivatives targets nicotinic receptors as the mechanism of action. These receptors include DES-2 and ACR-23 subunits which is located in the pharyngeal muscles, between the nerves throughout nerve cord and the sensory nerves. Subunits of nicotinic acetylcholine receptors sensitive to amino-acetonitrile derivatives have a mechanism that only affects nematodes, and so it is not toxic to mammals, insects and other vertebrates. In vitro studies on *Haemonchus contortus* have shown that two genes are effective on resistance. As a result of deletions at the intron-exon border in monepantel-1 (*Hco-mptl-1*, also called *Hc-acr-23H*) gene of resistant *H. concortus,* stop codon is located before the regular site. Another mutation is occured by 5' end insertional mutation in the *Hco-des-2H* gene and result in decreased susceptibility (Rufener et al. 2009, Kennedy and Harnett, 2013).

Famacha (Faffa Malan Chart)

Famacha is a low cost and easily applicable test which is developed by South African scientists to determine the anemia associated with haemonchosis in sheep and goats and it is aimed to avoid unnecessary use of anthelmintics. This test is widely used in Sub-saharan Africa and South America. The principle of this test is based on a comparison of the color of the eye conjunctiva of small ruminants with the Famacha card to determine the severity of the anemia (Malan et al. 2001).

Alternative Control Methods of Anthelmintic Resistance

Alternative treatment methods have been studied due to the problem of anthelmintic resistance in many regions of the world. The most common alternative treatment methods are; copper oxide wire particles, use of tannin-containing feeds, nematode-trapping fungi, vaccine, breeding for resistant animals, nutrition and using anthelmintic activities of medical plants (Fleming et al. 2006, Jabbar et al. 2006).

Anthelmintic Resistance in Turkey

Çırak et al. 2004 performed FECRT to detect the resistance status of strongylid nematodes on ten horse farms in Western Anatolia. Seven farms were found to be infected with the resistant cyathostomin population to benzimidazoles. Resistance of pyrantel embonate on five farms and macrocyclic lactone on six farms were investigated, but anthelmintic resistance was not detected.

Tınar et al. 2005 tested anthelmintic resistance in trichostrongylid nematodes of small ruminants by FECRT on twelve sheep and goat farms. Albendazole, tiabendazole, tetramisole and ivermectin resistance were tested and tetramisole resistance was detected in only one sheep farm.

Köse et al. 2007 tested albendazole, oxfendazole-oxyclozanide and ivermectin resistance by FECRT on seven sheep farms in Afyonkarahisar and found that ivermectin did not work at the desired level in five farms.

Çırak et al. 2010 found that macrocyclic lactone groups against *Parascaris equorum* in a horse farm were resistance.

Önder et al. 2016 determined the frequency of benzimidazole-sensitive and resistant alleles in the *H.concortus* population by 87.1% and 12.9%, respectively, and revealed the BZ resistance by the molecular method.

RESULTS

Consequently, as World Association for the Advancement of Veterinary Parasitology (WAAVP) has also noted, anthelmintic resistance is a very important and restrictive factor especially in livestock breeding. For this reason, the development of resistance to anthelmintics should not be overlooked in the selection and implementation of treatment and control options for helminth infections.

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REVIEW

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Rescue Glide Use in Emergency Large Animal Rescue

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ABSTRACT

The basis of animal rescue is to transfer an animal from a dangerous place to a safe place with the most appropriate method. The main principle is to use the simplest, safest, and most practical techniques while intervening in an incident, thus reducing the risk of injury to both the castaway animal and rescuers. As a general rule, the more detailed the procedure, the greater the risk to the rescuers' and victim's safety. Emergency rescue operations should optimize both rescuer safety and the victim's prognosis for post-incident recovery. Techniques and special equipment have been developed to rescue large animals more efficiently and safely. These techniques should be known without missing points. One of these rescue procedures is the transport of recumbent animals. This article deals with that transport of movement restricted animals and using the rescue glide, which is a basic procedure used in emergency rescue.

Keywords: animal, emergency rescue, rescue glide

Acil Büyük Hayvan Kurtarmada Kurtarma Kaydırağı Kullanımı

ÖZ

Hayvan kurtarma, bir hayvanı tehlikeli bir yerden güvenli bir yere, en uygun yöntemi kullanarak transfer etmek anlamına gelir. Hayvan kurtarmanın temel ilkesi, en basit, en güvenli ve en pratik teknikler kullanılarak, hayvanın ve personelin yaralanma riskini en aza indirecek şekilde müdahale etmektir. Genel bir kural olarak, prosedür ne kadar ayrıntılı olursa, kurtarıcıların ve mağdurun güvenliği için risk o kadar fazla olur. Acil kurtarma operasyonları hem personelin güvenliğini hem de kazazede hayvanın olay sonrası kurtarma için prognozunu optimize etmelidir. Büyük hayvanları daha verimli ve güvenli bir şekilde kurtarmak için teknikler ve özel ekipmanlar geliştirilmiştir. Bu tekniklerin net bir şekilde anlaşılması çok önemlidir. Bu kurtarma prosedürlerinden biri de yatan hayvanların taşınmasıdır. Bu makalede, yatan hayvanların taşınması ve acil kurtarma operasyonlarında kullanılan temel prosedürler olan kurtarma kaydırağının kullanılması açıklanmaktadır.

Anahtar Kelimeler: Hayvan, acil kurtarma, kurtarma kaydırağı

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INTRODUCTION

Disasters and catastrophies are important part of life. Situations that arise from natural or man-made incidents due to imprudence or unpredictable reasons and threatening health, life or the environment are defined as emergencies (Nikolovski et al. 2016). Emergencies are traumatic impacts with physiological, psychological and socio-economic dimensions and affect animals as much as people affect. In emergencies, people are sensitive to their own well-being. However, animal rescue and welfare work is not at the desired level when animals, our stakeholders in life are exposed to emergencies. It is common to be insensitive about an animal exposed to emergency situations and assume that it can found for itself in difficult circumstances. This is indicative of animal rescue training and equipment, which have been initiated and developed in recent years. Animals are our stakeholders in life and differ from us in that they cannot invent tools or instruments. Therefore, rescuing an animal exposed to emergency conditions by using technical opportunities and practical, safe methods is an area of responsibility for human beings as well as a scientific and ethical task.

Despite disasters and accidents affecting all animals, large, large animals have difficulties in reflexes such as escape, self defense and protection, and these animals require very serious consequences. Large animals often encounter accidents, such as falling into canals, pits, wells, creeks and streams and encounter life threatening risks. Common practices show that animals exposed to similar conditions are subjected to painful primitive methods during rescue efforts. Individuals who have not been trained can often lead to negative situations when they intervene with human emotions to an animal that has been exposed to an emergency. Interventions without regard to posture and animal-specific conditions, which are caused by the emotional state, physiological conditions, accidents, diseases and disasters regarding an animal exposed to emergency conditions and which must be protected, can lead to undesirable situations. Improving animal welfare such as not being timely intervened by a veterinarian or trained personnel exposed to an emergency, disaster or accident, not recovering the animal in a proper manner, using contemporary techniques during evacuation and transport, lifting animal welfare, increasing drowning, (Cengiz 2001), as well as the safety of individuals who want to help the animal. Emergencies, failure of animals exposed to disasters and accidents from interventions by a veterinary surgeon or trained personnel, failure to rescue animals according to proper procedure, failure to use modern techniques during evacuation and transport countermands animal welfare and increases animal losses, manifest serious tableaus such as drowning, cuts, broken and dislocated bones, ischemia, shock (Cengiz 2001**)** and endangers the lives of rescuers who want to help the animal.

Victim or survivor animal must receive treatment by an animal rescue team and subsequently be subjected to first aid. A rescuer with knowledge and experience must deliver first aid. We live in an era in which animal rights awareness and animal welfare practices are important and progressive, therefore, it is important to increase the number and capacity of specialized teams in emergency response (Biricik 2017, Fidan and Biricik 2018). It would be appropriate for veterinary faculties to deliver courses in such situations through protocols developed with relevant civil society and public organizations as well as include animal rescue and first aid in compulsory or elective curricula in 'Emergencies, Animal Rescue and First Aid.

Rescue operations require both the safety of the rescue team and well-managed post-event rescue and transport. Appropriate rescue procedures can be achieved through an organized approach between veterinary surgeons and other emergency response specialists (Biricik and Aksoy 2018).
The process starts with notification followed by first aid, transport, necessary treatment and rehabilitation and continues until the animal is fit to resume its normal life (Çakır et al. 2016). During this process, the equal importance of transport as well as rescue is manifested. The transport management of the injured animal is affected and varies as a result of numerous factors such as the number of injured animals, the species of the animal, the size of the injury, the health status of the rescued animal, the accessibility of the incident area as well as distance from health organizations (Nikolovskı et al. 2016).

As a different dimension of animal rescue, the use of rescue glides as an important rescue equipment in the transport of animals that cannot walk or stand up due to various reasons is addressed and technical information about application are given.

Transport in Animal Rescue

Transport which means to take something from one place to another, is an important part of first aid and animal rescue. The main objective of animal transport is to remove the victim from its current environment safely and deliver it to a health institution in the most appropriate way. Stabilization of the sick or injured animal is

ensured after first aid at the scene and subsequently it is delivered to the nearest health institution. When these procedures are carried out, the life of the animal and the first aid personnel should not be at risk and necessary precautions must be taken. Deciding how to transport the animal to the transporter once you are as confident as possible about the homeostasis of the victim's organism and using the equipment correctly and effectively during this procedure and acting according to the animal's physiological conditions and body mechanics are important parts of the transport procedure (MEB 2011, Çakır et al. 2016, Biricik 2017).

Transfer of the sick or injured animal takes place in three stages. 1) Removal of the animal from the site and transport to the ambulance (onsite transport). 2) Transfer of the animal taken to the ambulance to the health facility from the scene (primary transport). 3) Transportation of animals between health facilities (secondary transport) **(**MEB 2011). Every emergency situation has its own characteristics. Therefore, every situation for the transfer of animal must be assessed within itself and the method necessary for the transfer of the animal should be selected taking into consideration the basic principles. The method is determined according to the possible danger that could develop at the incident scene or the presence of hazardous substances, the ability of the personnel, the situation of the land and most importantly the status of the animal. Under normal conditions it is necessary to move the animal after stabilization at the scene. However, if there is a danger to the animal and the personnel, it is necessary to immediately remove the animal from the setting and take it to a safe place in case of unexpected and sudden developments such as disaster or accidents (MEB 2011).

The location where the incident has taken place may not always be easily accessible. An emergency may have occurred in a place that is difficult to reach, such as riverside, swamps, wells, inside vehicles, mountainous terrain, slopes, cliffs. In such cases, rescuing the animal from the conditions it requires special equipment and technical knowledge. The basic principle of animal rescue is to intervene with the most simple, safest, fastest, and most practical techniques to minimize the risk of injury to the animal and personnel. As the details of procedure and the number of required equipment increase, the safety risk to the animal and the personnel increases and the process becomes cumbersome and slow. For a successful rescue operation, it is important to provide a safe rescue medium for animals and personnel, to avoid iatrogenic injuries that cause permanent dysfunction or prolonged healing time and ensure that the staff at the scene are working with a team spirit (Gimenez et al. 2002).

Some basic principles for the transport of sick or injured animals are listed below (MEB 2011).

- Before deciding on how to transport the animal, an incident scene assessment shall be carried out to check for other possible hazards such as fire, explosion and necessary measures shall be taken.
- The injury status and area, the state of consciousness of the sick or injured animal shall be assessed.
- Stabilization of the animal, first aid before transport, the position of the victem during transport and the method of transport shall be planned within the available technical means.
- The most appropriate method for the transport of the animal is determined.
- The transport method is usually determined according by the clinical status of the victim, the distance of the incident scene to the nearest health facility, geography and available means.
- The transport is executed in an organized and unhurried manner.
- The animal is moved as little as possible during transport.
- The emergency relief team should know their capabilities and abilities. Necessary and appropriate equipment should be used during the transport process and applications which endanger the lives of the victim or the lives of the personnel.

Transport of Recumbent Animals

Small animals are also exposed to natural disasters and accident-based emergencies, but their small size facilitates recovery and transport. The difficulty of the rescue and transport of large animals is commensurate with their size, which is important in terms of process safety and success. Therefore, special equipment and technical know-how are needed to rescue all animals and especially for large animals. Large animals are often incapable of getting up after they have encountered a natural disaster or accident. This situation makes it difficult to transport recumbent animals. Transporting the animal safely from the incident scene to the ambulance, putting it into the ambulance, transporting it safely in the ambulance and countering all requirements is important for the transport.

First aid teams may be able to stabilize the animal at the scene; however transporting the animal in geographically difficult areas may be problematic. Problems such as the animal's current health condition, ability to walk, to stand or recumbency

and inability of the ambulance to access the incident scene due to geographical challenges are frequently encountered. Under the circumstances, the well-intentioned but erroneous behavior of those at the scene can endanger the lives of the animal as well as themselves. In such cases, technical personnel who can deal with the event and the necessary equipment must be available in the emergency call center. Therefore, the fire brigade and AFAD should provide necessary training to their personnel. It should not be forgotten that the basic principle of animal rescue is to intervene in the simplest, safest and least technological approach in a way with the least risk of injuring the animal and the personnel. Consequently, a simple and reliable method, which does not pose a threat to the health of the animal and personnel during transport of the animal from the incident scene to the ambulance, should be preferred. The role of veterinarian is rather important at this stage. When the decision to transport a sick or injured animal to the nearest health facility from the scene of the incident is made, the veterinarian has to organize the safe loading, transport and unloading of the animal. At this stage, the veterinary surgeon is not only responsible for the safety of the animal but the staff as well. It is always possible that the sick or injured animal develops a defense reflex that can cause harm. A veterinarian knows what reactions animals are capable of and is the person to take immediate measures at the scene. Therefore, efforts should not be made to transport the animal until it is calmed down and sedated under the control of a veterinarian. The animal must be under sedation before transport to avoid further injury during transport. The sick or injured animal's neurological and cardiovascular status should be carefully assessed before sedation or anesthetic protocol is selected. The equipment required to load the animal into the ambulance must be prepared and mounted before the animal is sedated. Once the necessary equipment is ready, sedation should be applied (Gimenez et al. 2002, Mccue et al. 2004, Biricik and Aksoy 2018).

The equipment to be used for transporting a recumbent animal with rescue glides is shown in Figure 1 below. These equipments should be prepared and mounted before the process.

Figure 1: 1. Rescue Glide (Anonymous 2018a:); 2. Glide Slip and J Hook for Glide Slip (Anonymous 2018b:); 3. Recovery Hood (Anonymous 2018c:); 4. Strap (Anonymous 2018d:); 5. Red Line Rescue Grade Nylon Rope (Anonymous 2018e); 6. Heavy Duty Ratchet Strap (Anonymous 2018f); 7. Fleece Pads For Ratchet Strap Comfort (Anonymous 2018g); 8. High Strength Nylon / Velcro Head Strap (Anonymous 2018h); 9. Nylon Hobbles (Anonymous 2018i); 10. Rescue Grade Heavy Duty Pulley For Nylon Rope (Anonymous 2018k); 11. Carabiner (Anonymous 2018l).

The procedures to be carried out during transport after the post-sedation phase are listed as follows (Gimenez et al. 2002, Gimenez et al. 2009, Leighton and Staples 2010,Thompson et al. 2015, Anonymous 2018m, Anonymous 2018n, Anonymous 2018o).

1. Even though the animal has been sedated, care must be taken against possible defensive reflexes when holding the animal's limbs. Since the animal will not be transported without being fully stabilized in the proper posture, the animal must be approached from the dorsal side and the extended limbs of the animal up to 45-degree angles should be avoided. This is important for the safety of the staff.

2. A recumbent animal must be fitted with recovery hood before transporting it to the ambulance as shown in Figure 2. Recovery hood designed to protect the heads of large animals is padded and designed for use during rescue operations or post-operative use. It can be used for rescue of large bulky animals, for protection during transport of injured animals or during recovery from some neurological conditions.

Figure 2: Protective headgear on a recumbent animal

3. Dragging sideways is the most practical and effective way of correctly positioning the animal on the rescue glide. Once the protective headgear has been fitted on the head of the sedated animal, a part of the glide slip, if any, is moved underneath the animal towards the back of the front legs, and the part underneath is moved over the animal towards the rear. This makes it easier to move the animal onto the Rescue Glide. During these operations, the J hook for Glide Slip can also be used (Figure 3). If there is no rescue glide, large plastic or woven belts or fire hoses can be used to carry out this operation. This will allow the recumbent animal to be moved safely.

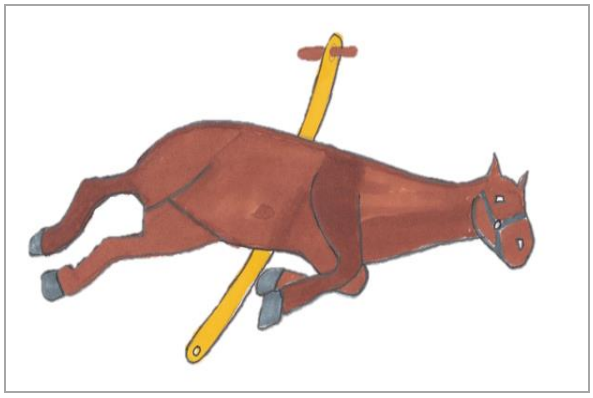

Figure 3: Using the Rescue Glide

4. The rescue glides is advanced underneath the dorsal part of the recumbent animal. By using the glide slip or wide straps, the animal is pulled over the animal rescue glide by lifting the animal's head and tail and pulled onto the glide (Figure 4). Necessary precautions shall be taken to prevent the slide from bending during pulling. During this process a staff member must always keep the animal's head in check. A rescue glide is a device used to remove a sick / injured animal on dry ground from the scene to be loaded into an ambulance. It is made of steel brackets with recessed bolts on the top and bottom for a smooth surface and is made of slip-promoting material such as soft Teflon or polyethylene. It usually measures 1.2 m x 2.4 m x 6 mm and modular joints can be made for the transport of larger animals. When the ring in the front is tied to a large pulley, the glide can be pulled into the ambulance with the animal.

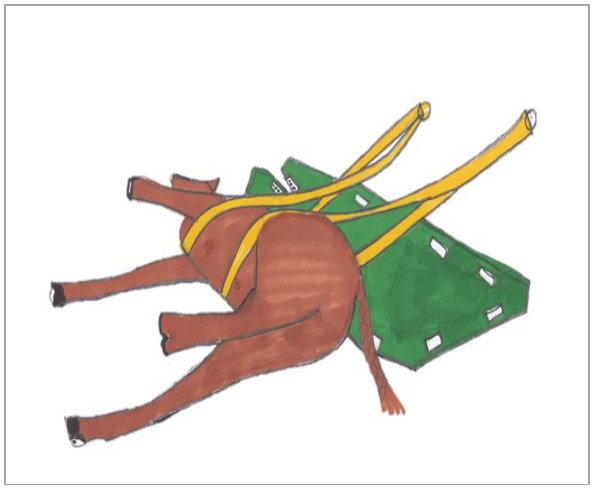

Figure 4: Hoisting an Animal onto the Rescue Glide

5. After being pulled onto the glide, the animal must be fixed on the slip. It is beneficial to use 2 ratchet straps when fixing the animal pulled onto the rescue glide. When attaching the strap to the rescue glide, the position to be taken is behind the animal's feet, the side with the feet must be

avoided. One of the ratchet belts is placed behind the front feet, both ends of the belt are passed through the holes on the glide, the ratchet belt is stretched until the sides of the rescue glide start to lift, and then it is fixed. However, an overly stretched strap can harm the animal. Therefore, felt pads are used to prevent the belts and the ratchets from harming the animal. The second ratchet strap is placed in front of the rear legs of the animal (Figure 5). An important issue here is that tightening a belt can cause the other one to loosen. Consequently, both belts must be checked for tightness and maintained that way.

Figure 5: Fixing the animal on the rescue glide

6. The next step is to fix the animal's legs. For this, the animal's feet must be tied to with a foot strap, pulled towards the body, and fixed. To do this, a staff member must position the animal's legs with the rescue hook without getting too close to the animal. Even if the animal is sedated it can kick spasmodically, so when the legs are fixed, a staff member approaches the back of the animal and ties the front legs first with foot ties and then the hind legs to each other. A rope is then passed through the rings on the foot ties after which the front and hind legs are bent and pulled as close to the body of the animal as possible with the help of the pulley and carabineer and the rope is fixed by passing it through the empty hole in the rescue glide (Figure 6). Felt pads should be used so that the equipment used during this process does not harm the animal. An important issue here is whether the animal has any injuries on its legs and feet. If the animal's leg is injured, only the uninjured legs are tied with the foot strap and the injured leg is left free, the injured leg is treated with bandages, splints, etc. During this process, the animal's tail must be positioned on top of the animal and tied with a strap to prevent it from getting caught in the rescue glide. The placement and fixing of a protective pad or pillow underneath the animal's shoulder is another important issue that must be accomplished prior to transport. If these procedures are carried out correctly, the animal is fixed on the glide.

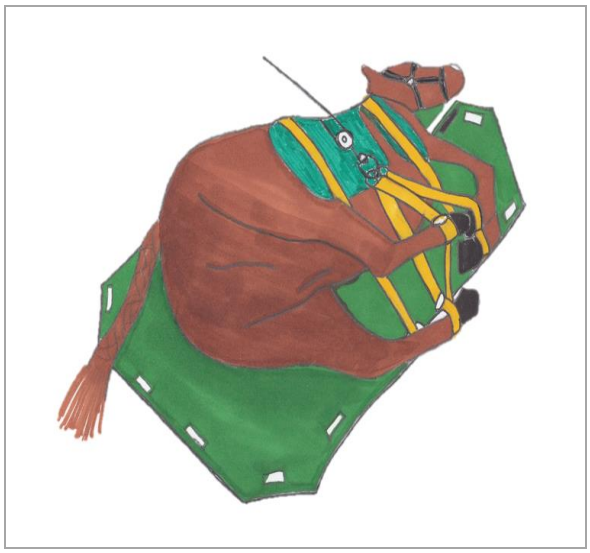

Figure 6: Fixing the Animal's Legs

7. Once the animal is fixed onto the glide it is ready to be moved. A rope is passed through the ring at the front of the glide and the glide is pulled while keeping the animal under control. In case the glide needs to be moved uphill, measures must be taken to prevent the animal from slipping backwards on the slide. Approaching the ambulance, the rope tied to the front of the glide is passed through the ring in the ambulance and the glide is loaded into the ambulance by pulling the glide into the ambulance manually or by means of a pulley (Figure 7).

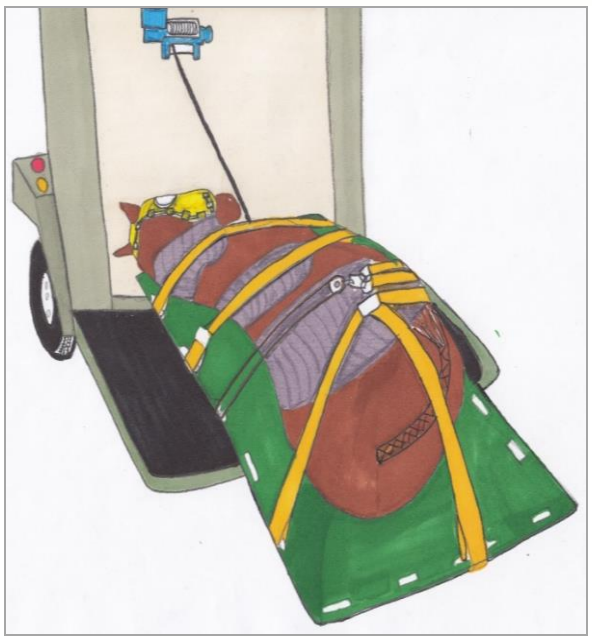

Figure 7: Moving the animal to the ambulance with rescue glides

8. After the animal is loaded into the ambulance, its posture is corrected and the animal is brought into a comfortable position. In order to protect this position, straw bales or other non-traumatic suitable materials are placed around to create a stable and safe environment.

CONCLUSION

Principle of whole times in animal rescue is to use the safest, the fastest, the most practical and easy techniques and equipments. Because rescue is against time. In this process, it is important to protect the homeostatic balance of the animal, prevent the deterioration of physiological and biochemical markers, and observe the safety of personnel. Experienced staff and appropriate equipment are essential to reduce the vital risks of animals and personnel to the minimum and for maximum safety, but to make them all so fast. For this reason, the most appropriate way to intervene in rescue and transport operations is to be sought and found as an option. The other important issue is personnel compliance and team work for rescue and transfer operations. A highly coordinated workflow is essential for emergencies. It is necessary to work quickly without forgetting that the race is against time, but without panic. It is important for the safety of the personnel to stand out from the hoof of the animal during restoration and transportation, to avoid the animal's mouth area which is prone to bite, to prevent the animal's claws which are known to be effective in this regard. Safety requirements should not be relaxed throughout the process. The vitality and health conditions of the animal should be constantly monitored. In order to complete the transfer process successfully, the capturing rapt, tightening of the loose belts and other fixing measures must be carried out without compromise. . This study is on the transport of restricted animals and the use of the rescue glide. Using rescue glide is the most important step for saving the animal health and staff safety in large animal rescue operations. For this reason, the article is an update that includes information, suggestions and techniques that can help researchers and institutions working on large animal rescue. .

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LETTER TO EDITOR

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Ehlers-Danlos Sendromlu Kedi ve Köpeklerde D Vitamini Düzeyleri: Nutrisyonel Bozukluk Hipotezine Dair Olgu Serisi

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ÖZ

Farklı hayvan türlerinde bildirilen ve derinin elastikiyetinde azalma, deride aşırı uzama, yumuşama bulgular gösteren Ehlers Danlos Sendromu (EDS) nadir görülen kalıtsal kollojen hastalığıdır. Hayvanlarda dermatosparaksis ve kutanöz astenia olarak da bilinen bu hastalığın tanısı klasik klinik bulgular, cilt uzayabilirlik indeksi ile histopatolojide kalın, düzensiz ayrılmış kollojen demetlerinin görülmesine dayanmaktadır. Farklı yaş (n=4 kedi; 1-3 yaşlı ile n=6 köpek 1-5 yaşlı), her 2 cinsiyetten kedi (2 erkek, 2 dişi) ve köpeğin (4 erkek, 2 dişi) dahil edildiği olgu serisinde serum 25 (OH) D3 seviyeleri ile ilişkileri sunulmaktadır. EDS tanısı konulan kedi ve köpeklerde bulunan düşük vitamin D seviyeleri (toplamda 8/10) düşünüldüğünde EDS' li kedi ve köpeklerde diğer beslenme eksikliklerinin yanı sıra vitamin D yetersizliğinin de göz önüne alınması gerektiği ve beşeri hekimlikte olduğu gibi oral vitamin D takviyesinin güvenle verilebileceği önerilmektedir.

Anahtar Kelimeler: Ehlers-Danlos Sendromu, kedi, köpek, vitamin D

Vitamin D Levels in Cat and Dogs With Ehlers-Danlos Syndrome: Case Series Touching Nutritional Deficiency Hypothesis

ABSTRACT

Ehlers Danlos syndrome (EDS) reported varied animal species is a rare hereditary collagen disorder standing out reduced strength of skin being hyperextensible, velvety and fragile. Diagnosis of also termed dermatosparaxis or cutaneous asthenia in animals is based on classic clinical symptoms, skin extensibility index with histopathologic examination included thin, unregular seperated collagen bundles. In the presented case series enrolled different ages (1-3 years old 4 cats; and 1-5 years old 6 dogs) with both gender cats (2 males, 2 females) and dogs (4 males, 2 females) has been conferred serum 25 (OH) D3 levels associations. Just as dog and cats diagnosed with EDS found lower vitamin D levels (total 8 of 10) are considered, it has recommended in cat and dogs with EDS taking into account of vitamin D deficiency as well as some other nutritional deficiencies and giving oral vitamin D supplements with safety as human medicine.

Keywords: Ehlers-Danlos Syndrome, cat, dog, vitamin D

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GİRİŞ

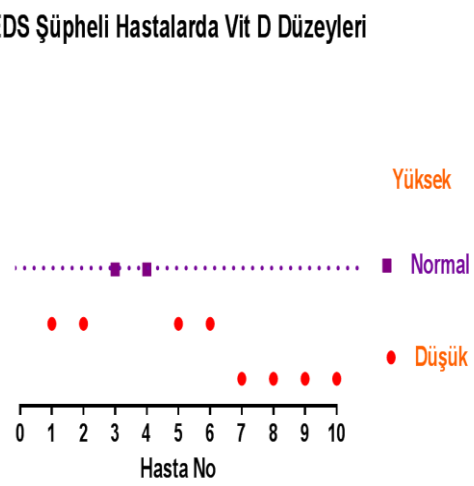
Bir grup kalıtsal bağ dokusu hastalığı olan Ehlers – Danlos sendromu (EDS), insanlarda olduğu kadar hayvanlarda da bilinmektedir (Pyeritz, 2012). Bu hastalığın doğal olarak ortaya çıkan formu atlar (De Paepe ve Malfait 2012, Beighton ve ark. 1998, Beighton ve ark. 1986), kediler (Germain 2007, De Felice ve ark. 2004, Lum ve ark. 2011, Oderich ve ark. 2005, Pepin ve ark. 2000), köpekler (Bergqvist ve ark. 2013, Busch ve ark. 2014, Horowitz ve ark. 2000, Malfait ve De Paepe 2009), buzağılar, koyunlar ve tavşanlar (De Paepe ve Malfait 2012, Malfait ve De Paepe 2009) olmak üzere farklı hayvan türlerinde bildirilmektedir. Bununla birlikte hastalık kedilerde nadiren görülür ve mevcut raporların çoğu bireysel vakaların tanımlarıdır (Beighton ve ark. 1998). Etkilenen hayvanlar hiperekstensibilite ve kırılgan bir cilde sahiptir (Pyeritz 2012, Oderich ve ark. 2005). Gecikmiş yara iyileşmesinin klinik ve histolojik kriterler kullanılarak insanlarda EDS'nin bir komplikasyonu olduğu, köpeklerde ve kedilerde yara iyileşmesinin EDS'ye ilişkin olduğu bildirilmiştir (Asherson ve ark. 2006). Bu bozukluklara hayvanlarda dermatosparaksis veya kutanöz asteni de denilmektedir (Pyeritz. 2012).

Tanı, histopatolojik incelemelerle doğrulanır, birbirinden uzak bir mesafede yer alan ve daha küçük parçalara ayrılan ve düzensiz bir model oluşturan kolajen liflerinin anormal yapısını açığa çıkarır (Calatzis ve ark. 2003). Rutin boyama tekniğinden ayrı olarak, bağ dokusunun yapılarını seçici bir şekilde ortaya koyan birkaç yardımcı yöntem vardır. Teşhis prosedüründe yararlı bir araçtır. Van Gieson, Mallori ve Masson boyama teknikleri en yaygın olarak kullanılmaktadır (Calatzis ve ark. 2003, Lindsay ve ark. 2015, Yenicesu ve ark. 2000). Kesin tanı, farklı bir kesit çapına sahip olan (Pyeritz 2012, De Paepe ve Malfait 2012, De Felice ve ark. 2004, Asherson ve ark. 2006, Lindsay ve ark. 2015) kollajen liflerinin düzensiz yapısını ortaya çıkaran bir elektron mikroskobu altında bir deri örneğini inceledikten sonra belirlenebilir.

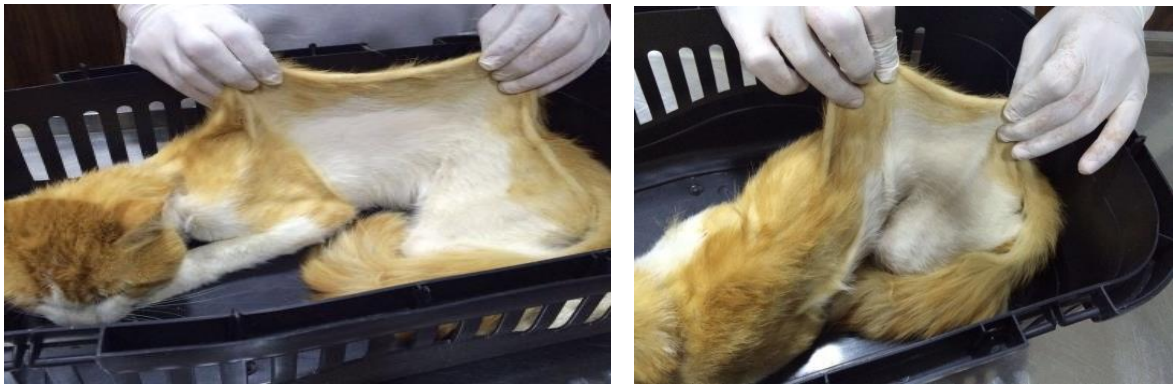
Hastalık için tedavi yoktur, ancak tutarlı yönetim, etkilenen kedilerin uzun ömürlü yaşaması sağlanabilir (Oderich ve ark. 2005). Hastalık transgenik farelerde deneysel olarak uyarılmıştır (Ong ve ark. 2010). Ehlers-Danlos Sendrom da insanlarda teşhis edilir. Bu durumda 10 türe ayrılır (Busch ve ark. 2014). Tek tek formlar dominant veya resesif bir genle veya kromozom X ile bağlantılıdır. İnsanlarda, hastalığın nedeninin, kollajen sentezindeki bazı kusurlar ile ilişkili olduğu (örn. Tip VIIc'de procollagen peptidazın aktivitesinde anormallikler) düşünülmektedir (Busch ve ark. 2014). Kedilerde, yakın zamana kadar, iki tip kutanöz asteni belirtilmiştir: baskın ve resesif kalıtım biçimi (Germain 2007, Pepin ve ark. 2000, Stine ve Becton 1997, Calatzis ve ark. 2003) köpeklerde ise, hastalık dominant bir gen ile bağlantılıdır. Ancak bu türlerde ortaya çıkan çekinik tip hakkında bazı öneriler vardır (Calatzis ve ark. 2003). Köpeklerde hastalık en sık Dachshunds, Boxers, St Bernard köpekleri, Alman çobanları, Springer spaniel, Greyhound, İrlanda setteri ve Poodles (Calatzis ve ark. 2003), kedilerde ise Himalaya (Beighton ve ark. 1998, Pepin ve ark. 2000, Calatzis ve ark. 2003) veya domestik kısa tüylülerde (Germain 2007, Stine ve Becton 1997, Calatzis ve ark. 2003) görülebilmektedir. Bazı yazarlar, kedilerin uzun tüylü ırklarının da bu cilt durumuna yatkın olduğunu iddia etmektedir (Germain 2007). Bu iki ırkın hiçbirinde cinsiyet eğilimi bulunmamaktadır (Germain 2007). Hastalık klinik bulgulara ve yardımcı testlere dayanarak teşhis edilir. Klinik muayenede cilt uzayabilirlik indeksi hesaplamasını içermesi çok önemlidir. Bu parametrenin ortalama değerleri köpeklerde% 14.5'in üzerinde, kedilerde% 19'un üzerinde ve tavşanlarda% 19.2'dir (Pyeritz 2012, De Felice ve ark. 2004, Bergqvist ve ark. 2013, Asherson ve ark. 2006, Calatzis ve ark. 2003).

Bu olgu serisinde farklı yaş (n=4 kedi; 1-3 yaşlı ile n=6 köpek 1-5 yaşlı), her 2 cinsiyetten kedi (2 erkek, 2 dişi) ve köpeklerde (4 erkek, 2 dişi) kutanöz asteni (EDS) ile serum 25 (OH) D3 seviyeleri arasındaki ilişki sunulmaktadır. Çalışmamızda yer alan olgulara ait sonuçlar aşağıda tabloda sunulmuştur.

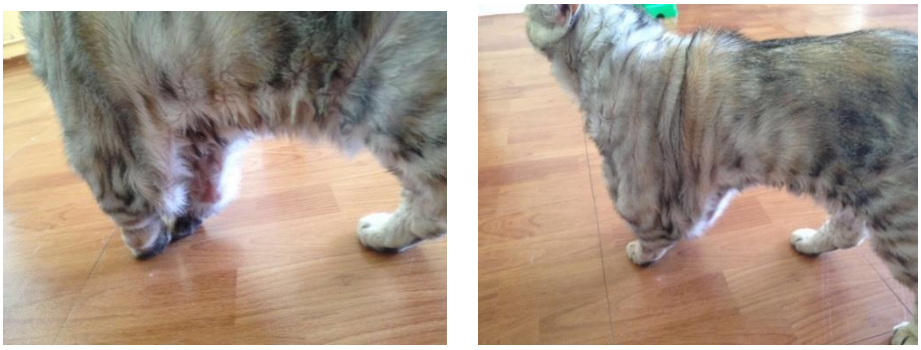
Tablo 1. Olgulara ait serum vitamin D sonuçları.
Table 1. Serum Vitamin D levels of cases.



*Olgu no: 1-4 kediler, 5-10 köpekler olarak yer almıştır.



Şekil 1. Olgu 1, 1 yaşlı erkek kedi. Derideki frajilite ve ektensiyon belirgin (Gaziemir Veteriner Kliniği, Veteriner Hekim Kemal Şimşek ile konsülte ve tedavi edilen olgu).
Figure 1. Case 1, a 1-years-old male cat. Explicit fragility and extensibility of skin (treated case was consulted with Veterinarian Kemal Simsek in Gaziemir Veterinary Clinic)



Şekil 2. Olgu 2, 2 yaşlı kedi. Deride sarkma, ekstensiyon dikkat çekici. Vetform Veteriner Kliniği, İstanbul'da Veteriner Hekim Gözde Çetin Kasap ile konsülte edilen olgu.
Figure 2. Case 2, a 2-years-old cat. Remarkable hanging and extension of skin. (case was consulted with Veterinarian Gözde Çetin Kasap in Vetform Veterinary Clinic)

Şekil 3. Olgu 3, 4 yaşlı köpek. Deride ekstensiyon ve fleksibilite artışı. Vetform Veteriner Kliniği, İstanbul'da Veteriner Hekim Gözde Çetin Kasap ile konsülte edilen olgu.
Figure 3. Case 3, 4-years-old dog. Extesion of skin and increased flexibility. (case was consulted with Veterinarian Gözde Çetin Kasap in Vetform Veterinary Clinic)

Ehlers-Danlos sendromu, kolajen sentezi ve metabolizmasındaki anormalliklerden kaynaklanan ve bağ dokusu bozukluklarından oluşan nadir görülen bir hastalıktır. Hastalık özellikle eklem hipermotilitesi, yaralanma ve artrite yatkınlık, deri ve vasküler problemleri (kolay morarma, kanama, varisli damarlar ve zayıf doku iyileşmesi) kardiyak mitral kapak prolapsları, iskelet ve kas problemleri (miyopati, miyalji, spinal skolioz, osteoporoz) ve peridontitlere sebep olabilen Ehlers-Danlos sendromunun klasik tipleri (Tip I-III) şeklinde karşımıza çıkmaktadır. Hastalık için günümüzde herhangi bir sağaltım mevcut değildir. Söz konusu hastalıkta ilişkili güncel yaklaşımların; (i) tek başına kusurlu genlerden kaynaklandığı düşünüldüğünde hastalığın patogenezinde beslenmenin önemine yönelik olarak bilimsel kanıtların artırılmasının, (ii) Ehlers-Danlos sendromu ile ilişkili semptomların çoğunun aynı zamanda beslenme eksikliklerinde de görülen karakteristik bulgular olduğunun, (iii) normal doku fonksiyonlarının sağlanmasında besin takviyeleri ile oluşturulan uygun kombinasyonların vücut ile sinerjistik etki göstermesi gibi temellere dayanmaktadır. Bu nedenle, Ehlers-Danlos sendromu ile ilişkili semptomların, kalsiyum, karnitin, koenzim Q, glukozamin, magnezyum, metil sülfonil metan, pnoginojen içeren spesifik (ve potansiyel olarak sinerjistik) bir besin takviyesi kombinasyonu kullanılarak başarılı bir şekilde ortadan kaldırılabileceğini varsayılmaktadır (Bergqvist ve ark. 2013). Bununla birlikte silika, C vitamini ve K vitamini, gibi ajanların belirtilen semptomlara karşı etkili olduğu kanıtlanmış durumdadır. Tüm bunlara karşın bu makalenin yazarları nezdinde yapılan literatür taramalarında vitamin d sviyelerinin gerek kedi gerekse köpeklerde incelenmediği saptanmıştır.

Tıp Hekimliği alanında gerçekleştirilen bir çalışmada vasküler tipte EDS bulunan 22 kişinin yarısından fazlasında d vitamini yetersizliği tespit edilmiş, gerekçe olarak vasküler damar bütünlüğünün bozulması gösterilmiştir (Busch ve ark. 2016). Bu makaleye konu olduğu üzere özel ilgi alanımıza girecek şekilde EDS'lu kedi ve köpeklerde (toplamda 8/10) belirlenen düşük vitamin d seviyeleri, bilgimiz ve literatür taramalarımız dahilinde daha önceden tespit edilmemiştir. Vitamin d teşhisinin çok pahalı olmaması, kolekalsiferol yada benzeri bir başka preparat ile oral takviyenin mümkün ve güvenilir (yan etkiye maruz bırakmaksızın) gibi tüm öne sürülebilecek gerekçelerden ötürü EDS'lu kedi ve köpeklerde diğer bazı nutriyonel eksikliklerin yanı sıra vitamin d eksikliğinin de göz önünde bulundurulması gerektiği söylenebilinir. Tıp hekimliği alanına benzer şekilde vasküler tipte EDS olgularda oral d vitamini takviyesinin yararlılığı antiinflamatuvar ve rejeneratif [kronik düşük dereceli inflamasyona karşı] etkilerinden kaynaklanabilir (Moriestte ve ark. 2014, Wong ve ark. 2014).

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