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Parvoviral enteritisli köpeklerde sepsis ve SİYS'e bağlı miyokardiyal fonksiyon değişikliklerinin ekokardiyografik değerlendirilmesi**

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ÖZET:

Bu çalışmada, parvoviral enteritisli köpeklerde sepsis ve SİYS'e bağlı miyokardiyal fonksiyon değişiklikleri ile standart ekokardiyografi ve iki boyutlu benek takibi ekokardiyografi (2DBTE) yöntemlerinin etkinliği karşılaştırılmıştır. Bulguların, kardiyak komplikasyonların erken teşhisine katkı sağlaması amaçlanmıştır. Çalışmaya, CPV enfeksiyonu teşhisi konulan ve SİYS kriterlerinden en az ikisini gösteren 16 yavru köpek (Grup A) ile sağlıklı 16 yavru köpek (Grup B) dâhil edildi. Grup A ve Grup B'de bulunan köpeklerden bir kez kan alınarak hematolojik muayene gerçekleştirildi. Her iki gruptaki yavru köpeklere standart ekokardiyografi ve 2DBTE uygulandı. 2DBTE ile sol ventrikül radyal gerilimi ve gerilim hızını belirlemek için görüntüler kaydedildi ve çevrimdışı analiz edildi. Grup A ve Grup B'nin radyal gerilim ve gerilim hızları kıyaslandığında istatistiksel olarak anlamlı bir farka rastlanmadı. Ancak Grup A'da ölen hayvanların sağlıklı hayvanlara kıyasla daha düşük radyal gerilim değerlerine sahip olduğu belirlendi. Standart ekokardiyografi ile belirlenen ejeksiyon fraksiyonunun (EF) ve fraksiyonel kısalmanın (FS) gruplar arasında fark oluşturmadığı görüldü. Sepsis ve SİYS görülen parvoviral enteritisli yavru köpeklerde sol ventrikül fonksiyonlarının etkilendiği ve bu fonksiyonların değerlendirilmesinde standart ekokardiyografi ile 2DBTE metodunun da kullanılması gerektiği kanısına varıldı.

Echocardiographic evaluation of myocardial function changes due to sepsis and SIRS in dogs with parvoviral enteritis

ABSTRACT:

In this study, the effectiveness of conventional echocardiography and two-dimensional speckle tracking echocardiography (2DSTE) methods were compared with changes in myocardial function due to sepsis and SIRS in dogs with parvoviral enteritis. The findings are aimed to contribute to the early diagnosis of cardiac complications. The study included 16 puppies diagnosed with CPV infection and meeting at least two of the SIRS criteria (Group A) and 16 healthy puppies (Group B). Blood sample was taken once from the dogs in Group A and Group B, and whole blood analysis was performed. Standard echocardiography and 2DSTE were performed to the puppies in both groups. Images were recorded and analyzed offline to determine left ventricular radial strain and strain rate with 2DSTE.When the radial strain and strain rate of Group A and Group B were compared, no statistically significant difference was found. However, it was determined that animals that died in Group A had lower radial strain values compared to healthy animals. Ejection fraction (EF) and fractional shortening (FS) determined by standard echocardiography did not differ between the groups. It was concluded that left ventricular functions are affected in puppies with parvoviral enteritis with sepsis and SIRS, and the 2DSTE method should be used together with standard echocardiography in the evaluation of these functions.

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** *''Parvoviral enteritisli köpeklerde kalp kası fonksiyonlarının ekokardiyografi ile değerlendirilmesi'' isimli doktora tezinden özetlenmiştir.*

1. Giriş

Canine parvovirus (CPV) enfeksiyonu köpeklerde yaygın olarak görülen önemli viral bir hastalıktır (1). Köpeklerde klasik parvoviral enteritisten sorumlu etken Canine parvovirus tip 2 (CPV-2)'dir (2). Virus intestinal kript, timus, lenf yumruları ve kemik iliği prekürsör hücreleri gibi hızlı bölünen hücrelerde yıkıma neden olur. Bunun sonucunda intestinal mukozal bariyerin bozulması, villöz atrofi, malabsorbsiyon, lökopeni (özellikle nötropeni ve/veya lenfopeni), diyare, kusma, şiddetli dehidrasyon/hipovolemi, metabolik asidoz (veya alkaloz) ve bakteriyel translokasyon görülebilir (2,3). Ölüm genellikle septisemi, endotoksemi, sistemik inflamatuvar yanıt sendromu (SİYS), yaygın intravasküler koagülasyon (DIC) ve çoklu organ yetmezliğinden kaynaklanmaktadır (4,5).

Miyokardiyal hücreler prenatal dönem ve doğumdan sonraki ilk birkaç haftalık dönemde hızlı bölünürler ve bu dönemde CPV için hedef oluştururlar. Miyokarditis şekillenen yavrularda ani ölüm görülmektedir (1). Miyokarditis sadece parvoviral enteritise bağlı değil aynı zamanda sepsis sonucu da oluşabilir. Bunun nedeninin multifaktöriyel olduğu düşünülmektedir (6,7,8). Miyokarditiste akut devreyi atlatan köpeklerde ilerleyen yaşlarda dilate kardiyomiyopati (DCM) ve konjestif kalp yetmezliği (CHF) gibi kronik kardiyak kalp hastalıkları görüldüğü bildirilmiştir (9,10,11). Mortalite yavru köpeklerde yüksek olabilirken (yaklaşık %70) erişkin köpeklerde %1'den daha azdır (3).

Sistemik inflamatuvar yanıt sendromu enfeksiyöz ya da nonenfeksiyöz nedenlerle (kanser, bağışıklık aracılı hastalıklar, diyabetik ketoasidozis gibi) ortaya çıkan geniş çaplı inflamatuvar bir yanıttır. Köpeklerde SİYS'ten söz edilebilmesi için Tablo 1'de belirtilen ölçütlerden iki veya daha fazlasının birlikte olması gerekmektedir (12). Sepsis, enfeksiyona karşı gelişen SİYS'tir ve konakçının enfeksiyona verdiği aşırı ve düzensiz tepki sonucu oluşan organ disfonksiyonunu ifade etmektedir (13,14). Sepsis sırasında dolaşıma geçen bakteriler direkt veya toksinleri aracılığı ile SİYS'i tetikler, bu durum çoklu organ yetmezliğine kadar giden patolojik olayları başlatır. Patolojik mekanizmaların aktivasyonu sonucunda ortaya çıkan endotel hasarı, kapiller permeabilite artışı, kanın mikro dolaşımda göllenmesi ve dolaşımdaki kan hacminin azalması çoklu organ yetmezliği, şok ve ölüme neden olmaktadır (15,16,17). Parvoviral enteritisin de köpeklerde sepsise neden olduğu bilinmektedir (12,18,19) ve ölüm çoğunlukla sepsisin neden olduğu komplikasyonlardan kaynaklanmaktadır (19). Sepsis şekillenen köpeklerde ölüm oranının ise %20-68 olduğu bildirilmiştir (20).

Tablo 1: Sistemik inflamatuvar yanıt sendromunun kriterleri
Table 1: *Criteria for systemic inflammatory response syndrome*

Parametre	**Kriter**
Vücut sıcaklığı (ºC)	< 37,8 °C ya da > 39,4°C
Kalp frekansı (dk)	> 140/dk
Solunum frekansı (dk)	> 20/dk
Lökosit sayısı (WBC) (x 10^9 /L)	< 6,0 ya da > 16,0
Band nötrofil (%)	> %3

**Alves F, Prata S, Nunes T, Gomes J, Aguiar S, Aires Da Silva F, et al. Canine parvovirus: a predicting canine model for sepsis. BMC Vet Res BioMed 2020;16:1–11.*

Sepsisin komplikasyonu olarak ortaya çıkan miyokardiyal disfonksiyon insanlarda ölümlerin başlıca sebebi olarak görülmektedir (21). Yoğun bakım ünitelerindeki kritik hastalarda prevalans %10-70'tir (22). Septik miyokardiyal disfonksiyonun kesin bir tanımı olmasa da sepsis sonucunda sağ ve sol ventriküllerde şekillenen, geri dönüşümlü, sistolik ve diyastolik fonksiyon bozukluğu olarak ifade edilebilir (23). Parvoviral enteritisli köpeklerde %38 oranında görülebilen miyokardiyal hasarın bu hastalıkta ölümlerin ana nedenlerinden olduğu

düşünülmektedir(24). Naseri ve ark.'a (17) göre sepsis ile indüklenen kardiyomiyopati, sitokin fırtınası, mitokondriyal disfonksiyon ve azalmış kalsiyum duyarlılığı gibi çeşitli patofizyolojik mekanizmalar sonucunda oluşmaktadır. Bu mekanizmaların parvoviral enteritisli sepsisli köpeklerde de benzer şekilde miyokardiyal disfonksiyona neden olması muhtemeldir.

Parvoviral enteritisli köpeklerde gelişen sepsis ve sistemik inflamatuvar yanıt sendromu (SİYS), sol ventrikül sistolik fonksiyonlarında bozulmaya yol açarak miyokardiyal hasarı artırır. İki boyutlu benek takibi ekokardiyografi (2DBTE) yöntemi sol ventrikül fonksiyonlarındaki değişiklikleri standart ekokardiyografik değerlendirmelere kıyasla daha hassas bir şekilde tespit edebilir. 2DBTE miyokardın belirli bölgelerine yerleştirilen benek desenlerinin kardiyak siklus boyunca hareketini analiz ederek gerilim (strain) ve gerilim hızı (strain rate) ölçümlerini sağlayan güncel bir ekokardiyografik metottur (25).

Bu çalışmanın amacı, parvoviral enteritisli köpeklerde sepsis ve SİYS'ye bağlı miyokardiyal fonksiyon değişikliklerini belirlemek ve bu değişikliklerin tespitinde standart ekokardiyografi ile 2DBTE yöntemlerinin etkinliğini karşılaştırmaktır. Bu doğrultuda, sağlıklı ve hasta köpeklerde sol ventrikül radyal gerilim ve gerilim hızları değerlendirilecek ve miyokardiyal fonksiyon bozuklukları araştırılacaktır. Elde edilen verilerin, parvoviral enteritisli köpeklerde kardiyak komplikasyonların erken teşhisi ve uygun tedavi stratejilerinin geliştirilmesine katkı sağlaması hedeflenmektedir.

2.Gereç ve Yöntem

Hayvan Materyali

Çalışmada hayvan hastanesine getirilen 4-16 haftalık yaşta, immunokromatografik analiz (Bionote Anigen Hızlı CPV test kiti, Sensitivite %100, Spesifite %98.8), klinik bulgular (halsizlik, iştahsızlık, kusma, kanlı ishal gibi) ve hemogram bulguları sonucu CPV enfeksiyonu tanısı konulan ve SİYS kriterlerinin (Tablo 1) iki veya daha fazlasını gösteren, daha önce tedavi uygulanmamış çeşitli ırk ve cinsiyette sahipli 16 hasta yavru köpek (Grup A) ile herhangi bir hastalık şikâyeti bulunmayan, yapılan klinik, hematolojik ve parazitolojik muayeneler sonucu sağlıklı olduğu belirlenen 4-16 haftalık yaşta, çeşitli ırk ve cinsiyette sahipli 16 sağlıklı yavru köpek (Grup B) olmak üzere toplam 32 yavru köpek kullanıldı.

Klinik Muayene ve Laboratuvar Analizleri

Köpeklerin kliniğe getirildikleri ilk gün vücut sıcaklıkları (°C), kalp ve solunum frekansları (frekans/dk) belirlendi ve fiziksel muayeneleri (kapiller dolum zamanının belirlenmesi, mukozaların kontrolü ve oskültasyon) yapıldı. Parazitolojik olarak dışkı muayenesi negatif köpekler (Grup A ve Grup B) çalışmaya dâhil edildi. Her iki gruptaki köpeklerin *vena cephalica antebrachii*'lerinden etilen diamin tetra asetik asit (EDTA) içeren tüplere alınan 1 ml kan örneğinde EXIGO VET otomatik kan hücre sayım cihazı ile hematolojik analiz gerçekleştirildi. Grup A'daki köpeklerden alınan rektal swap numunelerinde hızlı test kiti pozitif olan (parvovirus antijeni pozitif), klinik ve laboratuvar bulguları CPV ile uyumlu köpeklerde SİYS kriterleri (Tablo 1) değerlendirildi. İki ya da daha fazla kriterin bir arada bulunması SİYS olarak kabul edildi ve bu köpekler çalışmaya dahil edildi.

Ekokardiyografik Muayene

Her iki gruptaki köpeklere Hitachi Arietta 60 model ekokardiyografi cihazı ve S211 Sektör Prob (1-5 MHz) ile ekokardiyografi yapıldı. Çalışma grubundaki yavru köpeklere sedasyon uygulanmadı. Sağ lateral pozisyona alınan köpeklerde ekokardiyografi cihazının elektrotları sağ ön, sol ve sağ arka bacaklara takıldı. Köpeklerden standart sağ parasternal kısa ve uzun eksen ekokardiyografik görüntüler alındı. M-mod ekokardiyografi ile ejeksiyon fraksiyonu (EF) ve fraksiyonel kısalma (FS) değerleri hesaplandı. Klinik muayenede konjenital ya da edinsel bir kalp hastalığından şüphe edildiğinde (üfürüm, egzersiz intolerans, senkop gibi) veya sol ventrikül fonksiyonlarının daha iyi

değerlendirilmesi istendiğinde sol parasternal apikal görüntüler de alındı. Her bir ekokardiyografik görüntüleme sırasında eş zamanlı olarak ekokardiyografi cihazına entegre edilmiş elektrotlar aracılığıyla elektrokardiyografi (EKG) traseleri kaydedildi. EKG'de sinüs aritmi dışında patolojik aritmi görülen yavru köpekler çalışmadan çıkartıldı. Sağ lateral pozisyonda yatırılan köpeklerden papillar kas seviyesinde, parasternal kısa eksen ham görüntüler radyal gerilim ve gerilim hızlarının çevrimdışı tespiti için kaydedildi. Her bir muayenede ardı ardına en az 6 kardiyak siklusun (bir QRS kompleksinden diğer QRS kompleksine kadar) video görüntüleri değerlendirme yapılmak üzere kaydedildi. Muayeneden sonra radyal gerilim ve gerilim hızlarının belirlenmesi için kaydedilen 6 ardışık kardiyak siklusun her birinde kalp kasının diyastol sonu endokardiyal sınırı manuel olarak belirlendi ve işaretlendi. İşaretlenen alanların tüm kardiyak siklus sırasında miyokardiyal hareketle senkronize hareket edip etmediği kontrol edildi. Ardından kullanılan cihazın yazılım programı otomatik olarak epikard sınırındaki noktaları belirledi. Bu belirlemeden sonra yazılım programı ventrikülü eşit altı bölüme ayırdı ve bu bölümleri mid anteroseptal (MAS), mid anterior (MA), mid lateral (ML), mid posterior (MP), mid inferior (MI) ve mid septal (MS) olarak isimlendirdi (Şekil 1). Endokard ve epikardda belirlenen beneklerin kardiyak siklus sırasındaki hareketlerinin yazılım tarafından takip edilebilir olduğunu anlamak için test uygulandı. Yazılım programı tarafından yetersiz izleme kalitesi gösteren bölümler manuel olarak düzeltildi. Düzeltme girişimi üç kez başarısız olduğunda tüm görüntü analizden çıkarıldı. Sadece kardiyak siklus sırasında açıkça takip edilebilen benek hareketleri çalışmaya dâhil edildi. BTE muayeneleri sonrası sol ventrikül radyal gerilim ve gerilim hızı ile ilgili ölçümler ve hesaplamalarda Echolab DAS-RS1 programı kullanıldı. Bölgelerin her birinin verileri yazılım tarafından xml formatına aktarıldıktan sonra segmentlerin pik seviyelerinin ortalaması alındı. Dakikada 54 frame altı olan görüntüler çalışmaya dâhil edilmedi. Çalışma Laboratuvar Hayvanlarının Bakım ve Kullanımı Kılavuzuna göre planlanmış olup hayvanlar, insancıl muameleye tabi tutuldu. Ayrıca araştırma, Ankara Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (tarih: 17 Ekim 2018; no:2018-20-129) izni ile gerçekleştirildi.

Şekil 1: MAS, MA, ML, MP, MI ve MS bölgelerinin gösterimi.
Figure 1: *Demonstration of MAS, MA, ML, MP, MI and MS regions.*

İstatistiksel analiz

Elde edilen tüm değişkenler önemlilik testlerine geçilmeden önce normallik yönünden parametrik test varsayımlarından Shapiro Wilk ile, varyansların homojenliği yönünden ise Levene testi ile incelendi. Tüm değişkenlere ait tanımlayıcı istatistikler hesaplandı ve tablo şeklinde gösterildi. Parametrik varsayımların sağlandığı durumda tam kan, gerilim, gerilim hızı ve M-Mod parametrelerinin gruplar arası (hasta-sağlıklı ve yaşayan-ölen) farklılığın istatistiksel açıdan belirlenmesi Student t test ile, varsayımların sağlanmadığı durumda ise Mann-Whitney U testinden

yararlanıldı. Ölçülen parametreler arasındaki ilişkinin gücü ve yönü parametrik test varsayımların sağlandığı durumda Pearson korelasyon katsayısı, varsayımların sağlanmadığı durumda ise Spearman korelasyon katsayısı ile belirlendi. Tüm istatistiksel analizlerde anlamlılık $p<0,05$ olarak kabul edildi. Tüm analizler Stata 12/MP4 (Lisans No: 50120500264) istatistik paket programı ile yapıldı.

3. Bulgular

Grup A'daki köpeklerde en yaygın klinik belirtilerin kusma, kanlı ishal, mental depresyon ve anoreksi olduğu görüldü. Fiziksel muayenede ise şiddetli dehidrasyon, ateş, mukozalarda solgunluk, takipne ve taşikardi belirlendi. Grup A'daki köpeklerden 9'u (%56,25) iyileşirken 7'si (%43,75) farklı gün ve saatlerde öldüler.

Grup A'da yaşayan ve ölen köpeklerin yaş, vücut sıcaklığı, canlı ağırlık, solunum ve kalp frekansları karşılaştırıldığında sadece yaş ve canlı ağırlık değerlerinin anlamlı fark (sırasıyla $p=0,046$ ve $p=0,036$) oluşturduğu ve ölen hayvanların yaşayanlara kıyasla daha genç ve daha zayıf oldukları tespit edildi. Grup A'daki köpeklerin Grup B'deki köpeklerden anlamlı şekilde ($p=0,001$) daha yüksek vücut sıcaklığı, daha yüksek solunum ve kalp frekanslarına sahip oldukları belirlendi.

Grup A'da yaşayan ve ölen köpeklerin tam kan verileri karşılaştırıldığında ölen hayvanların yaşayanlara göre daha düşük hemoglobin ve hematokrit değerlerine sahip oldukları ve bu farkın anlamlı (sırasıyla $p=0,031$ ve $p=0,034$) olduğu tespit edildi. Grup A ve Grup B'deki köpeklerin hematolojik muayene verilerinin karşılaştırılması Tablo 2'de gösterildi.

Tablo 2: Grup A ve Grup B'deki köpeklerin hematolojik muayene verilerinin karşılaştırılması
Table 2*: Comparison of hematologic evaluation values of dogs in Group A and Group B*

Parametre	Grup	Arit. Ort.	Std. Hata	Std. Sapma	Medyan	Minimum	Maksimum	p
WBC (10^9/l)	A	4,06	1,26	5,05	2,75	0,70	22,00	<0,001
	B	11,35	0,59	2,35	11,25	6,00	16,20	
LYM (10^9/l)	A	0,86	0,15	0,61	0,70	0,40	3,00	<0,001
	B	2,64	0,26	1,03	2,90	0,90	4,10	
MONO (10^9/l)	A	0,33	0,15	0,59	0,20	0,00	2,50	<0,001
	B	0,99	0,07	0,29	0,90	0,40	1,50	
NEUT (10^9/l)	A	2,68	0,98	3,93	1,45	0,10	16,50	<0,001
	B	7,54	0,55	2,21	7,00	3,60	11,70	
RBC(10^{12}/l)	A	6,50	0,22	0,89	6,56	5,00	8,34	<0,001
	B	4,35	0,39	1,57	4,44	0,00	7,55	
HGB (g/dl)	A	15,06	0,56	2,22	14,90	11,20	20,40	<0,001
	B	10,05	0,72	2,90	10,20	4,25	16,10	
HCT (%)	A	41,43	1,42	5,67	41,10	33,10	51,90	<0,001
	B	28,04	2,01	8,04	28,90	8,70	43,90	
MCV (fl)	A	62,78	1,29	5,15	63,25	55,10	70,00	0,477
	B	63,98	1,06	4,24	63,80	53,80	71,00	
MCH (pg)	A	22,79	0,43	1,72	23,25	19,50	24,70	0,381
	B	22,52	0,37	1,48	23,25	19,30	24,40	
MCHC (g/l)	A	36,38	0,45	1,80	36,15	32,90	39,40	0,043
	B	35,24	0,29	1,17	35,35	33,50	36,80	
RDW (fl)	A	17,14	1,17	4,68	15,85	13,00	31,00	0,305
	B	17,31	0,64	2,56	17,30	13,50	22,80	
PLT (10^9/l)	A	339,31	37,21	148,83	349,50	37,00	632,00	0,037
	B	468,19	45,88	183,54	482,00	45,00	775,00	
MPV (fl)	A	8,20	0,36	1,46	8,05	5,50	10,40	0,047
	B	9,28	0,37	1,49	9,05	6,40	12,00	

WBC;total lökosit, LYM;lenfosit, MONO;monosit, NEUT;nötrofilik lökosit, EOS;eozinofilik lökosit, RBC; eritrosit, HGB;hemoglobin, HCT;hematokrit, MCV;ortalama eritrosit hacmi, MCH;eritrositlerdeki hemoglobin miktarı, MCHC;eritrositlerdeki ortalama hemoglobin konsantrasyonu, RDW;eritrositlerin dağılım genişliği, PLT; platelet, MPV;ortalama trombosit hacmi.

Grup A ve Grup B'deki köpeklerin elektrokardiyografilerinde herhangi bir anormalliğe rastlanmadı. Grup A'daki yaşayanların ölenlere kıyasla daha yüksek sol ventrikül serbest duvarı diyastol sonu kalınlığına (LVPWd) sahip oldukları belirlenirken (p=0,016), ölen ve yaşayanların M-mod ile elde edilen EF ve FS değerleri arasında önemli bir fark olmadığı görüldü.

Grup A ve Grup B'deki köpeklere ait ekokardiyografik veriler karşılaştırıldığında ise diyastol sonu sol ventrikül iç çapı (LVIDd) ve LVPWd'de anlamlı fark belirlenirken (sırasıyla p=0,003 ve p=0,007), her iki grubun EF ve FS değerleri arasında önemli bir farklılığa rastlanmadı.

Grup A ve Grup B'deki köpeklerin sol ventrikül radyal gerilim verileri kıyaslandığında ise sadece MI ile ifade edilen bölgede anlamlı bir fark oluştuğu belirlendi (p=0,039) (Tablo 3).

Tablo 3: Grup A ve Grup B'deki köpeklerin MAS, MA, ML, MP, MI ve MS bölgelerindeki radyal gerilim verilerinin karşılaştırılması.
Table 3: *Comparison of radial strain values in the MAS, MA, ML, MP, MI and MS regions of dogs in Group A and Group B.*

Bölge	Grup	Arit. Ort.	Std. Hata	Std. Sapma	Medyan	Minimum	Maksimum	p
MAS	A	24,40	3,70	14,81	23,16	4,80	61,14	0,119
	B	30,91	2,72	10,88	29,40	18,40	50,65	
MA	A	24,76	4,43	17,71	19,45	3,58	62,68	0,613
	B	27,68	3,62	14,48	27,58	3,45	55,84	
ML	A	26,26	2,77	11,08	25,67	9,44	46,82	0,232
	B	31,52	3,30	13,22	29,88	12,81	59,33	
MP	A	26,84	2,83	11,31	26,97	8,28	44,33	0,086
	B	33,90	2,78	11,14	31,88	22,19	64,70	
MI	A	25,21	2,85	11,39	21,51	10,48	53,30	0,039
	B	34,08	2,97	11,87	36,88	15,29	53,87	
MS	A	27,57	3,38	13,53	26,84	5,75	59,44	0,143
	B	35,63	4,15	16,60	32,83	11,67	76,06	

Grup A'daki ölen ve Grup B'deki sağlıklı köpeklerin sol ventrikül radyal gerilim verilerinin ve Grup A'daki ölen ve yaşayan köpeklerin sol ventrikül radyal gerilim verilerinin karşılaştırılması Tablo 4 ve Tablo 5'te gösterildi.

Sol ventrikül radyal gerilim hızları gruplar (Grup A'daki yaşayan ve ölen köpekler; Grup A ve Grup B'deki köpekler; Grup A 'daki ölen köpekler ve Grup B'deki köpekler) arasında karşılaştırıldığında ise herhangi bir anlamlı fark bulunamadı.

Tablo 4: Grup A'daki ölen köpeklerin ve Grup B'deki sağlıklı köpeklerin MAS, MA, ML, MP, MI ve MS bölgelerinin gerilim verilerinin karşılaştırılması.
Table 4: *Comparison of the strain values of MAS, MA, ML, MP, MI and MS regions of deceased dogs in Group A and healthy dogs in Group B.*

Bölge	Grup	Arit. Ort.	Std. Hata	Std. Sapma	Medyan	Minimum	Maksimum	p
MAS	B	30,91	2,72	10,88	29,40	18,40	50,65	0,004
	Ölen	15,22	2,84	7,52	15,07	4,80	25,58	
MA	B	27,68	3,62	14,48	27,58	3,45	55,84	0,011
	Ölen	11,46	2,82	7,45	9,59	3,58	26,17	
ML	B	31,52	3,30	13,22	29,88	12,81	59,33	0,026
	Ölen	18,54	3,05	8,08	18,67	9,44	33,97	
MP	B	33,90	2,78	11,14	31,88	22,19	64,70	0,008
	Ölen	20,09	4,37	11,55	16,93	8,28	42,31	
MI	B	34,08	2,97	11,87	36,88	15,29	53,87	0,010
	Ölen	20,12	2,81	7,43	18,26	10,48	31,75	
MS	B	35,63	4,15	16,60	32,83	11,67	76,06	0,027
	Ölen	23,02	3,26	8,63	21,46	9,69	34,71	

Tablo 5: Grup A'daki ölen ve yaşayan köpeklerin MAS, MA, ML, MP, MI ve MS bölgelerinin gerilim verilerinin karşılaştırılması.
Table 5: Comparison of strain values of MAS, MA, ML, MP, MI and MS regions of living and deceased dogs in Group A.

Bölge	Grup A	Arit. Ort.	Std. Hata	Std. Sapma	Medyan	Minimum	Maksimum	p
MAS	Yaşayan	31,54	5,14	15,42	33,06	8,92	61,14	0,023
	Ölen	15,22	2,84	7,52	15,07	4,80	25,58	
MA	Yaşayan	35,10	5,49	16,47	38,50	17,11	62,68	0,003
	Ölen	11,46	2,82	7,45	9,59	3,58	26,17	
ML	Yaşayan	32,27	3,14	9,41	31,11	19,25	46,82	0,008
	Ölen	18,54	3,05	8,08	18,67	9,44	33,97	
MP	Yaşayan	32,08	2,77	8,30	31,07	20,15	44,33	0,030
	Ölen	20,09	4,37	11,55	16,93	8,28	42,31	
MI	Yaşayan	29,17	4,23	12,70	28,77	12,81	53,30	0,117
	Ölen	20,12	2,81	7,43	18,26	10,48	31,75	
MS	Yaşayan	31,11	5,33	15,98	30,72	5,75	59,44	0,248
	Ölen	23,02	3,26	8,63	21,46	9,69	34,71	

4. Tartışma ve Sonuç

Bu çalışmada parvoviral enteritisli sepsis ve SİYS bulguları gösteren yavru köpekler ile sağlıklı köpeklerin sol ventrikül sistolik fonksiyonları, standart ekokardiyografi ve 2DBTE metodu kullanılarak karşılaştırıldı. Parvoviral enteritisten ölen köpeklerin sol ventrikül kısa eksen radyal gerilim değerlerinde sağlıklılara kıyasla anlamlı bir düşüş olduğu görüldü. Gerilim hızının ise gruplar arasında bir fark oluşturmadığı belirlendi.

Abreu ve ark. (24), parvoviral enteritisli köpekleri enfeksiyonun şiddetine göre üç gruba ayırmış ve bu gruplarda 2DBTE ile sistolik disfonksiyonu araştırmışlardır. Araştırmanın sonucunda sağlıklılara kıyasla hasta köpeklerde radyal gerilim ve gerilim hızlarının azaldığını bildirmişlerdir. Altı bölümde incelenen kalp kasında, her bir grupta farklı sayıda bölümün etkilendiği, hafif enfeksiyon görülen grupta kalp kasının dört bölümünün, şiddetli enfeksiyon görülen grupta altı bölümünün ve ölen grupta beş bölümünün radyal geriliminin azaldığını bildirmişlerdir.

Bu çalışmada da ölen hayvanların sol ventrikül radyal gerilimlerinin sağlıklılara kıyasla azalması söz konusu araştırma ile uyumlu bulunmuştur. Ancak yapılan bu çalışmada hasta ve sağlıklı gruplar arasında sol ventrikül radyal gerilimde anlamlı bir fark olmadığı da belirlenmiştir. Çalışmalar arasındaki bu farkın enfeksiyonun aşamasına ve şiddetine bağlı olarak şekillendiği düşünülmektedir.

Sepsis ve septik şok görülen insanlarda miyokardiyal disfonksiyonun belirlenmesi için yapılan bir çalışmada hastalarda sadece uzunlamasına gerilimin azaldığı, radyal ve çevresel gerilimin değişmediği ve EF'nin sistolik disfonksiyonun tespitinde yetersiz olduğu belirtilmiştir (26). Araştırmacılar radyal ve çevresel miyokardiyal liflerin kompenzasyona uğradığını ve bu nedenle bu yönlerdeki gerilimin değişmediğini bildirmişlerdir.

Standart ekokardiyografide M-Mod ile elde edilen EF ve FS değerleri köpeklerde miyokardiyal performansı değerlendirmek için en sık kullanılan parametrelerdir (27). EF ve FS değerlerinin kalp atım sayısından, ön ve ard yükten etkilenmeleri ve FS'nin standart ekokardiyografik metod ile elde edildiğinde sadece radyal yönü yansıtması bu parametrelerin kullanımını kısıtlamaktadır (6,25,28-30). Yapılan bu araştırmada standart ekokardiyografimj ile elde edilen EF ve FS'nin gruplar arasında istatistiksel olarak anlamlı bir fark oluşturmadığı ve sistolik disfonksiyonu değerlendirmede tek başına yeterli olmadıkları kanısına varıldı. Bu durumun söz konusu parametrelerin ön ve ard yük gibi çeşitli faktörlerden etkilenmelerinden kaynaklandığı düşünülmektedir. Abreu ve ark. (24) da benzer şekilde parvoviral enteritisli köpeklerde EF ve FS'nin sistolik disfonksiyonu değerlendirmede yetersiz kaldığını ve dehidre hayvanlarda ön yükün bu parametreler üzerinde etkili olduğunu bildirmişlerdir. SİYS görülen köpeklerde yapılan başka bir çalışmada da benzer şekilde EF ve FS'nin sağlıklı ve hasta hayvanlar arasında herhangi bir fark oluşturmadığı bildirilmiştir (6). İnce ve ark. (31), şiddetli sepsis ve septik şokun sistolik ve diyastolik fonksiyonlara etkisini araştırdıkları çalışmalarında ise hasta köpeklerde EF değerinin normal aralıkta olduğunu belirlemişlerdir. Bunun nedeninin azalan ön ve ard yük, düşük kan basıncı ve adrenerjik uyarımdan kaynaklandığını, düşük EF değerinin normal vasküler tonusu gösterdiğini ve iyi bir prognostik indikatör olduğunu öne sürmüşlerdir. Sepsisli ve ağır travmalı insanlarda sistolik fonksiyonları değerlendiren araştırmacılar standart ekokardiyografi ile elde edilen EF değerinin gruplar arasında anlamlı fark oluşturmadığını ancak hastaların uzunlamasına gerilim ve gerilim hızlarının kontrol grubuna kıyasla daha düşük olduğu bildirilmişlerdir (32). Araştırmacılar EF değerinin tek başına değerlendirilmesinin sistolik disfonksiyonu göstermede yetersiz olacağını öne sürmüşlerdir. Septik şok görülen insanlarla sağlıklı insanların miyokardiyal performansının karşılaştırıldığı bir araştırmada, EF ve FS değerlerinin iki grup arasında farklı olmadığı, uzunlamasına ve çevresel gerilim değerlerinin septik şok görülenlerde sağlıklılara kıyasla daha az olduğu bildirilmiştir (33).

Kontrol grubu köpeklerde LVIDd'nin parvoviral enteritisli köpeklerden daha fazla, LVPWd'nin ise hasta köpeklerde kontrol grubu köpeklere göre daha fazla olduğu belirlendi. Bu farklılıkların istatistiksel olarak anlamlı ($p<0,05$) olduğu görüldü. Hestenes ve ark. (29), sepsis ile ilgili yaptıkları deneysel çalışmada farelerde sistol sonu ve diyastol sonu duvar kalınlıklarının arttığını, Nielsen ve ark. (34) ise sepsise bağlı olarak akciğer, bağırsak ve kaslarda ödem şekillenebileceğini göstermişlerdir. Benzer şekilde kalp kasında da ödem şekillenebileceği ve bunun miyokardiyal performansı etkileyebileceği ileri sürülmektedir (29,34). Sol ventrikül duvar kalınlıklarının, sepsis sırasında kapillar geçirgenliğin artmasıyla şekillenen ödeme bağlı olarak artacağı düşünülse de sol ventrikül hacminin azalması ve sistemik vasküler direncin azalması gibi hemodinamik değişikliklerin de sürece katkıda bulunduğu düşünülmektedir.

Bu çalışmada 2DBTE ile miyokardiyal radyal gerilim değerlendirildiğinde, hasta grubun gerilim verilerinin sağlıklılara kıyasla daha düşük olduğu ancak anlamlı bir fark ifade etmediği belirlendi. Ölen köpeklerle sağlıklı köpeklerin gerilim verileri kıyaslandığında ise ölenlerin daha düşük radyal gerilime sahip oldukları ve bu farkın istatistiksel olarak anlamlı ($p<0,05$) olduğu tespit edildi. Bu farkın temel nedeninin, hastalığın ilerleyen evrelerinde sepsis ve hemodinamik instabilitenin miyokardiyal fonksiyon üzerindeki etkilerinin artması olduğu düşünülmektedir. Sepsis sırasında SİYS'e bağlı olarak vazodilatasyon, hipovolemi ve hipotansiyon gelişebilir (34). Bu durum, koroner perfüzyonun azalmasına, miyokardiyal oksijen ihtiyacının artmasına ve sonuç olarak miyokardiyal disfonksiyonun derinleşmesine neden olabilir (29). Özellikle ileri evrelerdeki hastalarda kardiyak depresyonun daha belirgin hale gelmesi, ölen gruptaki düşük radyal gerilim değerlerini açıklayabilir. Daha önce yapılan çalışmalar da sepsisin erken

evrelerinde miyokardiyal kompenzasyon mekanizmalarının devrede olduğunu, ancak hastalığın ilerlemesiyle birlikte miyokardiyal kontraktilitenin giderek bozulduğunu göstermektedir (6). Dolayısıyla, çalışmamızda hasta ve sağlıklı gruplar arasında belirgin bir fark görülmemesi, hastalığın erken ve orta evrelerinde miyokardiyal fonksiyonların korunabilmesine bağlı olabilir. Ancak hastalık ilerledikçe miyokardiyal yetmezlik belirgin hale gelmekte ve bu durum ölen hayvanlarda daha düşük radyal gerilim değerleri ile yansımaktadır. Radyal gerilim değerinin klinik kararları etkileyecek eşik değerinin belirlenmesi ve prognostik öneminin gösterilmesi için daha geniş çaplı çalışmalara ihtiyaç duyulmaktadır. Çalışmamızın sonuçları, ileriye dönük daha büyük hasta gruplarında kritik eşik değerlerin belirlenmesine zemin hazırlayacak niteliktedir.

Bu çalışmada sağlıklı ve hasta köpeklerin radyal gerilim hızları karşılaştırıldığında gruplar arasında anlamlı bir fark olmadığı belirlendi. Corda ve ark. (6) da SİYS olan köpeklerde gerilim hızının sağlıklılarla anlamlı bir fark oluşturmadığını bildirmişlerdir. Bu durumu, kullanılan teknolojinin, SİYS'e bağlı olarak artan kalp atım sayısı nedeniyle yeterli zamansal çözünürlüğe erişememesiyle açıklamışlardır. CPV-2 görülen köpeklerde kalp kasının sistolik fonksiyonunu değerlendiren bir çalışmada (24) sol ventrikül radyal gerilim hızının, enfeksiyonun şiddetine göre sınıflandırılan gruplarda daha çok bölgesel etkilendiği bildirilmiştir. Aynı çalışmada parvoviral enteritisli tüm gruplarda ortak olarak endokardiyal ve epikardiyal seviyede uzunlamasına gerilim hızının azaldığını belirtmişlerdir. Sol ventrikül çevresel ve radyal gerilim hızı farklı gruplarda ve farklı bölgelerde azalmıştır. Tüm gruplarda ortak olarak görülen uzunlamasına gerilim hızının azalmasının nedeni ise uzunlamasına liflerin diğer liflere kıyasla kardiyak hasara daha duyarlı olmalarıdır. Chetboul ve ark. (35), miyokardiyal disfonksiyonun tespitinde gerilimin, gerilim hızından daha duyarlı bir ölçek olduğunu ifade etmişlerdir. Bu çalışmada da gerilim hızlarının gruplar arasında fark oluşturmaması bu görüşü destekler niteliktedir.

Mevcut çalışmada parvoviral enteritise bağlı sepsisin, miyokardiyal hasara neden olarak ölümün nedenlerinden biri olduğu görüldü. Ayrıca standart ekokardiyografi ile elde edilen verilerin gruplar arasında fark oluşturmaması, 2DBTE metodunun sol ventrikül sistolik fonksiyonlarını değerlendirmede daha hassas bir yöntem olduğunu gösterdi. Ölen köpeklerde radyal gerilim değerlerinin anlamlı derecede düşük olması, düşük radyal gerilim değerlerinin mortalite ile ilişkili olabileceğini düşündürmektedir. Ancak, tek bir ölçüm değerine dayanarak hasta yönetimini değiştirmek yerine, hastaların seri ölçümlerle takip edilmesi gerektiği düşünülmektedir. Sepsis gelişen hastalarda düşük radyal gerilim tespit edildiğinde, bu hastaların daha yakından izlenmesi ve inotropik destek ihtiyacının değerlendirilmesi önerilebilir. Bu çalışma, parvoviral enteritise bağlı sepsis ile ilişkili kardiyak komplikasyonların daha iyi anlaşılmasına ve uygun tedavi stratejilerinin geliştirilmesine yardımcı olacağı düşünülmektedir.

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Araştırma Makalesi / Research Article

Evaluation of the change in the economic and purchasing power of the welfare of cattle breeding in Türkiye

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ABSTRACT

In this study, economic welfare changes in cattle fattening in Türkiye in 2010-2022 were analyzed with the Net internal terms of trade (NITOT) index and purchasing power changes were analyzed with the Income internal terms of trade (INTOT) index. Accordingly, the sustainability status of the enterprises was analyzed multidimensionally on the axis of parameters such as meat imports, agriculture and livestock subsidies and milk-feed parity. When meat import data are included, it can be seen that INTOT, the purchasing power parity indicator, changed little between 2010 and 2013, increased between 2014 and 2017, and decreased from 2018 for cattle farms. On the other hand, it is found that INTOT data decreased by 12.7%, 13.6%, and 19.1% in 2011, 2014, and 2018, respectively, when meat import data are excluded. According to the regression results of the study, with everything else held constant, a 1% increase in meat imports results in a 0.0861 unit decrease in the cattle farm economic welfare index (NITOT) in the short run, a 0.2129 unit decrease in the medium run, and a 0.2833 unit decrease in the long run. A 1-unit increase in consumer price index (CPI) results in a 0.7264-unit decrease, while a 1-unit increase in the Agriculture and Livestock support index (ASDI) causes a 0.6156-unit increase. As a result, inflation problem and meat imports in Türkiye affect the economic welfare of fattening enterprises negatively and subsidies positively. The regression model obtained as a result of the research will be guiding in animal husbandry policies.

Türkiye'de büyükbaş hayvancılığın, ekonomik refahının ve satın alma gücündeki değişimin değerlendirilmesi

ÖZET

Bu çalışmada, 2010-2022 yılları arasında Türkiye'de sığır besiciliğinde ekonomik refah değişimleri, Net iç ticaret hadleri (NİTOT) endeksi ile satın alma gücü değişimleri ise Gelir iç ticaret hadleri (INTOT) endeksi ile analiz edilmiştir. Bu doğrultuda işletmelerin sürdürülebilirlik durumu et ithalatı, tarım ve hayvancılık destekleri ve süt-yem paritesi gibi parametreler ekseninde çok boyutlu olarak analiz edilmiştir. Et ithalatı verileri dahil edildiğinde, satın alma gücü paritesi göstergesi olan INTOT'un büyükbaş hayvan çiftlikleri için 2010-2013 yılları arasında çok az değiştiği, 2014-2017 yılları arasında arttığı, 2018 yılından itibaren ise azaldığı görülmektedir. Öte yandan, et ithalatı verileri hariç tutulduğunda INTOT verilerinin 2011, 2014 ve 2018 yıllarında sırasıyla %12,7, %13,6 ve %19,1 oranında azaldığı tespit edilmiştir. Çalışmanın regresyon sonuçlarına göre, diğer her şey sabit tutulduğunda, et ithalatındaki %1'lik bir artış, sığır çiftliği ekonomik refah endeksinde (NITOT) kısa vadede 0,0861 birim, orta vadede 0,2129 birim ve uzun vadede 0,2833 birim azalmaya neden olmaktadır. Tüketici fiyat endeksindeki (TÜFE) 1 birimlik artış 0,7264 birimlik azalışa neden olurken, Tarım ve Hayvancılık destek endeksindeki (ASDI) 1 birimlik artış 0,6156 birimlik artışa neden olmaktadır. Sonuç olarak, Türkiye'de enflasyon sorunu ve et ithalatı besi işletmelerinin ekonomik refahını negatif, sübvansiyonlar ise pozitif yönde etkilemektedir. Araştırma sonucunda elde edilen regresyon modeli hayvancılık politikalarında yol gösterici olacaktır

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1. Introduction

After the 1980s, the word "sustainability" has become a frequently used title in developed and developing countries. Although the origin of the term sustainability belongs to the science of ecology, it is examined using many subheadings such as economic, environmental and social sustainability (1).

When the concept of sustainability in business economics is mentioned, businesses with regular, stable cash flow and profitability come to mind. In other words, it is the set of activities that provide the greatest amount of economic welfare with the least amount of resource use and environmental damage.

In studies where sustainability is evaluated from an economic point of view, profitability, efficiency, economic welfare change and purchasing power indicator are used (2). Among the mentioned parameters, economic welfare change and purchasing power indicator are two of the most striking parameters (3,4).

In studies examining economic sustainability, various methods are used to calculate changes in purchasing power and economic welfare. Accordingly, while variables such as personal income and disposable income have been the basic indicators of purchasing power at the household level since the 1920s, various studies on purchasing power have been conducted at both the household and enterprise levels using alternative parameters such as domestic trade conditions (5,6) since the 1950s. These studies have focused particularly on resource transfers between the agricultural and industrial sectors and the resulting changes in purchasing power. In this context, studies have been deepened by also taking into account imbalances in production factors. Eckaus (7) in Asia, the Middle East and Italy, Harris and Todaro (8) in Central Africa and Corden and Findlay (9) in the USA have examined changes in resource transfers between sectors caused by factor imbalances.

In the 1970s, the internal terms of trade focused on the relationship between the prices that agricultural enterprises received as a result of their sales and the prices they paid for production, which made the internal terms of trade a useful tool for researchers to analyse purchasing power and economic welfare indicators (10). In Türkiye, Keyder (11), Kip (12) and Çetinkaya (13) analysed the terms of trade and price differentials between the agricultural and industrial sectors and the effects of resource transfers between these two sectors on economic welfare and purchasing power. In the following years, sectoral studies on single products have increased in the literature and these studies have turned into specific analyses on one or a few specific products. In this context, studies on internal terms of trade focusing on single products are frequently encountered. Especially in studies on internal terms of trade in agriculture and animal husbandry, Uzunöz et al. (14) on milk, Uzunöz (15) on legumes, Mencet Yelboğa et al. (16) on tomatoes, Menşet Yelboğa et al. (17) on citrus fruits, Kızılaslan et al. (18) on sunflower and Tuncel and Cevger (19) on cattle fattening enterprises are examples of single product-based studies. In this framework, the Net Internal Terms of Trade (NITOT) is used as an indicator of economic welfare in analysing internal terms of trade for the livestock sector. NITOT is an index value obtained by dividing the prices obtained by the breeder from the sale of carcass meat by an index showing the production costs (agri-food index, PPI, etc.), while the Income Internal Terms of Trade (INTOT) is defined as an index value obtained by multiplying the NITOT index by the carcass production index (19). These indicators provide an important analysis tool for understanding the economic dynamics in the livestock sector. Within the scope of this research, the factors affecting the economic welfare level and purchasing power index of cattle fattening enterprises in Turkey between 2010 and 2022 are analysed using internal terms of trade (NITOT and INTOT). In this study, unlike other studies, the effects of meat imports, inflation and agricultural-livestock subsidies on cattle fattening enterprises are analysed with the model and estimation results of the internal terms of trade.

2. Material and Methods

Among the data and variables used in the study, the Net domestic terms of trade index (NITOT) was used as an indicator of economic welfare loss, the Income domestic terms of trade index (INTOT) was used as a purchasing power index, the Consumer Price Index (CPI) was used for the change in the general level of prices, and the agriculture and livestock support payment index was used for support in the agriculture and livestock sector. Accordingly, since livestock farms in Turkey benefit from both agricultural and livestock subsidies together, a composite index covering agricultural and

livestock subsidies is constructed in the study. In addition, data on meat imports are included in the study. Data on meat imports are obtained by summing the data on imports of slaughter animals and breeding meat imports in kg at annual frequency and converting them into an index.

The definitions of the variables used in the study are given in Table 1.

Table 1: Definitions of the variables used in the study

Tablo 1: *Araştırmada kullanılan değişkenlerin tanımları*

Variables	Definitions
NITOT	Net internal terms of trade
INTOT	Income internal terms of trade
CPI	Consumer price index
ASDI	Agricultural and livestock supports index
Limport	Natural logarithm of cattle import

Internal Terms of Trade

It is a concept that shows the relationship between the price received by the producer and the prices paid by the producer to the industrial sector. In this study, the "Breeder's Price Index Received" (BPIR), which shows the monetary amount received by the breeders after carcass sales in cattle farm, and the "Breeder's Price Index Paid" (BPIP), which shows the costs incurred to realize this production, are used. NITOT index and INTOT index are calculated with these two variables (19).

Net Internal Terms of Trade (NITOT)

It is the ratio of BPIR to BPIP. In the research, the NITOT index was calculated by using the Carcass Price Index (CAPI) for the "Price Received by the Grower" and the Agri-Food Index (AFI) index for the Price Index paid by the Grower (12, 20).

NITOT = CAPI / AFI *100

Income Internal Terms of Trade (INTOT)

The INTOT index, known as the "purchasing power index", also takes into account changes in demand for beef. Accordingly, the "Income Internal Terms of Trade Index" is calculated by multiplying the NITOT with the meat production volume (Q) of cattle farms (12)

INTOT = NITOT x Q/100

INTOT = CAPI / AFI x Q /100

Statistical analysis

In the analysis stage of the research, econometric analysis was conducted using the INTOT and NITOT indices from the internal terms of trade. In the study, the effects of meat imports, subsidies and inflation on the terms of trade index between 2010 and 2022 are analysed by regression analysis. Among the data used in the research, the data on agriculture and livestock subsidies were obtained from the Ministry of Treasury and Finance of the Republic of Turkey (THMB), while all other data were obtained from Turkstat (21).

Due to the change in Turkish Statistical Institute (TSI) data collection methodology in 2010, data from 2010 and subsequent years were used in the study. Accordingly, until 2010, the red meat production statistics published by

the Turkish Statistical Institute were based on data obtained from the slaughter of bovine and ovine animals recorded in slaughterhouses and the sacrificial skins received by the Turkish Aeronautical Association. However, the metadology was changed after 2010 (22).

Finally, although not directly included in the analysis in the regression model within the scope of the research, UKON (national milk council) milk/feed parity data are included and interpreted in Graphs 1 for a more consistent assessment of the current situation of the sector (23).

3.Results

Within the scope of the research, the values of beef production index (Q), Carcass Price Index (CAPI), Agri-Food Index (AFI), Net Internal Terms of Trade (NITOT), Purchasing Index (INTOT), Consumer Price Index (CPI), Inflation-Adjusted Agricultural and Livestock Support Index (ASDI) and Meat Import Index (MII) values are given in Table 2 (23-28).

Table 2: Q, CAPI, AFI, NITOT, INTOT, CPI, ASDI and MII indices
Table 2: *Q, CAPI, AFI, NITOT, INTOT, CPI, ASDI ve MII endeksleri*

Time	Q	CAPI	AFI	NITOT	INTOT	CPI	ASDI	MII
2010	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2011	106.90	96.39	106.47	90.53	99.43	110.45	94.11	277.14
2012	113.12	98.59	115.94	85.04	103.83	117.25	107.07	284.13
2013	118.31	102.17	124.63	81.98	101.20	125.93	93.40	96.05
2014	125.88	117.77	135.66	86.81	109.44	136.22	94.45	333.03
2015	138.25	148.38	146.07	101.59	135.35	148.22	105.83	104.71
2016	153.69	159.14	157.01	101.35	149.78	160.86	114.34	236.11
2017	155.39	172.43	172.47	99.97	169.01	180.04	124.87	325.67
2018	158.68	178.56	208.54	85.63	169.55	216.59	132.68	691.98
2019	167.71	189.05	238.48	79.27	162.97	242.23	137.96	305.27
2020	186.01	212.82	263.92	80.64	167.18	277.59	121.97	185.72
2021	212.79	259.55	346.24	74.96	169.23	377.75	120.65	123.82
2022	249.25	519.22	760.69	68.26	165.91	620.53	109.83	60.34

1 Since the AFI index was calculated by the Turkish Statistical Institute (TURKSTAT) after 2015, the AFI calculation between 2010 and 2014 was calculated retrospectively using the Turkish Producer Price Index (PPI).
2 MII (Meat import index) data is the annual value in kg obtained from the sum of breeding and butchery meat imports. Based on 2010 and converted to index value.
*3 ASDI: Nominal agricultural livestock subsidy value/ Nominal livestock subsidy value*100*

When the NITOT index is analysed in Table 2, it shows a downward trend since 2010, except for 2015 and 2016. Especially since 2018, the CAPI index, which shows the prices received by cattle breeding enterprises after their sales, has remained far below the AFI index, which shows the prices they pay to realize their production. In other words, the income of breeding enterprises has remained below their costs.

When the INTOT index data in Table 2 are analysed, it is seen that the purchasing power, which is stagnant in 2010-2013, increased by 8.1%, 23.7%, 10.6% and 12.8% between 2014 and 2017, respectively, and remains stagnant again in 2018 and beyond. The CPI index, which is 100 in 2010, increased 3.7 times by 2021 and 6.2 times by 2022. Moreover, support items, which started to increase in real terms in 2015, reached the highest level in 2019, but have been decreasing since 2020.

The study also constructed a meat import data index (Table 2). Meat import data are converted into indices by summing the data on imports of slaughter animals and breeding meat imports in kg at annual frequency. Table 2 shows that meat imports, which reached a maximum level in 2018, entered a downtrend with the decision of the public authority to restrict meat imports.

In the study, Figure 1 presents the milk/feed parity, NITOT, INTOT, INTOT (excluding imports) and agricultural and livestock support indices (ASDI) together (12).

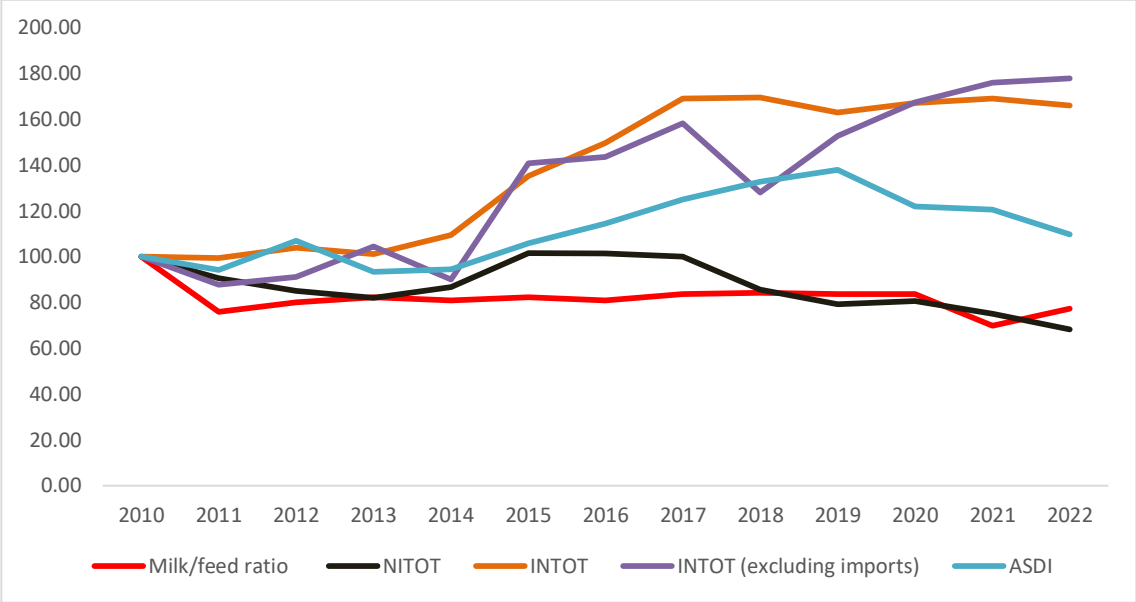

Figure 1: Milk/feed parity, NITOT, INTOT, INTOT (excluding imports), ASDI

Şekil 1: *Süt /yem paritesi, NITOT, INTOT, INTOT (ithalat hariç), ASDI*

Figure 1 reveals that sharp breaks in the purchasing power of INTOT (excluding imports) are not evident in the INTOT index. In line with this data, it is clear that the rise in the INTOT index is due to meat imports rather than a real increase in the level of meat production.

Figure 1 shows that the NITOT, which declines in 2013, increases in 2015 and remains constant until 2018, and starts to decline again after 2018. Accordingly, it is evident that since 2018, carcass meat prices have been well below the production costs of the breeders.

An analysis of the ASDI index data in Figure 1 reveals that the index, which is on an upward trend until 2019 except for 2011 and 2013, has been on a downward trend since 2020.

The natural logarithm value of meat imports (Limport) is given in Figure 2a, Consumer Price Index (CPI) is given Figure 2b, Net internal terms of trade (NITOT) index is given in Figure 2c in Figure 2 for the years understudy.

Figure 2 shows that meat imports decrease in 2019 and beyond, while the Consumer Price Index (CPI) increases and the NITOT index decreases as an indicator of the rapidly rising inflation problem.

Table 3 presents the model and estimation results of the regression analysis to determine the effect of red meat imports, inflation, agricultural and livestock subsidies on the terms of trade.

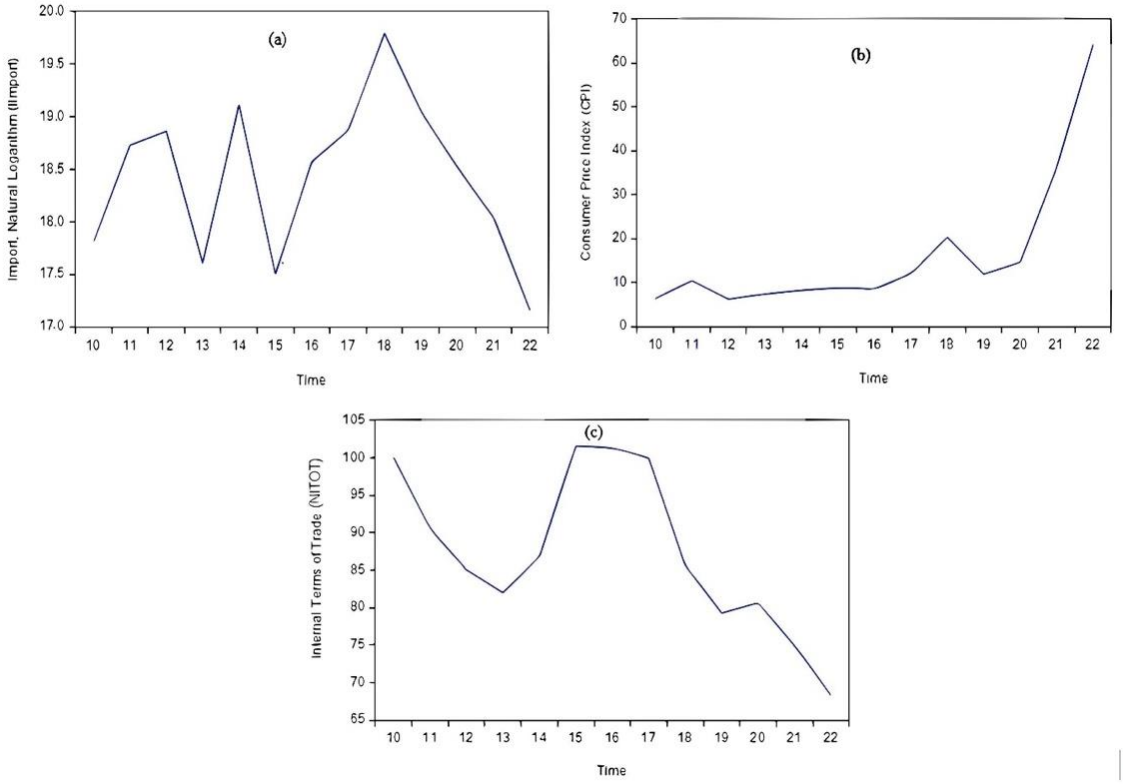

Figure 2: The figure in a is Natural logarithm figure of imports, the figure in b is Consumer Price Index (CPI) figure, the figure in c is Net domestic terms of trade (NITOT) figure.

Şekil 2: *İthalatının doğal logaritma grafiği(a), Tüketici fiyat endeksi (CPI) grafiği(b), Net iç ticaret haddi (NITOT) grafiği(c)*

Table 3: NITOT regression analysis results

Table 3: *NITOT regresyon analiz sonuçları*

Variables	**Results**
Limport	-8.6123*
Limport(-1)	-12.6790**
Limport(-2)	-7.0379*
CPI	-0.7264**
ASDI	0.6156**
Constant	554.0427***
Statistics and Diagnostics Test Results	
R^2	0.9032
$\bar{R}^2$, Adjusted R^2	0.8064
N, Number of Observations	11
F, Statistical Significance Test of Regression	9.3294**
F, Autocorrelation	0.8409
F, Heterocedasticity	0.8653
F, Model Specificarion	0.0562
Jacque-Berra Normality test	0.5230

****, ** and * denote statistical significance at 1%, 5% and 10% significance levels, respectively.*

When the results of the model are analysed in the regression analysis,

R-square Statistic: The explanatory variables in the model explain 90% of the variability in the dependent variable. This shows that the model is significant.

F Statistic: The F statistic is 9.3294, which indicates that the model is generally significant at 5% significance level.

Autocorrelation Test: The F test was used for the autocorrelation test and the result was 0.8409. In this case, the null hypothesis that there is no autocorrelation is not rejected, so there is no autocorrelation problem in the model.

Homoscedasticity Test: The Breusch-Pagan-Godfrey test is used to test whether the variance is constant and the F statistic is 0.8653. This indicates that the null hypothesis that the variance is constant (homoscedasticity) is not rejected. Accordingly, it is determined that the variance does not vary in the model and therefore there is no heteroscedasticity problem.

Model Setup Error Test: The Ramsey RESET Test was used to test the correctness of the model and the F statistic was found to be 0.0562. This result indicates that there is no setup error in the model.

Normal Distribution of Residuals Test: Jacque-Bera test is used to test whether the residuals are normally distributed, and the test statistic is 0.5230. This result indicates that the null hypothesis that the residuals are normally distributed is not rejected, in other words, the residuals are normally distributed.

According to the results of the regression analysis, the coefficient of the current import variable (Limport) among the independent variables is -8.6123 and statistically significant. This result indicates that, with all other variables held constant, a 1% increase in imports leads to a decrease of 0.0861 units in the short-run terms of trade (INTOT). Similarly, the coefficient obtained for the one-period lagged import variable Limport(-1) is -12.6790, indicating that a 1% increase in imports has a stronger downward effect on the short-run terms of trade (INTOT). Accordingly, past imports have a more pronounced negative effect than current imports. For the two-period lagged import variable Limport (-2), the coefficient is -7.0379, indicating that the lagged effect of imports is again negative. These findings suggest that the impact of imports continues to diminish over time, but in any case, it continues to have a negative impact on the terms of trade in the long run.

In the model, a 1% increase in the Consumer Price Index (CPI) inflation variable leads to a decrease of -0.7264 units in the short-term terms of trade, while the coefficient of the variable related to agricultural and livestock supports (ASDI) is positive and it is found that a 1% increase increases the terms of trade by 0.6156 units. This finding indicates that the increase in inflation has a negative impact on economic welfare, whereas agricultural and livestock subsidies have a positive impact on economic welfare.

In line with all these findings, the model has a high level of explanatory power and the estimated coefficients are statistically significant.

Sample: 2010 2022
Included observations: 11

Autocorrelation	Partial Correlation		AC	PAC	Q-Stat	Prob
		1	-0.232	-0.232	0.7700	0.380
		2	0.008	-0.048	0.7710	0.680
		3	-0.150	-0.169	1.1726	0.760
		4	-0.052	-0.139	1.2273	0.874
		5	0.082	0.025	1.3886	0.926

Figure 3: Correlogram of the estimated regression equation
Şekil 3: *Tahmin edilen regresyon denkleminin korelogram*

Figure 3 shows the autocorrelation (AC) and partial autocorrelation (PAC) coefficients for the analysed time series and their corresponding Ljung-Box Q-statistics (Q-Stat) and p-values. In this way, it is shown whether the time series has a statistically significant autocorrelation relationship with its past values at different lag levels. Lag (1-5) in

Figure 3 represents the first to fifth lag of the time series. In this context, the AC (Autocorrelation) coefficient of each lag reflects the direct correlation of the series with a particular past value. The values of these AC coefficients are close to zero, indicating that there is no significant autocorrelation. Indeed, Figure 3 shows that the AC coefficients are low and the series does not exhibit a strong autocorrelation structure.

Similarly, the PAC (Partial Autocorrelation) coefficients indicate the extent to which the relationship at a given lag explains the series after subtracting the effect of other lags. The fact that the PAC values are close to zero indicates that there is no strong partial autocorrelation in the series.

The Ljung-Box Q-statistics (Q-Stat) in the fifth column test the cumulative statistical significance of autocorrelations up to the lag. The p-values (Prob) in the last column indicate the significance levels of the respective Q-statistics. Values above 0.05 or 0.10 indicate that the autocorrelation is not statistically significant.

According to the findings, the time series does not have a significant autocorrelation for the first five lags. This indicates that the series does not have a strong relationship with its past values and there is no significant autoregressive (AR) structure. In the literature, such structures are interpreted as “exhibiting white noise characteristics”.

As a result, the AC and PAC coefficients in Figure 3 and the p-values of the Ljung-Box test reveal that there is no autocorrelation relationship in lags 1 to 5 of the analysed series.

4. Discussion and Conclusion

The terms of trade are used to monitor changes in the purchasing power and income of the rural sector and to determine the transfer of resources between sectors and the impact of inflation (29). The terms of trade can be calculated on a sectoral basis such as agricultural and industrial sectors or on a single product. As a matter of fact, Uzunöz and Esengün's (14) study on dairy cattle farms, Tuncel and Cevger’s (19) study on cattle breeding establishments, and Yelboğa et al.'s (30) study on tomato producers are examples of studies conducted on a single product. However, in this study, internal terms of trade, meat imports, subsidies, inflation, and milk/feed parity data were analysed in a multifaceted manner.

The economic survival of enterprises is closely related to their production costs and the sales price and sales volume of this product. However, Türkiye has intensively implemented policies to increase the import of carcasses and live animals in order to reduce the price increases in red meat prices and to ensure the supply-demand balance between 2010 and 2018 (31, 32). In 2019 and afterwards, the pandemic process and the disruptions in production caused by this process, the rapid upward change in foreign exchange prices and the accompanying increase in inflation caused the costs of breeding establishments to increase rapidly, while their incomes did not increase at the same rate. As a matter of fact, in the study, NITOT index is an indicator of economic welfare, has been at a low level since 2018 (As seen at Figure 2). Since the Carcass Price Index (CAPI) remained below the Agricultural Food Index (AFI) in this period, it can be said that the situation was unfavorable to the breeders operating in the sector and the income obtained by the producers did not cover the production costs. The analysis shows that the terms of trade have changed to the detriment of livestock enterprises. In his study, Demir Ayvazoğlu (33) reports that producers suffered losses due to the decrease in profit margin in the face of increasing costs in the same periods and withdrawals from production started. Similarly, in another study conducted in the agricultural sector, it is reported that the terms of trade developed against producers (29). In his study, Hossain (34) reports that especially in developing countries, long-run terms of trade will develop against enterprises producing agricultural and livestock products.

Within the scope of the research, it was found that although the price changes between input and output prices are realized to the detriment of breeding establishments, the purchasing power of breeding establishments did not change due to the increase in production. In other words, while the NITOT index, which is an indicator of economic welfare, decreased, the INTOT index, which is the purchasing power of breeding enterprises between 2017 and 2022, remained constant. Accordingly, it was determined that there was a horizontal course in the economic welfare of cattle fattening enterprises, in other words, there was no sharp change in a positive or negative direction, and economic welfare did not change in terms of cattle fattening enterprises. However, it is clear that this situation is misleading. It can be stated that the recent increase in meat production in Türkiye is due to meat imports and slaughter of cows rather

than a real increase in production (35). As a matter of fact, in this study, it is revealed that the purchasing power of breeding establishments decreased by 12.7%, 13.6% and 19.1% in 2011, 2014 and 2018 when the data on meat imports excluded, which caused an artificial increase.

Apart from meat imports, another factor that causes an artificial increase in meat production is the slaughter of breeding dairy animals. Changes in milk and feed price parity affect livestock prices and meat production. In years when milk-feed parity decreases, the number of female and milking cows sent to slaughter increases as the cost of keeping live animals increases for producers (33, 36). This situation causes an increase in artificial production outside the activities of breeding establishments. Especially in 2008 and 2021, when the milk-feed parity was below 1 in Türkiye, dairy animals were slaughtered, leading to an increase in meat production (35). Therefore, the INTOT index increased in these periods.

The study finds that the purchasing power (INTOT) of breeding farms increases in 2020-2021, when the Covid 19 pandemic occurred, but the economic welfare power (NITOT) of farms decreased. In parallel with the results of this study, Tuncel (37) reports that a 1% increase in restrictions during the pandemic period in Türkiye causes an 11% decrease in meat demand.

In the study, it is found that a 1-unit increase in agricultural and livestock subsidies (ASDI) causes an increase of 0.6156 units on NITOT. Accordingly, subsidies affect the economic welfare of the farms not only directly but also indirectly through terms of trade. There is no study in the literature that focuses on the relationship between terms of trade and subsidies. However, Demir and Yavuz (38) report that there is a statistically significant relationship between livestock subsidies and the number of animals and animal production. In the study, it is also found that a 1 unit increase in the consumer price index causes a 0.7264 unit decrease unit in the terms of trade. Accordingly, an increase in the inflation level causes a decrease in the terms of trade and consequently a decrease in the economic welfare of breeding establishments. In parallel with the finding of this study, Aral et al. (39) reports in their study that an increase in an income of breeding establishments remains below the cost increases due to the inflation in Türkiye after 2018. It can be said that the fact that the changes in the price of carcasses have remained far below the increase in costs over the last 10 years is an important obstacle to the economic sustainability of establishments. In the research, it can be said that inflation, meat imports, changes in milk/feed parity and livestock supports have affected the economic welfare levels of cattle breeding establishments, and in this context, it can be said that the establishments that had to produce by being squeezed between price and cost pressure in the market have turned into structures that can survive with livestock supports (credit, feed, etc.) over time.

In conclusion, although practices such as improvement in livestock support and import restriction program provide significant economic contributions to breeders, it is important to reduce production costs for the sustainability of a farm. In this context, in the livestock policies to be taken in this context, on the one hand, public authorities should support the economic welfare and purchasing power of cattle breeding enterprises, on the other hand, it is necessary to realize the necessary structural transformation that will increase the demand of the consumer and enable the market to return to its normal functioning.

The findings of this study are based on secondary data; when compared to future studies with primary data, they will provide an important reference point for policymakers to develop more accurate and data-driven strategies.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Authors' Contributions

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Ethical Approval

There is no need for ethics committee and other ethics committee decisions regarding the use of experimental animals.

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Araştırma Makalesi / Research Article

Investigation of individuals' perception of pet adoption and the effect of animal criteria on adoption

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ABSTRACT

Pet adoption is the assumption of full responsibility for an animal. Social preferences directly affect the adoption rate and preference. The main channels used in adoption are shelters, breeders, neighbors and friends with pets, but there is no precise data on how and why an animal is preferred when adopting an animal from a shelter. The main purpose of the study is to determine the adoption perception and preference parameters of individuals who adopt animals from shelters. Three hundred four participants were included in the study who adopted cats or dogs from the shelter between June 2023 and January 2024. 26.6% of the individuals included in the study were male, 73.4% were female, and proportionally, the age range in which the highest number of adoptions occurred was over 45 years old with 33%. In addition, it was determined that 18.2% of the individuals were between the ages of 18-25, 18.2% between 26-35 and 21.9% between 26-45, and 43.1% of them owned cats and 56.9% owned dogs. While the rate of cat adoption was higher among women, men preferred dog adoption. As a result of the study, it was revealed that the feeling of pity was dominant due to the poor health status of the adopted animals and this situation affected the adoption behavior of women. Although fear / excitement / nervousness behaviors are exhibited in the first encounter, it differs from other studies in terms of adoption. It was revealed that the training of shelter staff is a key point in adoption.

Bireylerin evcil hayvan sahiplenme algısı ve hayvan kriterlerinin sahiplenme üzerindeki etkisinin incelenmesi

ÖZET

Evcil hayvan sahiplenme, bir hayvanın tüm sorumluluğunun üstlenilmesidir. Sosyal tercihler, sahiplenme oranını ve tercihini doğrudan etkilemektedir. Hayvan sahiplenmede kullanılan başlıca kanallar barınaklar, yetiştiriciler, komşular ve evcil hayvanı olan arkadaşlardır ancak barınaktan hayvan sahiplenirken bir hayvanın nasıl ve neden tercih edildiğine dair kesin bir veri bulunmamaktadır. Çalışmanın temel amacı, barınaklardan hayvan sahiplenen bireylerin sahiplenme algısı ve tercih parametrelerini belirlemektir. Çalışmaya Haziran 2023 - Ocak 2024 tarihleri arasında barınaktan kedi veya köpek sahiplenen 344 katılımcı dahil edilmiştir. Çalışmaya dahil edilen bireylerin %26,6'sı erkek, %73,4'ü kadındır ve oransal olarak en fazla sahiplenmenin gerçekleştiği yaş aralığı %33 ile 45 yaş üstüdür. Ayrıca bireylerin %18,2'sinin 18-25, %18,2'sinin 26-35 ve %21,9'unun 26-45 yaş aralığında olduğu ve %43,1'inin kedi, %56,9'unun köpek sahibi olduğu belirlendi. Kadınlar arasında kedi sahiplenme oranı daha yüksekken, erkekler köpek sahiplenmeyi tercih etmiştir. Yapılan çalışma sonucunda sahiplenilen hayvanların sağlık durumlarının kötü olması nedeniyle acıma duygusunun baskın olduğu ve bu durumun kadınların sahiplenme davranışını etkilediği ortaya çıkmıştır. İlk karşılaşmada korku/heyecan/sinirlilik davranışları sergilense de sahiplenme açısından diğer çalışmalardan farklılık göstermektedir. Barınak personelinin eğitiminin sahiplenmede kilit nokta olduğu ortaya çıkmıştır.

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1. Introduction

For many years, countless families have shared their homes with many cats and dogs and have formed close emotional bonds with them. The human meaning of pet ownership is to make them feel at serenity and to keep them company. In addition, adoption is known to reduce stress, anxiety, and depression in humans. Moreover, under proper training and discipline, these animals show their loyalty to protect and obey their owners, with whom they live in symbiosis (1).

Pet adoption is the assumption of full responsibility for an animal. Adopting a living being is a sensitive issue that cannot be simplified, and we are obligated to provide the love, trust, and patience it deserves. Today, the main channels used for adoption are shelters, breeders, neighbors, or friends with pets (2). Significant increases in the pet population have left animal shelters across the country with the challenge of finding solutions to increase adoption rates (3). According to the Humane Society of the United States, adoption not only gives the animal a second chance but also helps to provide better conditions for the remaining animals by reducing shelter costs (4, 5).

Studies evaluating anthropological literature reviews support that social preferences affect ownership. In addition, increasing disposable income, attitude change, and urbanization have significantly increased pet ownership in Turkey, Brazil, China and Thailand (6). In recent years, it has been observed that the cat population has increased considerably in many countries, and the number of cats living as pets in the USA is higher than the number of pet dogs (7).

In the United States, an estimated 3.2 million animals are adopted from animal shelters annually and the adoption rate is increasing yearly (8). Although most owners report high levels of satisfaction with their newly adopted animals (9, 10). It is known that a significant proportion of adopted animals are returned to shelters for various reasons (11). Although there are studies investigating the change in the bond between humans and animal in animals left in shelters in the USA (12, 13) there is no clear data on how and why an animal is preferred when adopting an animal from a shelter. However, it is known that various factors affect the choice of a cat or a dog in pet adoption. In Turkey, there is no study on the abandonment or adoption behavior of individuals.

The primary objective of the study is to determine the preference parameters of individuals who adopt animals from shelters. The secondary aim of the study is to determine the reasons for the preference for shelter animals and the animal behaviors and characteristics that play a role in the selection process and to reveal the importance of information collection methods.

2. Material and Methods

This study was supported by the Ondokuz Mayıs University Scientific Research Project with the code PYO.VET.1908.23.003.

Study Population

The questionnaire applications were completed online by individuals who were adopted from the shelter between June 2023 and January 2024. The questionnaire consisted of 7 questions, including four questions to determine the reason for pet preference, what the reaction was at the first encounter, and from whom the necessary information about the animals was obtained, and three questions containing the demographic information of the pet adopter. Participation in the survey was voluntary, and the questionnaire was filled out after the adoption to ensure that it did not affect adoption behavior. Individuals who adopted more than one pet were asked to complete a questionnaire for only last adopted pet.

Question Categories

Question 1. What is the most important reason for choosing your pet friend?

Category	Examples of Answers
Behaviour	"The way its behaviour towards me"
Appearance	"Physical appearance"
Temperament	"It's character" "being energetic and playful"
A feeling of pity/Health status	"It needed us." "It needed a home." "Compassion"
For child/Family friendly	'My children needed to learn compassion'
Being friends	"Being friends" "the death of my life mate"

Question 2. What are the Other Important Factors in Choosing Your Pet Friend?

Category	Examples of Answers
Appearance	"Physical Appearance (colour, breed, size, coat, body shape, appearance, expression, posture)"
Temperament	"Being playful" "Having a calm temperament"
A feeling of pity/Health status	"Health status" "Unclaimed for a long time" "I adopted it out of pity'
For child/Family friendly	"For child"
Being friends	"To be my friends"

Question 3. How did your pet friend react the first time you met it?

Category	Examples of Answers
Approach/Contact	"It came into our laps, kissed my wife's chin." "It endeared itself by running and jumping" "It licked my hands"
Excitement/Enjoyment	"Very joyful" "very happy" "amazement and joy"
Irritability/Response	"It behaved aggressively" "hissed"
Fear/Anxiety	"Scared" "It got scared and didn't come to us for a while." "It was a bit nervous because it was so small."
Bashful/ Abstention	"Stayed away" "withdrawn" "at first it was unfamiliar, then got used to it'

Question 4. What is the source of information that influences you in choosing your pet friend?

Category	Examples of Answers
Shelter Staff	"Shelter" "Shelter Staff"
Close Friends	"Close Friends"
Social Media/Internet	"Social Media" "Internet advertisement"
Breeder/Petshop	"Breeder" "Petshop"

Statistical Analysis

Statistical analyses of the data were performed in the IBM SPSS v24 program. Statistical analyses of categorical data between gender and species were evaluated with a chi-square test. And also, One-sample Chi-squared test of goodness-of-fit was applied to evaluate the compatibility of categorical data with the theoretical (expected) distribution. The expected distribution was determined according to the equal frequency assumption. Descriptive statistics were presented as frequency and percentage. Statistical significance was accepted as $p<0.05$.

3. Results

A total of 304 participants were included in the questionnaire, and those who left any data blank (n=3) and those who could not be categorized (n=3) were excluded from the study, and evaluations were made on 298 participants. The responses to the survey questions are summarised in Table 1. It was found that 26.6% (n=79) of the respondents were male, 73.4% (n=297) were female, and the age range with the highest rate of adoption was 33% (n=98) over 45 years of age. It was determined that 18.2% (n=54) of the participants were 18-25 years old, 18.2% (n=54) were 26-35 years old and 21.9% (n=65) were 26-45 years old. Regarding the type of animal adopted, it was determined that 43.1% (n=128) of the respondents adopted cats and 56.9% (n=169) adopted dogs, while cat adoption was higher in women, men preferred to adopt dogs.

While the most important reason for adopting animals from the shelter was pity for animals and poor health, it was determined that this situation was higher in women than men. The second most important reason people prefer their pets is the expectation of a friend, poor health, and pity. It was seen that the need for animals in need of care surpassed other preferences (Table 2a).

Tablo 1: Anket cevaplarına göre pet tercihlerinin kadın ve erkek dağılımı.

Table 1: *Gender distribution of pet preferences according to survey responses.*

	Pet Preference			
		Man	Woman	
	Frequency (%)	**Frequency (%)**	**Frequency (%)**	
Cat	128 (43.1)	25 (31.6)^{Ax}	103 (47.2)^{B}	P<0.05
Dog	169 (56.9)	54(68.4)^{y}	115 (52.8)	

A, B: Refers to the difference between the column percentages in the same row (p<0.05). x, y: means the difference between the column percentages in the same column (p<0.05).

When asked about their first reactions to the newly adopted animals, 105 (35.4%) people reported fear and uneasiness, 88 (29.6%) reported excitement and joy, and 53 (17.8%) reported that contact with the animal was effective in adoption. It was observed that the first information about the adopted pets was received from the shelter staff at a high rate (68.4%) (Table 2b).

Tablo 2a: Anket sorularına verilen cevapların frekansları ve istatistiksel anlamlılıkları.

Table 2a: *Frequencies and statistical significance of the answers given to the survey questions.*

Question 1. What do you think is the most important reason for choosing your pet friend?				
		Man	Woman	
Category	**Frequency (%)**	**Frequency (%)**	**Frequency (%)**	
Behaviour	16 (5.4)	4 (5.1)	12 (5.5)	
Appearance	17 (5.7)	7 (8.9)	10 (4.6)	
Temperament	29 (9.8)	12 (15.2)	17 (7.8)	
A feeling of pity/Health status	220 (74.1)*	50 (63.3)^{A}	170 (78.0)^{B}	P<0.05
For child/Family friendly	5 (1.7)	2 (2.5)	3 (1.4)	
Being friends	10 (3.4)	4 (5.1)	6 (2.8)	

A,B: It expresses the difference between the column percentages in the same row (p<0.05). Frequency with an asterisk (*) in the same column for each question title expresses the difference between the percentages of total frequency distributions (p<0.05).

Tablo 2b: Anket sorularına verilen cevapların frekansları ve istatistiksel anlamlılıkları.

Table 2b: *Frequencies and statistical significance of the answers given to the survey questions.*

Question 2. What are the other important factors in choosing your pet friend?	
Category	**Frequency (%)**
Appearance	9 (3)
Temperament	8(2.7)
A feeling of pity/Health status	86 (29)
For child/Family friendly	46 (15.5)
Being friends	148 (49.8)*
Question 3. What was your reaction when you first met your pet friend?	
Approach/Contact	53 (17.8)
Excitement/Enjoyment	88 (29.6)
Irritability/Response	17 (5.7)
Fear/Anxiety	105 (35.4)
Bashful/ Abstention	34 (11.4)
Question 4. What is your source of information that influenced you to choose your pet friend?	
Shelter Staff	203 (68.4)*
Close Friends	21 (7.1)
Social Media/Internet	45 (15.2)
Breeder/Petshop	28 (9.4)

Frequency with an asterisk (*) in the same column for each question title expresses the difference between the percentages of total frequency distributions ($p<0.05$).

4. Discussion and Conclusion

This study aimed to reveal the preferences of individuals in adoptions made from animal shelters/nurseries, which are based on rehabilitating stray animals and protecting public health as well as animal health. Cat adoption rates are reportedly higher than other domestic animals worldwide (14). It is seen that dogs are more common in populations in animal shelters due to reasons such as the fact that cats are better adapted to the street individually, and their home care and costs are more affordable than dogs (15). These characteristics of cats also reduce their abandonment rates compared to dogs. These data explain why dogs were adopted more from shelters in this study. In addition, it is known that male individuals prioritize appearance and breed qualities in dog preference. This situation can be accepted as normal due to the diversity of different sizes, breeds, and purposes (protection, hunting, companionship). In women, it is seen that emotional attachment (pity, possessiveness, protection) plays a role in adoption. In the study, it was revealed that the feeling of pity was predominant due to the poor health status of the adopted animals, and this situation affected the adoption behaviour of women.

A study determined that the rate of adoption from the shelter was 4.8%, 23.7% from the street, and 37.1% from pet shops in total adoption (n=704). In the same study, when the reason for adoption was investigated, it was revealed that the majority, 72.6%, adopted a dog ‘because I like it’ and 7.2% adopted a dog ‘because of loneliness and friendship’. As a general finding, those who want to adopt a dog prefer breeders and pet shops, while those who wish to adopt a cat prefer shelters (16). This is because dogs can be used for relatively qualified/educated jobs such as protection, hunting, and guarding. The fact that shelters in our country have a dog-dominated population explains individuals' high rate of dog adoption (17). According to Hirschman (18), one reason for adoption is that pets are perceived as siblings, family members, or friends and associated with people. In the results obtained from the study, the fact that the animal adopted from the shelter has a high expectation of ‘being a friend’ and that these animals are

approached with a feeling of pity reveals that individuals exhibit adoption behaviour with a sense of empathy. In addition, considering that the people who show the most adoption behaviour are over 45 years old, the high expectations of pity and friendship (19) show that the feeling of empathy and pity for shelter animals is intense in society. While communication and physical appearance come to the fore in the first contact when adopting a cat or dog, stray animals have recently become a problem, and the subsequent culling discourses have increased the adoption rate of animals confined to shelters with a sense of pity.

Across species and age groups, the most important reasons for adoption are the physical appearance of the animal, its social behaviour with its relatives, and personality. A related study determined a positive correlation between contact, play, or greeting/voicing behaviours and adoption in the first encounter between the individual and the animal (20). However, this study differs from other studies in that adoption is shaped, although fear/excitement/nervousness behaviours are exhibited in the first encounter.

It has been reported that the information about the animal conveyed by the staff or volunteer is generally more effective in adoption than the data on the cage cards and that the health and temperament information of the animal is also important. That interaction with the animal is more valuable than seeing it in its kennel or cage (20). Related studies show that the shelter staff mainly obtains information about the animals to be adopted (21). For this reason, shelter personnel positively affect the adoption rates by recognizing the adopted animals and providing sufficient and qualified information about them. In addition, these personnel are the only individuals who will evaluate the suitability of the family to be adopted, as well as the adopters. Although most animal owners report a high level of satisfaction with their newly adopted animals (9, 10), it is known that a significant portion of the adopted animals are returned to shelters for various reasons. Behavioural and health problems rank high among the reasons for abandonment (11), and it is important to convey these situations to the prospective owners correctly and to shape the adoption by establishing a connection with different parameters other than just appearance or pity feelings, in order to prevent possible abandonment. For this reason, factors such as the fact that the shelter personnel from whom the first information is received have sufficient knowledge about domestic animals and have a good command of breed characteristics will increase the possibility of animal adoption from shelters. In the study conducted, it was revealed that the information about the adopted animals mainly (68.4%) was received from the shelter staff; therefore, as stated above, the shelter staff is the key point in adoption.

Legal culling decisions regarding stray animals and dogs have become a problem that is increasing daily and disturbing society (22, 23). As a result, it is important to prevent abandonment, increase adoption from animal shelters, and make correct potential owner-pet matches for the welfare of pets and society. The results obtained from this study can be used to create better matches in shelters, prioritize shelter resources and staff training, determine the temperament of animals, match them with suitable families, and increase potential adoption. In addition, simple training techniques can be developed to ensure that animals in the shelter exhibit adopter-friendly behaviours, which can lead to stronger bonds between the owner and the animal.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

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Ethical Approval

This study was approved by the HADYEK- Local Ethics Committee for Animal Experiments office of Ondokuz Mayıs University with 24.02.2023/2023-4 approval number.

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Investigation of *Brucella* agents in soil-fertilizer mixtures and animal feed samples from cattle farms after extinguished brucellosis outbreaks

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ABSTRACT

Although brucellosis is under control in developed countries, it still threatens the health of animals and humans in the endemic regions. In these endemic regions although the outbreaks in some cattle farms have been officially extinguished, the farmers have wondered whether the bacteria are still present in some farm materials after extinguishment processes. The situation must be clarified so that farm production activities can continue, and new animals can be procured in time. With this study, it was aimed to investigate the presence of *Brucella* bacteria in critical materials that may effectively transmit the disease after extinguishment procedures. For this reason, soil-fertilizer mixtures and animal feed were selected and investigated in three cattle farms where the disease was officially extinguished after the brucellosis outbreaks. The samples were collected approximately twenty days after completing extinction procedures for brucellosis. The soil-fertilizer mixtures and animal feed samples were collected in 68 and 55 pieces, respectively. The classic culture methods for the bacterial diagnosis of brucellosis were used with some modifications. After the growth of bacterial colonies on selective media, they were diagnosed by genus and species-specific PCRs. Five positive results were obtained by genus-specific PCR, but only one could be confirmed with species-specific multiplex PCR. For the remaining four, it was thought that they could belong to some soil bacteria genetically close to the *Brucella* genus. As a result, the brucellosis extinguishment procedures implemented could be considered adequate, and the farms were safe regarding the contamination. Although the official rules for disease extinction are fully implemented after outbreaks, similar studies are needed on more farms for more robust evaluations.

Söndürülen bruselloz salgınlarından sonra sığır çiftliklerinden alınan toprak-gübre karışımları ve hayvan yemi örneklerinde Brucella etkenlerinin araştırılması

ÖZET

Bruselloz gelişmiş ülkelerde kontrol altına alınmış olsa da endemik bölgelerde hayvan ve insan sağlığını olumsuz etkilemektedir. Bu bölgelerdeki sığır çiftliklerinde çıkan hastalıklar resmi olarak söndürülmüş olsa da, çiftçiler söndürme işlemlerinden sonra çiftliklerinde bulunan bazı materyallerde bakterilerin mevcut olup olmadığını merak etmektedir. Çiftlik üretim faaliyetlerinin devam edebilmesi ve yeni hayvanların zamanında tedarik edilebilmesi için bu hassas durumun açığa kavuşturulması bir ihtiyaç haline gelmiştir. Bu çalışmada, söndürme işlemlerinden sonra hastalığı etkili bir şekilde bulaştırabilecek kritik materyallerde *Brucella* bakterilerinin varlığının araştırılması amaçlamıştır. Bu sebeple, bruselloz salgınlarından sonra hastalığın resmi olarak sonlandırıldığı üç sığır çiftliğinde toprak-gübre karışımları ve hayvan yemleri seçildi ve incelendi. Örnekler, bruselloz için söndürme prosedürleri tamamlandıktan yaklaşık yirmişer gün sonra, toprak-gübre karışımlarından 68, hayvan yemlerinden 55 adet toplandı. Brusella bakterilerinin teşhisi için klasik kültür yöntemleri bazı modifikasyonlarla kullanıldı. Bakteri kolonileri seçici besiyerlerinde üretildikten sonra, Brusella bakterileri için cins ve tür spesifik PCR testleriyle araştırıldı. Cins-spesifik PCR ile 5 pozitiflik elde edildi. Bunlardan sadece bir tanesi tür-spesifik multipleks PCR ile doğrulanabildi. Kalan dördünün, genetik olarak yakın bazı toprak bakterilerine ait olabilecekleri düşünüldü. Sonuç olarak, bruselloz söndürme prosedürlerinin uygun olduğu ve çiftliklerin bulaş açısından güvenli sayılabileceği kanaatine varıldı. Hastalık çıkışlarından sonra hastalığın yok edilmesine yönelik resmi kurallar tam olarak uygulansa da, daha güvenilir değerlendirmeler için daha fazla çiftlikte benzer çalışmalara ihtiyaç duyulmaktadır.

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1. Introduction

Brucellosis is the general name for animal and human infections (1). The disease is caused by different bacterial species affiliated with the genus *Brucella* (2), mainly *B. melitensis*, *B. abortus*, and *B. suis* (1). Cattle are usually infected by *B. abortus*, less often by *B. melitensis*, and sometimes by *B. suis.* In animals, the disease is identified by abortion and infertility (3). There are limited symptoms to suspect the presence of the disease; however, many diagnostic tests can be used to make a diagnosis. Conventional culture can be used as a gold standard method. With this, molecular and serological methods are the most common diagnostic methods for brucellosis (1).

Many endemic countries use different fighting strategies to control and eradicate brucellosis (4, 5). These strategies are formed according to the conditions of countries, such as geographic borders, disease prevalence, the budget for the control of disease, infrastructure of farms, organizations of veterinary services, feasible diagnostic tests, etc. (6). In Türkiye, the "test and slaughtered" strategy with vaccination has been conducted with various projects since the 1980s. According to the valid regulation when the study was carried out in Türkiye (Circular No: 2019-6), in case of any abortion in any herd, the culture method was used to determine *Brucella* bacteria in the herd. For bacterial cultures, some organs and abomasal fluid of aborted fetuses, samples of vaginal discharges, placenta, and milk were critical materials for isolations. In those times, if any *Brucella* agent was determined with conventional methods, all animals in the same herd were tested serologically (Rose Bengal rapid agglutination test and Complement fixation test), and seropositive animals were slaughtered in the contracted slaughterhouses. This practice was continued until negative results were determined twice in the same herd at two-month intervals. After taking two consecutive negative test results for each animal, disinfection was made, and the disease was considered extinguished within the enterprises.

Some researchers reported that *Brucella* bacteria could also be shed in urine and feces except in aborted fetuses, birthing and aborting fluids, placenta, semen, and milk (7). After shedding, the bacteria can be transmitted to animals or humans through digestion, inhalation, conjunctiva, and even intact skin (1, 8, 9). This uncontrolled shedding and transmission have led many researchers to investigate the persistence of *Brucella* bacteria in soil and other materials. It was reported that the bacteria can survive up to 250 days in soil (7, 10), although contamination varies according to many factors. Temperature, direct sunlight, and soil moisture could play a prominent role in the persistence of the bacterium in soil (11). All these cause question marks in the farmers' minds and lead to uneasiness about whether the danger of contamination is still continuous after the official disease extinction processes in the farms.

This study aimed to investigate the presence of *Brucella* bacteria in the soil-fertilizer mixture and animal feed at the cattle farms where the disease had been officially extinguished and evaluate these materials for the risk of contamination by *Brucella* agents.

2. Material and Methods

Samples

The study was conducted in three cattle farms where authorized units carried out official eradication procedures for brucellosis in 2020. The extinction procedures were continuous for each farm for approximately eight months. The morbidity rates of the disease in these farms were 65%, 60%, and 42%, respectively. Almost twenty days following the official extinction date for each farm, samples were chosen from the feed stored in the farms' areas and from risky regions in terms of contamination, such as birthing paddocks and walking areas. Pieces of 32, 21, and 15 soil-fertilizer mixtures and 20, 22, and 14 feed samples were taken from the first, second, and third farms, respectively. Samples for the soil-fertilizer mixture were collected from the topsoil surface at about 0 to 5 cm depth. Approximately 400-500 g samples were taken using a sterile spatula and gloves. After being placed into sterile containers, all samples were brought to the laboratory under cold chain conditions as soon as possible.

Standard strains

Standard strains were obtained from the strain collection of Harran University, Faculty of Veterinary Medicine, Department of Microbiology. *B. abortus* Tulya (ATCC 23450), the reference strain of *B. abortus* biotype 3, was used in the tests as the reference strain.

Bacterial isolation

All tests were carried out in the Laboratory of the Department of Microbiology in the Faculty of Veterinary Medicine of Harran University. *Brucella* selective broth and agar (Farrell's media) were prepared using the base media tryptic soy broth and agar (Oxoid, England) with adding a commercial *Brucella* selective supplement (Oxoid, England). Farrell's media were used for conventional bacterial isolation (1, 9, 12).

Each sample was mixed thoroughly before inoculations. Twenty-five g of each sample was inoculated into selective broth at a ratio of 1/10. Inoculated media were incubated at 37 °C in microaerobic conditions (Panasonic, MCO-18AC-PE, Japan) for six weeks. During the incubation period, cultures were refreshed weekly with the additional medium at a ratio of 1/5. Then, 200 µl from these media was passed onto selective agars every two weeks. These agars were incubated under the same conditions with broth media for five days. After five days, DNA extractions were performed from the colonies for the molecular diagnosis of *Brucella* bacteria. These PCRs were repeated every two weeks up to 6 weeks.

Molecular identification from bacteria

The genomic DNAs were isolated from the cultures using boiling (13). The isolated DNAs were stored at -20ºC until analyzed by genus and species-specific PCRs.

The method of Queipo-Ortuno et al. (14) was used with slight modifications for the genus-level PCRs. B4 and B5 primers (Sentebiolab, Türkiye) were preferred from the gene region encoding *Brucella* cell surface salt extractable protein 31 (Bcsp31) (15). Briefly, five µl of 10 X PCR solution containing a mixture of 100 mM Tris-HCl (pH:8.4, 500 mM KCl, and 15 mM $MgCl_2$,) 38.25 µl of PCR water, 1µl of primer mix stock (Final concentration 0.2 µM), dNTP mix stock 0.5 µl (Final concentration 0.2 mM), 0.25 µl of enzyme (5 IU/µl) (Final concentration 1.25 IU). Finally, five µl of DNA template extracted directly from the cultures was added to the tubes to complete the PCR mixture. The PCR tubes were placed, and the amplification process was completed in the thermal cycler by applying a total of 35 cycles, following pre-denaturation at 93 ºC for 5 minutes, one minute at 90 ºC (denaturation), 30 seconds at 60 ºC (annealing), one minute at 72 ºC (extension) and 7 minutes at 72 ºC (last extension) at the end of the cycles.

The species-specific multiplex PCR was carried out using the method of Mayer-Scholl et al. (16). In the PCRs, nine pair primers (Sentebiolab, Türkiye) were used (16, 17) with a commercial ready-to-use master mix kit (Qiagen, Germany).

In all tests, the genomic DNA of *B. abortus* bt 3 and the PCR-grade water were used as positive and negative (NTC) controls respectively.

The obtained amplicons were electrophoresed into 1.5% agarose gel at 100 volts for 45 minutes. The band formations were investigated after visualizing with the UV illuminator (Vilber-Lourmat, France).

3. Results

After the incubation periods of the samples, none of the cultures were pure, although the selective media had been used. So, mixed cultures, including various bacterial colonies, were used for molecular analyses.

Five positive results were determined at the end of the first two weeks (Table 1, Figure 1).

Table 1: The samples and results of the molecular tests
Tablo 1: *Örnekler ve moleküler test sonuçları*

Farms	Samples	Number of Samples	Results of genus-specific PCR		Results of Species-specific PCR	
			Positive	Negative	Positive	Negative
1. Farm	Soil-fertilizer mixture	32	3	29	1	31
	Animal feed	20	0	20	0	20
2. Farm	Soil-fertilizer mixture	24	1	23	0	24
	Animal feed	22	0	22	0	22
3. Farm	Soil-fertilizer mixture	15	1	14	0	15
	Animal feed	14	0	14	0	14

Among the five positive samples for genus-specific PCR, only one *B. abortus* field strain was identified by multiplex PCR from the DNAs of the soil-fertilizer mixture sample taken from the grazing area of the first farm. The other four samples were found to be negative by species-specific PCR. (Table 1, Figure 2).

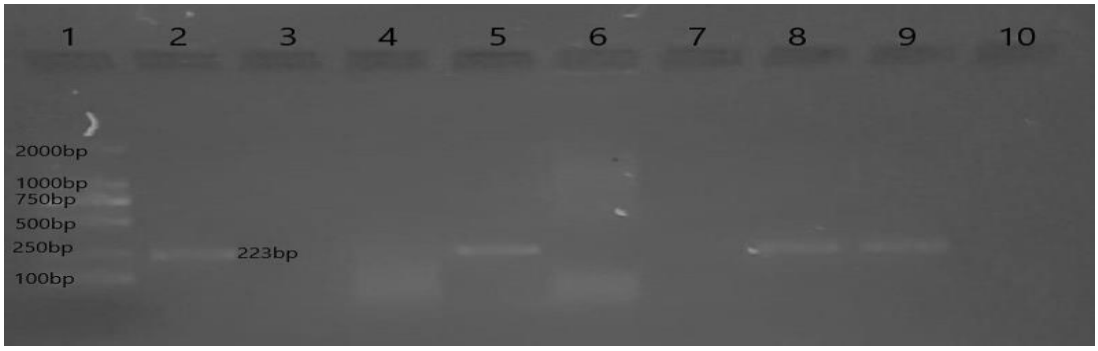

Figure 1: Agarose gel view for the genus-specific PCR
Şekil 1: *Cins spesifik PCR için agaroz jel görüntüsü*
Line 1: Marker 2000bp, Line 2, 5, 8, 9: Some positive results; Line 3, 4, 6, 7, 10: Some negative results.

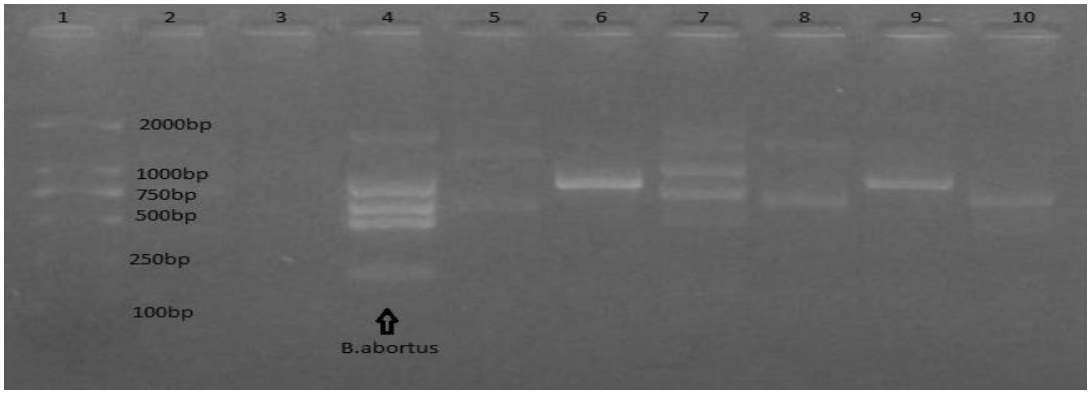

Figure 2: Agarose gel view for the species-specific PCR
Şekil 2: *Tür spesifik PCR için agaroz jel görüntüsü*
Line 1: Marker 2000bp, Line 2, 3, 8, 10: Some negative results, Line 4: Positive results (*B.abortus*-Band sizes: 1682, 794, 587, 450, and 152 bp), Line 5, 6, 7, 9: Genus-specific PCR positive but species-specific PCR negative results.

4. Discussion and Conclusion

Brucella agents can be shed in many different ways according to the clinical symptoms of diseased and porter animals during their lifetime. The most common way of spreading the agents of brucellosis is via aborted fetuses and their membranes. The others are vaginal discharges, semen, milk, hygroma, or arthritis fluids (1). Although many researchers and institutions of international health organizations consider the excretion of *Brucella* agents in feces possible, the transmission of the disease in this way is generally evaluated as insignificant in the epidemiology of the disease. On the other hand, studies show that brucellosis-bearing animals can also spread the agent through feces. Morales-Estradaa et al. (7) isolated *B. abortus*, *B. melitensis*, and *B. suis* species, a total of ten pieces from cattle and goat feces. They reported that contaminated feces is a risk factor in endemic areas. In this study, soil-fertilizer mixtures were chosen as the research subject because they carry a high risk of contamination. Both feces and secretions-excretions of animals, as well as abortion or postpartum shedding, were considered to be high risk for transmission.

Because the spread of *Brucella* agents in the environment by a latent carrier animal might last a lifetime, the possibility of transmission of these bacteria through soil has been a matter of curiosity, and many studies have been conducted on this topic. In the studies conducted, it has been reported from the past that *Brucella* species scattered with feces or by other means can remain in soil and dust for several weeks (18-21). Leski et al. (22) took soil samples from 19 locations in Kuwait and Iraq and analyzed samples molecularly. The researchers reported that bacteria from the *Brucella* genus were detected in 8 locations. Aune et al. (23) reported in their study conducted in the Greater Yellowstone Area that the persistence of *Brucella* bacteria in soil lasted up to 43 days in the places where contamination occurred through bison births or their waste, and they reported that the persistence of bacteria in soil was directly related to exposures of temperature and sunlight. Ahmed et al. (24) reported collecting 1280 soil samples from 256 villages in 9 regions of the Punjab province of Pakistan and investigating the DNA of *Brucella* bacteria. They detected positive results in 27 samples from the DNAs obtained using a soil DNA extraction kit. As a result, researchers investigated not only soil but also potential infectious materials such as fomites (25) and water (19), and they concluded that these materials could be a risk factor for transmission. In this study, animal feedstuffs were not taken off the farms' area, so they were considered a potential risk source for new animals. So, in addition to the soil-fertilizer mixture, contamination of stored feeds by various means was also considered possible, and these feeds were included as research material in the study. Essential carriers of the agent of brucellosis, such as soil and animal feeds, have the potential to harbor, preserve, and transmit the agent under appropriate conditions; they were made the subject of this research in the farms where the disease emerged.

Researchers were used isolation methods with molecular methods to identify *Brucella* agents at the genus and/or species level. Garrido-Haro et al. (26) performed bacterial isolation from samples obtained from cattle populations in Ecuador in the last 3 years. They confirmed the diagnosis by using genus-specific and species-specific PCRs. Mazwi et al. (27) performed bacterial isolation from animals at a slaughterhouse in South Africa and confirmed these isolates by PCR. In a study conducted in Northeastern Ethiopia Tekle et al. (28) isolated *Brucella* bacteria from eight out of 64 samples. After using species-specific PCR, they reported that four goat isolates were *B. melitensis*. Similar to previous studies, classical isolation methods were used with together a molecular diagnostic method, PCR, in this study.

The genus *Brucella* phylogenetically belongs to the family Rhizobiaceae in the α-2 subgroup of the class Proteobacteria. The genera of *Rhizobium* and *Ochrobactrum* are also in the same family (29). Researchers reported that these species are abundant in the soil and that soil bacteria are the closest relatives of *Brucella* agents. (30). Researchers reported that members of the *Ochrobactrum* genus are the closest phylogenetic relatives of *Brucella* agents and that the 16S rRNA gene shows more than 97% similarity to the *Brucella* genome sequence. Species such as *O. antrophi* and *O. intermedium* are closer to *Brucella* species than other species in the genus. This striking similarity requires attention to identify *Brucella* agents and diagnose infection correctly (31). Moreover, some researchers have reclassified the genus *Ochrobactrum* and included its species in the genus *Brucella* based on extensive genome comparisons using advanced molecular technology (32, 33).

PCR methods targeting the highly conserved 16S rRNA, the gene region encoding the Bcsp31 protein, and other genetically conserved regions have been developed to differentiate the genus *Brucella* (15, 34, 35). However, it should be noted that these regions may cross-react with species genetically close to the *Brucella* genus, such as *Ochrobactrum anthropi* and *Ochrobactrum intermedium* (34, 36, 37). For the results that were positive according to the genus level PCR but could not be confirmed by species-specific PCR, it was thought that these positive PCR results may be caused by saprophytic soil bacteria that are genetically close to the *Brucella* genus. On the other hand, this result may be due to the ability of the PCRs with different minimum detection limits to detect different amounts of specific DNA (38). Other bacteria can grow with *Brucella* agents, and they can limit the growth of the specific agents. So, the amount of specific DNA obtained may have been below the detection limit for species-specific PCR.

According to the results of this study, all positive samples were found in areas with soil-fertilizer mixtures. No positivity was detected in sampling from feed storage areas. These results were considered reasonable regarding the possibility of contamination of the materials. In addition, the preference of the culture method in the analyses was critical in obtaining viable microorganisms. Molecular methods cannot give us information about viability.

One of the most critical limitations of this study is that similar samples could not be taken from the farms where the study was conducted during the active disease period. Unfortunately, the farm owners requested analyses after the disease was extinguished, so comparisons of samples before and after the disease was extinguished could not be made.

It is also crucial for farm workers to follow biosafety protocols to prevent human infections, especially in case of outbreaks, since *Brucella* species are zoonotic pathogens.

With this study, the idea was obtained, especially in these or similar farms, how long the areas where new animals will be bought could be safe and whether the disease control methods applied in the farms could be effective after achieving disease extinction. The results of this study suggest that in-house risky areas can be considered epidemiologically safe for the spread of brucellosis on farms where the official rules for disease extinction are fully implemented after outbreaks. However, carrying out similar studies on more farms will make the evaluations on this subject even more reliable.

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Conflict of Interest

The author declared that there is no conflict of interest

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Authors' Contributions

Motivation/Concept: Ahmet Murat SAYTEKİN, Sevil ERDENLİĞ GÜRBİLEK
Design: Ahmet Murat SAYTEKİN, Sevil ERDENLİĞ GÜRBİLEK
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Ethical Statement

The ethical declaration has been obtained from the authors that the data, information and documents presented in this article have been obtained within the framework of academic and ethical rules, and that all information, documents, evaluations and results are presented in accordance with the rules of scientific ethics and ethics.

According to the Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees (Article 2 - paragraph 2), this study does not require ethics committee approval. Because the study involves "non-experimental clinical veterinary medicine practices".

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Olgu Sunumu / Case Report

Hemangiosarcoma with brain metastasis in a cocker spaniel in Türkiye

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ABSTRACT:

A 2.5 years old male English Cocker Spaniel dog was admitted to our clinic with symptoms of left axis circling, increased vocalization and vestibular ataxia. The patient's medical history revealed that the left hind limb was amputated due to a malignant tumor and the tumor type was confirmed as hemangiosarcoma by pathology report. As a result of physical, neurological and radiographic examinations, intracranial tumor metastasis of primary origin in the left hind limb was suspected. No abnormal findings were found in the cerebrospinal fluid obtained from the patient. Advanced imaging techniques (computed tomography and magnetic resonance imaging) and biopsy procedures required for treatment planning were refused by the owner and the patient died 4 days later. Macroscopic necropsy findings revealed mass foci of varying sizes in the brain, spleen, liver, diaphragm and lung. Histopathologic examination revealed hemangiosarcoma metastasis of 0.2.x0.1 cm in the brain. The post-mortem findings were consistent with the clinical findings. Brain metastasis of hemangiosarcoma in a cocker spaniel dog shows that our case is rare.

Türkiye'de cocker spaniel ırkı bir köpekte beyin metastazı ile seyreden hemanjiosarkom

ÖZET:

2.5 yaşlı erkek, English Cocker Spaniel ırkı bir köpek sol eksenli kendi etrafında dönme, vokalizasyonda artış, vestibular ataksi semptomları ile kliniğimize başvurdu. Hastanın sağlık geçmişinde sol arka ekstremitenin malign tümör kaynaklı ampute edildiği, tümör tipinin patoloji raporu ile hemanjiyosarkom olarak doğrulandığı bilgisi alındı. Yapılan fiziksel, nörolojik ve radyografik muayeneler sonucunda hastada sol arka ekstremite primer kökenli, intrakraniyal tümör metastazından şüphelenildi. Hastadan alınan beyin omurilik sıvısının yapılan incelemelerinde anormal bulguya rastlanılmadı. Tedavinin planlanması için gereken ileri görüntüleme teknikleri (bilgisayarlı tomografi ve manyetik rezonans görüntüleme) ve biyopsi işlemleri hasta sahibi tarafından reddedildi ve 4 gün sonra hasta ex oldu. Hastanın makroskobik nekropsi bulgularında başta beyin olmak üzere, dalak, karaciğer, diyafram ve akciğerde değişken boyutlarda kitlesel odaklar olduğu görüldü. Yapılan histopatolojik incelemede beyinde 0.2.x0.1 cm boyularında hemanjiosarkom metastazı görüldü. Elde edilen post-mortem bulgular klinik bulgular ile eşleşmektedir. Cocker spaniel ırkı bir köpekte hemanjiyosarkomun beyin metastazının görülmesi, olgumuzun ender olduğunu göstermektedir.

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1. Introduction

Hemangiosarcoma “hem/hemo- angio- sarcoma” is a malignant tumor that forms on the lining of blood vessels. Derived from an endothelial cell line, this highly malignant tumor is characterized by severe metastases in a short period of time (1). Commonly described in dogs, but less common in other species (2). Although hemangiosarcoma is usually reported to occur in older animals, it has also been reported to occur less frequently in younger animals (3). Although hemangiosarcoma in dogs is commonly reported in breeds such as Golden Retriever, Pointer, Boxer, Labrador Retreiver, Poodle, Siberian Husky, English Setter (1), all large breed dogs can be considered at risk. Limited success in prolonging life with surgical interventions and chemotherapeutic agents (4, 5). Hemangiosarcoma has the inherent risk of arising in any organ with blood vessels, but the most common sites reported to date are the spleen (50-65%), heart and right atrium (3-25%), subcutaneous tissues (cutaneous hemangiosarcoma) (13-17%) and liver (5-6%) (6). In a study of 85 dogs diagnosed with hemangiosarcoma, 14% of the patients were reported to have intracranial metastases and these patients usually had metastatic findings in other organs (7). Secondary brain metastases arising from primary hemangiosarcoma tumors and reaching the brain via hematogenous route are known to be rare in dogs. (8). Hemangiosarcoma in dogs is less common in cocker spaniels (9). It was aimed to contribute to the literature by presenting the clinical and pathomorphological findings of a rare case of hemangiosarcoma with brain metastasis in a cocker spaniel dog.

2. Case Story

A 2.5 years old (2 years and 7 months) English Cocker breed male dog with neurological symptoms such as circling around the left axis, inability to walk, tipping to one side, behavioral changes (increased aggression, increased vocalization) brought to Ondokuz Mayıs University Veterinary Teaching Hospital on September 2024. The dog's left posterior limb had previously been amputated. Physical examination revealed bilateral hyperplasic submandibular lymph nodes and dyspnea. Increased vocalization and moaning findings were associated with pain. On neurologic examination, threat response reflexes were negative and pupillary and palpebral reflex findings were normal. The patient had unilateral vestibular ataxia. Optic nerve (CN: II) and vestibulocochlear nerve (CN: VIII) findings were abnormal, while other cranial nerve findings were normal. Hematologic findings included mild neutrophilia and monocytosis. Urea (BUN), creatine (CREA) and alanine aminotransferase (ALT) were decreased. Mild hyperglycemia was associated with stress. Electrolyte panel (Na, K, Cl) was normal. The patient was sedated with propofol for cerebrospinal fluid (CSF) analysis (Propofol 200 mg/20 mL, Fresenius Kabi Pharmaceuticals, Istanbul, Türkiye) and intubated. CSF sample was collected with a 22-gauge black syringe tip (Tıbset®, Istanbul, Türkiye). Microscopic examination of the CSF sample revealed no pathology. Radiographic imaging revealed severe metastatic masses in the lung (Figure 1.B).

He was previously admitted on March 2024 with the complaint of limping, physical examination revealed swelling around the left femur that felt hard with palpation, and radiological examination revealed a periosteal reaction and increased surrounding soft tissue opacity in the distal left femur (Figure 1, C). A fine-needle biopsy (22-gauge black syringe tip (Tıbset®, Istanbul, Türkiye) was taken from the lesion site with suspicion of osteosarcoma. The examination showed that round or oval nucleated, shuttle-shaped neoplastic cells with limited eosinophilic cytoplasm formed irregular structures and the tumor was determined to be a sarcoma with malignant character; the origin and exact diagnosis of the tumor could not be made in the biopsy sample. For definitive diagnosis, the leg was amputated and evaluated as an excisional biopsy sample.

Shuttle-shaped neoplastic endothelial cells with oval or round nuclei, prominent multinuclei and eosinophilic cytoplasm were observed to form irregular large and small vascular structures. However, some newly formed vessels

were filled with erythrocytes and some of them were empty. After this examination, it was determined that the tumor was a hemangiosarcoma.

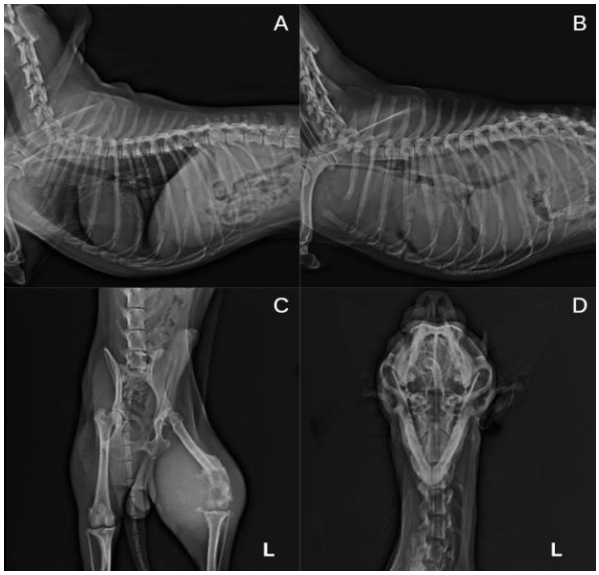

Figure 1: (A) Thoracic radiograph taken on the day the patient presented with limping. Images A and C are from the same time **(B)** Radiograph of the thorax at the last examination shows metastatic neoplastic nodules spreading to all lobes **(C)** radiograph of the left femur, before amputation **(D)** Radiography of the bulla tympanica for the differential diagnosis of vestibular syndrome caused by otitis media

Şekil 1: ***(A)*** *Hastanın topallama şikayetiyle başvurduğu gün çekilen toraks radyografisi. A ve C görüntüleri aynı zamana aittir* ***(B)*** *Hastanın son muayenedeki toraks radyografisi, tüm loblara yayılan metastatik neoplastik nodüller görülmektedir* ***(C)*** *Amputasyon öncesi sol femur radyografisi* ***(D)*** *Otitis media kaynaklı vestibüler sendromun ayırıcı tanısı için bulla timpanika radyografisi*

At our examination in September, we suspected that the main source of the patient's clinical symptoms was the primary leg-focused (Figure 1.C) intracranial metastasis diagnosed in March. The findings indicated that computed tomography (CT) and cranial-focused magnetic resonance imaging (MRI) were necessary to understand the spread and severity of the metastasis to other organs. The imaging and biopsy procedures required to determine whether the newly formed mass foci were hemangiosarcoma metastases or a different mass formation were refused by the owner and the patient died 4 days after admission to our hospital.

Post-mortem findings

The abdominal cavity was opened and nodular, dark red masses of varying sizes and diameters were observed on the spleen (Figure 2.A) and liver (Figure 2.B). Adhesions between the liver and diaphragm were noted. When the thoracal cavity was opened, a large, white and firm masses of varying sizes were seen in the lung (Figure 2.D). There was also a dark structure on the apex of the heart. Skull was opened and 2 bilateral masses were detected dorsally in the hemispheres (Figure 2.C). Other brain regions appeared normal.

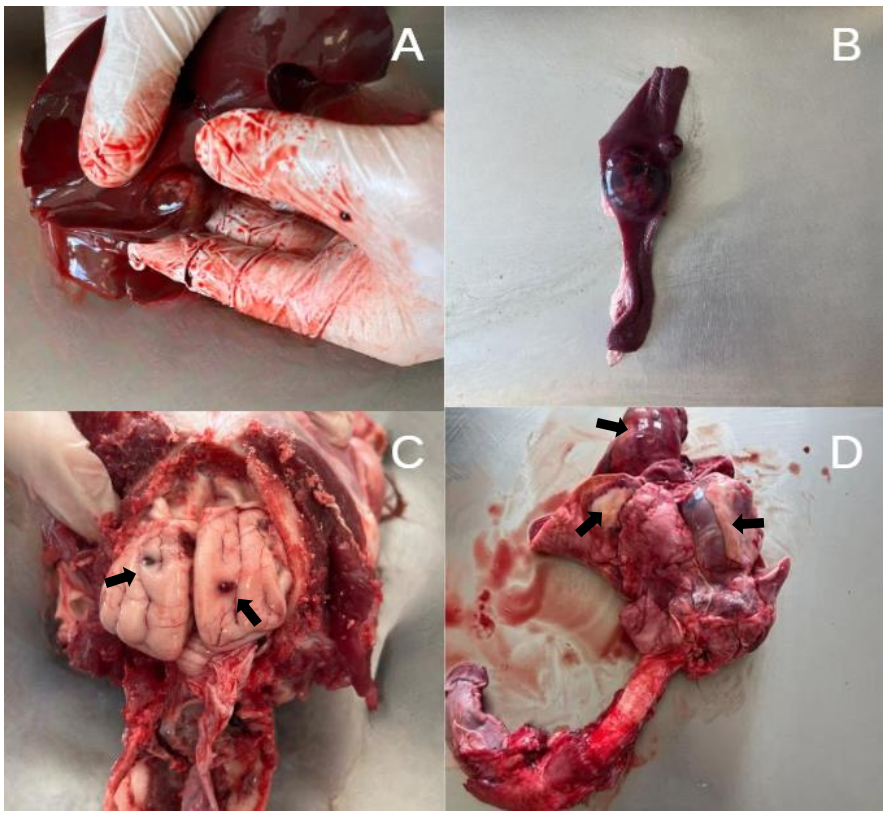

Figure 2: (A) Approximately 1x1 cm massive node localized in the right anterior lobe of the liver **(B)** Two masses on the parietal surface of the spleen, approximately 2x1.5 and 0.5x0.5 cm in the mid-anterior region **(C)** Superficial, approximately 0.2x0.1cm masses in the dorsal part of the brain, on both hemispheres, superficial to the sulcus marginalis (arrows) **(D)** Variable size of masses in the lung (arrows)

***Şekil 2**: **(A)** Karaciğerin sağ ön lobunda lokalize yaklaşık 1x1 cm'lik kitlesel nodül **(B)** Dalağın parietal yüzeyinde, orta-ön bölgede yaklaşık 2x1,5 ve 0,5x0,5 cm'lik iki kitle **(C)** Beynin dorsal kısmında yüzeysel, yaklaşık 0,2x01. Beynin dorsal kısmında, her iki hemisferde, sulkus marginalis yüzeyinde 1 cm'lik kitleler (oklar) **(D)** Akciğerde değişken büyüklükte kitleler (oklar)*

After necropsy, tissue samples were fixed in 10% formaldehyde, implanted in paraffin, sectioned at 5 µm thickness and stained with Hematoxylin-Eosin (HxE). The histopathologic appearance of the masses seen microscopically in the liver (Figure 3.A), lung (Figure 3.B), diaphragm (Figure 3.C) and brain (Figure 3.D) was determined as hemangiosarcoma as in the previous biopsy sample. Accordingly, the tumor in the leg region was the primary focus and the structures in the organs examined were determined to be metastatic foci of this tumor.

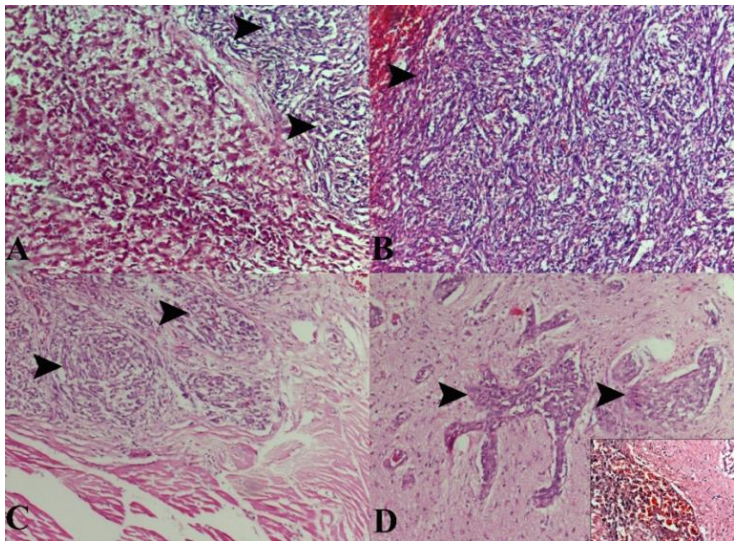

Figure 3: (A) Focus of metastatic tumor cells in the liver (arrowheads). Intact hepatocytes are seen in the right border, HxE **(B)** Tumor cells diffusely infiltrated into lung tissue (arrowhead), HxE **(C)** Tumor cell focus (arrowheads) located in the periphery of the diaphragm muscles, HxE. **(D)** Focal focuses of metastases in the brain (arrowheads) HxE. X10 objective magnification. Inset: Neoplastic vascular structures filled with erythrocytes. Brain, HxE. X20 objective magnification.

Şekil 3: (A)*Karaciğerde bulunan metastatik tümör hücresi odağı (okbaşları). Hemen komşu sınırında bulunan sağlam kalmış hepatositler görülmektedir, HxE* ***(B)*** *Akciğer dokusuna diffuz şekilde infiltre olmuş tümör hücreleri (okbaşı), HxE* ***(C)*** *Diyafram kaslarının periferinde bulunan tümör hücresi odağı (okbaşları), HxE* ***(D)*** *Beyinde fokal metastaz odakları (okbaşları) HxE. X10 objektif büyütme. İnset: İçleri eritrositle dolu neoplastik damar yapıları. Beyin, HxE. X20 objektif büyütme*

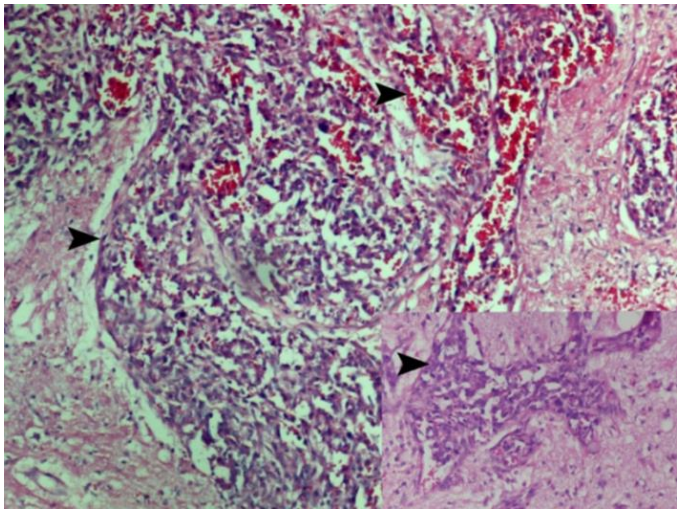

Figure 4: Brain metastasis of haemangiosarcoma. Neoplastic vessels filled with erythrocytes or empty in the lumen (arrows). x10 magnification. Inset: Neoplastic cells form capillary-like vessels (arrow). x20 magnification.

Şekil 4: *Hemanjiosarkomun beyin metastazı. Eritrositlerle dolu veya lümeni boş neoplastik damarlar (oklar). x10 büyütme. İnset: Neoplastik hücreler kapiller benzeri damarlar oluşturur (ok). x20 büyütme.*

3. Discussion and Conclusion

Hemangiosarcoma has an aggressive metastatic character. Survival is not long and prognosis is poor despite medical and operative treatments. Although hemangiosarcoma is reported to be more common in German Shepherd dogs, Boxers and Golden Retrievers and less common in English Cocker Spaniels (9), our case was reported in a Cocker Spaniel. In a study published in 1985, the mean age of 104 dogs diagnosed with hemangiosarcoma was 10 years (1). In the study published in 2013, the mean age of 51 dogs diagnosed with hemangiosarcoma was 10.7 years, the youngest patient was 5 years old (10). In the most recent report, it was stated that although the cases are mostly seen in the age range of 7-15 years, it can also be seen less frequently in young animals 2 years and older (11). In fact, a case of primary cerebrum-focused hemangiosarcoma in a 6-week-old dog has been reported and it is clear that the tumor can be seen at any age (12). In the studies conducted since 1985, the median age of patients diagnosed with hemangiosarcoma has not changed. In addition, the fact that our patient was a 2.5-year-old is rare case according to the current literature. In hemangiosarcoma in the leg, which was the primary tumor focus, metastasized to other abdominal organs, lung and the brain within 6 months, confirming the severe metastatic character of the tumor and the poor prognosis. It can be considered as stage- III in the clinical classification of hemangiosarcoma, especially because the mass foci in the lung are 5 cm in diameter, have long distance metastasis from the primary focus, and have a short life expectancy (13). In dogs with splenic hemangiosarcoma, the mean survival time of patients who underwent splenectomy was reported to be between 19 and 86 days. Considering that 31% of the patients had a survival time of 2 months and 7% had a survival time of 1 year in the postoperative period, it is clear that the current treatment options are inadequate (2).

The fact that hemangiosarcoma originates from endothelial cells on the inner surface of blood vessels and lymph vessels and can easily metastasize through the circulation leads to the risk of intracranial metastasis. In 2022, 14.2% of reported hemangiosarcoma cases had intracranial metastases (14), and 14% of reported hemangiosarcoma cases had intracranial metastases in a retrospective study conducted in 1985 (1), it can be assumed that brain metastasis rates have not changed from past to present and brain metastases can be seen in 14%-14.2% of hemangiosarcoma patients. Our patient had left-axis circling movement and vestibular ataxia, absence of threat response reflex, CN: II and CN: VIII cranial nerves were abnormal. The intracranial lesion sites were located in the pars orbitalis of the procencephalon, at the level of the parietal lobes of both hemispheres, and medial to the sulcus marginalis, explaining the neurological symptoms seen. increased vocalization was associated with mental impairment and pain due to vestibular dysfunction. Similar neurological symptoms to our case were noted in a case with lesions in the left ventricle and cerebrum and cerebral hemorrhage due to hemangiosarcoma (15). The presence of neurological symptoms in 6 of 17 patients with intracranial hemangiosarcoma (7), indicates that neurological symptoms may vary according to the localization and size of metastatic lesions in the brain.

In recent years, cases of perivulvar (Rottweiler/11 years/ Female) (16), perianal (Doberman Pinscher/5 years/ Male) and mesenterial (mongrel/6 years/ Female) (17), perianal (Doberman Pinscher/5 years/ Male) and mesenterial (mongrel/6 years/ Female) (18) hemangiosarcoma have been reported in Türkiye. Our case is rare because it was observed in a cocker spaniel, a breed of dog in which hemangiosarcoma is less common, and it was accompanied by brain metastasis.

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Conflict of Interests

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Olgu Sunumu / Case Report

First record of *Kathlania leptura* (Rudolphi, 1819) and *Tonaudia tonaudia* (Lane, 1914) (Nematoda) in a loggerhead sea turtle (*Caretta caretta*) from the Eastern Mediterranean, Türkiye

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ABSTRACT:

The Aegean and Mediterranean coasts of Türkiye are home to the most important feeding, breeding and nesting areas in the Mediterranean for the migratory loggerhead sea turtles (*Caretta caretta*). These sea turtles, which are carnivorous and have a complex life cycle, attract attention with their population density in the Mediterranean and are being investigated parasitologically. The material of this case report submitted consists of nematode specimens obtained from the gastrointestinal tract of a stranded adult female loggerhead sea turtle during necropsy. Two species of kathlanid nematodes (*Kathlania leptura* and *Tonaudia tonaudia*) are reported from the small intestine of a loggerhead sea turtle *Caretta caretta*, found stranded on Demre Beach (Antalya), Türkiye, in August 2024. Both species represent new locality records for Türkiye and in general for the Eastern Mediterranean. In addition, necropsy revealed that the potential cause of death was malnutrition due to two fish hooks penetrating the esophagus and intestinal plication caused by fishing line traction. In conclusion, this study is the first detailed parasitological report on sea turtles in Türkiye and will set an example for future research.

Türkiye Doğu Akdeniz'den iri başlı deniz kaplumbağası (Caretta caretta)'ndan Kathlania leptura (Rudolphi, 1819) ve Tonaudia tonaudia (Lane, 1914) (Nematoda)'nın ilk kaydı

ÖZET:

Türkiye'nin Ege ve Akdeniz kıyıları, göçmen iri başlı deniz kaplumbağaları (*Caretta caretta*) için Akdeniz'deki en önemli beslenme, üreme ve yuvalama alanlarına ev sahipliği yapmaktadır. Karnivor ve karmaşık bir yaşam döngüsüne sahip olan bu deniz kaplumbağaları, Akdeniz'deki popülasyon yoğunluklarıyla dikkat çekmekte olup parazitolojik olarak araştırılmaktadır. Sunulan bu olgu sunumunun materyali, karaya vurmuş yetişkin bir dişi iri başlı deniz kaplumbağasının gastrointestinal sisteminden nekropsi sırasında elde edilen nematod örneklerinden oluşmaktadır. Ağustos 2024'te Türkiye'nin Demre Plajı'nda (Antalya) karaya vuran iri başlı deniz kaplumbağasının (*Caretta caretta*) ince bağırsağından iki tür kathlanid nematod (*Kathlania leptura* ve *Tonaudia tonaudia*) rapor edilmiştir. Her iki tür de Türkiye ve genel olarak Doğu Akdeniz için yeni kayıt niteliği taşımaktadır. Ayrıca nekropsi sonucunda potansiyel ölüm nedeninin yemek borusuna iki adet balık kancasının batması sonucu oluşan yetersiz beslenme ve olta misinanın çekilmesi sonucu oluşan bağırsak düğümlenmesi olduğu belirlendi. Sonuç olarak bu çalışma Türkiye'de deniz kaplumbağaları hakkında yapılmış ilk detaylı parazitolojik çalışma olup, gelecekte yapılacak araştırmalara örnek teşkil edecektir.

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1. Introduction

The loggerhead sea turtle *Caretta caretta* (Linnaeus, 1758) is the most abundant sea turtle species in the Mediterranean Sea (1). It is a carnivorous and highly migratory species with a complex life cycle. In early development, they feed on plankton and small arthropods, while at the adult stage, they feed prevalently on crabs, molluscs, and fish (2). Because of their long-life span, ability to live in large and diverse areas such as pelagic, neritic and benthic areas, and wide range of dietary preferences, they are exposed to many helminth parasites (3).

In the eastern Mediterranean, the Aegean waters of Türkiye host important foraging, breeding and nesting areas for loggerhead sea turtles (4,5). Despite this, there is only one report of loggerhead sea turtle parasites from Türkiye, where the parasites were identified as Trematoda, Nematoda and Annelida (*Ozobranchus* sp.) (6). Herein, we report a case of coinfection with *Kathlania leptura* and *Tonaudia tonaudia* in a loggerhead sea turtle found stranded on Demre Beach (Antalya), Türkiye.

2. Case Story

In August 21, 2024, a fresh carcass of an adult female loggerhead sea turtle ranging from approximately 85 cm curved carapace length (CCL) was found stranded on the Demre Beach (Antalya). The carcass was brought to the Mediterranean Fisheries Research, Production and Education Institute Training Facility Beach where a complete necropsy was performed to determine the cause of death using methods described in the relevant literature (7). The gastrointestinal tract was also examined for helminth parasites. When macroscopically observed, parasites were collected, counted, washed in tap water and preserved in ethanol 70% for morphological identification. For the light microscopy study, a representative number of nematodes were cleared with Amman's lactophenol, mounted using Hoyer's solution and dried at room temperature for 1–2 weeks. Then, they were studied using a compound microscope equipped with a digital camera (CX23 Binocular Microscope, Olympus Corporation, Tokyo, Japan). Nematodes were identified using identification keys provided in (8) and (9).

At necropsy, there were no signs of trauma on the external body surfaces of the carcass. During the examination of gastrointestinal viscera, two fish hooks penetrating the oesophagus and intestinal plication caused by fishing line traction were found. The small intestine was filled with a large amount of nematode parasites (n: 76). Nematodes were well agreed with the diagnostic morphological features of the family Kathlaniidae Lane, 1914 as described in the (9). Two species of Kathlaniidae were identified including 48 specimens of *Kathlania leptura* (Rudolphi, 1819) (Figure: A, B, and E) and 28 specimens of *Tonaudia tonaudia* (Lane, 1914) (Figure: C, D, and F) were identified. The main morphological characters used to discriminate among species were the lips of the cephalic extremity, and characters of spicule and gubernaculum. In particular, males with spicules of about 500 µm were identified as *K. leptura* and males with longer spicules (sometimes ending just posterior to the esophageal bulb) were identified as *T. tonaudia*. (see reference 10).

3. Discussion and Conclusion

In terms of number of species, the majority of helminths infecting loggerhead sea turtles comprise trematodes and nematodes (3,11,12). Currently, there are only five nematode species infecting the Mediterranean loggerhead sea turtles. These are *Cucullanus carettae* (Cucullanidae), *Sulcascaris sulcata*, *Anisakis pegreffii* (Anisakidae), *Kathlania leptura*, and *Tonaudia tonaudia* (Kathlaniidae). Except *A. pegreffii* that represents an accidental finding (see reference 13), all other nematode species use the loggerhead sea turtle as definitive host.

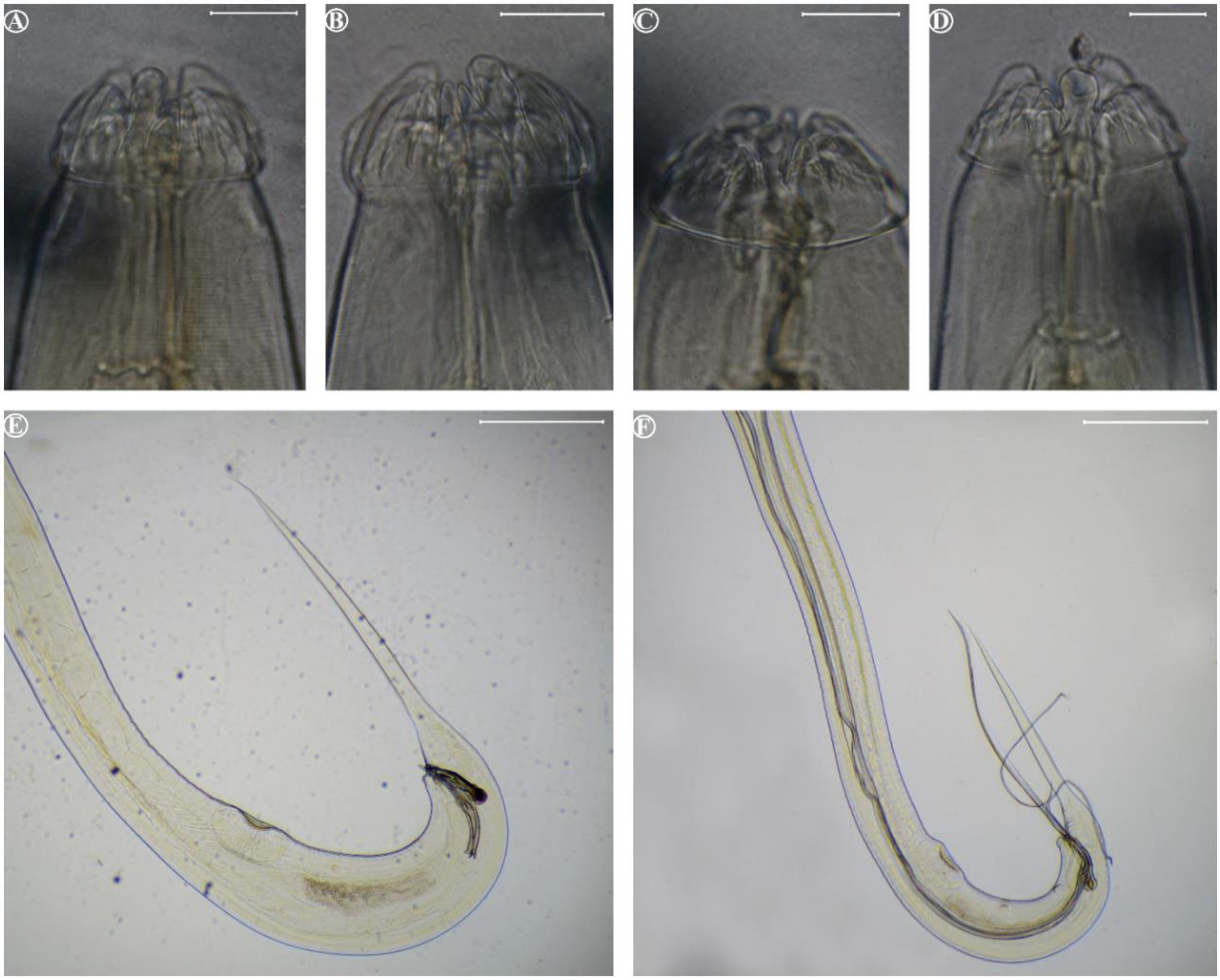

Figure 1: Anterior extremities of *Kathlania leptura* male **(A)** and female **(B),** *Tonaudia tonaudia* male **(C)** and female **(D),** in dorsolateral view; tails and spicules of *Kathlania leptura* **(E)** and *Tonaudia tonaudia* **(F)** in lateral view (scala bars: 250 µm).

Şekil 1: *Dorsolateralden Kathlania leptura* erkek ***(A)*** *ve dişi* ***(B)*** *anterior uç*, *Tonaudia tonaudia* erkek ***(C)*** *ve dişi* ***(D)*** *anterior uç*; *lateralden Kathlania leptura* ***(E)*** *ve Tonaudia tonaudia* ***(F)*** *kuyruk ve spikülümler* (ölçek çizgileri: 250 µm).

Both *Kathlania* and *Tonaudia* are monotypic genera which infect only sea turtles. *Kathlania leptura* is known from the loggerhead sea turtle, the green sea turtle (*Chelonia mydas*) and the olive ridley sea turtle (*Lepidochelys olivacea*), while the latter only from the loggerhead sea turtle. Regarding their geographical distribution, *Kathlania leptura* has been reported from the Nearctic Region (including Brasil, Costa Rica, and the United States of America) (10,14,15), the Western Mediterranean (including Spain, Italy, Egypt, and Tunisia) (11,12,16,17), the Oriental Region (Sri Lanka) (18), the Afrotropical Region (including Mauritania and Zanzibar) (19), and the Australian Region (20). In contrast, *T. tonaudia* has been reported from the Atlantic of the United States of America (10), the Western Mediterranean (Egypt and Tunisia) (8,16,17) and the Indian Ocean (Sri Lanka) (18).

Then, according to previous cited studies, the finding of both species represents new locality records for Türkiye and in general for the Eastern Mediterranean.

Conflict of Interests

The authors declared that there is no conflict of interest.

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Veteriner doğum ve jinekoloji araştırmalarında kullanılan moleküler analizler için doku örnekleme, saklama ve taşıma yöntemleri

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Doku saklama

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ÖZET:

Veteriner doğum ve jinekoloji alanında yapılan moleküler çalışmalar hem temel hem de klinik bilimler açısından büyük bir öneme sahiptir. Bu alanda gerçekleştirilen araştırmalar, fizyolojik süreçlerin moleküler mekanizmalarını aydınlatmak bakımından önemli bir rol oynamaktadır. Yapılan bu çalışmaların başarısı, doku örneklerinin doğru bir şekilde ve araştırma hipotezine uygun olan fonksiyonel organ bölümünden alınması, muhafazası ve laboratuvara ulaştırılmasına bağlıdır. Alınan doku örneklerinin zarar görmeden muhafaza edilmesi ileride yapılacak olan moleküler analizlerin güvenilirliğini artırmakta ve elde edilen sonuçların doğruluğunu sağlamada belirleyici bir etken olmaktadır. Bu derlemede, veteriner doğum ve jinekoloji alanındaki moleküler çalışmalar için doku örneği alma yöntemleri, dokuların muhafaza yöntemleri, bu örneklerin laboratuvara ulaştırılması sürecine ilişkin bilgiler verilmesi amaçlanmıştır. Ayrıca, bu süreçte örnek toplama ve taşıma aşamalarında karşılaşılan zorluklar, örneklerin taşınması sırasında dikkat edilmesi gereken hususlar ve laboratuvar sonuçlarının doğruluğunu sağlamak için alınması gereken önlemler de ele alınmıştır. Sonuç olarak bu makalede, araştırmaların kalitesi ve elde edilecek verilerin doğruluğu için örnek toplama, muhafaza ve taşınma protokollerinin titizlikle uygulanması gerekliliği üzerinde durulmuştur.

Tissue sampling, storage and transport methods for molecular analyses used in veterinary obstetrics and gynecology research

ABSTRACT:

Molecular studies conducted in the field of veterinary obstetrics and gynecology hold significant importance for both basic and clinical sciences. Research in this area plays a crucial role in elucidating the molecular mechanisms underlying physiological processes. The success of these studies depends on the accurate collection of tissue samples from the functionally relevant sections of organs that align with the research hypothesis, as well as on their proper preservation and timely transportation to the laboratory. Preserving tissue samples without any damage not only enhances the reliability of subsequent molecular analyses but also serves as a key factor in ensuring the accuracy of the results obtained. This review aims to provide a comprehensive overview of the methods employed for tissue sample collection, the techniques used for tissue preservation, and the processes involved in transporting these samples to the laboratory for molecular studies in veterinary obstetrics and gynecology. In addition, the review discusses the challenges encountered during the collection and transportation stages, highlights the critical considerations that must be addressed during sample transfer, and outlines the necessary precautions to ensure the precision of laboratory results. In conclusion, this paper emphasizes the imperative need for the rigorous implementation of protocols regarding sample collection, preservation, and transportation to safeguard the quality of the research and the accuracy of the data produced.

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1. Giriş

Veteriner hekimliğinde klinik ve temel araştırmalarda son yıllarda yoğun bir şekilde moleküler analiz yöntemlerinden yararlanılmaktadır. Moleküler analizlerde yaşanan gelişmeler kendisini veteriner doğum ve jinekoloji ve reprodüksiyon alanında da göstermiştir. Bu bağlamda Web of Science'de "animal reproduction, gene expression, molecular analyses, transcriptional changes" anahtar kelimeleri ile 2020-2024 yıllarını kapsayacak şekilde yapılan literatür taramasında toplam 4365 makaleye rastlanmış olması bu durumu doğrular niteliktedir.

Birçok alanda olduğu gibi veteriner jinekoloji alanında yapılan araştırmalarda kullanılan moleküler yöntemler farklı bilim alanlarında uzmanlık gerektiren ve önemli bir laboratuvar alt yapısına ihtiyaç duyan spesifik çalışma alanlarıdır ve sürecin laboratuvar ve değerlendirme kısmını kapsamaktadır (1). Ancak süreçte en az laboratuvar kısmı kadar önemli olan başka bir aşama daha bulunmaktadır. Örnek toplama olarak isimlendirilebilecek olan bu aşama; çalışmanın klinik veya pratik kısmı içerisinde yer aldığı için doğrudan veteriner doğum ve jinekoloji alanını ilgilendirmektedir. Söz konusu örnekleme yönteminde bir hata olması, laboratuvar sonuçlarının hatalı elde edilmesine ve araştırmanın yanlış sonuçlanmasına yol açacaktır. Bu nedenle moleküler analiz yöntemlerinin kullanılacağı çalışmalarda doku örneği alma aşamasının dikkatli bir şekilde planlanması gerekmektedir (2). Kullanılacak olan moleküler analiz yöntemleri ile deoksiribonükleik asit (DNA), ribonükleik asit (RNA) ve protein varlığının veya miktarının belirlenebilmesi için öncelikle uygun dokudan örnek alınması gerekmektedir. Bu bağlamda yapılacak olan araştırmanın sorusuna uygun olan organın fonksiyonel bölümünden, uygun miktarda, uygun koşullarda örnekleme yapılması ve alınan doku örneklerinin incelenmesi planlanan moleküllerin bozulmadan kalmasını sağlayacak şekilde muhafaza edilmesi yapılacak analizlerin doğruluğu için büyük önem taşımaktadır (3).

Sunulan derlemede, veteriner doğum ve jinekoloji alanında yapılan araştırmalarda kullanılabilecek olan farklı moleküler yöntemlerde kullanmak üzere alınacak doku örneklerinin; nereden ve hangi yöntemlerle alınacağı, analizler yapılana kadar bozulmadan nasıl muhafaza edilecekleri ve laboratuvara nasıl ulaştırılacakları hakkında bilgi verilmesi amaçlanmıştır.

2. Veteriner Doğum ve Jinekoloji Alanında Kullanılan Moleküler Analizler İçin Örneklerin Toplanması

Veteriner doğum ve jinekoloji alanında yapılması planlanan çalışmalar için alınabilecek örnekler; uterus ve ovaryum dokusu, folikül sıvısı, vaginal, servikal veya endometriyal akıntılar, vaginal veya endometriyal sürüntüler, endometriyal biyopsi, yavru zarları, meme tümörü dokusu, kan ve idrar örnekleridir. Bu örnekler, canlı hayvanlardan noninvaziv, minimal invaziv veya invaziv yöntemlerle; kadavradan ise sakrifikasyon (laboratuvar hayvanları) veya mezbaha materyali (çiftlik hayvanları) yoluyla elde edilmektedir. Küçük biyopsiler genellikle minimal invaziv tekniklerle alınırken, daha büyük doku örnekleri rutin cerrahi operasyonlarla veya mezbahada kesim sırasında alınabilmektedir (4). Doku örneklerinin, çalışmanın amacı doğrultusunda örnekleme yapılacak olan organın uygun bir bölgesinden ve yeterli miktarda alınması gerekmektedir (5).

Noninvaziv örnek alma yöntemleri: Endometriyal, servikal veya vaginal akıntı veya sürüntü örneği alma. Doğum sırasında yavru zarlarının alınması (6).

Minimal invaziv örnek alma yöntemleri: Kan örneği alma, endometriyal (7), vaginal, servikal biyopsi alma; (21); folikül sıvısı aspirasyonu (8,9), meme tümörlerinden ince ve kalın iğne aspirasyonu (10,11)

İnvaziv örnek alma yöntemleri: Ovaryum, uterus veya meme tümörü dokusunun intraoperatif alınması.

Sakrifikasyon ile örnek alma yöntemleri: Derin anestezi altında sakrifiye edilen dişi laboratuvar hayvanlarının ovaryum, uterus veya vaginalarından örnek alma (12).

Mezbaha materyalinden örnek alma yöntemleri: Mezbahalarda kesilen dişi hayvanların; ovaryum, uterus veya vaginalarından örnek alma (4).

Noninvaziv örnek alma yöntemleri

Moleküler analizlerde kullanılan noninvaziv örnekleme yöntemleri; cerrahi müdahale gerektirmeden, hayvan sağlığında risk oluşturmadan ve stres yaratmadan örnek alınmasını sağlamaktadır. Jinekolojik uygulamalarda endometriyal, servikal veya vaginal akıntı ve sürüntü örneklerinin yanı sıra doğum sırasında yavru zarlarının toplanması bu yöntemlere örnek olarak verilebilir (6). Veteriner jinekoloji alanında bu akıntı ve sürüntülerin makroskopik muayenesi oldukça önemlidir ve moleküler analizlerde kullanılabilmektedir. Örnekler, hayvan türüne ve akıntı miktarına göre mikropipetlere veya uygun tüplere alınabilmektedir. Örnekleme sırasında antisepsi koşullarının sağlanması, araştırmacının dikkat etmesi gereken önemli bir husustur.

Özellikle büyük ruminant veya tek tırnaklı hayvanlarda örnekleme öncesi hayvanın güvenli şekilde zapturapt altına alınması, kuyruğun bağlanması, perineal bölgenin temizlenip antisepsisinin sağlanması gerekmektedir. Endometriyal örnek alınırken; fırça, eküvyon çubuğu veya kateter gibi ekipmanlar steril kılıf veya spekulum kullanılarak vulva, vagina ve servikse temas ettirilmeden özenle uygulanmalıdır. Serviks veya vaginal örnek alınırken de benzer sterilite önlemleri alınmalıdır (13).

Alınacak olan örnek akıntı formunda ise, steril numune kaplarına hızla alınmalı, üzerine örneğin bilgilerini içeren bir etiket eklenmeli ve yapılması planlanan analizin gerektirdiği koşullara göre saklanmalıdır. İneklerden servikal sıvı toplanırken ise rektum yoluyla hafif baskı uygulanarak serviksten vaginal açıklığa sıvı akışı sağlanabilmektedir. Bu işlem sırasında piyometra veya mukometra gibi hastalık durumları tespit edilirse bu örnekler analiz için kullanılmamalıdır. Sadece berrak servikal mukusa sahip örnekler toplanmalıdır (14).

Uterus sıvılarının alınmasında Merocel swab yöntemi kullanılabilmektedir. Bu süngerimsi materyal uterus içindeki sıvıyı emmekte ve alınan örnek buz üzerinde soğutulmuş ekstraksiyon tamponu içeren tüplere aktarılmaktadır. Örneklerin en fazla üç saat içinde Spin X Santrifüj tüplerine konulması gereklidir. Bu işlemler sonucunda elde edilen süpernatant -80 °C'de saklanabilmektedir (15,16). Merocel swab alma yöntemi, uterus yıkama yöntemine göre daha çok hayvandan numune alınmasına olanak sağlamakta ve uterus yıkamasıyla sıvı alınamayan durumlar (17) için alternatif sunmaktadır. Merocel swab yöntemi ile alınan uterus sıvısı örnekleri, yapılacak olan proteomik analizler için rahatlıkla kullanılabilmektedir (18).

Sürüntü örneği alma yöntemi uygun maliyetli ve noninvaziv bir yaklaşımdır. Alınan örnekler, koruyucu solüsyon içeren steril tüplere hızla konmakta ve yapılması planlanan analizin gerektirdiği koşullarda saklanmaktadır (19). Vaginal sürüntü örneği ise vaginal spekulum kullanılarak, vaginanın kraniyal kısmından, izotonik sodyum klorür ile ıslatılıp doygunluğu sağlanmış pamuklu eküvyon çubukları ile dairesel hareketlerle alınabilmektedir (20). Servikal sürüntü alınırken eküvyon çubuklarının vagina duvarına değmeden geri çıkartılmasına dikkat edilmelidir (6).

Doğum sırasında yavru zarlarının toplanması için ise, kontaminasyona karşı steril bir çalışma alanı hazırlanmalıdır. Alınacak olan örneklerin dışkı veya diğer vücut sıvılarıyla temasından kaçınılmalıdır. Doğumdan hemen sonra koryon, amniyon ve allantois zarları steril pens veya forsepsler ile alınabilmektedir. Plasentanın fonksiyonel bölgelerinden alınan örnekler, hayvan türüne göre villi koryalis dağılımı açısından farklılık göstermektedir. Kedi ve köpeklerde zoner bant denilen alanlar, ruminantlarda kotiledonlar, domuz ve kısraklarda ise plasentanın tüm yüzeyine yayılan mikrovilluslar fonksiyonel bağlantı bölgelerini oluşturmaktadır (21).

Minimal invaziv örnek alma yöntemleri

Kan örneği alma (22); endometriyal, vaginal, servikal biyopsi alma (23); folikül sıvısı aspirasyonu (8) gibi yöntemler minimal invaziv örnek alma yöntemleri olarak sınıflandırılmaktadır.

Kan örneği alma: Kan örneklerinin analizinden elde edilen bilgiler moleküler çalışmalarda kullanılabilmektedir. Kan tüplerinde antikoagülan madde ile kanın yeterince karışmaması, yanlış veya gecikmiş ve uygun olmayan sıcaklıklarda yapılan santrifüj işlemleri, kan tüplerinin yetersiz doldurulması, toplanan örneklerin uygun olmayan koşullarda saklanması gibi hatalı işlemler, örneğin bütünlüğü ve kalitesi üzerinde olumsuz etkiler yaratabilmektedir. Kan örneklerinin toplanması ve işlenmesi sırasında dikkate alınması gereken çeşitli noktalar vardır (22). Örnek

alınırken; kan alınacak bölgedeki kılların tıraş edilip, derinin %70'lik alkolle silinmesi gerekmektedir. Örnek alacak kişinin kan alma sırasında eldiven kullanması sağlanmalıdır. Arteriyel kan, steril koşullarda doğrudan arterin punksiyonu ile yapılabilmektedir. Bunun için femoral, brakiyal, radial ve karotis arteri tercih edilebilmektedir. Arteriyel kan yüksek oksijen içeriği ve basıncı nedeniyle hızlı bir şekilde pıhtılaşabileceğinden örnek alma işlemi heparinize edilmiş enjektörler kullanılarak yapılmalıdır (24). Venöz kan örnekleri ise; steril kanül, intravenöz kateter veya vacutainer kullanılarak alınabilmektedir (25).

Kan örneği alırken hayvan türüne göre kan örneği alınacak olan damar farklılık göstermektedir (Tablo 1).

Tablo 1: Hayvan türüne göre kan örneği alınacak venöz damarlar (26,27).

Table 1: *Venous vessels for blood sampling according to animal species* (*26,27).*

Hayvan Türü	Örnek Alınacak Damar İsimleri
Büyük ruminant	v. jugularis, v. abdominalis subcutanea, v. coccygea
Küçük ruminant	v. jugularis, v. coccygea
Kedi, köpek	v. cephalica antebrachii, v. saphena, v. jugularis, v. femoralis
Sıçan, fare	v. coccygea
Tek tırnaklı	v. coccygea
Domuz	v. jugularis
Kanatlı	v. jugularis, v. cutanea ulnaris
Tavşan	v. auricularis

Yapılması planlanan çalışmada kullanılacak olan örneğe göre farklı kan alma tüpleri kullanılmalıdır (Tablo 2).

Tablo 2: Çalışmada kullanılacak olan örneğe göre kan alma tüplerinin özellikleri (22).

Table 2: *Characteristics of blood collection tubes according to the sample to be used in the stud (22).*

Alınacak Örneğe Göre Kullanılacak Tüp Özellikleri
Serum: Sarı kapaklı tüplerin içinde bulunan ayırıcı jel tabakası sayesinde santrifüj sonrasında serum pıhtıdan ayrılmaktadır. Kırmızı kapaklı tüplerde de antikoagülan madde bulunmadığından kan pıhtılaşmakta ve santrifüj sonrası serum eldesi sağlanabilmektedir. Serum numuneleri için istenilen miktarın yaklaşık iki katı kan alınmalıdır. Serum örnekleri özellikle ELISA ve Western Blot gibi moleküler tekniklerde protein analizi için kullanılabilmektedir.
Plazma: İçerisinde antikoagülan madde olarak Etilendiamintetraasetik asit (EDTA) bulunduran mor kapaklı tüpler ve içerisinde heparin bulunduran yeşil kapaklı tüpler kanın pıhtılaşmasını engellemektedir. Santrifüj sonrasında tüpün üst kısmında bulunan, hücresel bileşenlerinden ayrılmış olan plazma ile DNA ve RNA analizleri yapılabilmektedir. Polimeraz Zincir Reaksiyonu (PCR) gibi moleküler tekniklerde plazma kullanılabilmektedir. Fakat heparin PCR için inhibitör özelliktedir. Bu yüzden antikoagülan olarak kullanılmamalıdır. EDTA, DNAazları inhibe ettiğinden çoğunlukla tüm PCR çeşitlerinde EDTA'lı tam kan tercih edilmektedir.
Tam Kan: İçerisinde sodyum sitrat bulunduran mavi kapaklı tüpler ve EDTA bulunduran mor kapaklı tüpler ile kanın pıhtılaşması önlenerek elde edilmektedir. Kan içerisinde bulunan şekilli elamanlardan özellikle lökositlerin çekirdek içermesi sebebiyle tam kandan hücresel DNA elde edilebilmektedir. Ayrıca flowsitometri gibi moleküler tekniklerde de tam kan kullanılabilmektedir.

Endometriyal biyopsi: Endometriyal epitelin yanı sıra uterus lumenine en yakın olan stratum compactum gibi daha derin katmanların da endometriyal değişikliklerinin değerlendirilmesine olanak sağlamaktadır (28). Endometriyal biyopside ince bir kateter yardımıyla uterus lümeninden doku örneği alınabilmektedir. Bazı araştırmacılar tarafından uterus biyopsisinin ineklerde sonraki fertilite üzerinde zararlı bir etkisi olduğu bildirilirken (29), bazı araştırmacılar ise doğru şekilde uygulandığında güvenli bir teknik olduğunu bildirmektedirler (30).

Endometriyal biyopsi sıklıkla kısraklarda daha nadir olarak da ineklerde kullanılan bir yöntemdir. Kedi, köpek ve laboratuvar hayvanlarında endometriyal biyopsi örneği almak anatomik olarak mümkün değildir. İnek ve kısraklardan endometriyal örnek alma öncesinde, örnekleme yapılacak olan hayvanların travaya alınması, alt epidural anestezi uygulanması, kuyruğun bağlanması ve vulvanın asepsisinin sağlanması gerekmektedir (31).

Kısrak ve büyük ruminantlarda endometriyal biyopsi almak için kullanılan biyopsi pensleri; uzun saplı, ince uçlu ve uterusun derin kısımlarına ulaşabilecek şekilde tasarlanmış olmalıdır. Bu amaçla biyopsi alınacak olan hayvanın türü ve vücut ölçülerine göre; Kevorkian, Miltex, Tischler, Jackson biyopsi pensleri gibi farklı tip ve boylarda biyopsi forsepsleri kullanılabilmektedir (32,33,34). Örnekleme sırasına; steril biyopsi aletleri steril kılıf içine alınmakta ve rektovaginal yöntem ile vulva, vagina veya servikse değmeden uterusa ulaşması sağlanmaktadır (35). Kısraklarda dorsal ve ineklerde dorsal ve interkornual alandan endometriyal katları içine alacak şekilde örnekleme yapılmalıdır. Sonrasında örnek, yapılacak analiz yöntemine uygun saklama koşullarına alınmalıdır.

Servikal biyopsi: Alınacak olan örneğin boyutu küçük ise lokal anestezi tercih edilirken daha geniş çaplı ve derin dokulardan biyopsi örneği alınacaksa alt epidural anestezi tercih edilmektedir. Yapılacak olan işlem öncesinde perineal bölge öncelikle fiziksel olarak temizlendikten sonra, bölgenin asepsi ve antisepsisi çeşitli antiseptik solüsyonlar ile sağlanarak biyopsi alma işlemi gerçekleştirilmektedir (23).

Vaginal biyopsi: Lidokain ile lokal anestezi veya alt epidural anestezi uygulanarak yapılabilmektedir (36). Örnek toplanmadan önce, perineal bölge ılık suyla yıkanmalı ve tek kullanımlık kağıt havlularla kurulanmalıdır. Vulvanın asepsisi povidon iyot veya klorheksidin gibi antiseptik solüsyonlar ile sağlandıktan sonra timsah ağızlı biyopsi pensi ile dorsal vaginal alandan biyopsi örneği alınabilmektedir (31). Alınan örnek, yapılacak olan analiz yöntemine uygun saklama koşullarında saklanmalıdır.

Folikül sıvısı aspirasyonu: Sıklıkla tek tırnaklı ve büyük ruminantlarda kullanılan bir yöntemdir. Ovum pick up (OPU) aparatı olan bir ultrasonografi cihazı eşliğinde ve epidural anestezi uygulanarak örnekleme yapılabilmektedir. OPU aparatı bulunan ultrasonografi probu ile vaginadan görüntülenene foliküllerden aspirasyon yöntemi ile folikül sıvısı toplanabilmektedir. Aspire edilen folikül sıvısı, yapılacak olan analize uygun koşullarda saklanmalıdır (37).

İnce ve kalın iğne aspirasyonu: Köpek veya kedi meme tümörlerinden ince iğne aspirasyon yöntemi ile örnekler alınabilmektedir. Bu yöntemler daha çok genel anestezi altında pre-operatif dönemde tümörün karakterizasyonu için kullanılmaktadır (10,38). Aynı zamanda bu yöntemler ile alınan aspiratlarda moleküler analizler de yapılabilmektedir (11). İnce iğne aspirasyonunda; aspirat alınmadan önce örnek alınacak olan meme tümörünün yüzeyinde bulunan kıllar tıraş edilmeli, derinin antisepsisi sağlanmalıdır. Daha sonra tümörün boyutuna ve hayvan türüne göre değişen büyüklüklerde steril kanül takılı enjektörler yardımı ile içerisinde tümör ve meme hücrelerinin olduğu aspiratlar alınabilmektedir. Alınan aspirat hızlı bir şekilde yapılacak olan analize uygun koşullarda saklanmalıdır (10,39). Kalın iğne biyopsisi veya tru-cut biyopsisi olarak adlandırılan diğer yöntem ise daha çok kadınlarda meme tümörlerinin teşhisinde yaygın olarak kullanılan minimal invaziv bir yöntemdir (40,41). Köpek ve kedilerde de kullanılabilen bir yöntem olan kalın iğne biyopsisi, ince iğne biyopsisine göre daha fazla doku örneği alınmasına olanak vermektedir. Bu nedenle bu yöntemin örnekleme açısından daha uygun olduğu bildirilmektedir (38). Kalın iğne biyopsisi ile alınan biyopsi örneklerinin hızlı bir şekilde yapılması planlanan analize uygun saklama koşullarına alınması gerekmektedir.

İnvaziv örnek alma yöntemleri

Örnek alınacak hayvan türüne göre farklı yöntemler ile ilgili dokulardan örnekler alınabilmektedir. Laboratuvar hayvanlarında genellikle derin anestezi altında sakrifiye edilen hayvanlardan doku örnekleri hızlı bir şekilde alınmaktadır. Çiftlik hayvanlarında mezbahada kesim sırasında, tek tırnaklı ve pet hayvanlarında ise intraoperatif olarak örnekleme yapılabilmektedir (42).

Örnekleme yapılmadan önce alınacak olan örneklerin hızlı bir şekilde uygun saklama koşullarına alınabilmesi için gerekli hazırlık yapılmalıdır. Örneklerin alınacağı kaplar, kasetler etiketlenmeli, fiksasyon solüsyonları veya hızlı dondurma yapılacaksa sıvı azot hazırlanmalıdır (43).

Yapılması planlanan çalışmada incelenecek dokunun işlevsel bölümü organ diseke edilerek ortaya çıkartılmalı ve mümkün olduğu kadar büyük örnekler alınmalıdır. Örneğin yapılması planlanan çalışma korpus luteum ile ilgili ise ovaryum üzerindeki korpus luteumlar diseke edilmeli veya çalışma gebelik ile ilgili planlanıyorsa örnekler plasental bölgelerden alınmalı, çevre dokular ve kan damarları uzaklaştırılmalıdır (44).

Sakrifikasyon ile örnek alma yöntemleri

Moleküler çalışmalarda kullanılacak olan deney hayvanlarına, derin anestezi altında uygun yöntemlerle yapılan ötenazi uygulamasına sakrifikasyon adı verilmektedir. Sakrifiye edilen hayvanlardan bir veya daha fazla doku örneği toplanabilmektedir. Sakrifiye edilen deney hayvanından tüm üreme sistemi eksize edilebilmektedir. Bu organlar genellikle moleküler düzeyde doku analizleri ve histopatolojik incelemeler için kullanılabilmektedir. Kolay ve ekonomik bir örnek alma yöntemi olmak ile birlikte tekrarlayan örneklemeler yapmak mümkün değildir (45).

Mezbaha materyalinden örnek alma yöntemleri

Bu yöntem genel olarak kesimhanelerden elde edilen; uterus, ovaryum ve diğer genital organlardan alınan doku örneklerine dayanmaktadır (42). Ovaryumlar üzerindeki fonksiyonel yapılar veya folikül sıvıları incelenmek üzere toplanabilmektedir (4). Örnek alma işlemi steril aletlerle yapılmalı ve kontaminasyon şekillenmemesi için dikkat edilmelidir. Örnekleme öncesinde örnek doku alınacak olan organın çevre dokulardan titizlikle ayrılması gerekmektedir. Elde edilen doku örnekleri yapılması planlanan analiz yöntemine uygun saklama koşullarında saklanmalıdır (46).

Embriyonik veya fötal doku örneği alma yöntemleri

Hayvan türüne göre embriyonik örnek alma yöntemleri değişiklik göstermektedir. Kedi (47), köpek (48) veya küçük ruminantlarda (49) pre-implantasyon dönemindeki embriyolar operatif olarak toplanabiliyorken, büyük ruminant ve tek tırnaklı hayvanlarda servikal yoldan intrauterin yıkama yapılarak toplanabilmekte veya in vitro olarak üretilen embriyolar kullanılabilmektedir (50). Toplanan veya in vitro olarak üretilen embriyolar, total olarak kullanılabildiği gibi embriyo biyopsisi yapılarak; kutup cisimcikleri, blastomerler, trofoektoderm hücreleri veya blastosel sıvısı gibi embriyonik biyopsi örnekleri de analizlerde kullanılabilmektedir (50).

Fötal dönemde hayvan türüne göre farkı yöntemler kullanılarak örneklemeler yapılabilmektedir (51,52,53). Amnion veya allatois gibi yavru sıvılarından alınan örneklerin steril tüplere aktarılarak yapılması planlanan analize uygun saklama koşullarına alınması gerekmektedir.

3. Uygulanacak Olan Moleküler Analiz Yöntemine Göre Örneklerin Saklama Koşulları

ELISA

ELISA yöntemi ile kan, serum, plazma, doku ve folikül sıvısı gibi örneklerde analiz yapılabilmektedir. Kan örnekleri doğrudan, serum ve plazma örnekleri ise +4°C'de santrifüj edilerek -20°C'de analizler yapılana kadar saklanabilmektedir (54). Diğer doku örneklerinde ise biyomoleküllerin korunması için sıvı azotla şok dondurma yapılması gerekmektedir. Daha sonra doku örnekleri -86°C'de veya sıvı azotta saklanarak moleküllerin stabilitesi sağlanmaktadır (55). Alınan folikül sıvısı örnekleri de -20°C'de saklanabilmektedirler (8,56).

Dondurulmuş örneklerin çözdürülüp yeniden dondurulması protein denatürasyonuna yol açabileceği için analizler için örneklerin uygun porsiyonlara bölünerek donrurulması gerekmektedir (57).

İmmunohistokimya

İmmunohistokimyasal analizler için alınan doku örneklerinin soğuk koşullarda hızla işlenmesi, örneklerin kuruma ve bozulmasını önlemek için büyük önem taşımaktadır. Toplanan doku örneklerinden laboratuvara gönderilmeden önce hızlı bir şekilde uygun kalınlıkta kesitler alınmalıdır. Alınan bu kesitler doku kasetlerine yerleştirilerek hacimlerinin en az 5 katı miktarda %10'luk formol içerisine alınmalı ve laboratuvara bu koşullarda 24 saat içinde ulaştırılmalıdır. Laboratuvara ulaştırılan dokular alkol ile dehidre edilip parafin bloklara gömülmekte ve +4°C'de analizler yapılana kadar saklanabilmektedir (58).

İmmunohistokimyasal analizler için dokuların parafin bloklara gömülereke saklanması altın standart olarak kabul edilmektedir. Bu yöntem dokuların uzun süre yapılarını kaybetmeden korunmasını sağlamaktadır (59).

Western blot

Western blot doku örneklerinde ilgilenilen proteinlerin tespiti ve miktarlarının belirlenmesi için kullanılan moleküler bir tekniktir. Alınan kan, sürüntü, serum, plazma veya doku örnekleri uygun örnekleme ortamlarına alınarak, biyomoleküllerin korunması için sıvı azot ile şok dondurma işlemine tabi tutulmaktadırlar. Alınan örneklerin çözdürülüp tekrar dondurulması proteinlerin denatüre olmasına neden olacağı için örneklerin uygun boyutlarda porsiyonlara ayrılarak dondurulması gerekmektedir (60).

Analiz yapılana kadar uzun süre saklanacak protein örneklerinin yapılarının korunması ve enzimatik aktivitelerin durdurulabilmesi için -86°C'de saklanmalıdır (58).

DNA ve RNA analizleri

DNA ve RNA analizleri için alınan doku örneklerinin saklanmasında dikkat edilmesi gerekli olan en önemli husus nükleik asitlerin bozulmasını önleyici tedbirler almaktır. DNA molekülü görece daha stabildir ve farklı yöntemler ile işlenmiş örneklerden izole edilebilmektedir. DNA analizi yapılması amacıyla alınan örneklerde enzimatik aktiviteleri durdurabilmek için sıvı azotla şok dondurma yapılması ve analizler yapılana kadar doku örneklerinin -86°C'de saklanması önerilmektedir (55, 61). RNA, DNA ile karşılaştırıldığında çok daha kırılgan bir yapıya sahiptir ve çok çabukk bozulabilmektedir. Bu nedenle RNA analizlerinde, RNA stabilizasyon reaktifleri (örn. RNAlater®) kullanılarak RNA kalitesi korunması gerekmektedir. Bu reaktifler saha çalışmaları gibi hızlı işlem gerektiren durumlarda avantaj sağlamaktadır (62). RNAlater® gibi solüsyonlar RNA ve DNA stabilitesini koruyarak uzun süreli saklama imkânı tanımaktadır. Bu tür solüsyonlar, RNA miktarının belirlendiği gen ifadesi analizlerinde tercih edilmektedir. Bu solüsyonlar ile muamele edilen örnekler -20°C veya -86°C'de saklanabilmektedir (63, 64).

Özellikle RNA analizlerinin yapılacağı çalışmalarda, RNA'nın bozulmasını en aza indirmek için dikkat edilmesi gereken diğer bir hususu; örnek alımı ile saklama arasındaki geçen süredir. Bu süre ne kadar uzun olursa enzimatik bozulma da o kadar fazla olmakta ve analiz sonuçları olumsuz etkilenmektedir. Bu nedenle alınan doku örneklerinin en fazla 15 dakika içerisinde saklama için uygun koşullara alınmış olması gerekmektedir. RNA analizi yapılacak olan dokular için ideal olan sıvı azotla hemen şok dondurma işleminin yapılmasıdır (65).

4. Muhafaza Edilen Dokuların Laboratuvara Ulaştırma Koşulları

RNA veya DNA analizleri için kullanılacak olan doku örneklerinin sağlıklı bir şekildde laboratuvara ulaştırılabilmesi için içerisinde -78,5 °C'de kuru buz bulunan termokutular, sıvı azot içeren özel taşıma kapları veya içeerisinde RNAlater® gibi stabilizatörler bulunan solüsyonlar kullanılabilmektedir (58). Dokuların laboratuvara taşınması sırasında kullanılan taşıyıcı kap veya kutuların darbe ve kırılmalara karşı dayanıklı, güvenli ve sızdırmaz olmasına dikkat edilmelidir. Laboratuvar uzak bir lokasayonda ise numune taşıma kapları üzerine içinde bulunan maddenin ne olduğu, soğuk zincir ile taşınması gerektiği gibi bilgilerin yazılması gereklidir. Laboratuvarda yapılacak analizler sonucunda elde edilecek verilerin doğru bir şekilde dökümante edilebilmesi için; taşınacak olan her bir doku

örneği doğru bir şekilde etiketlenmeli ve örneklerin alındığı hayvanın türü, doku örneğinin hangi organdan alındığı, örnek alma tarihi gibi bilgileri içeren bir belge hazırlanmalı ve bu belge dokular ile birlikte laboratuvara gönderilmelidir (66). Dondurulmuş doku örnekleri laboratuvara gönderilirken gerekli bilgilerin yazıldığı etiketlerin ıslanarak düşme veya silinme riski bulunmaktaddır. Bu nedenle örnek kaplarının üzerine sudan etkilenmeyen silinmeyen mürekkepli kalemler ile gerekli bilgilerin yazılması daha uygun olmaktadır. Örneklerin laboratuvara ulaştırılması sürecinde biyomoleküllerin korunması bakımından taşıma işleminin en kısa sürede ve örneklerin türüne göre; içinde buz konteyneri bulunan termo taşıma çantası (+4, -20 °C), içinde kuru buz bulunan termo taşıma kutusu (-78,5 °C) veya sıvı azot (-196 °C) kullanılarak gerçekleştirilmesi gerekmektedir. Eğer alınan doku örnekleri fikse edilmeden laboratuvara ulaştırılacak ise doku 1 saat içinde soğutulmuş bir izotonik solüsyon içerisinde laboratuvara taşınmalıdır.

İmmunohistokimyasal analizler için alınmış doku örnekleri ise %10 formol solüsyonu içine alınarak 24 saat içinde laboratuvara ulaştırılmalıdır.

5. Sonuç

Bu derlemede veteriner doğum ve jinekoloji alanında yapılan araştırmalarda kullanılan moleküler analizler için uygulanacak olan örnek toplama ve saklama yöntemleri ele almıştır. Yapılması planlanan çalışmalarda incelenecek olan biyomoleküller ve kullanılacak olan yöntemlere göre farklı metodolojiler ile doku örneklerinin toplanması ve saklanması gerekmektedir. Örneğin alınan doku örneği; yapılması planlanan analize göre; +4, -20, -86 veya -196 °C'de, RNA later gibi koruyucu çözeltiler içerisinde stabilize edilerek veya %10 formol gibi solüsyonlar içinde fikse edilerek parafin bloklar içerisine alınarak saklanabilmektedir. Doku örneklerinin saklanmasında kullanılabilecek olan bu yöntemlerin hangisinin tercih edilmesi gerektiği alınan doku örneğinin türüne ve yapılması planlanan analiz yöntemine göre değişmektedir.

Sonuç olarak, yapılması planlanan moleküler analizler için en uygun doku örneği saklama yöntemi, çalışmanın amacına, analiz türüne ve laboratuvarın olanaklarına bağlı olarak değişmektedir. Örneklerin doğru alınması, uygun koşullarda saklanması ve hızlı bir şekilde laboratuvara ulaştırılması, moleküler analizlerin güvenilirliğini artırmaktadır. Bu nedenle yapılması planlanan çalışmalarda bu hususların göz önünde tutulması, çalışma plan ve takviminin bu bilgiler doğrultusunda dizayn edilmesi, çalışmanın sağlıklı bir şekilde tamamlanması ve elde edilecek olan verilerin güvenilirliğinin yüksek olması açısından büyük önem taşımaktadır.

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Microalgae as a new resource in the food industry

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ABSTRACT:

The increasing global population, environmental pollution, energy consumption, and climate change have emphasized the need for sustainable food sources. Microalgae have emerged as an eco-friendly and sustainable alternative, with applications in food, pharmaceuticals, animal feed, biofertilizers, wastewater treatment, and bioenergy. With over 50,000 classified species, microalgae thrive in nutrient-rich waters, recycling nutrients while offering sustainable benefits like wastewater treatment and environmental improvement. Their high photosynthetic efficiency also supports biofuel and biomass production, promoting sustainable practices. Key microalgal species used in the food industry include *Arthospira platensis (Spirulina), Chlorella vulgaris,* and *Duniella salina*, cultivated globally for various applications. *Arthrospira platensis* contains up to 70% protein in its biomass, while algal species such as *Euglena gracilis* and *Chlorella vulgaris* contain up to 40% protein. Besides primary metabolites such as proteins, carbohydrates, and polyunsaturated fatty acids, microalgae produce secondary metabolites like pigments and phytosterols with known health benefits, supporting their use as functional foods. Microalgae cultivation is a sustainable approach to biomass production, characterized by its low land requirement, adaptability to non-arable regions, and high productivity. Its rapid growth rate and frequent harvesting potential make it a viable and resource-efficient alternative to conventional agricultural practices. Commercial cultivation began with *Chlorella* in Japan, followed by *Spirulina* in Mexico and *Duniella salina* in the U.S. for beta-carotene production. In India, cyanobacteria and *Haematococcus pluvialis* are used for astaxanthin. With high efficiency, cost-effectiveness, and adaptability, microalgae hold significant potential as a sustainable alternative food source for the future.

Gıda endüstrisinde yeni bir kaynak olarak mikroalgler

ÖZET:

Artan dünya nüfusu, çevre kirliliği, enerji tüketimi ve iklim değişikliği, sürdürülebilir gıda kaynaklarına olan ihtiyacı vurgulamaktadır. Mikroalgler, gıda, ilaç, hayvan yemi, biyogübre, atık su arıtma ve biyoenerji gibi çeşitli alanlarda uygulamalarıyla çevre dostu ve sürdürülebilir bir alternatif olarak ortaya çıkmıştır. 50.000'den fazla sınıflandırılmış türüyle mikroalgler, besince zengin sularda gelişerek besinleri geri dönüştürürken, atık su arıtımı ve çevresel iyileştirme gibi sürdürülebilir faydalar sunar. Yüksek fotosentez verimliliği sayesinde biyoyakıt ve biyokütle üretimini destekleyerek sürdürülebilir uygulamaları teşvik eder. Gıda endüstrisinde yaygın olarak kullanılan temel mikroalg türleri arasında *Arthospira platensis (Spirulina), Chlorella* vulgaris ve *Duniella salina* bulunmaktadır ve bu türler dünya genelinde çeşitli uygulamalar için yetiştirilmektedir. *Arthospira platensis* biyokütlesinde %70'e kadar protein içeriği barındırırken, *Euglena gracilis* ve *Chlorella* vulgaris gibi türler yaklaşık %40 protein içeriği sunar. Mikroalgler, protein, karbonhidrat ve çoklu doymamış yağ asitleri gibi birincil metabolitlerin yanı sıra, pigmentler ve fitosteroller gibi sağlık açısından faydalı ikincil metabolitler üretir ve fonksiyonel gıda olarak kullanılmalarını destekler. Mikroalglerin yetiştirilmesi sürdürülebilir bir yöntemdir; daha az alan gerektirir, tarım için uygun olmayan bölgelerde yetiştirilebilir ve hızlı büyüme ile sık hasat avantajı sunar. Ticari üretim Japonya'da *Chlorella* ile başlamış, ardından Meksika'da *Spirulina* ve ABD'de *Duniella salina*'nın beta-karoten üretimi için kullanılmasıyla devam etmiştir. Hindistan'da ise siyanobakteriler ve *Haematococcus pluvialis* astaksantin üretimi için kullanılmaktadır. Yüksek verimliliği, maliyet etkinliği ve uyarlanabilirliği ile mikroalgler, geleceğin sürdürülebilir alternatif gıda kaynağı olarak büyük potansiyele sahiptir.

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1. Introduction

As the world population continues to expand, consumer demand for food also rises. Two billion people do not have sufficient access to nutritious food. As awareness of the relationship between food and population growth increases, alternative food sources are needed (1, 2). Global warming, increased environmental pollution and escalated energy consumption of energy causing significant problems worldwide. Climate change is also affecting agriculture (3). Microalgae are seen as a sustainable alternative food source for the growing population and insufficient food resources (4). Microalgae have many industrial applications such as pharmaceuticals, functional foods, animal and fish feed, cosmetics, chemicals and polymers, pollution control, bio-fertilisers, waste water treatment systems, bio-energy and many others (5).

The term algae is a polyphyletic grouping of a wide variety of organisms (6 ,7). The term microalgae refers to eukaryotic unicellular organisms and includes prokaryotic Cyanobacteria (6, 8).

The first unialgal cultures were obtained by Beijernick (1890) with *Chlorella* vulsynthetic gas exchangers for use as a microbial source of protein in space travel. The cultivation of *Chlorella* in Japan in the early 1960s led to a wide range of commercially available cultures. In the 1970s, a *Spirulina* harvesting and cultivation facility was established in Mexico. Later in the 1980s, over 1000 kg of microalgae per month were traded in Asia. From 1986, facilities were established in Australia and the USA to trade *Duniella salina* as a source of beta-carotene (9, 10). More recently, the production of *Haematococcus pluvalis* as a source of astaxanthin has been established in the USA and India. The biomass market for microalgae produces about 5000 tonnes of dry matter per year (8).

Besides high levels of primary metabolites such as protein, carbohydrates, polyunsaturated fatty acids and vitamins, secondary metabolites produced by microalgae are associated with beneficial health effects. Studies show microalgae as functional food with effective concentrations of various secondary metabolites including pigments, phytosterols and mycosporin-like amino acids (11).

Microalgae can be grown more quickly than other crops in terms of sustainability, and more frequent harvesting with a faster growth rate leads to an increase in overall productivity. Microalgae have the ability to adapt to different environments. As a result, microalgae production has a broader perspective and contributes to a reduction in land use. Their high photosynthetic efficiency makes them an important source for biofuel and biomass production. There are more than 50,000 classified species. They can grow in nutrient-rich waters and have the ability to recycle nutrients (2,12).

The entire biomass of some microalgae species has been incorporated into all foods for human consumption. The high quality metabolites obtained are more effective than synthetic alternatives in food applications due to their chemical structure. As food supplements and nutraceuticals, the concentration of metabolites contained in microalgae is higher than in other sources and has a positive effect on human health, and microalgae have gained a place in the food industry (8).

There are several species of microalgae that have been granted Generally Recognised as Safe (GRAS) status by the Food and Drug Administration (FDA). *Arthospira platensis, Chlamdomonas reinhardtii, Chlorella*, *Chlorella vulgaris*, *Dunaliella bardawill* and *Euglana gracilis* are among the species with GRAS status. GRAS only applies to the United States of America and differs from regulations in other countries. In the European Union (EU), the European Food Safety Authority (EFSA) oversees EU food regulations. *Haematococcus pluvialis, Odontella aurita, Schizochytrium spp., Tetraselmis chui* and *Ulkenia spp*. added to new EU food list. EFSA's risk assessment for the 2019 update of the list of biological agents added to food for Qualified Presumption of Safety (QPS) status includes *Aurantiochytrium limacinum*, *Euglena gracilis* and *Tetraselmis chui*. β-carotene from *Dunaliella salina* is also authorised for use as a food additive in the EU. *Dunaliella salina* is marketed as *Dunaliella* powder as a component of dietary supplements and functional foods for human consumption. *Chlorella* contains β-1,3-glucan, an active immune stimulator, a free radical inhibitor and a blood lipid lowering agent, which can also be used as a food additive and as a flavour modifier. *Chlamydomonas reinhardtii* is considered unsafe in Canada, Europe, Japan, India and China.

Dunaliella bardawil is also not considered safe in any of these countries, whereas *Dunaliella salina* is considered safe in China and Canada (7, 13).

2. Macro and Micro Nutritional Factors in Microalgae

Microalgae are a rich source of nutritional factors and bioactive compounds, including high value metabolites. They have positive effects on human and animal health. They also offer a sustainable and environmentally friendly alternative for potential food and feed applications (14).

Protein content varies between microalgal species. Genetic traits play an important role in determining protein content. Environmental factors also influence this variation. In general, 40-60% of microalgae dry matter can contain protein as demonstrated in Table 1 (15). The nutritional quality of proteins is usually determined by their amino acid composition. Most microalgae proteins consist of certain essential amino acids such as leucine, arginine and lysine, which make up 7% of the protein content. Other amino acids such as isoleucine, phenylalanine and threonine make up 4% of the protein content. Non-essential amino acids are amino acids such as aspartic acid and glutamic acid, which constitute 20% to 30% of the protein content (16). Mycosporine-like amino acids are involved in protection from salt stress and photosynthetic activities by stabilising osmotic pressure in microalgae. Mycosporine-like amino acids are found in approximately 152 species of microalgae and find important areas of use due to their potential benefits such as antioxidants and supporting cell proliferation (7) The amino acid content (g/100 g protein) and the amino acid profile of some proteins found in microalgae are presented in Table 2 (17).

Table 1: Protein content of some commercially important microalgae biomass.

Tablo 1: *Bazı ticari açıdan önemli olan mikroalg türlerinin biyokütlelerindeki protein içeriği.*

	Average protein content % DW	References
Chlorella vulgaris	51-58	15
Dunaliella salina	57	18
Arthospira platensis	46-63	15
Aphanizomenon flos-aquae	62	19
Tetraselmis chuii	35-40	20

Table 2: Amino acid content (g/ 100 g protein) and the amino acid profile of some proteins found in microalgae.

Tablo 2*: Mikroalglere ait bazı proteinlerin amino asit içeriği (g/100 g protein) ve amino asit profili.*

	Alanine	Arginine	Lysine	Tryptophan	Histidine	Phenylalanine
Chlorella vulgaris	10.82 ± 0.32	7.33 ± 0.21	5.35 ± 0.16	0.21 ± 0.01	1.52 ± 0.04	6.17 ± 0.18
Dunaliella salina	10.99 ± 0.32	8.16 ± 0.24	5.99 ± 0.17	0.18 ± 0.01	1.73 ± 0.05	6.98 ± 0.20
Arthospira platensis	11.48 ± 0.34	6.02 ± 0.18	7.11 ± 0.21	1.16 ± 0.03	2.19 ± 0.06	7.85 ± 0.23

The fatty acids found in microalgae contain higher levels of linoleic acid (C18:2) and alpha/gamma-linolenic acid (C18:3) than those found in commonly used vegetable oils. Phytosterols, similar to animal cholesterol but derived from microalgae, have anti-cancer, antioxidant, anti-inflammatory, anti-atherogenic and cholesterol-lowering properties. The phytosterol yield of microalgae including species such as *Pavlova lutheri, Tetrasellimis spp*. is high. The sustainability potential of microalgae as a source of phytosterols is higher when compared to other plants (21). Microalgae's lipid accumulation capacity has many potential uses. For example, various potential uses such as food,

animal feed and food additives are being investigated (22).

The most abundant carbohydrates in microalgae are glucose, rhamnose, mannose and xylose. Hemicellulose and lignin are absent from microalgal biomass. The carbohydrate content of the biomass depends on the microalgal species and the environmental conditions. Microalgae typically contain high concentrations of carbohydrates, in excess of 50% of their dry weight. The specific composition of stored carbohydrates also varies between species. For example, Cyanobacteria produce glycogen (α-1,4-linked glucan). Green algae produce amylopectin-like polysaccharides (starch). Depending on their diversity, microalgae can be used for the production of biofuels and for different industries (23).

Microalgae are a potential source of a wide range of vitamins. *Dunaliella spp.* contains high levels of provitamin A and carotene, while *Chlorella spp.* contains three times the amount of carotene found in grass meal. According to studies, high levels of carotene, ascorbic acid and tocopherols are associated with *Dunaliella* spp. species and the carotene content can reach up to 1100 mg %. The efficiency and vitamin content of microalgae grown under open mass cultivation conditions are shown in Table 3 (7).

Table 3: Efficiency and vitamin content in microalgae that grown under the conditions of open mass cultivation (of absolute dried mass)
Tablo 3: *Açık havuz sisteminde yetiştirilen mikroalglerin verimliliği ve vitamin içeriği (kuru ağırlık bazında)*

	Productivity (g/m^2 per day)	**Carotene, mg%**	**Ascorbic acid, mg%**	**Tocopherol, mg%**
Chlorella pyrenoidosa	22.0	220.3	68.5	25.2
Dunaliella salina	21.0	815.7	120.0	123.4
Euglena gracillis	18.0	137.0	182.7	67.8

Microalgae are important photosynthesizers and produce important pigments such as chlorophyll a, b and c, ß-carotene. The pigments contained in microalgae play an important role in photosynthesis and energy transfer processes. They also colour the organism. Environmental conditions such as temperature, light intensity and wavelength affect pigment production. Also, their adaptation to the environment, absorption spectra, ecological roles and various applications such as biotechnology and biofuels vary according to the pigments they contain. The main classes of pigments include chlorophylls, carotenoids and phycobiliproteins (24).

Phycobiliproteins are mainly found in Cyanobacteria. Although their spectral properties vary, phycobiliproteins can be analysed in four main groups as allophycocyanin, phycocyanin, phycoerythrin and phycoerythrocyanin. Phycocyanin extracted from *Spirulina* platensis is primarily used as a protein dye in the food industry. Phycocyanin is commercially known as lina blue and has fluorescent properties. *Phorphyridium aerugineum* produces a blue colour that is unaffected by pH changes. This property makes it important in the food industry. In particular, pigments extracted from *Phorphyridium aerugineum* are incorporated into beverages without heat treatment. Phycoerythrin has a yellow fluorescence (25). Microalgae contain one or more types of chlorophyll. The amount of chlorophyll they contain varies according to environmental conditions. For example, *S. platensis* contains only chlorophyll a. Chlorophyll a is used as a dye because of its stability. Microalgal species can accumulate 8-14% of their biomass from carotenoids. For example, in β-carotene-accumulating species of *Dunaliella salina*, astaxanthin accumulation is observed in addition to long-term exposure to UV radiation (26,27).

Lutein has food colouring, anticancer and antioxidant effects and covers approximately 4% of the dry weight in *Chlorella* spp. species. It can also be extracted from *Dunaliella salina* and *Muriellopsis spp*. microalgae species (28). The high antioxidant capacity of astaxanthin, a red coloured xanthophyll, which is the second most widely used

microalgal carotenoid after β-carotene, is very important and has anti-inflammatory properties. It is used as a natural food colouring in both animal and human diets (29).

3. Uses of Microalgae in Food Industry

Microalgae can be used in various industries, from food to biofuel production. The use of microalgae in the renewable energy, biopharmaceutical and nutraceutical industries is increasing worldwide. Microalgae have multiple applications as value-added products in pharmacological and food formulation, feed production, cosmetics, fertilizers, wastewater treatment. Microalgae are considered to be a source of various bioactive compounds with antioxidant and anti-cancer properties, showing beneficial health effects. Their use in food formulations demonstrates the potential of microalgae as a new raw material for the food industry. The compounds are generally used in powder or tablet form. For example, *Arthrospira platensis* and *Chlorella* vulgaris are available as food ingredients in powder and capsule form (7, 30).

By 2050, the increase in total food consumption from animal sources is expected to lead to an increase in the global livestock population, resulting in an increase in greenhouse gas emissions from the livestock industry. According to FAO reports that methane emissions from ruminants, which are the largest source of greenhouse gases from agriculture, need to be reduced by 11-30 per cent by 2030 and 24-47 per cent by 2050 (31, 32). There are studies showing that microalgae can also be effective in this area. For example, Sucu's (33) study on the effect of adding *Chlorella vulgaris* and *C. variabilis* to diets on rumen fermentation showed that microalgae reduced methane production.

Global production and market of microalgae

Chlorella and *Spirulina* have become globally marketed nutraceuticals. Major production centres are located in countries such as Australia, China, South Korea, Malaysia, Singapore, Taiwan, USA, Netherlands, Spain, Portugal, France, Denmark, Japan and several African countries. In particular, *Chlorella* and *Spirulina* have been incorporated into a variety of food products such as pasta, biscuits and juices in order to improve their nutritional profile. In addition, the use of *Spirulina* as a natural colour additive in foods has been approved by the FDA in the United States. *Arthrospira*, and more commonly *Spirulina*, are protein-rich microalgae that have been known and present in the human diet for thousands of years. Historical sources show that the Aztecs consumed a blue cake made from *Arthrospira*. In the food industry, the cultivation of *Arthrospira* began in Mexico in the 1970s, and commercial production of *Chlorella* began in Japan in 1952 (34).

Microalgae in food and nutritional applications

Spirulina platensis is an important source of protein for malnourished people. Chocolate biscuits enriched with *Spirulina* platensis, as well as its addition to bread ingredients, have improved the protein content and digestibility of foods. In particular, *Chlorella* and *Spirulina* have been incorporated into a variety of foods, including noodles, cookies, nutritional bars and fruit juices, to improve their nutritional profile (34). Table 4 presents an overview of nutraceutical products formulated with microalgae or their components, along with their applications in different food and supplement forms (35).

Isochrysis galbana microalgae has been added to some foods to provide the effects of omega-3 polyunsaturated fatty acids. *Arthospira platensis* is added to increase the protein content of products. They are used to improve the nutritional and health benefits of snacks. *Dunaliealla sp.* and *Arthrospira* species are added to bread varieties, including gluten-free ones, to increase the protein content (3).

Table 4: Applications of microalgae in food, pharmaceuticals, and animal feed, and their use as fertilizers and biostimulants.
Tablo 4: *Mikroalglerin gıda, ilaç ve yemlerdeki uygulamaları, gübre ve biyostimülan olarak kullanımı.*

Bioactive Compound	Microalgae Source	Product Examples (Brand)	Manufacturers
Carotenoids	Dunaliella salina	Supplement – Patented Betatene® β-carotene	Cyanotech, Mera Pharmaceuticals, AstraReal AB, Jingzhou Naturals
Beta-carotene	Chlorella vulgaris	Supplements – Dr. Mercola, Chlorophyll-Fermented Chlorella, 450 Tablets	Mercola, Florida
Lutein	Chlorella pyrenoidosa	Terranova fresh freze dried Chlorella pyrenoidosa	Terranova Synergenistic Nutrition, UK
Protein & Vitamin B12	Chlorella sp.	Food additives – Algal proteins or whole biomass	Sophie's BioNutrients, Singapore
Astaxanthin	Haematococcus pluvialis	Supplement – Max Botanics astaxanthin supplements/sunscreens	Mera Pharmaceuticals, AstraReal AB, Jingzhou Naturals Astaxanthin Inc., Max Botanics UK & Europe

Meat samples from broilers fed *Spirulina* platensis showed many positive effects, including longer shelf-life and increased antioxidant capacity with reduced lipid oxidation. In fatty acid profiles, increased levels of omega-3 fatty acids provide nutritional benefits in humans (37).

Microalgae such as *Chlorella vulgaris*, *Arthrospira platensis* and *Spirulina* are also used in aquaculture. Pigments are particularly important in maintaining flesh colour in species such as salmon, sea bream and koi/ornamental fish. Pigment supplementation, including carotenoids, has been observed to improve product quality in fish such as rainbow trout. For example, *Haematococcus pluvialis* is used to provide pigmentation (38).

Cattle were fed a diet containing microalgae to increase the levels of omega-3 polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The microalgae species *Schizochytrium* and *Nannochloropsis sp.* show that their cell walls prevent the omega-3 fatty acids in the algae from becoming unavailable in the small intestine after they have been dispersed from the rumen and hydronised. This has led to studies to increase omega-3 levels in beef production due to economic factors and consumer preferences (39).

Studies have shown the bioavailability of fatty acids such as PUFA and eicosapentaenoic acid (EPA) extracted from *Phaeodactylum tricornutum*, including fucoxanthin and its metabolites and β-carotene. In this case, *P. tricornutum* can potentially be a sustainable and valuable food source, as it can be a low-cost, nutrient-rich food. It has also been observed that *P. tricornutum* is richer in EPA and n-3 PUFA than fish (40). The incorporation of microalgae-based ingredients into various food products, such as baked goods, enhances their nutritional and techno-functional properties, as demonstrated in Table 5.

Table 5: Nutritional and techno-functional properties of microalgae-based formulated food products.

Tablo 5: *Mikroalg bazlı formüle edilmiş gıda ürünlerinin besinsel ve teknolojik-fonksiyonel özellikleri.*

Bioactive Compounds	Microalgae Source	Food Product	Reference
Chlorophyll-a and total carotenoids	*Isochrysis galbana* and *Nannochloropsis oculata*	Natural green colorant in chewing gum	41
Astaxanthin	*Haematococcus pluvialis*	Wholemeal cookies with enhanced antioxidant properties and reduced glycaemic response	42
High protein content (80%), iron (49.8 mg/100 g), polyunsaturated fatty acids (γ-linolenic acid - 13.8%), and pigments (chlorophylls, β-carotene, phycocyanin, allophycocyanin)	*Spirulina maxima*	Nutritionally enriched biscuits with improved protein, iron content, and sensory acceptance through spray-drying microencapsulation	43
High protein content (60–70%), vitamin B12, gamma-linolenic acid (GLA), calcium, iron, magnesium, chlorophyll-a, phycocyanin, and carotenoids	*Spirulina platensis*	Nutritionally enriched bread with *Spirulina platensis* (10% incorporation)	44

4. Regulations

Although food safety is important for regulatory organizations worldwide, potential hazards associated with the consumption of algae-based food require assessment. Various nations, like the European Union and the USA, enforce specific laws for food products that contain microalgae (45).

There are some challenges in the marketing of microalgae-based products in the European Union, such as production costs, technological developments and regulatory compliance. Despite regulatory barriers such as climatic variability, limited consumer demand and the EU Novel Food Regulation, the EU is considered to have the potential to strengthen its market position in the coming periods. The share of microalgae products in the EU covers approximately 5 % of the global market (46).

Microalgae-based products used in the food industry are subject to food safety regulations, namely the European Community Food Safety Regulation (EC 178/2002). The placing on the market of food containing microalgae or microalgal ingredients is primarily governed by the Food Safety Regulation (EC 178/2002) and the suitability of algae for human consumption by the Novel Food Regulation (EC 2015/2283). For example, *Chlorella* vulgaris is not recognised as a novel food. However, it can be used as a food ingredient under the Novel Food Regulation (EC) 2015/2283. Under this regulation, health claims for food and feed must be generally recognised under (EC) 1924/2006. The European Food Safety Authority (EFSA), Panel on Nutrition and Allergies (NDA), carries out all scientific assessments of food and feed (46, 47).

In the USA, the Federal Drug and Cosmetic Act and the Dietary Supplement Health and Education Act apply to the use of microalgae. Generally Recognised as Safe (GRAS) status is granted by the FDA. GRAS status defines what is safe for human consumption. Certain microalgae species such as *Spirulina* spp., *Chlorella* spp., *Dunaliella spp., Haematococcus spp., Schizochytrium spp., Porphyridium cruentum* and *Crypthecodinium cohnii* have GRAS status. In addition, specific oils from *Schizochytrium* and Ulkenia and specific microalgal protein powder and lipid content from *Chlorella* spp. have been granted GRAS status in the USA (47, 48).

China and Japan are leading the way in creating sustainable market production for microalgae-based products, with Japan particularly focused on securing its energy supply through algae-derived biofuels. Algae consumption is more widespread in Asia than in Western countries. Consumers have been using algae for a long time. Additionally, the lower cultivation and labor costs in Asian nations, coupled with fewer regulatory constraints, are driving the rapid development of the microalgae industry across various sectors, including food, feed, and energy diversification (49, 50). In Japan, food safety inspections are overseen by the Minister of Health, Labour and Welfare, primarily through the Department of Pharmaceutical and Food Safety. The Basic Food Safety Act serves as the principal legal framework regulating food quality, supplemented by additional statutes such as the Food Sanitation Act (51). Microalgae-based products are subject to regulatory oversight under Foods with Function Claims (FFC) and Foods for Specified Health Uses (FOSHU), which encompass products containing bioactive components with scientifically validated physiological benefits and biological activities (52).

Although algae consumption is not widespread in our country, there is no food regulation in force. However, microalgae preparations as food additives are evaluated within the food categories specified in Annex-II Part D of the Turkish Food Codex Food Additives Regulation (53). In the legal legislation, the regulation for the Turkish Food Codex 'Regulation on plants, fungi, algae and lichens that can be used in foods' was submitted as a draft in 2022 and has not yet entered into force (54). Furthermore, the Turkish Food Codex Novel Foods Regulation remains in the draft stage and has not yet been officially published in the Official Gazette. Microalgae are classified as novel foods, necessitating comprehensive safety and quality assessments prior to market approval to ensure compliance with regulatory standards. This regulation establishes the framework for evaluating the safety of foods that have not been historically consumed on a significant scale. For instance, the regulation defines the usage conditions, specifications, and labeling requirements for β-glucan derived from *Euglena gracilis*. It mandates that product labels explicitly state "Beta-glucan (derived from *Euglena gracilis* microalgae)", while dietary supplements containing this ingredient must include consumption warnings tailored to specific age groups. These provisions are designed to facilitate the safe incorporation of microalgae-based ingredients into the food industry while ensuring transparent consumer information and regulatory compliance (55).

5. Challanges and Future Perpective

Arthospira, *Chlorella*, *Dunaliella salina*, and *Scenedesmus sp*. are the most common species utilized in the commercial cultivation of microalgae for the food industry. *Arthospira* produces 12,000 tonnes annually, *Chlorella spp.* 5,000 tonnes, and *Dunaliella salina* 3,000 tonnes (12). The United States, Asia, and Europe are engaged in the microalgae-based products market (56). Although the industry is expanding, the costs of producing and processing of microalgae limit their popularity as food alternatives, technological developments and automation may mitigate these challenges. Improvements such as strict contamination control, efficient energy use, and optimized harvesting techniques are essential for enhancing production. Additionally, addressing taste and odor issues, along with promoting eco-friendly products through public incentives and consumer awareness initiatives, is vital for the successful market integration of microalgae-derived foods (57).

Challenges exist in the production and processing of microalgae within the food industry. These include isues such as regulation, cost, and food safety. There are a number of strategies which are being developed in response to these challenges. These include taking measures to control contamination through the use of photobioreactors, improving microalgae production and processing stages, and developing more sustainable methods (44).

The production of microalgae can be categorized into two categories: open pond systems and closed cultivation systems, such as photobioreactor units (58). Open pond systems are cost-effective and suitable for fast-growing species; however, they are prone to contamination (59). Periodic inspection and monitoring are required. Furthermore, the quality of water utilized in biomass production must be examined for contaminants like heavy metals, pesticides, and antibiotics (60). Photobioreactors have advantages over open systems. These include the ability to utilise light more effectively, produce a high volume of biomass, have a low risk of contamination and water loss, facilitate gas transfer and incorporate mixing. In addition, the investment costs are higher. However, algae production in outdoor open ponds

is cost-effective, but is only compatible with fast-growing species and is associated with risks related to climatic conditions (61).

In the production of microalgal biocultures, it is essential to detect potential hazards. Including biological, chemical, and physical risks. Critical points include the presence of naturally occurring toxins, contamination with heavy metals and levels of pathogenic microorganisms. For example, Arsenic (As) pollution and eutrophication in aquatic environments increase the risk of heavy metal contamination in edible microalgae, leading to potentially harmful effects in algal products. Limited studies have reported As levels in algae products; for example, the addition of 150 µg/L As(V) in to Zarrouk medium resulted in *Spirulina platensis* surprassing the Chinese National Standard for Health Functional Food (25). Hazard analysis is important in production to identify critical control points that require monitoring. Protocols established by regulatory organisations such as EFSA and FDA are important in ensuring the quality and safety of products (62).

Toxins found in microalgae are mainly domoic acid, pectenotoxins, microcystins, saxitoxin, okadaic acid, brevetoxin. In the study, no toxicity was observed when 10% of the algal biomass from C. protothecoides was included in the diet for 28 days. This supports the absence of toxicity at the highest No Observable Adverse Effect Level (NOAEL) dose tested (63).

Blue-green algaes (Cyanobacteria) are capable of producing toxins. Among these, anatoxin-a is a neurotoxin that known for its fast absorption and abilty to cause acute paralysis and respiratory collapse. These toxins can be contamine the algae-based dietary supplements, presenting significant public health risks. To address this issue, effecient water treatment methods are required to reducing toxin presence in recreational and drinking water sources (64). Also, *Spirulina* spp. and in particular *Aphanizomenon flos aquae*, are mainly used in the production of blue-green algae supplements (BGAS). However, these supplements can be contaminated with microcystins, presenting a significant health risk to consumers. The variability in contamination levels, even between batches of the same brand, and uncertainties about daily consumption, highlights the need for strict monitoring by BGAS producers and increased vigilance by health authorities to ensure consumer safety (65).

There is a potential risk of allergy associated with the consumption of microalgae-based products. While limited research exists on the frequency of allergenicity of edible algae species, some studies indicate high IgE activity specific to algae, while more research is needed on consumer exposure and potential contamination from seafood-related allergies (66). Particularly *Spirulina*, poses potential allergenic risks. Le et al., (67) reported a case of a 17-year-old male who experienced anaphylaxis after ingesting *Spirulina*, with skin prick testing confirming that the allergy was specific to the microalgae and not to its additives. Due to the increasing popularity of *Spirulina* as a dietary supplement, it is essential that comprehensive allergenicity risk assessments to determine the potential for cross-reactivity with known allergens prior to its widespread consumption.

Despite the potential health benefits of microalgae, their inclusion in food products is limited, mainly due to sensory concerns. There are sensory barriers in their daily use, especially in terms of colour and taste. Furthermore, processing and packaging technology advances, such as microencapsulation or nanoemulsion, offer opportunities for encapsulation of microalgae extracts, thus reducing their sensory impact while maintaining nutritional enrichment (68). Overcoming these organoleptic difficulties will be important to the effective incorporation of microalgae into conventional food products, thus revealing their potential health benefits and nutritional values (50).

Legislation and regulations related to food additives and innovative foods, including nutraceuticals and functional foods, differ between countries. Microalgae products intended for human and animal nutrition are subject to a range of regulations that affect production processes and labeling requirements, leading to potentially lengthy and costly market introduction processes. This regulatory complexity can result in protracted and expensive routes for introducing these products to the market (69). Challenges to the commercialisation of microalgae products in the European Union include biomass production costs, technology and regulatory compliances. Despite challenges such as climatic conditions and regulatory barriers, the microalgae industry has taken off in the European Union (56).

Advances in genetics are enabling the use of transgenic microalgae in bioactive production, and gene modification in various microalgae species is attracting attention. Transgenic microalgae expressing dsRNA have the potential for disease management by targeting specific pathogens (70).

Recent advancements in CRISPR-Cas systems have facilitated the genetic optimization of microalgae and cyanobacteria, enabling significant progress in various biotechnological applications. Cas9 and Cas12a (Cpf1) enzymes precisely target specific DNA sequences, thereby enhancing the efficiency of genome editing processes. Moreover, species such as *Chlorella*, *Phaeodactylum*, *Tetraselmis* have been successfully engineered using CRISPR technology for genetic modifications and enhanced lipid production. These developments significantly expand the biotechnological and industrial potential of microalgae (71). However, certain technical challenges, such as Cas9 toxicity, have limited the advancement of CRISPR-based genetic modifications in microalgae (72).

The work by Qv et al. emphasi the efficacy of microalgae-bacteria consortia (MBC) in the elimination of fluoroquinolone antibiotics and the horizontal transmission of resistance genes. The results highlight the importance of environmental monitoring and the role that transposons and integrons play in gene transmission. MBC-based therapies should be improved in future studies to increase effectiveness and prevent the spread of resistance genes (73).

6. Conclusion

Microalgae have great potential for various industries, from food to medicine, from animal feed to fuel, if produced on a commercial scale. Research and development studies on biomass production and processing of microalgae are also very important in terms of economic sustainability.

Microalgae-based products can play an important role in the food industry. With their high quality metabolites, they can contribute to the demand for various food ingredients. However, there are only a few microalgae-based products available on the market. Microalgae are produced as nutraceuticals and food supplements. To fully harness their potential, it is essential to improve social acceptance of microalgae-based foods. The provision of legal capacity will expand their use in the food industry. Again, as research into the use of microalgae in food is carried out, it is expected that the market for these products will develop.

Innovative design and technology should be developed to address the taste and odour problems caused by microalgae in food. There is a need for public incentives for the consumption of environmentally friendly products. Design is also important for market integration of foods containing microalgae. Support for innovative designs is important for the promotion of algae products. Consumer awareness programmes and the positive health properties of the products should be demonstrated.

Advanced design and technology must be developed to resolve the flavour and aroma issues associated with microalgae in food. Public incentives are necessary to promote the consumption of environmentally sustainable products. The design is crucial for the market integration of foods including microalgae. Advocacy for novel designs is essential for the advancement of algal products. Consumer awareness initiatives and the beneficial health benefits associated with the products should be demonstrated

In addition to the nutrient composition of microalgae and expand their utility in the food sector, biotechnological and genetic research should be undertaken to elevate their protein synthesis capabilities and reduce anti-nutritional factors. Advanced gene editing technologies, such as as CRISPR-Cas, are essential for enhancing the functional components of microalgae. Additionally, the advancement of flavour and odour neutralisation methods is crucial for enhancing the sensory qualities of microalgae-derived foods. Innovative technologies, such as microencapsulation and nanoemulsion, can enhance the flavour profiles of algal-based food products, hence improving their market acceptance.

Progress in genetic research facilitates the use of transgenic microalgae for bioactive synthesis, underscoring the significance of gene modifications across diverse microalgae species. The production of double-stranded RNA (dsRNA) by transgenic microalgae has possibilities for disease management through the targeted elimination of specific pathogens.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Ethical Approval

The data, information and documents presented in this article have been obtained within the framework of academic and ethical standards. Ethical statements have been obtained from the authors, affirming that all information, documents, evaluations, and conclusions are presented in accordance with scientific ethical and moral principles.

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Hip joint of cats and dogs: anatomy and biomechanics, fractures and treatment methods

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ABSTRACT

Fractures of the proximal femur and acetabulum, which together constitute the hip joint in cats and dogs, are typically severe injuries resulting from high-energy trauma. These injuries are often accompanied by concomitant orthopedic damage and, due to their anatomical location, intrapelvic organ damage may also be encountered. Surgical treatment is recommended rather than conservative treatment because long-term complications such as osteoarthritis and chronic pain may occur in intra-articular fractures. The primary objectives of surgical management are to restore extremity function, achieve precise anatomical reduction, and ensure rigid fixation of the fracture fragments within the shortest possible timeframe to minimize the risk of adverse outcomes. In young animals, preservation of the vascular supply to the proximal femur and protection of the growth plates are critical factors in surgical planning. For acetabular fractures, an understanding of the biomechanical forces acting on the region is essential for appropriate implant selection and effective stabilization. Equally important is an in-depth knowledge of the biomechanical characteristics of the implants themselves, including their respective advantages and limitations. This understanding directly influences implant choice and postoperative outcomes. Detailed knowledge of the vascular anatomy of the proximal femur, as well as the localization of the sciatic nerve, which lies in close proximity to the acetabulum, is imperative to minimize iatrogenic complications and ensure optimal surgical approach. Differentiating traumatic fractures from underlying pathological conditions is critical in the diagnostic process. Accurate identification of pre-existing disorders not only aids in planning the surgical approach but also allows for better prediction and management of potential postoperative complications. This review aims to provide a comprehensive overview of the current principles and considerations in the surgical management of proximal femur and acetabular fractures in cats and dogs, emphasizing the integration of biomechanical, and anatomical knowledge for successful outcomes.

Kedi ve köpeklerin kalça eklemi: anatomi ve biyomekanik, kırıklar ve tedavi yöntemleri

ÖZET

Kedi ve köpeklerde kalça eklemini oluşturan proksimal femur ve asetabulum kırıkları, genellikle yüksek enerjili travmalar sonucu oluşan ciddi yaralanmalardır. Bu yaralanmalara genellikle eş zamanlı ortopedik hasarlar eşlik eder ve anatomik konumları gereği intrapelvik organ hasarları ile de karşılaşılabilir. Eklem içi kırıklarda osteoartrit ve kronik ağrı gibi uzun vadeli komplikasyonlar oluşabileceği için konservatif sağaltımdan ziyade cerrahi sağaltım önerilir. Cerrahi sağaltımın primer hedefleri, ekstremite fonksiyonunu geri kazandırmak, tam anatomik redüksiyon elde etmek ve olumsuz sonuçları en aza indirmek için kırık fragmentlerinin mümkün olan en kısa sürede rijit bir şekilde sabitlenmesini sağlamaktır. Genç hayvanlarda, proksimal femurun vasküler beslemenin ve büyüme plakalarının korunması cerrahi planlamada kritik faktörlerdir. Asetabular kırıklar için, bölgeye etki eden biyomekanik kuvvetlerin anlaşılması, uygun implant seçimi ve etkili stabilizasyon için esastır. Aynı derecede önemli olan, implantların kendi avantajları ve sınırlamaları dahil olmak üzere, biyomekanik özelliklerinin derinlemesine bilinmesidir. Bu anlayış, implant seçimini ve postoperatif sonuçları doğrudan etkiler. Proksimal femurun vasküler anatomisinin ve asetabuluma yakın mesafede bulunan siyatik sinirin lokalizasyonunun ayrıntılı bilgisi, iatrojenik komplikasyonları en aza indirmek ve optimum cerrahi yaklaşımı sağlamak için zorunludur. Travmatik kırıkları altta yatan patolojik durumlardan ayırt etmek, tanı sürecinde kritik öneme sahiptir. Önceden var olan bozuklukların doğru bir şekilde tanımlanması, yalnızca cerrahi yaklaşımın planlanmasına yardımcı olmakla kalmaz, aynı zamanda olası postoperatif komplikasyonların daha iyi tahmin edilmesine ve yönetilmesine de olanak tanır. Bu derlemenin amacı, kedi ve köpeklerde proksimal femur ve asetabular kırıklarının cerrahi tedavisinde güncel prensipler ve değerlendirmeler hakkında kapsamlı bir genel bakış sunmak ve başarılı sonuçlar için biyomekanik ve anatomik bilginin bütünleştirilmesinin önemini vurgulamaktır.

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1. Anatomy of The Hip Joint

The hip joint is composed of the hemispherical-shaped acetabular fossa and the caput ossis femoris, which articulates within it. The primary stabilizers of the hip joint include the ligamentum capitis ossis femoris, the joint capsule, the dorsal acetabular rim, and the ligamentum transversum acetabuli, the latter of which completes the acetabular fossa ventrally. The ligamentum capitis ossis femoris arises from the acetabular fossa and inserts into the fovea capitis of the femur, contributing significantly to the joint's stability. The joint capsule extends from the acetabular rim to the base of the femoral neck (1, 2).

The medial wall of the acetabulum is formed by the ilium, ischium, and acetabular bone. Notably, the pubis is not part of the acetabular wall. Radiographic studies on the closure times of acetabular physis in juvenile animals are limited, with the ilioischial physis being the only one reported. In cats, the closure occurs between 20-25 weeks, while in dogs, this occurs between 18-20 weeks (3). Due to the superposition femoral head, the ilioacetabular, ischioacetabular, and puboacetabular physes are not visible on standard radiographs, making the closure time for these regions difficult to determine. As a result, definitive data regarding the closure of the ilioischial and iliopubic acetabular physes are lacking (4).

The proximal femur initially grows as a single physis until around 2-3 months of age, after which it divides into the capital and trochanteric physes, as a result of the pull of the muscles attached to the region. The capital physis is responsible for approximately 30-40% of the longitudinal growth of the femur in animals. In transverse cross-section, the capital physis exhibits an L-shaped profile, which confers resistance to both shear and rotational forces encountered during growth (5). The growth plate of the greater trochanter contributes to the overall development of the proximal femur but does not influence its longitudinal growth (6). In cats, the capital physis typically closes between 30-40 weeks (7), whereas in dogs, this closure occurs between 6-12 months (8).

The vascular supply to the proximal femur can be categorized into extraosseous, intracapsular, and intraosseous vessels. The extraosseous vessels, in order of importance, include the lateral and medial circumflex femoral arteries, the caudal and cranial gluteal arteries, and the iliolumbar artery. These vessels contribute to the formation of an extracapsular vascular ring located at the base of the femoral neck, which gives rise to the intracapsular and intraosseous vascular networks. This vascular ring enters the joint capsule distally and spreads toward the epiphysis, where it forms an intracapsular ring near the capital physis. Branches from this ring penetrate the physis and develop into an intraosseous arcuate network that supplies both the epiphysis and proximal femoral neck (5, 6, 9, 10).

Since these arteries originate from a single ring, any traumatic damage to the region may disrupt the blood circulation of the femoral neck and head. This is especially concerning in juvenile animals, where incomplete bone development may predispose them to vascular damage. Such disruption, whether due to trauma or surgical intervention, can result in complications such as abnormal femoral head and neck development, femoral head resorption, and the onset of degenerative joint disease (5).

In young cats, in addition to the vascular network surrounding the femoral neck, the femoral head receives blood supply from a branch of the medial circumflex femoral artery, which traverses from the acetabulum to the femoral head alongside the ligamentum capitis ossis femoris (11). In contrast, this artery does not contribute to the femoral head's blood supply in dogs, making them more susceptible to avascular necrosis of the femoral head and neck following trauma compared to cats (8, 12).

2. Classification of Fractures

Acetabular fractures are classified as cranial, central and caudal. If the fracture line includes the area in front of the acetabular notch, it is called "cranial", if it includes the area behind the acetabular notch, it is called "caudal", and if it includes the acetabular notch on both sides, it is called "central" fracture (13) (Figure 1A).

Proximal femur fractures are generally classified as intracapsular or extracapsular according to the location of the fracture in relation to the joint capsule. Intracapsular fractures include epiphyseal, physeal, subcapital, and transcervical fractures (Figure 1B). Ekstracapsular fractures include cervical, intratrochanteric, subtrochanteric

fractures. This classification system is related to the vascular supply of the femoral head and neck. Accordingly, it has been stated that intracapsular fractures are more likely to result in avascular necrosis and that the classification system can potentially be used as a prognostic indicator (5).

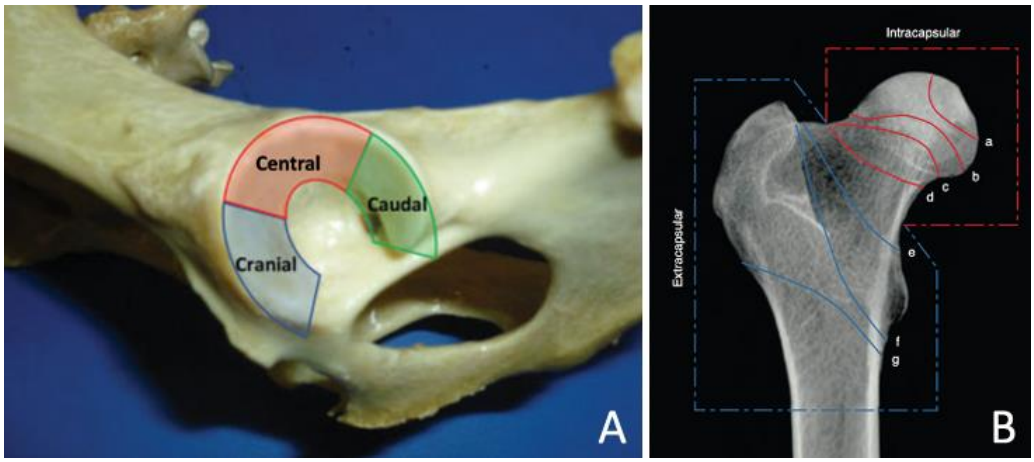

Figure 1: (A) Classification of acetabular fractures (14). (B) Classification of proksimal femoral fractures. Intracapsular fractures include epiphyseal (capital) (a), physeal (b), subcapital (c) and transcervical (d) fractures (5).
Şekil 1: *(A) Acetabular kırıkların sınıflandırılması (14). (B) Proksimal femoral kırıkların sınıflandırılması. İntrakapsüler kırıklar epifizeal (capital) (a), fizeal (b), subcapital (c) ve transcervical (d) kırıkları içerir (5).*

3. Incidence of Acetabular And Proximal Femoral Fractures

Acetabular fractures represent the lowest incidence among pelvic fractures and typically occur unilaterally (15). Pelvic fractures as a whole account for approximately 25% of all fractures in dogs, with acetabular fractures constituting 12–20% of these cases (16, 17, 18). In cats, pelvic fractures comprise 20–22% of all fractures, with acetabular fractures contributing 17.5–26% of these pelvic fractures (15, 19).

The development of acetabular fractures is often attributed to the force generated during trauma. This force may originate laterally at the greater trochanter or from the extremity in extension, with energy being transmitted proximally along the femur. Therefore, acetabular fractures are frequently observed alongside other pelvic fractures (17, 20). Unlike stress fractures, which occur in racing greyhounds due to repetitive or excessive microtrauma to the musculoskeletal system, trauma-induced acetabular fractures are typically associated with significant displacement of bone fragments. Stress fractures, by contrast, are limited to the acetabulum and exhibit minimal displacement, as they arise without external trauma (21).

Proximal femur fractures constitute approximately 25% of femur fractures. In young animals, the majority of proximal femur fractures (70%) are capital physeal fractures. Although rare, capital epiphyseal fractures involve the avulsion of the epiphyseal fragment, which often remains attached to the ligament of the femoral head (5). In cats, proximal femur fractures predominantly involve the femoral neck until the first 6 months of age, while between 6-10 months they mostly occur in the epiphysis of the femoral head. This transition reflects differences in bone strength during growth, as the femoral neck is more susceptible to fracture in younger animals. After the closure of the growth plates, which typically occurs by one year of age, epiphyseal fractures of the femoral head become uncommon (22). Physeal fractures of the proximal femur are most often classified as Salter-Harris types I and II and represent 16% of all physeal fractures. Proximal femur fractures may also occur spontaneously as a result of pathological disorders (23).

Capital physeal dysplasia, also referred to as spontaneous capital physeal fractures, is characterized by the unilateral or bilateral separation of the femoral head epiphysis from the proximal femoral metaphysis along the physeal growth plate, occurring in the absence of trauma. These lesions, commonly associated with acute epiphysiolysis observed radiographically, predominantly affect cats under two years of age. Differentiating these fractures from

trauma-induced physeal injuries can be challenging due to their radiographic similarities (14). Although more frequently reported in cats, this condition has also been documented in dogs (24).

The condition is most prevalent in young (<2 years old), neutered, obese male cats (6, 25, 26). The underlying cause is multicentric chondrocyte disorder (dysplasia) in the physis, leading to physeal separation (10). Androgens, which play a critical role in initiating and terminating physeal closure and growth. In males, androgens, predominantly testosterone, are produced in the Leydig cells of the testicles, while in females, this androgen is mainly produced in the zona reticularis region of the adrenal cortex. Following neutering in males, the removal of the testicles significantly reduces androgen levels, resulting in delayed physeal closure when compared to intact males or neutered females (27). This hormonal deficiency slows cartilage maturation and prolongs the period during which the physis remains open, making it structurally weaker and more susceptible to mechanical forces (28, 29, 30). As the dysplastic growth plate fails to tolerate biomechanical stresses, it separates from the femoral neck. Over time, remodeling and sclerosis occur, eventually leading to pseudoarthrosis formation (26, 28, 31). Neutering is also associated with secondary physiological changes, including elevated insulin levels, which have been implicated in weight gain (32). Additionally, obesity is exacerbated by increased caloric intake and reduced energy expenditure. As a result, obesity is a frequently observed comorbidity in cats diagnosed with capital physeal dysplasia (33).

Metaphyseal osteopathy represents another pathological condition affecting the proximal femur. It remains unclear whether this condition constitutes an independent pathology or arises secondary to a primary fracture, followed by subsequent new bone formation. Some authors propose that initial bone loss, as observed in Legg-Calvé-Perthes disease in dogs, may predispose to secondary femoral neck fractures (2).

Increased or decreased vascularization in the metaphysis (such as Legg Calve Perthes) can result in necrosis, collapse or fracture of the femoral neck. Radiographic features of this condition often include osteolysis and remodeling of the femoral neck (11). Interestingly, in cats, changes in metaphyseal circulation do not typically affect the epiphysis, as the epiphysis receives additional vascular support from the ligamentum capitis ossis femoris. This contrasts with the vascular anatomy of dogs, where the epiphysis lacks such auxiliary support, leading to different pathological outcomes (2). Chronic fractures can often be differentiated from acute traumatic ones based on the presence of metaphyseal remodeling and the typically older age of affected patients (14).

Both capital physeal dysplasia and metaphyseal osteopathy of the femoral neck result from conditions such as dysplasia of the physis or impaired vascularization in the proximal femur. These pathological disorders share common radiographic findings, including sclerosis, osteolysis, and remodeling, which can make distinguishing between them challenging, particularly in chronic cases (2). Accurate differentiation of these pathological fractures from trauma-induced fractures is critically important. Pathological fractures occur in structurally compromised bone, increasing the likelihood of complications such as nonunion following surgical intervention. In contrast, traumatic fractures typically carry a lower postoperative risk of nonunion due to the relatively healthier bone structure (14).

4. Biomechanics of The Hip Joint

Healthy bone tissue possesses inherent material properties and is resistant to specific force modalities. As long as the applied forces remain below the bone's resistance threshold, fractures do not occur. Instead, the bone absorbs the load, undergoes elastic deformation, temporarily alters its shape, and reverts to its original form once the force is removed. Under normal physiological conditions, such as those imposed by gravity and routine movements, healthy bone tissue can withstand these forces without fracturing. When subjected to repetitive forces, bones adapt through a dynamic process involving bone resorption and subsequent new bone formation. However, if the magnitude and duration of stress on the bone exceed the remodeling capacity, excessive resorption may occur. Without sufficient time for remodeling, stress fractures can develop (21). This process represents an adaptive mechanism wherein the organism attempts to strengthen the bone by deconstructing and reconstructing it under persistent stress. However, this destruction-reconstruction cycle relies on a delicate balance. If adequate rest periods are not provided to allow for the necessary reconstruction, bone tissue weakens, increasing the risk of fractures even under normal physiological loads. Studies in humans have demonstrated that individuals undergoing continuous training subject their bones to repetitive

stress, leading to plastic deformation and structural changes in the musculoskeletal system (34).

Biomechanical analysis of the canine hip has revealed that during running, the primary forces transmitted from the extremities to the hip act within the horizontal plane and are directed cranially (35). Based on this, some authors have hypothesized that caudal acetabular fractures occur in areas subjected to minimal force, making non-displaced fractures potentially amenable to conservative management (13, 36, 37, 38). In an experimental study, canine acetabulum were divided into cranial, central, caudal and axial, middle, abaxial regions and subjected to load testing. Results showed that the cranial and caudal one-third portions of the acetabulum bore loads 7.9 and 13.1 times greater, respectively, than the central region. Similarly, the abaxial and middle portions of the acetabulum carried loads 72.4 and 351 times greater than the axial portion, respectively (39). A similar study on cat acetabulum divided the acetabulum into cranial, central, and caudal segments, filled the regions with fast-setting alginate, and subjected them to load testing. The findings indicated that the central and caudal regions bore significantly higher loads than the cranial region, though the cranial portion still contributed to load-bearing (40).

Given these findings, it has been argued that the fracture location does not significantly influence treatment options, as all acetabular regions contribute to weight-bearing (40, 41). This supports the recommendation that no acetabular fracture should be treated conservatively (36). However, conservative management may be suitable for stable, minimally displaced, or non-displaced fractures that do not involve the facies lunata, especially in young dogs. Even in such cases, stabilization using an Ehmer sling is advised (17).

The femoral neck provides structural support to the femoral head and aligns the diaphysis laterally to the body wall, allowing for a wide range of motion in the hip joint. However, this configuration also exposes the femoral neck to significant bending stresses during daily activities. The proximal femur’s trabecular architecture is specifically adapted to withstand these forces and is reinforced cranially by the linea transversa, a bony ridge extending from the femoral head base to the greater trochanter (17).

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5. Complication of Acetabular and Proximal Femoral Fractures

Osteonecrosis and nonunion are significant complications following femoral neck fractures, with osteonecrosis occurring in approximately 20% of cases (42, 43, 44). In animals with incomplete bone development, outcomes such as femoral neck shortening and hip joint instability may result after repairing femoral neck fractures or epiphyseal separations, potentially leading to hip joint dysplasia. Radiographically, narrowing of the collum femoris, often described as an “apple core” shape, occurs in about 70% of cases undergoing internal fixation. However, this rarely leads to collapse of the femoral neck (45, 46).

It was previously thought that Salter-Harris type I fractures occur in the hypertrophic zone of the growth plate, allowing continued growth if the epiphyseal blood supply remains intact. However, more recent histopathological studies show that in over 75% of traumatic fractures, the fracture line traverses the physis, separating the reserve zone from the epiphysis (8). This is critical since the proliferative zone's vascular networks are limited, and the hypertrophic zone is avascular. Damage to the epiphyseal blood supply or proliferative cells may lead to premature growth plate closure, ossification, and the formation of nonunion or pseudoarthrosis (23, 47).

Prompt surgical intervention is generally believed to reduce the risk of osteonecrosis in femoral neck fractures, though evidence from randomized controlled trials is lacking (48, 49). Although early surgery does not appear to have a beneficial effect on mortality or function in patients with a hip fracture alone, early surgery has been associated with less pain, shorter hospital stay, and fewer postoperative complications in stable patients (50). Delayed fracture repair can lead to fibrous adhesions and muscle contractions, making fracture reduction more difficult and increasing morbidity (51). deally, surgical treatment should be performed within 24 hours, provided the patient's condition permits (46).

In human studies, the incidence of postoperative avascular necrosis varies widely, ranging from 3-47%. Factors such as screw placement within the epiphysis, the number of screws used, fracture displacement, and difficulty in reduction significantly affect the risk of avascular necrosis. There is a strong correlation between the degree of epiphyseal displacement and the likelihood of necrosis. Reduction during the operation increases the likelihood of avascular necrosis by causing the vessels that adapt to the fracture area to stretch. Interestingly, patients with bilateral fractures exhibit lower rates of avascular necrosis, likely due to decreased mobility compared to those with unilateral fractures (52).

Postoperative complications usually manifest within six weeks and may include transient demineralization or slight narrowing of the femoral neck, though the final outcome of the femoral head and neck may take up to six months to evaluate fully. In cases of coxofemoral luxation, epiphysiolysis, or femoral neck fractures, narrowing of the femoral neck is common but rarely leads to functional issues (46).

Conservative management of proximal femur fractures is often associated with chronic pain, lameness, muscle atrophy, secondary osteoarthritis (OA), and hypertrophic pseudoarthrosis. Several factors complicate surgical repair, including vascular trauma, residual growth potential in the capital physis, eccentric loading of the femoral head, limited bone stock for stabilization, and potential for joint locking (5).

In animals with open acetabular physes, premature physeal closure due to trauma can result in reduced acetabular depth, sclerosis, and subluxation of the femoral head (4). Although conservative management of acetabular fractures may be appropriate in skeletally immature animals due to their greater bone remodeling capacity and higher healing potential, surgical intervention is typically indicated for all types of acetabular fractures in skeletally mature animals (53).

Pelvic fractures, in the absence of accompanying thoracic or cranial trauma, are infrequently fatal. Nonetheless, these injuries are associated with significant morbidity, primarily due to soft tissue damage. Critical structures, such as the urinary bladder or the intrapelvic urethra, are particularly vulnerable, leading to complications such as rupture (41). Mortality from high-energy trauma involving the pelvis typically arises from secondary systemic effects, including multi-organ failure and sepsis (54).

Acetabular fractures, as intra-articular injuries, are inherently predisposed to long-term complications, most notably osteoarthritis (OA), regardless of the quality of anatomical reduction achieved (17). This inevitability is attributed to the high kinetic energy absorbed by both osseous and cartilaginous structures during trauma. However, early anatomical reduction has been shown to attenuate the progression of OA (55, 56). Despite this benefit, surgical intervention may be delayed in the presence of life-threatening injuries, which complicates and often precludes achieving optimal reduction (20). Notably, the extent of fragment displacement—a parameter reflecting the trauma's severity—plays a more critical role in prognosticating outcomes than the timing of surgical intervention (17).

The development of degenerative joint disease (DJD) following acetabular trauma stems from two primary mechanisms. The first is direct mechanical damage to the joint at the time of injury, while the second involves a progressive deterioration of articular cartilage due to abnormal pressure distributions caused by joint incongruity (36). Malalignment within the joint leads to accelerated wear of articular cartilage and irregular intra-articular pressure gradients. As such, the primary surgical objective in acetabular fractures is to restore joint congruity. Conservative management, while potentially alleviating symptoms temporarily, fails to stabilize the joint adequately, thus perpetuating intra-articular incongruity and exacerbating the risk of OA. Consequently, surgical intervention significantly lowers the incidence of OA compared to non-operative approaches (17, 57, 58).

Cats' lower body weight and efficient locomotor system may make them more amenable to conservative treatment of acetabular fractures. However, even in such cases, long-term complications, including a painful hip joint and muscle atrophy, may occur (59). Recent studies have revealed that only 50% of cats diagnosed with acetabular fractures undergo surgical intervention, despite the clear indication for surgery in these cases (15). Displaced fractures necessitate surgical intervention to prevent severe complications, such as narrowing of the pelvic canal, constipation, or degenerative joint disease (4, 46 60).

Pelvic fractures typically result from high-energy traumatic events, necessitated by the robust protective role

of the surrounding musculature. The extensive muscle masses that envelop the pelvic region act as a buffer, absorbing lower-energy impacts and thereby reducing the likelihood of fractures under normal circumstances. However, the high energy required to breach this protective mechanism often produces significant comorbidities alongside the pelvic fracture itself. Research conducted in 2002 identified that between 59% and 72% of patients with pelvic fractures also presented with non-orthopedic injuries. These injuries most commonly involved the urinary system and neurological structures, underscoring the systemic implications of pelvic trauma (61). Among the structures at heightened risk in pelvic trauma are the urinary bladder and the urinary tract. Their vulnerability can be attributed to their anatomical position and relative exposure during high-energy impacts (37). Studies have consistently demonstrated that urethral injuries are disproportionately prevalent in male patients, a phenomenon that may be explained by the unique anatomical configuration of the male urethra. Specifically, the *urethra masculina* is anatomically tethered to the ischial structures via the *ischiocavernosus* and *ischiourethralis* muscles, which creates a biomechanical predisposition to injury under high-impact conditions (62).

Sciatic nerve injury, although more commonly associated with ilial fractures and sacroiliac luxations, can also occur in acetabular fractures. The nerve is highly susceptible to compression from fracture fragments or manipulation during reduction, as it closely contacts the medial surface of the ilium. Anatomical reduction and rigid internal fixation can minimize callus formation and reduce the risk of secondary nerve compression (60, 63). Neurological evaluation in animals with pelvic fractures may be challenging, but absence of withdrawal reflex or deep pain perception can indicate significant sciatic nerve damage, negatively affecting the prognosis for limb function recovery (38).

6. Fixation Methods

The surgical management of acetabular fractures necessitates meticulous anatomic reduction, rigid and stable fixation, and the restoration of joint surface congruity to prevent secondary complications such as degenerative joint disease. Achieving these objectives minimizes the risk of callus formation through primary bone healing mechanisms. Among the fixation implants available, plates are uniquely capable of providing the structural conditions required for optimal outcomes (14, 64, 65, 66).

Fixation methods for acetabular fractures

Plates: The standard approach for repairing acetabular fractures involves securing an acetabular plate along the dorsal surface of the acetabulum (46). However, the inherently curved anatomy of the acetabulum poses challenges to achieving accurate plate contouring. Inadequate shaping of the plate can lead to poor bone-implant contact, compromising the reduction and potentially resulting in a loss of stability during screw tightening (64). The caudal acetabulum is particularly vulnerable due to limited bone stock in this region, increasing the likelihood of screw loosening in fractures affecting the caudal fragment. In such cases, extending the implant toward the ischium can enhance stabilization by securely anchoring the caudal fragment (67). Reconstruction plates are preferred over standard veterinary acetabular plates for their adaptability in these complex scenarios.

The primary implants used in acetabular fracture management include standard reconstruction plates, "String of Pearl" (SOP) plates, and “C”-shaped veterinary acetabular plates (VAP). Although dynamic compression plates (DCP), screw-wire-polymethylmethacrylate (VTP) constructs, and external fixators have been historically used, their application is now generally limited to situations where the aforementioned implants are unavailable or due to the surgeon’s familiarity with alternative methods.

Locking plates, which function biomechanically as internally placed external fixators, offer significant advantages in certain fracture patterns. These plates reduce the need for precise shaping—a critical challenge in acetabular fracture repair—and provide enhanced stability in comminuted fractures. By avoiding compression between the bone and plate, locking systems preserve periosteal blood flow and reduce the risk of vascular compromise (68). Additionally, locking systems distribute axial forces through the screws, minimizing the stress on individual screws and reducing the risk of screw loosening or failure (69). Comparative studies have shown no significant differences between locking and non-locking plate systems in terms of achieving and maintaining anatomic reduction or structural

strength. However, these findings may be influenced by the fact that both plate types were precisely contoured to the bone in the study designs. In cases where ideal plate-bone congruency cannot be achieved, locking plates demonstrate superior biomechanical performance, particularly in osteoporotic or osteopenic bone models (68).

Veterinary acetabular plates, with their characteristic "C" shape, are specifically designed to conform to the dorsal acetabular surface, simplifying their application. However, achieving anatomic reduction remains a challenge, and reduction loss can occur during screw tightening (41, 46). Locking versions of these plates may provide additional stability in cases where ideal contact between the implant and bone is less feasible. While C-shaped plates require less contouring than straight implants, they may not be suitable for fractures extending cranially along the *corpus ossis ilium*, caudal acetabular fractures, or comminuted patterns. These plates are most effective for treating fractures confined to the middle one-thirds of the dorsal acetabular surface (60). In veterinary practice, acetabular plates are more commonly utilized in dogs compared to cats. This disparity is attributed to anatomical and size differences between the two species. The broader adoption of these implants in canine patients highlights their utility in accommodating the unique biomechanical demands of larger species (70).

String of Pearl Plates (SOP) plate system represents a versatile locking plate design, characterized by a series of spherical components ("pearls" or "nodes") interconnected by cylindrical segments ("internodes"). This unique geometry allows the SOP plates to be contoured in three planes without compromising their locking capability. Specifically, these plates can tolerate bending up to 40° and rotation up to 20° along their longitudinal axis while maintaining structural integrity and mechanical locking properties. This adaptability makes SOP plates particularly suitable for securing fixation on the irregularly curved dorsal surface of the acetabulum (65). A significant advantage of SOP plates is their relatively narrow profile compared to conventional plate systems, simplifying their placement on the limited dorsal width of the acetabulum (67).

A significant advantage of SOP plates is their relatively narrow profile compared to conventional plate systems, simplifying their placement on the limited dorsal width of the acetabulum. Furthermore, SOP plates demonstrate superior bending strength in vitro compared to commonly used implants such as Limited Contact Plates (LCP), Dynamic Compression Plates (DCP), and Limited Contact Dynamic Compression Plates (LC-DCP). Research suggests that SOP plates retain their mechanical strength and rigidity after shaping, performing comparably to unshaped, conventional DCPs in terms of resistance to bending forces (71). Despite their favorable bending characteristics, SOP plates may be less effective under torsional forces compared to locking compression plates (LCP). In static and cyclic load testing, LCPs demonstrated superior torsional strength, while SOP plates exhibited higher resistance to bending forces (72). This disparity could have clinical implications, as certain acetabular fractures are prone to failure due to ventrolateral rotation of the caudal segment (65, 73).

A biomechanical comparison of SOP plates with Veterinary Tension Plates (VTP) and Veterinary Acetabular Plates (VAP) revealed that SOP plates offer superior load-bearing capacity. Specifically, SOP plates demonstrated 16% and 30% greater flexural strength compared to 3.5 mm LCPs and LC-DCPs, respectively (74). However, it is noteworthy that the failure loads in these studies exceeded physiologic forces encountered in vivo, suggesting that all systems would be sufficient to maintain fracture reduction in clinical scenarios (75).

Reconstruction plate, crafted from relatively less rigid materials, are characterized by "V"-shaped grooves positioned between the screw holes. Due to these features, they are relatively easy to shape to fit irregular and curved bone surfaces, but due to these same features, they are weaker than similarly sized dynamic compression plates. These plates are designed to be used in less load-bearing bones such as the pelvis and mandible (5, 76). When 3.5 mm reconstruction plates were compared to 2.7 mm acetabular plates, the latter provided superior performance in terms of ease of shaping and precision of fracture fragment reduction. While the reconstruction plate showed no measurable stiffness advantage, the acetabular plate was inherently better suited for achieving and maintaining accurate reduction in complex acetabular fractures (77, 78).

Recent developments in minimally invasive plate osteosynthesis (MIPO) have introduced novel techniques for managing acetabular fractures. One such approach utilizes preoperative 3D modeling to optimize implant design and placement. In this technique, computed tomography (CT) scans are used to generate a three-dimensional model of the

intact acetabulum, enabling precise pre-contouring of locking compression plates. During surgery, the pre-shaped plates are introduced through an epiperiosteal tunnel created via small incisions at the ilium and ischium. This method minimizes surgical exposure, preserves soft tissue integrity, and potentially reduces perioperative complications associated with extensive surgical dissections (57).

Other Methods: The use of a Type IA external fixator (EF) has been reported in the management of acetabular fractures in animals with unclosed physeal plates (3). This approach offers several advantages, including minimally invasive application, reduced disruption to surrounding tissues, and a lower risk of infection, which collectively contribute to faster healing times (53). However, evidence regarding whether EF provides definitive benefits over alternative fixation methods remains inconclusive and requires further investigation.

The Screw/Wire/Polymethylmethacrylate (SWP) technique involves a systematic approach to stabilize acetabular fractures. Following anatomic reduction of the fracture fragments, Kirschner wires (K-wires) are strategically placed within the fragments to maintain alignment. Screws are subsequently positioned in the cranial and caudal acetabular fragments. A cerclage wire, typically arranged in a figure-of-eight configuration, is then passed around these screws and tensioned to neutralize tensile forces along the dorsal acetabular margin. The screws and cerclage wire are subsequently encased in polymethylmethacrylate (PMMA), enhancing fixation strength and stability (78). The dorsal acetabulum is suitable for tension band application because it is a tension surface. However, this technique is less appropriate for oblique fractures, as the tension bands may inadvertently draw fragments together, resulting in fragment overlap (4).

This fixation method can be used with or without composite (PMMA), but naturally the use of composite significantly enhances fixation quality. In biomechanical studies comparing constructs with and without PMMA, the non-composite group demonstrated stability against ventral distraction forces alone. Conversely, constructs incorporating PMMA displayed additional resistance to rotational, dorsal, medial, and lateral distraction forces. This increase in stabilization was attributed to the rotational and distraction forces being absorbed and eliminated by PMMA (73).

In acetabular fractures, the caudal fragment is frequently displaced caudally, ventrally, and medially, primarily due to the mechanical pull exerted by the biceps femoris, semitendinosus, and semimembranosus muscles. To counteract these forces and prevent reduction loss, it has been recommended to insert 2-3 Kirschner wires before the application of PMMA (65). While increasing the number of K-wires enhances stabilization, the placement of additional wires poses technical challenges due to the limited space and potential for interference with other implants (58). Biomechanical analyses have shown that even a single K-wire contributes significantly to stabilization after PMMA application. However, the placement of multiple K-wires can complicate screw positioning and increase the risk of implant conflicts (65).

The SWP technique is associated with several potential complications, including, abduction deformities caused by contact between the femoral head and PMMA, restricted joint range of motion, penetration of K-wires into the pelvic canal, ventrolateral bending of K-wires, fracture or failure of PMMA, thermal damage to adjacent tissues during the exothermic polymerization of PMMA, challenges in removing PMMA in cases of infection, impaired radiographic evaluation of fractures due to PMMA opacity (4, 58, 65, 73, 78).

Plate luting, an alternative fixation method, involves the application of PMMA between the plate and bone or between the screw heads and plate. This technique increases the contact area between the implant and the underlying bone, facilitating precise fracture reduction and minimizing implant movement during screw tightening. Consequently, plate luting helps maintain anatomic alignment and enhances stabilization (64).

Fixation methods used in proximal femur fractures

Conservative treatment of fractures in the proximal femur is generally associated with a high incidence of complications, including nonunion, malunion, pseudoarthrosis, and secondary osteoarthritis (OA). These outcomes underscore the importance of surgical intervention for fractures in this region. Surgical approaches are broadly classified into primary repair techniques and salvage procedures, with the choice of method depending on factors such

as the patient's age, the time between trauma and treatment, vascular supply to the area, fracture location, and the surgeon's expertise. In cases where pre-existing hip joint arthritis is present, primary repair is typically unnecessary, and salvage methods such as total hip replacement (THR) or femoral head and neck excision arthroplasty are recommended (5, 23).

Open reduction and internal fixation (ORIF) has historically been a preferred method for managing femoral head and neck fractures. This technique typically involves the use of multiple Kirschner wires (K-wires), pins, or screws to stabilize the fracture fragments. However, ORIF carries potential complications, particularly in young animals. These include premature closure of the proximal femoral physis, femoral head or neck deformities, loss of reduction, resorption of the femoral neck, and the progression of degenerative joint disease (79). To maximize mechanical strength and minimize interference with normal growth, K-wires should ideally be placed parallel to each other. This configuration not only ensures greater stability but also allows for continued physeal growth in young animals (14). Parallel pin placement also distributes mechanical forces evenly between the pins, thereby reducing the likelihood of implant failure. In contrast, divergent pin placement may impair physeal growth by creating a locking effect on the growth plate, potentially resulting in premature closure of the physis. Divergent pinning also unevenly distributes forces across the implants, weakening the construct and predisposing it to failure (5).

Proper alignment of pins or wires during placement is critical to the success of ORIF. Implants must be oriented to match the inclination and anteversion angles of the femoral neck to maximize mechanical stability. Parallel pins or screws positioned to follow these angles ensure optimal force distribution across the fracture site and reduce the risk of reduction loss. Additionally, correct orientation facilitates dynamic compression of the fracture, promoting more efficient and stable healing (46).

While three-pin fixations are biomechanically stronger and more rigid compared to one- or two-pin constructs, the positioning of the implants is a more critical determinant of success than their number or diameter. This is primarily due to the significant role of local blood supply preservation in fracture healing. Disruption of vascularity, especially in young animals with open growth plates, can impair healing and compromise long-term outcomes. Pins that are well-distributed across the fracture site improve stability without excessively interfering with local vascularity, while implants that are clustered or poorly oriented may exacerbate complications (80).

Parallel pin placement provides several advantages, including uniform load distribution and minimal disruption to the physeal growth plate. Forces applied to parallel pins are transmitted equally, allowing dynamic compression across the fracture and ensuring that physiological loading of the growth plate continues uninterrupted. In contrast, divergent pins unevenly distribute loads, leading to implant fatigue and failure. Furthermore, divergent pin placement often results in excessive stress concentration on individual implants, weakening the overall construct and increasing the likelihood of mechanical complications (5).

Fluoroscopy-guided percutaneous pinning represents a less invasive alternative to open reduction and internal fixation (ORIF) for managing epiphyseal fractures in dogs and cats. This technique minimizes iatrogenic damage to local soft tissues and vascular structures, promoting faster postoperative recovery. However, the success of this method depends on achieving an adequate reduction prior to fixation, as it is best suited for fractures with minimal displacement between fragments. The use of closed reduction avoids unnecessary trauma to the surrounding tissue, but complications such as intra-articular pin migration, nonunion, and malunion remain significant risks (79).

Radiographic modalities like fluoroscopy and computed tomography (CT) may overestimate the distance between the screw tip and the articular surface, potentially increasing the likelihood of pin migration into the joint space (81). Furthermore, fluoroscopy-guided percutaneous pinning is a technically demanding procedure, requiring advanced skills in both intraoperative imaging and minimally invasive techniques—skills that are not yet widely adopted in veterinary surgical practice (14). Repeated attempts at fracture reduction, often necessitated by poor intraoperative visualization or inadequate reduction, may inadvertently exacerbate local trauma and disrupt blood flow, further complicating healing.

In skeletally mature animals, fixation with anti-rotational Kirschner wires (K-wires) or threaded cortical screws can provide stable fixation. However, these implants are contraindicated in young, skeletally immature patients, as they

can cause physeal compression and premature closure of the growth plate, leading to long-term developmental complications (14). Studies have shown that interfragmentary compression screws, while effective in certain contexts, are associated with high risks of premature physeal closure, femoral neck resorption, and degenerative joint changes in the acetabulum when used in young animals (5). Due to these risks and the technical challenges associated with their application, compression screws are rarely preferred for these cases, especially given their lack of mechanical superiority over pins or wires. Regardless of the implant type, trauma incurred during the surgical procedure itself increases the likelihood of premature growth plate closure. Comparative studies have demonstrated that both a single lag screw and three parallel 2.0 mm K-wires can withstand forces equivalent to three times an animal's body weight. These methods are generally considered suitable for large-breed patients, while two parallel or divergent K-wires are more appropriate for smaller breeds (46).

Cannulated screws are characterized by a central channel through the screw body and head, allowing the passage of a guide K-wire. The guide wire is first placed across the fracture site to achieve and temporarily stabilize the reduction. The cannulated screw is then advanced over the guide wire and fixed into the bone using a specialized screwdriver. Notably, cannulated screws are equipped with self-drilling tips, reducing the need for pre-drilling and thereby minimizing bone trauma.

The smaller diameter of the guide wire, relative to the screw, ensures minimal disruption to the bone during insertion. However, improper orientation of the screw during placement may necessitate its removal and reinsertion. This repeated manipulation can create larger voids in the cancellous bone, potentially disrupting local circulation and weakening the fixation. Biomechanically, cannulated screws are slightly inferior to non-cannulated screws of the same size due to their hollow structure, but studies have shown no significant differences in clinical performance between the two types (82).

Salvage Procedure

Femoral head and neck excision (FHNE), involving resection of the femoral head and neck, serves as a vital salvage procedure for patients in which conventional repair methods are contraindicated, unsuccessful, or associated with severe complications. This procedure is particularly effective in relieving pain and restoring mobility in small patients (weighing <15 kg) with acetabular, femoral head, or neck fractures. In larger patients (weighing >15 kg) or those with contralateral hip pathology, total hip replacement (THR) is typically the preferred salvage option.

When appropriately selected and performed according to established surgical principles, excision arthroplasty yields favorable outcomes, particularly in small-breed dogs and cats. However, in larger patients, biomechanical limitations may result in suboptimal recovery, and total hip replacement may provide superior functional outcomes. Despite its advantages, THR is associated with risks, including prosthesis-related complications and technical challenges during implantation. For cases where neither excision arthroplasty nor total hip replacement can resolve the issue, **amputation** becomes the final treatment option. While amputation is rarely necessary in cats due to their smaller size and adaptability (83), it may be required in severe or refractory cases in dogs.

7. Surgical Approach

Various surgical techniques are available to address acetabular and proximal femoral fractures in dogs and cats. Commonly utilized approaches include:

- Craniodorsal approach with craniolateral incision
- Craniodorsal and caudodorsal approach with trochanter major osteotomy (Gorman method)
- Craniodorsal and caudodorsal approach with tenotomy of gluteal muscles can be used (47).

Each approach has unique advantages and limitations, and their impact on epiphyseal circulation remains a subject of ongoing debate. The craniolateral approach offers excellent access to the proximal femoral epiphysis, physis, and femoral neck. To enhance visibility, particularly in complex cases, it may be combined with greater trochanter osteotomy or gluteal muscle tenotomy. Despite its utility, the craniolateral approach is associated with potential disruption of critical vascular structures. Specifically, it can cause trauma to vessels supplying the extracapsular

vascular ring, including the caudal gluteal artery and the lateral femoral circumflex artery. These same vessels are also at risk of damage during a greater trochanter osteotomy, as performed in the Gorman approach (5).

The dorsal approach with osteotomy of the greater trochanter is widely regarded as the gold standard for providing optimal surgical visualization of acetabular and proximal femoral fractures. This approach involves detaching the gluteus medius and gluteus profundus muscles from the greater trochanter and retracting them dorsally to expose the joint capsule. Once the capsule is incised and extended cranially and caudally, the femoral head and neck become visible, facilitating fracture reduction and fixation. However, ligamentum capitis femoris transection, often necessary to facilitate reduction of femoral head or neck fractures, compromises joint stability and disrupts blood flow to the femoral head in cats. This vascular compromise significantly increases the risk of avascular necrosis (46, 84).

Sciatic nerve protection is paramount during this approach, as the nerve lies just caudal to the greater trochanter. Care must be taken to avoid iatrogenic damage during osteotomy and throughout the procedure (38). This method allows comprehensive exposure of the dorsal acetabulum, the caudal ilium, and the cranial ischium, providing excellent access for manipulation of the femoral head and neck as well as plate placement.

For juvenile and young animals, where iatrogenic injury to the physis of the greater trochanter is a concern, tenotomy of the gluteus medius and gluteus profundus muscles serves as a viable alternative to greater trochanter osteotomy. This approach avoids potential physeal damage while providing equivalent surgical visibility of the acetabulum and sciatic nerve (46, 85). Comparative studies have shown no significant difference between the two techniques regarding surgical exposure or outcomes (60, 86).

The intergluteal approach provides access to the acetabulum by retracting the gluteal muscles without detachment or osteotomy. While this method can be effective in cats and small dogs, its utility in larger dogs is limited due to inadequate surgical exposure. In large dogs, visualization and manipulation of fracture fragments, as well as plate placement, are significantly more challenging (87).

The dorsal acetabular rim has a triangular cross-sectional shape that tapers laterally, necessitating careful plate placement. Plates should be positioned as medially as possible, closer to the cavum pelvis, to maximize the depth and bone volume available for screw fixation. This positioning reduces the likelihood of screw penetration into the acetabulum and enhances fixation stability. Proper placement ensures sufficient cortical bone engagement while minimizing the risk of intra-articular complications.

Conflict of Interest

The authors declared that there is no conflict of interest.

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2. Derginin yayım dili Türkçe veya İngilizce'dir.
3. Dergide, tamamı daha önce başka bir yerde yayımlanmamış güncel konulara ilişkin özgün bilimsel araştırmalar, derlemeler, olgu sunumları ve kısa bilimsel çalışmalar yayımlanır. Derleme niteliğindeki çalışmalar, ilgili bilim insanlarından davet usulü ile talep edilir.
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7. Makalenin başlığı kısa ve açık olmalı; ilk sözcüğün başlangıcı büyük, diğerleri küçük harflerle olacak şekilde, yazılmalıdır ("Köpek ve kedilerde uterus patolojileri" gibi). Varsa çalışmaya ilişkin açıklama dipnot işareti ile gösterilmelidir.
8. Yazar/yazarların, ad ve soyadları makale başlığının altına yazılmalıdır; adresleri ve unvanları ilk sayfada dipnot şeklinde belirtilmelidir. Yazarların ORCID numaralarını belirtmeleri zorunludur.
9. Özet, makalenin önemli noktalarını içerecek tarzda kısa ve açık olmalıdır. Türkçe Özet, en az 150, en fazla 250 sözcük olmalıdır. Anahtar sözcükler MeSH (Medical Subject Headings) terimlerine uygunluk açısından Türkiye Bilim Terimleri'nden seçilmeli ve en az 3, en fazla 5 adet olacak şekilde alfabetik olarak sıralanmalıdır. Yabancı dilde Özet (Abstract), en az 200, en fazla 300 sözcük olmalıdır. Yabancı dilde anahtar sözcükler MeSH terimlerine uygun olmalı ve en az 3, en fazla 5 adet olacak şekilde alfabetik olarak sıralanmalıdır. Anadili Türkçe olmayan yazarlardan Türkçe özet istenmez.
10. Giriş bölümünde, çalışma ile doğrudan ilgili kısa literatür bilgisi ve çalışmanın orijinalliği ile ilgili bilgi verildikten sonra, son paragrafta çalışmanın amacı vurgulanmalıdır. Bu bölüm 2 sayfayı geçmemelidir.
11. Gereç ve Yöntem, gereksiz ayrıntıya girilmeden, öz ve anlaşılır biçimde yazılmalıdır. Etik kurul izni gerekli ise mutlak suretle belirtilmelidir. (Kurum, Tarih, sayı numarası ile)
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15. Yazarlar her bir bilimsel kısaltmanın açılımını metinde ilk geçtiği yerde açıklamalıdırlar. Latince cins ve tür isimleri italik yazı tipi ile yazılmalıdır. Tüm ölçüler SI (Systeme Internationale)'ye göre verilmelidir.
16. Tartışma ve Sonuç bölümünde, veriler literatür bilgilerinin ışığında tartışılmalı ve yorumlanmalıdır.
17. Kaynakça gösteriminde Vancouver stili kullanılmalıdır. Kaynakça gösterimi ile ilgili detaylara aşağıda yer verilmiştir. (Dergi yazım kuralları ile uyumlu Endnote stili, dergi web sitesinden indirilebilir)
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National Organization for Rare Diseases [Online]. 1999 Aug 16 [cited 1999 Aug 21]; Available from: URL:http://www.rarediseases.org/
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