MAKÜ BURDUR MEHMET AKİF ERSOY ÜNİVERSİTESİ

VETERİNER PATOLOJİ DERNEĞİ

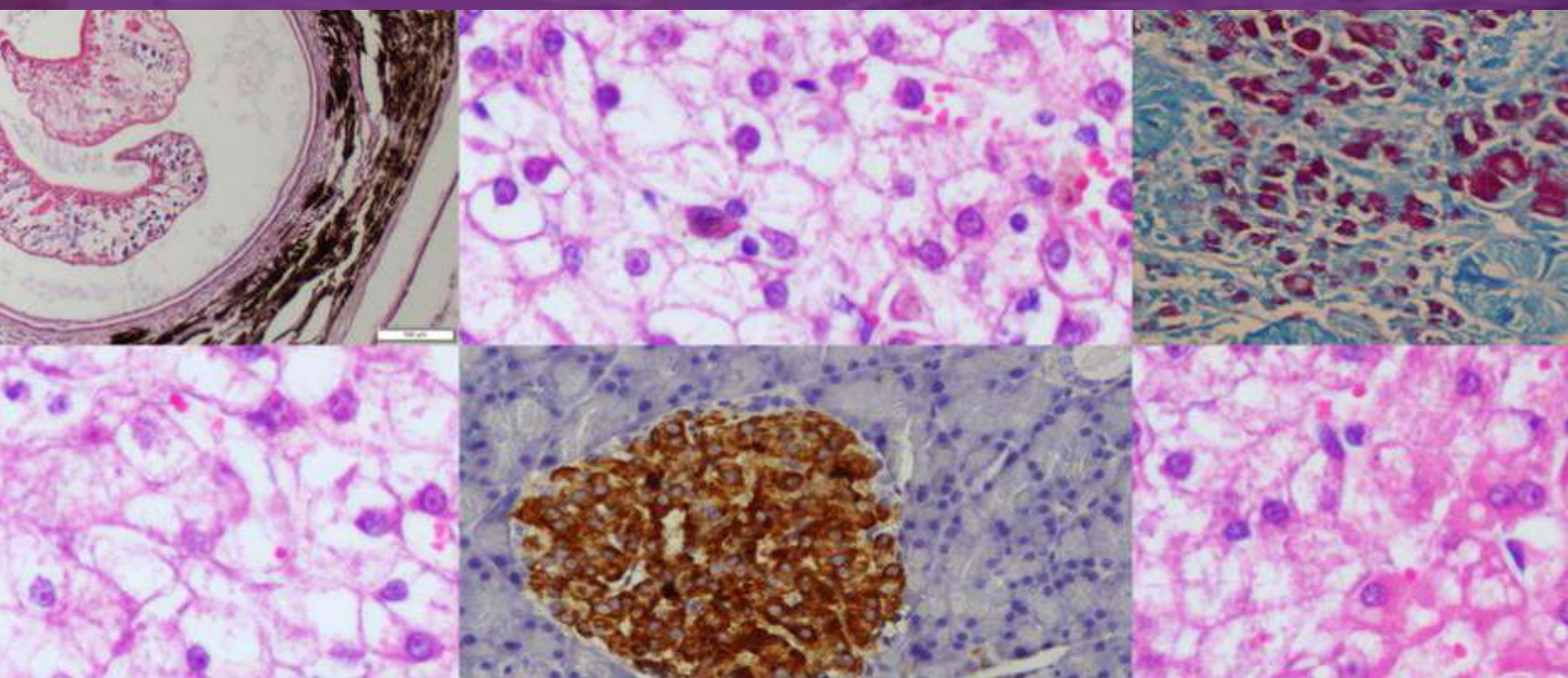
VETPAT-2020
Burdur/TÜRKİYE

VET PAT 2020

X. NATIONAL & I. INTERNATIONAL
VETERINARY PATHOLOGY
CONGRESS (Online)

27-31 OCTOBER 2020 BURDUR/TÜRKİYE

ABSTRACT AND FULL TEXT BOOK



SCIENTIFIC PROGRAM

27.10.2020 Time	Congress Opening
10:00-11:00	 Prof. Dr. Özlem ÖZMEN Chair of the VETPAT2020  Prof. Dr. Rıfkı HAZIROĞLU Chair of the Veterinary Pathology Association  Prof. Dr. Hakan ÖNER Dean of the MAKU Veterinary Faculty  Prof. Dr. Adem KORKMAZ Rector of the MAKU

27.10.2020 11:15-12:30	Oral Presentations Session-1		
Time	**Topic**	**Speaker**	**Chairs of Session**
11:15-11:30	Pathological and Immunohistochemical Findings in Chukar Partridge (Alectoris Chukar) in Turkey	Özlem ÖZMEN	Prof Dr Osman KUTSAL Prof Dr Sevil ATALAY VURAL
11:35-11:50	Pathological and Immunohistochemical Investigations on the Prophylactic Effects of Oleuropein in Streptozotocin Induced Diabetes mellitus	Özlem ÖZMEN	
11:55-12:10	Diabetogenic Effects of Ochratoxin A in Female Rats	Firdevs MOR SAVAŞ	
12:15-12:30	The histopathological and molecular diagnosis of myocardial sarcocystosis in sheep and the correlation of parasitic lesions with MMP - 2 and MMP – 9	Rahşan YILMAZ	
12:30-13:50	Lunch Break		

27.10.2020 14:00-15:55	Oral Presentations Session-2		
Time	Topic	Speaker	Chairs of Session
14:00-14:15	Pathological effects of Eustrongylides excisus (Nematoda) in some fish species (Aphanius iconii, Atherina boyeri, Knipowitschia caucasica and Sander lucioperca) of Eğirdir Lake	Özlem ÖZMEN	Prof Dr M. Kemal ÇİFTÇİ Prof Dr Zafer ÖZYILDIZ
14:20-14:35	Investigation of Inducible Nitric Oxide Synthase, CD3, And CD8 Immunoexpressions in Lamb Pneumonia With Respiratory Syncytial Virus Infection	Turan YAMAN	
14:40-14:55	Expression of epithelial-mesenchymal transition markers in bovine chronic mastitis	Ayşe Meriç MUTLU	
15:00-15:15	Identification of mast cells in acute and chronic bovine mastitis in the context of fibrosis	Ayşe Meriç MUTLU	
15:20-15:35	Combined effect of fat graft and platelet-rich fibrin on tissue recovery in temporomandibular joint defects	Senem Esin YAVAŞ	
15:40-15:55	Combined Effect of Fat Graft and Platelet-Rich Fibrin on Perineural Adhesion and Scar Formation in a Rat Model	Senem Esin YAVAŞ	

27.10.2020 16:00-17:10	Invited Speakers Session-1		
Time	**Topic**	**Speaker**	**Chairs of Session**
16:00-16:30	Human cancers challenge a pet model: the case of Canine Oral Melanoma	 Prof Dr Massimo CASTAGNARO -  Ginevra BROCCA	Prof Dr Özlem ÖZMEN
16:40-17:10	Infectious Disease of Parrots with Special Emphasis on Patho(physio)logical Changes	 Prof Dr Gerry DORRESTEIN	Prof Dr Mahmut SÖZMEN

28.10.2020 10:00-11:55	Oral Presentations Session-3		
Time	**Topic**	**Speaker**	**Chairs of Session**
10:00-10:15	Microbiological and Pathomorphological Examination of Scopulariopsis Brevicaulis Infection in Lungs and Beaks in a Canary Herd	Arda Selin TUNÇ	Prof Dr M. Yavuz GÜLBAHAR Prof Dr Şule YURDAGÜL ÖZSOY
10:20-10:35	Gastrointestinal stromal tumor in a female Terrier dog	Özkan YAVAŞ	
10:40-10:55	Nervous system and skull malformations on Pomeranian dog: Anencephaly, bifid tongue, and cleft palate	Özkan YAVAŞ	
11:00-11:15	Mandibular Changes in Rats With Type I Postmenopozal Osteoporosis	Kürşat FİLİKCİ	
11:20-11:35	Methods of Inducing Allergic Contact Dermatitis with 2,4-dinitrofluorobenzene in BALB/c Mice	Büşra GÜLBENLİ	
11:40-11:55	The Role of Chemokine (TARC / CCL17) Regulated by Thymus Activation in Atopic Dermatitis	Büşra GÜLBENLİ	
12:00-13:15	Lunch Break		

28.10.2020 13:30-15:55	Oral Presentations Session-4		
Time	Topic	Speaker	Chairs of Session
13:30-13:45	A Case of Tuberculosis in a Mackerel (Scomber scombrus)	Özlem ÖZMEN	Prof Dr Rıfkı HAZIROĞLU
14:50-14:05	A Case of Fibromyxolipoma in a Golden Fish	Özmen ÖZMEN	
14:10-14:35	Extracellular Vesicles: New Players in the Field of Veterinary Medicine	Valentina MOCCIA	
14:40-14:55	Histopatological and Electron Microscopic Studies on Porcine Proliferative Ileitis	Kiril K. DIMITROV	
15:00-15:25	Herpesvirus antigen detection in paraffinized lung sections of pneumonic sheep lung using immunohistochemistry	Keivan JAMSHIDI	Prof Dr Mahmut SÖZMEN
15:20-15:35	Histopathogic evaluation of neuroprotective effect of chrysin in cerebellar neurotoxicity induced by acrylamid in rat	Keivan JAMSHIDI	
15:40-15:55	Osteochondrosis in a new-born calf: a case report	Davide PINTUS	

28.10.2020 ***16:00-17:40***	***Invited Speakers Session-2***		
Time	***Topic***	***Speaker***	***Chairs of Session***
16:00-17:00	***Diagnosis and Prognosis of canine and feline proliferative histiocytic diseases***	 ***Prof Dr Habil Matti KIUPEL***	***Prof Dr Rıfkı HAZIROĞLU***
17:10-17:40	***Foodborne mycotoxicoses: risk assessment and underestimated hazard for animals or humans (review)***	 ***Prof Dr Stoycho Dimitrov STOEV***	***Prof Dr M. Yavuz GÜLBAHAR***

29.10.2020 10:00-11:55	Oral Presentations Session-5		
Time	Topic	Speaker	Chairs of Session
10:00-10:15	Diastematomyelia and Hydromyelia Case in a Goat Kid	Mehmet HALIGÜR	Prof Dr Tolga GÜVENÇ Prof Dr Recai TUNCA
10:20-10:35	Investigation of p53, perp and e-cadherin expressions in bovine skin papilloma	Zafer ÖZYILDIZ	
10:40-10:55	Ethylene Glycol Toxicity in Calves	Zafer ÖZYILDIZ	
11:00-11:15	Investigation of Neuroprotective Effect of Kefir in Experimental Spinal Cord Injury	Şule YURDAGÜL ÖZSOY	
11:20-11:35	The first case of fibrosarcoma detected in a sultan's parrot	Timuçin Çağdaş KURT	
11:40-11:55	Investigation of Antiproliferative Effects of Grape Vinegar on Myeloma Cells	Muhammet Mükerrem KAYA	
12:00-13:15	Lunch Break		

29.10.2020 13:20-15:55	Oral Presentations Session-6		
Time	**Topic**	**Speaker**	**Chairs of Session**
13:20-13:35	A Case of Monkey Pox in a Baby Monkey	Özlem ÖZMEN	Prof Dr Zafer ÖZYILDIZ Assoc Prof Dr Rahşan YILMAZ
13:40-13:55	A Case of Renal Adenocarcinoma in a Male Rat	Özlem ÖZMEN	
14:00-14:15	Pathological Investigations in Tench (Tinca tinca (L., 1758)) Naturally Infected with Ligula intestinalis Plerocercoids	Ahmet AYDOĞAN	
14:20-14:35	The Immunohistochemical Detection of Toll Like Receptors in Mycoplasma Pneumonia	Gözde YÜCEL TENEKECİ	
14:40-14:55	Cardioprotective Effects of Fetal Kidney Derived Mesenchymal Stem Cells on Doxorubicin-induced Cardiotoxicity in Rats	Orhan YAVUZ	
15:00-15:25	Immunohistochemically investigation of oxidative stress-induced DNA damage in papillomas and fibropapillomas	Emin KARAKURT	
15:20-15:35	Mammary Squamous Cell Carcinoma in a Dog	Tuncer KUTLU	
15:40-15:55	Avocado (Persea americana) toxicity in six rabbits	Onur KIZILGÜN	

29.10.2020 16:00-17:40	Invited Speakers Session-3		
Time	Topic	Speaker	Chairs of Session
16:00-17:00	Practical Rodent Pathology	 Assoc Prof Dr Cory BRAYTON	Prof Dr Tolga GÜVENÇ
17:10-17:40	Reproductive pathologies in farm animals:the use of the ultrasonography as diagnostic tool	 Prof Dr Calogero STELLETTA	Prof Dr Oğuz KUL

30.10.2020 10:00-11:55	Oral Presentations Session-7		
Time	**Topic**	**Speaker**	**Chairs of Session**
10:00-10:15	Histoprotective effect of Resveratrol and Avocado Oil against Acetaminophen related acute nephrotoxicity in rats	Şükriye YEŞİLOT	Prof Dr Oğuz KUL
10:20-10:35	Septicemia in a Salamander	Aynur ÇINAR	
10:40-10:55	Pulmonary Aspergillosis in a 20 Day Old Suckling Lamb	Emre KURTBAŞ	
11:00-11:15	A Case of Enzootic Nasal Adenocarcinoma (ENA) in a Honamli Goat	Merve GÜNDÜZ	Prof Dr M. Özgür ÖZYİĞİT
11:20-11:35	Investigation of the presence of coccidiosis by histopathological methods in geese in the Kars region	Ayfer YILDIZ	
11:40-11:55	A Case of Spinal Nephroblastoma in a Dog	Oğuzhan KAPLAN	
12:00-13:15	Lunch Break		

30.10.2020 13:20-15:55	Oral Presentations Session-8		
Time	**Topic**	**Speaker**	**Chairs of Session**
13:20-13:35	Experimental animal model 'concept' and special experimental models	Oğuz KUL	Prof Dr Özlem ÖZMEN
13:40-13:55	Hepatic Encephalopathy in Van Cats: Clinical, Hematological and Pathological Findings	Tuğçe SÜMER	
14:00-14:15	Investigation of Vascular Endothelial Growth Factor (Vegf) Activity in Ascites Syndrome of Meat Type Chicken	Ömer ARDA	
14:20-14:35	Visceral cavernous hemangiosarcoma with common metastasis in a captive graywolf (Canis lupus): A case report	Aysun SARIÇETİN	
14:40-14:55	Effects of Nanoparticle Ozone Solutions to Laparatomy Incision Line Injection in Rats: An Experimental Research	Aysun SARIÇETİN	Prof Dr Ahmet AYDOĞAN
15:00-15:25	A Case of Granulosa Cell Tumor in a Female Iguana	Melike ALTINTAŞ	
15:20-15:35	Trials of Histochemical Methods on Epon-Embedded Semithin Sections	Ayşe AKBAŞ	
15:40-15:55	Confocal Microscope and Its Usage in Pathology	Özgür ÖZÖNER	

30.10.2020 16:00-17:50	Invited Speakers Session-4		
Time	**Topic**	**Speaker**	**Chairs of Session**
16:00-16:30	International Veterinary Pathology Coalition (IVCP)	 Prof Dr Leonardo LEONARDI	Prof Dr M. Özgür ÖZYİĞİT
16:40-17:10	Imaging sublingual microcirculation in horses anaesthetized for elective surgery and colic surgery: a pilot study.	 Dr Christelle MANSOUR	Prof Dr Recai TUNCA
17:20-17:50	Place and Importance of Micro-RNAs in Scientific Studies	 Assoc Prof Dr Mustafa ÖZKARACA	Prof Dr Mehmet HALIGÜR

31.10.2020 10:00-11:55	Oral Presentations Session-9		
Time	Topic	Speaker	Chairs of Session
10:00-10:15	Caseous Lymphadenitis in a Llama	Ozan AHLAT	Assoc Prof Dr Ahmet AKKOÇ
10:20-10:35	The Effect of Sepsis on the Lung Tissue; the Role of Agomelatin	Mustafa SAYGIN	
10:40-10:55	Investigation of the Protective and Therapeutic Effects of Thiamine in Thioacetamide-Induced Liver Injury	Şerife AĞIRCA	
11:00-11:15	Exocrine Pancreatic Atrophy in a Dog	Oya Burçin DEMİRTAŞ	Assoc Prof Dr Mehmet Eray ALÇIĞIR
11:20-11:35	Effects of Subcutaneous Injection of Nano Particle Ozone Solution on Tissue in Rats: An Experimental Study	Zehra AVCI KÜPELİ	
11:40-11:55	A Case of Calcinosis Circumscripta in a Belgian Malinois Dog	Zehra AVCI KÜPELİ	
12:00-13:15	Lunch Break		

31.10.2020 13:20-16:15	Oral Presentations Session-10		
Time	Topic	Speaker	Chairs of Session
13:20-13:35	Investigation of the harmful effects of Electric Field (50 Hz) on Female Rat Ovaries, Uterus and Vagina and the role of Astaxanthin	İlker GÜNYELİ	Prof Dr Mehmet HALIGÜR
13:40-13:55	Evaluation of Some Immunohistochemical Markers in the Diagnosis of Canine Cutaneous and Subcutaneous Round Cell and Soft Tissue Tumors	Volkan İPEK	
14:00-14:15	Golden Retriever Irkı Bir Köpekte Burunda Transisyonel Karsinom Olgusu	Leyla Elif Özgü AYÖZGER	
14:20-14:35	Tricuspid Valve Endocardiosis in a Puppy with Distemper	Leyla Elif Özgü AYÖZGER	
14:40-14:55	The Effect of Experimental Sepsis Model Created with Lipopolysaccharide on Nasal and Oral Mucosa and the Role of Astaxanthin	Yücel KURT	
15:00-15:15	A Case of Generalized Tuberculosis in a Domestic Pigeon	Adem MİLLETSEVER	Assoc Prof Dr Turan YAMAN
15:20-15:35	The Relationship Between Histopathological Evaluation and Prognosis in Canine Cutaneous Mast Cell Tumors	Işıl Aytemiz DANYER	
15:40-15:55	Examination of Ruminant Cases with Botulismus Suspected by Immunohistochemical Method	Mehmet Burak ATEŞ	
16:00-16:15	Investigation of the therapeutic effect of poppy oil in bovine dermatophytosis by pathological methods	Şaban YÖNTER	

31.10.2020 16:20-18:10	Invited Speakers Session-5		
Time	**Topic**	**Speaker**	**Chairs of Session**
16:20-16:50	The Role Of Chronic Exposure To Amoxicillin/Clavulanic Acid On The Developmental Enamel Defects In Mice	 Assoc Prof Dr Aurelian Sorin PASCA	Prof Dr Özlem ÖZMEN
17:00-17:30	Immunohistochemical Evaluation of Hepatic Progenitor Cells in Different Types of Feline Liver Diseases	 Dr Yanad Abou MONSEF	Prof Dr Ahmet AYDOĞAN

31.10.2020 18:00-19:00	Closing Session
Closing Speech and Final Statement Determining the Best Oral and Poster Presentations Wishes and Requests Planning the Next Congress	Prof Dr Rıfkı HAZIROĞLU Chair of the Veterinary Pathology Association Prof Dr Özlem ÖZMEN Chair of the VETPAT2020

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CONTENTS

ORAL PRESENTATIONS

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ORAL PRESENTATIONS

Pathological and Immunohistochemical Findings in Chukar Partridge (*Alectoris Chukar*) in Turkey*

Ozlem OZMEN[1], Tamer ALBAYRAK[2]

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[2]Burdur Mehmet Akif Ersoy University, Faculty of Arts and Science, Department of Biology, Burdur, Turkey

Abstract

In this study, samples for pathological examination were collected from 232 Chuckar partridges from the field and selected from breeding stations. Sections were taken from tissues that underwent routine tissue procedures for histopathological and immunohistochemical examinations, and were stained and examined under a microscope. Samples collected from hunters from the field were directly evaluated. Birds with evidence of disease or disability were selected to collect pathological samples from breeding stations. First of all, the clinical findings of these partridges were evaluated and noted. Subsequently, they were euthanized, necropsied, and blood and tissue samples were collected. Traumatic lesions and inflammatory reactions were mostly observed in the eyes and extremities of the partridge in the production station. In necropsies, the most common finding was enteritis, and liver lesions in some small number of partridges were found. At the histopathological examination, pneumonia, anthracosis, inflammatory changes in the digestive system were among the most common findings. Interestingly, liver parasites were found commonly in field samples. At the immunohistochemical examinations of the tissues evaluated for Tuberculosis, Marek's Disease, Adenovirus and Aspergillosis, it was determined that Marek diseases and Tuberculosis were not found in the breeding stations, Aspergillosis in only 1 partridge and Adenovirus antigens in 1 partridge in breeding stations. In this study, Marek's Disease was detected in 3 cases, Tuberculosis disease in 1 partridge and Aspergillosis in 2 cases in the field samples. As a result of the study, the high rate of hepatitis in the breeding stations (6 of 12 cases in the breeding stations) suggested that attention should be paid to feeding and feed quality. In addition, the high rate of pneumonia and anthracosis cases detected in the Kahramanmaraş breeding station showed that there was a problem with air quality in this region. Again, the high eye problems in the Kahramanmaraş breeding station made us think that it was caused by the crowded and

congested breeding at this station. However, it was thought that the samples taken from breeding stations were collected only from animals showing symptoms of disease, which was also effective on this rate. As a result, the data of this study showed that partridge from both breeding stations and nature are not an important source of diseases, but especially partridge released from breeding stations can carry some microorganisms.

Keywords: Partridge, disease, diagnosis.

*This research was supported by grants from the Scientific and Technological Research Council of Turkey (Grant Number: TUBITAK-117O580).

Türkiye'deki Kekliklerde (*Alectoris Chukar*) Patolojik ve İmmunohistokimyasal İncelemeler*

Özlem ÖZMEN[1], Tamer ALBAYRAK[2]

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[2]Burdur Mehmet Akif Ersoy Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Burdur, Türkiye

Özet

Bu çalışmada sahadan ve üretme istasyonlarından seçilen 232 kınalı keklikten patolojik inceleme için örnekler toplandı. Rutin doku prosedüründen geçirilen dokulardan histopatolojik ve immunohistokimyasal incelemeler için kesitler alınarak boyandı ve mikroskopta incelendi. Sahadan avcılardan toplanan örneklerin doğrudan değerlendirilmesine geçildi. Üretme istasyonlarından patolojik örnek toplamak için hastalık bulgusu gösteren veya sakat olan kuşlar seçildi. Bu kekliklerin öncelikle klinik bulguları değerlendirildi ve not edildi. Ardından ötenazileri gerçekleştirilerek, nekropsileri yapıldı, kan ve doku örnekleri toplandı. Üretme istasyonundaki kekliklerde en fazla göz ve ekstremitelerde travmatik lezyonlar ve yangısal reaksiyonlar gözlendi. Nekropsilerde ise en sıklıkla enteritis ve bazı az sayıdaki keklikte karaciğer lezyonları saptandı. Histopatolojik incelemede ise pnömoni, antrakozis, sindirim sisteminde yangısal değişiklikler en sık rastlanan bulgulardandı. Saha örneklerinde ilginç bir şekilde yaygın karaciğer parazitlerine rastlandı. Dokuların Tüberküloz, Marek, Adenovirus ve Aspergillozis yönünden yapılan immunohistokimyasal incelemelerinde üretme istasyonlarında Marek ve Tüberküloz hastalıklarının bulunmadığı, bir üretme istasyonunda sadece 1 keklikte Aspergillozis ve bir üretme istasyonunda 1 keklikte de Adenovirus antijenleri bulunduğu tespit edildi. Bu çalışmada saha numunelerinde Marek Hastalığına 3 olguda, Tüberküloz hastalığına 1 keklikte ve Aspergillozise ise 2 olguda rastlandı. Çalışma sonucunda üretme istasyonlarında hepatitise yüksek oranda rastlanması (12 olgunun 6'sı üretme istasyonlarında) yemleme ve yem kalitesine dikkat edilmesi gerektiği fikrini uyandırdı. Ayrıca pnömoni ve antrakozis olgularının Kahramanmaraş üretme istasyonunda yoğun olarak saptanması bu bölgede hava kalitesi ile ilgili bir problemin olduğunu gösterdi. Yine Kahramanmaraş üretme istasyonunda göz problemlerinin yüksek olması bu istasyonda kalabalık ve sıkışık yetiştirmeden kaynaklandığı düşündürdü. Ancak özellikle üretme istasyonlarından alınan örneklerin sadece hastalık

semptomu gösteren hayvanlardan toplanmış olmasının da bu oran üzerinde etkili olduğu düşünüldü. Sonuç olarak bu çalışmanın verileri gerek üretme istasyonlarında gerekse doğadaki kekliklerin incelediğimiz hastalıklar yönünden önemli bir kaynak olmadığını ancak özellikle üretme istasyonlarından sahaya salınan kekliklerin bazı mikroorganizmaları taşıyabileceğini göstermiştir.

Anahtar kelimeler: Keklik, hastalık, teşhis.

*Bu çalışma TÜBİTAK tarafından 17O580 numara ile desteklenmiştir.

Pathological and Immunohistochemical Investigations on the Prophylactic Effects of Oleuropein in Streptozotocin Induced Diabetes mellitus

Ceren DOLU[1], Özlem ÖZMEN[2]

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Abstract

Diabetes Mellitus (DM); the most common endocrine disorder characterized by impaired glucose tolerance or insulin deficiency. patients with Diabetes have to use insulin or antidiabetic drugs throughout their lives. The difficulty of administration of insulin injections increased the interest in oral antidiabetic use. There are several studies that suggest that antioxidant plants reduce the oxidative effects of diabetes. Among these, oleuropein, which is one of the components of olive leaf, has an antioxidant property. The aim of this study was to investigate the effects of oleuropein in experimentally induced diabetes with streptozotocin in mice. In the study, 40 mice were divided into 4 groups of 10 animals each and oleuropein was administered by oral gavage for one month and then diabetes was induced. Five days after the administration of streptozotocin, all mice were euthanized and urine, blood and pancreas samples were collected. Insulin and glucose levels in serum and presence of glycosuria in urine samples were investigated. Pancreas samples were evaluated histopathologically and immunohistochemically. Streptozotocin administration was shown to decrease insulin and increase in glucose levels in serum samples, and glycosuria in urine. Histopathological examination of pancreas samples showed that streptozotocin administration caused to hyperemia, vacuolar degeneration and small necrosis in Langerhans islets. Immunohistochemical evaluation showed that streptozocin caused decreased in insulin and insulin receptor and increased glucagon expression. Oleuropein was observed to cause improvement in both biochemical and pathological findings. This study showed that oleuropein is effective in the prophylaxis of diabetes.

Keywords: Diabetes mellitus, immunohistochemistry, oleuropein, pathology, prophylaxis.

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Streptozotosin ile İndüklenen Diabetes Mellitus'ta Oleuropeinin Profilaktik Etkileri Üzerine Patolojik ve İmmunohistokimyasal İncelemeler

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Özet

Diabetes mellitus (DM); en yaygın görülen endokrin bozukluk olup bozulmuş glikoz toleransı veya insülin eksikliğiyle karakterizdir. Diyabet hastaları hayatları boyunca insülin ya da antidiyabetik ilaç kullanmak zorundadır. İnsülin enjeksiyonlarının uygulama zorluğu oral antidiyabetik kullanımlarına olan ilgiyi artırmıştır. Antioksidan özellik taşıyan bitkilerin, diyabetin oksidatif etkilerini azalttığı yönünde çeşitli araştırmalar mevcuttur. Bunlar arasında zeytin yaprağının bileşenlerinden biri olan oleuropeinin antioksidan özelliğe sahip olduğuyla ilgili görüşler vardır. Bu çalışmanın amacı; farelerde streptozotosinle deneysel olarak oluşturulan diyabet modelinde oleuropeinin etkilerinin incelenmesidir. Çalışmada toplamda 40 fare 10'ar hayvandan oluşan 4 gruba ayrılmış ve bir ay süreyle oleuropein oral gavaj yoluyla verilip ardından diyabet oluşturuldu. Streptozotosin uygulamasından 5 gün sonra tüm farelere ötenazi uygulanarak idrar, kan ve pankreas örnekleri toplandı. Serum örneklerinde insülin ve glikoz düzeyleri, idrarda glikozüri varlığı incelendi. Pankreas örnekleri ise histopatolojik ve immunohistokimyasal olarak değerlendirildi. Streptozotosin uygulamasının serum örneklerinde insülin düzeylerinde düşüş, glikoz düzeylerinde ise artışa ve idrarda glikozüriye sebep olduğu gözlendi. Pankreas örneklerinin histopatolojik incelemesinde streptozotosin uygulamasının hiperemi, Langerhans adacıklarındaki hücrelerde vakuoler dejenerasyon ve küçük nekrozlara yol açtığı gözlendi. İmmunohistokimyasal değerlendirmede streptozosinin insülin ve insülin reseptör aktivitesinde azalma, glukagon ekspresyonlarında artışa sebep olduğu gözlendi. Oleuropeinin hem biyokimyasal hem de patolojik bulgularda düzelmeye sebep olduğu görüldü. Bu çalışma oleuropeinin diyabetin profilaksisinde etkili olduğunu gösterdi.

Anahtar kelimeler: Diabetes mellitus, immunohistokimya, oleuropein, patoloji, proflaksi

Bu çalışma Burdur Mehmet Akif Ersoy Üniversitesi Bilimsel Araştırma Projeleri Komisyonu tarafından desteklenmiştir (Proje No: 0504-YL-18).

Diabetogenic Effects of Ochratoxin A in Female Rats

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Abstract

In this study, the diabetogenic effects of long term Ochratoxin A (OTA) administration in rats were investigated, and its role in the etiology of diabetes mellitus (DM) was examined utilizing 42 female Wistar rats for these purposes. The rats were divided into three different study and control groups according to the duration of the OTA administration. The rats received 45 mu g OTA daily in their feed for 6, 9 and 24 weeks, respectively. Three control groups were also used for the same time periods. Blood and pancreatic tissue samples were collected during the necropsy at the end of the 6, 9 and 24 weeks. The plasma values of insulin, glucagon and glucose were determined for the study and control groups. Pancreatic lesions were evaluated via histopathological examination and insulin and glucagon expression in these lesions was subsequently determined using immunohistochemical methods. Statistically significant decreases in insulin levels were observed, in contrast to increases in blood glucagon and glucose levels. Histopathological examinations revealed slight to moderate degeneration in Langerhans islet cells in all OTA-treated groups. Immunohistochemistry of pancreatic tissue revealed decreased insulin and increased glucagon expression. This study demonstrated that OTA may cause pancreatic damage in the Langerhans islet and predispose rats to DM.

Keywords: Ochratoxin A; insulin; glucagon; glucose; rat plasma; pathology; immunohistochemistry

Dişi Ratlarda Okratoksin A'nın Diyabetojenik Etkileri

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Özet

Bu çalışmada, ratlarda uzun süreli Okratoksin A (OTA) uygulamasının diyabetojenik etkileri araştırıldı ve bu amaçla 42 dişi Wistar rat kullanılarak OTA'nın diabetes mellitus (DM) etiyolojisindeki rolü incelendi. Ratlar, OTA uygulama süresine göre üç farklı çalışma ve kontrol grubuna ayrıldı. Sırasıyla 6, 9 ve 24 hafta boyunca ratların yemlerinde 45 ug OTA katıldı. Aynı zaman dilimleri için üç kontrol grubu da kullanıldı. Çalışmada 6, 9 ve 24 haftalar sonunda ratlara ötenazi uygulandı, nekropsi sırasında kan ve pankreas doku örnekleri alındı. Çalışma ve kontrol grupları için plazma insülin, glukagon ve glikoz değerleri ölçüldü. Pankreaslar histopatolojik olarak değerlendirildi ve daha sonra insülin ve glukagon ekspresyonları immünohistokimyasal yöntemlerle belirlendi. Kan glukagon ve glukoz seviyelerindeki artışların aksine, insülin seviyelerinde istatistiksel olarak anlamlı düşüşler gözlendi. Histopatolojik incelemelerde, OTA ile tedavi edilen tüm gruplarda Langerhans adacıklarındaki hücrelerde hafif ila orta derecede dejenerasyonlar gözlendi. Pankreas dokusunun immunohistokimyasal incelemesinde, insülin ekspresyonunda azalma ve glukagon ekspresyonunda artma gözlendi. Bu çalışma, OTA'nın Langerhans adacıklarında hasarına neden olabileceğini ve ratları DM'ye yatkın hale getirebileceğini gösterdi.

Anahtar Kelimeler: Okratoksin A; insülin; glukagon; glikoz; rat plazması; patoloji; immünohistokimya

The Histopathological and Molecular Diagnosis of Myocardial Sarcocystosis in Sheep and The Correlation of Parasitic Lesions with MMP - 2 and MMP – 9

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Abstract

This study was aimed at the histopathological and immunohistochemical investigation of cardiac tissue lesions caused by *Sarcocystis spp.* in sheep, and thereby, the collection of detailed information on the presence of these parasites and the pathogenesis of sarcocystosis in ovine animals. Besides PCR was used for the identification of the parasite species. The microscopic examination revealed that, out of the 45 ovine cardiac tissue specimens examined, 23 contained *Sarcocystis spp.* cysts. Based on PCR analysis results, out of the 23 cardiac tissue specimens infected with sarcocysts, 7 were determined to be infected with *Sarcocystis gigantea* and 16 were ascertained to be infected with *Sarcocystis tenella*. Immunohistochemical examination demonstrated that, semiquantitatively, MMP - 9 staining was stronger than MMP - 2 staining in the periphery of the parasite cysts.

***Keywords*:** Myocardial sarcocystosis, PCR, immunohistochemistry, MMP - 2, MMP - 9, sheep

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Koyunlarda Miyokardiyal Sarkositozun Histopatolojik ve Moleküler Tanısı ve Paraziter Lezyonların MMP - 2 ve MMP - 9 ile Ilişkisi

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Özet

Bu çalışma ile koyunlarda, *Sarcocystis spp.*'nin kalp dokusunda oluşturduğu lezyonlar histopatolojik ve immunohistokimyasal yöntemler ile incelenerek, hem parazitin varlığı hem de patogenezi hakkında ayrıntılı bilgi edinilmesi amaçlanmıştır. Ayrıca parazitin tür tayini için PCR kullanıldı. Toplam 45 koyuna ait kalp dokusunun 23'ünde mikroskobik olarak *Sarcocystis spp.* kistine rastlandı. PCR sekans analiz sonuçlarına göre sarkokistli 23 kalp dokusundan 7'sinde etkenin *Sarcocystis gigantea*, 16'sında *Sarcocystis tenella* olduğu belirlendi. İmmunohistokimyasal incelemede parazit kisti çevresinde semikantitatif olarak MMP-9 boyamalarının MMP-2'ye göre daha yoğun olduğu gözlendi.

***Anahtar kelimeler*:** Miyokardiyal sarkositozis, PCR, İmmunohistokimya, MMP - 2, MMP - 9, koyun

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Pathological Effects of *Eustrongylides excisus* (Nematoda) in Some Fish Species (*Aphanius iconii, Atherina boyeri, Knipowitschia caucasica* and *Sander lucioperca*) of Eğirdir Lake

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Abstract

This study was carried out to determine infection of *Eustrongylides excisus* in fish species inhabiting Egirdir Lake between January and October 2018. Host specimens belonging to *Aphanius iconii, Atherina boyeri, Knipowitschia caucasica* and *Sander lucioperca* were analyzed to investigate the parasite infection. The highest prevalence of infection was determined in pike-perch. The nematode *Eustrongylides excisus* was recorded for the first time on *Knipowitschia caucasica*, a non-native fish species in Eğirdir Lake. Moreover, the present study provides data concerning histopathological aspects of *E. excisus* encysted in four fish species. At the histopathological examination, hyperemia, edema, microhemorrhage, inflammatory reaction and necrosis were observed around the parasites.

Keywords: Alien species, dioctophymatidae, nematoda, histopathology

Eğirdir Gölü'nün Bazı Balık Türlerinde (Aphanius iconii, Atherina boyeri, Knipowitschia caucasica ve Sander lucioperca) Eustrongylides excisus (Nematoda)'un Patolojik Etkileri

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Özet

Bu çalışmada Ocak-Ekim 2018 tarihleri arasında Eğirdir Gölü'nde yaşayan balık türlerinde *Eustrongylides excisus* enfeksiyonu araştırılmıştır. *Aphanius iconii, Atherina boyeri, Knipowitschia caucasica* ve *Sander lucioperca*'ya ait örnekler parazit enfeksiyonunu araştırmak için analiz edilmiştir. En yüksek enfeksiyon yoğunluğu sudakta gözlenmiştir. Nematod *Eustrongylides excisus*, Eğirdir Gölü'ndeki yabancı balık türü *Knipowitschia caucasica*'da ilk kez kaydedilmiştir. Ayrıca, bu çalışmada, dört balık türünde *E. excisus*'un histopatolojik yönlerine ilişkin veriler sunmaktadır. Histopatolojik incelemede parazitlerin çevresinde hiperemi, ödem, mikroskobik kanama, yangısal reaksiyon ve nekroz gözlenmiştir.

Anahtar kelimeler: Yabancı türler, dioctophymatidae, nematoda, histopatoloji

Investigation of Inducible Nitric Oxide Synthase, CD3, and CD8 Immunoexpressions in Lamb Pneumonia with Respiratory Syncytial Virus Infection

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Abstract

Respiratory syncytial virus (RSV) is an RNA virus that belongs to the *Pneumovirus* genus of the Paramyxoviridae family. The aim of this study was evaluating the expressions of inducible nitric oxide synthetase (iNOS), CD3 (pan T cells), and CD8 (cytotoxic T cells) in lamb lungs naturally infected with RSV using the immunohistochemistry (IHC). For this purpose, 100 pneumonia and 10 control lung tissue samples were taken from lambs slaughtered in the slaughterhouse after macroscopic examination. The samples were fixed in 10% neutral formalin and embedded in paraffin using routine methods. Sections of 5 μm in thickness were cut. The streptavidin–peroxidase method (ABC) was used for staining the sections for IHC examination, which revealed RSV positivity in 18 tissues. These positive cases were then evaluated for iNOS, CD3, and CD8 immunostaining and compared to controls. In all these cases, an increase in iNOS expression was detected, whereas $CD3^+$ T lymphocytes were detected in 14 cases and $CD8^+$ T lymphocytes were detected in five cases. Given the increase of iNOS immunoexpression in all RSV-positive cases and increase in the number of CD3 T lymphocytes in most cases, it was concluded that iNOS and $CD3^+$ T lymphocytes play an important role in the immune response in lamb pneumonia arising with natural occurring RSV infection. In addition, these findings inform our understanding of RSV pathogenesis and may simplify the development of new approaches for prevention and treatment.

Key words: CD3, CD8, iNOS, lamb, RSV

This research was supported by the Van Yuzuncu Yil University Scientific Research Projects Foundation (Project number; TYL-2019-8309).

Respiratuar Sinsityal Virus ile Doğal Enfekte Kuzu Pnömonilerinde İndüklenebilir Nitrik Oksit Sentetaz, CD3 ve CD8 İmmun-Ekspresyonlarının Araştırılması

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Özet

Respiratorik Sinsityal Virus (RSV), Paramyxoviridae familyasının Pneumovirus genusuna dahil bir RNA virusudur. Sunulan bu çalışmanın amacı, RSV ile doğal enfekte kuzu akciğer dokularında indüklenebilir nitrik oksit sentetaz (iNOS), CD3 (Pan T hücre) ve CD8 (sitotoksik T hücre) ekspresyonlarını immunohistokimya (IHC) metodu ile değerlendirmektir. Bu amaçla, mezbahanede kesilen kuzulardan, 100 adet pnömonili ve 10 adet kontrol akciğer doku örnekleri makroskobik olarak incelendikten sonra alındı ve %10'luk tamponlu formaldehitte tespit edildi. Rutin doku takibinden geçirilen dokular, histopatolojik incelemeler için Hematoksilen & Eozin (H&E) ile boyandı. Doku kesitleri ayrıca immünohistokimyasal incelemeler için poliklonal antikorlar kullanılarak Streptavidin-peroksidaz (ABC) prosedürüne göre boyandı. İmmunohistokimyasal incelemeler sonucunda onsekiz (18) dokuda RSV pozitif olarak tespit edildi. Daha sonra bu pozitif vakalar iNOS, CD3 ve CD8 immun-ekspresyonları açısından değerlendirildi. Bu vakaların tamamında iNOS ekspresyonunda kontrole göre artış belirlenirken, ondört (14) vakada CD3+ T lenfositlerin sayısında artış tespit edildi. CD8+ T lenfositler ise beş (5) vakada belirlendi. Sonuç olarak, RSV ile pozitif vakalarda iNOS immun-ekspresyonunda artış olması ve vakalrın çoğunda CD3+ T lenfositlerin sayısında artış tespit edilmesi, RSV ile doğal enfekte kuzu pnömonilerinde meydana gelen immun yanıtta, iNOS ve CD3+ T lenfositlerin önemli rol aldıklarını gösterdi.

Anahtar Kelimeler: CD3, CD8, iNOS, RSV, Kuzu.

Bu Araştırma Van Yüzüncü Yıl Üniversitesi Bilimsel Araştırma Projeleri Başkanlığı Tarafından (TYL-2019-8309) No'lu proje olarak desteklenmiştir.

Expression of Epithelial-Mesenchymal Transition Markers in Bovine Chronic Mastitis

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Abstract

Chronic mastitis is characterized by progressive fibrosis and atrophy of the mammary gland. Recently, epithelial-mesenchymal transition has been approved as an important mechanism in the pathogenesis of fibrosis. During this process, epithelial cells undergo morphological and biochemical changes in which cells lose their epithelial markers and cell-cell adhesion complexes and upregulate mesenchymal marker proteins. Cells gain ability to degrade basement membrane, migrate to interstitial tissue and produce extracellular matrix components. The aim of this study was to evaluate important epithelial-mesenchymal transition markers in bovine mammary tissues by immunohistochemistry. Tissue sections from mammary tissues (healthy, acute, and chronic mastitis) were stained immunohistochemically with primary antibodies against E-cadherin, cytokeratin, alpha smooth muscle actin, collagen, β-catenin, and vimentin. For the evaluation of each epithelial-mesenchymal transition markers, ten randomly selected fields were photographed under x400 magnification and 3, 3 – diaminobenzidine (DAB) stained areas in photomicrographs were quantified with Olympus Stream Image Analysis. Results were expressed as mean number of positively stained areas for each marker. E-cadherin staining intensity was significantly decreased in tissues with chronic mastitis (mean: 1.03), compared with healthy (mean: 7.31 and $p<0,001$) and acute mastitis tissues (mean: 6.28 and $p<0,001$). Myoepithelial cells, myofibroblasts and vascular smooth muscle cells stained positively with alpha smooth muscle actin. In chronic mastitis tissues, alpha smooth muscle actin was highly expressed (mean: 11.02), compared with healthy (mean: 6.65 and $p<0,001$) and acute mastitis tissues (mean: 7.38 and p: 0.043). A strong staining for collagen was detected in chronic mastitis tissues (mean: 11.88). In contrast, collagen immunopositivity was decreased

in healthy (mean 6.77 and p: 0,013) and acute mastitis tissues (mean: 6.51 and p: 0,008). Mean vimentin staining intensity was significantly increased in chronic mastitis tissues (11.5), compared with healthy (mean: 3.17 and p<0,001) and acute mastitis tissues (mean: 5.3 and p<0,001). No statistically important differences were found in β-catenin and cytokeratin staining intensity between groups. These results strongly suggest that epithelial-mesenchymal transition contributes fibrogenesis in bovine chronic mastitis.

***Keywords*:** Bovine mastitis, fibrosis, epithelial-mesenchymal transition, immunohistochemistry

This study was supported by The Scientific & Technological Research Council of Turkey (TUBITAK) (Project number: 118O893).

İneklerin Kronik Mastitislerinde Epitelyal-Mezenkimal Geçiş Belirteçlerin İncelenmesi

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Özet

Kronik mastitis ilerleyici fibrosis ve meme dokusunun körelmesi ile karakterizedir. Son yıllarda epitelyal-mezenkimal geçişin fibrozis patojenezinde önemli bir mekanizma olduğu gösterilmiştir. Bu süreç esnasında, epitel hücreleri morfolojik ve biyokimyasal değişiklikler geçirerek epitelyal belirteçlerini ve hücre-hücre adezyon komplekslerini kaybederler ve mezenkimal belirteç proteinlerinin up-regülasyonunu gerçekleştirirler. Hücreler bazal membranı yıkımlama yeteneği kazanır, interstisyel dokuya göç ederler ve ekstraselüler matriks bileşenlerinin üretimini gerçekleştirirler. Bu çalışmanın amacı, sığır meme dokularında epitelyal-mezenkimal geçiş yönünden önemli olan belirteçleri immunohistokimyasal olarak değerlendirmektir. Meme dokularından (sağlıklı, akut ve kronik mastitisli) hazırlanan doku kesitleri, E-kaderin, sitokeratin, alfa-düz kas aktini, kollajen, β-katenin ve vimentine karşı oluşturulmuş primer antikorlar kullanılarak immunohistokimyasal olarak boyanmıştır. Her bir epitelyal-mezenkimal geçiş belirtecinin değerlendirilmesi için, x400'lük büyütmede rastgele seçilen 10 mikroskop sahasından çekilen fotoğraflarda 3,3 – diaminobenzidin (DAB) ile boyanan alanlar Olympus Stream İmaj Analiz Sistemi kullanılarak hesaplanmıştır. Sonuçlar her bir belirteç için pozitif boyanan sahaların ortalaması şeklinde ifade edilmiştir. Sağlıklı (ortalama: 7,31 ve $p<0,001$) ve akut mastitisli dokular (ortalama: 6,28 ve $p<0,001$) ile kıyaslandığında, kronik mastitisli meme dokularındaki E-kaderin boyanma yoğunluğunun (ortalama: 1,03) belirgin derecede azaldığı gözlenmiştir. Myoepitel hücreleri, myofibroblastlar ve vasküler düz kas hücreleri alfa-düz kas aktini ile pozitif boyanmıştır. Sağlıklı (ortalama: 6,65 ve $p<0,001$) ve akut mastitisli meme dokuları ile kıyaslandığında (ortalama: 7,38 ve p: 0,043),

kronik mastitisli dokularda alfa-düz kas aktininin oldukça yüksek miktarda ekprese edildiği (ortalama: 11,02) saptanmıştır. Kronik mastitisli dokularda oldukça yoğun bir kollajen boyanması (ortalama 11,88) tespit edilmiştir. Buna karşılık olarak, kollajen immunopozitivitesinin sağlıklı (ortalama 6,77 ve p: 0,013) ve akut mastitisli dokularda (ortalama: 6,51 ve p: 0,008) azaldığı gözlenmiştir. Vimentin boyanma yoğunluğunun sağlıklı (ortalama: 3,17 ve p<0,001) ve akut mastitisli dokular (ortalama: 5,3 and p<0,001) ile kıyaslandığında kronik mastitisli meme dokularında (11,5) belirgin derecede artış gösterdiği saptanmıştır. Gruplar arasında β-katenin ve sitokeratin boyanma yoğunluğu açısından istatistiksel olarak anlamlı bir farklılık saptanamamıştır. Bu bulgular epitelyal-mezenkimal geçişin sığırların kronik mastitislerinde fibrozis oluşumuna katkıda bulunduğuna işaret etmektedir.

***Anahtar Kelimeler*:** İnek mastitisi, fibrozis, epitelyal-mezenkimal geçiş, immunohistokimya

Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından desteklenmiştir (Proje numarası 118O893).

Identification of Mast Cells in Acute and Chronic Bovine Mastitis in The Context of Fibrosis

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Abstract

Mast cells are immune cells bearing important roles in many physiological and pathological processes. They are classified according to their cytoplasmic granule contents as tryptase (+), chymase (+), and tryptase-chymase (+) mast cells. The aim of the present study is to evaluate mast cells in bovine mastitis. A total of 264 bovine mammary samples were investigated, all slides stained with Hematoxylin&Eosin, Masson's trichrome, Toluidine blue and immunohistochemically for the classification of mammary inflammation, visualization of fibrosis, demonstration of mast cells and their immune identity, respectively. Mast cells were observed in interlobular and intralobular areas in a close proximity to blood vessels. The number and activity of mast cells were significantly increased in inflamed tissue samples. The mean number of intact and degranulated mast cells in 10 randomly selected microscopic fields under x400 magnification were significantly increased in acutely (mean number: 10.05; $p<0.001$) and chronically (mean number: 32.7; $p<0.001$) inflamed tissues when compared with the healthy controls (mean number: 4.14). Furthermore, when compared with the healthy controls, the average numbers of degranulated mast cells in acute and chronic mastitis were 2.5 and 7.1-fold higher, respectively. Three different mast cell immunophenotypes were detected in both healthy and inflamed mammary tissues. Mean numbers of tryptase positive mast cells were increased in chronic mastitis tissues (mean number: 19), compared with acute mastitis tissues (mean number: 4.4 and $p<0,001$) and healthy controls (mean number: 1.8; $p<0.001$). Compared with healthy controls (mean number: 1.0), chymase positive mast cell numbers were significantly increased in acutely (mean number 2.7; p: 0.015) and chronically inflamed tissues

(mean number: 7.8; p<0,001). The mean numbers of tryptase-chymase positive subset of mast cells were also higher in acutely and chronically inflamed tissues (2.0 and 5.8, respectively) when compared with healthy controls (1.4). Masson's trichrome staining results clearly demonstrated that increased number of mast cells was positively correlated with increased fibrosis (r: 0.760; p: 0.047). The average percent of connective tissue in healthy, acutely and chronically inflamed samples were as follows; 9.9, 12.35, and 29.83. Results of the study clearly indicate mast cells contribute in the pathogenesis of bovine mastitis and mammary fibrosis.

***Keywords*:** Mast cell, tryptase, chymase, bovine mastitis, fibrosis

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Mast Hücrelerinin Akut ve Kronik İnek Mastitislerinde Fibrosis Açısından Ortaya Konması

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Özet

Mast hücreleri, pek çok fizyolojik ve patolojik süreçte önemli roller oynayan önemli immun hücrelerdir. Sitoplazmik granül içeriklerine göre triptaz (+), kimaz (+) ve triptaz-kimaz (+) mast hücreleri olarak sınıflandırılırlar. Bu çalışmanın amacı sığır mastitislerinde mast hücrelerini ve bu hücrelerin olası fonksiyonlarını değerlendirmektir. Toplam 264 adet sığır meme dokusu incelenmiştir, tüm kesitler Hematoksilen&Eozin, Masson's trichrome, Toluidine mavisi ile ve immunohistokimyasal olarak boyanarak sırasıyla meme dokusundaki yangının sınıflandırılması, fibrozisin gösterilmesi, mast hücrelerinin ve immunofenotiplerinin demonstrasyonu gerçekleştirilmiştir. Mast hücrelerinin interlobüler ve intralobüler alanlarda ve kan damarlarına yakın yerleşim gösterdiği tespit edilmiştir. Yangılı doku örneklerinde mast hücre sayısı ve aktivitesi önemli derecede artış göstermişti. x400'lük büyütmede rastgele olarak seçilen 10 mikroskop sahasında sayılan ortalama intakt ve degranüle mast hücre sayısının, sağlıklı kontrol grubu (ortalama: 4,14) ile kıyaslandığında akut (ortalama: 10,05; $p<0,001$) ve kronik (ortalama: 32,7; $p<0,001$) yangılı dokularda artış gösterdiği tespit edilmiştir. Dahası, sağlıklı kontrol grubu ile kıyaslandığında ortalama degranüle mast hücre sayısı akut ve kronik mastitisli dokularda sırası ile 2,5 ve 7,1 kat artmıştı. Sağlıklı ve yangılı meme dokularında üç farklı mast hücre immunofenotipi de tespit edilmiştir. Kronik mastitisli meme dokularındaki ortalama triptaz pozitif mast hücre sayısı (ortalama: 19), akut mastitisli (ortalama: 4,4; $p<0,001$) ve sağlıklı kontrol dokuları (ortalama 1,8; $p<0,001$) ile kıyaslandığında artış göstermişti. Akut (ortalama 2,7; p: 0,015) ve kronik (ortalama: 7,8; $p<0,001$) mastitisli meme dokularındaki ortalama kimaz pozitif mast hücre sayısının, sağlıklı kontrol dokuları ile kıyaslandığında

(ortalama: 1,0) belirgin derecede artış gösterdiği gözlenmiştir. Ortalama triptaz-kimaz pozitif mast hücre sayısı da kontrol grubu (1,4) ile kıyaslandığında akut ve kronik yangılı dokularda (sırası ile 2,0 ve 5,8) artış göstermişti. Masson's trichrome boyama sonuçları, artmış mast hücre sayısının şiddetli fibrosis ile pozitif korelasyon gösterdiğini açıkça ortaya koymuştur (r: 0,760; p: 0,047). Sağlıklı, akut ve kronik yangılı dokulardaki ortalama konnektif doku yüzdesi 9,9, 12,35 ve 29,83 idi. Bu çalışmanın sonuçları, mast hücrelerinin sığır mastitisi ve meme fibrozisinin patojenezine katkıda bulunduğunu göstermektedir.

***Anahtar Kelimeler*:** Mast hücresi, triptaz, kimaz, inek mastitisi, fibrosis

Bu çalışma Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TÜBİTAK) tarafından desteklenmiştir (Proje numarası 118O893).

Combined Effect of Fat Graft and Platelet-Rich Fibrin on Tissue Recovery in Temporomandibular Joint Defects

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Abstract

Temporomandibular joint, is a synovial joint necessary for the movements of mammal chin and one of the most frequently utilized joints of the body. Osteoarthritis that is formed primarily on elder patients or secondarily on some pathological cases (trauma and internal derangement) is the most common type of arthritis of Temporomandibular joint. Recovery potential of Temporomandibular joint pathologies is low due to the histological structure of the cartilage tissue and therefore, clinical treatment of these pathologies is limited. In this study, the effects of fat tissue-derived stem cells that have the ability to differantiate into different cells and is stimulated with Platelet-Rich Fibrin that contains many growth factors on artificially damaged Temporomandibular joint is investigated. Defects of 3mm were created on the lateral sides of both of the Temporomandibular joint discs of the rabbits in the study. Fat grafts obtained from the neck-dorsal region and Platelet-Rich Fibrin prepared from the rabbits' own blood were placed on the right side and the left side of each rabbit was planned as the sham group and closed without further manipulation. After eight weeks, joint condyles and discs were removed, dyed with Haematoxylen&Eosin and Safranin O/Fast Green and histologically examined. Joint surface structures, chondrocyte densities, cell aggregations and losses of Safranin O/Fast Green staining of both of the groups were scored histopathologically. There was no significant difference between investigated parameters and thus, fat graft and Platelet-Rich Fibrin combination wasn't concluded to be effective on experimental Temporomandibular joint defect model recovery.

***Keywords*:** Temporomandibular Joint, Fat Graft, Platelet-Rich Fibrin

Temporomandibular Eklem Bozukluklarında Yağ Grefti ve Trombositten Zengin Fibrinin Doku Onarımı Üzerine Kombine Etkisi

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ÖZET

Temporomandibular eklem, memeli çenesinin hareketleri için gerekli olan sinoviyal bir eklemdir, vücutta en sık kullanılan eklemlerden biridir. Yaşlı hastalarda primer olarak görülebilen, bazı patolojik durumlarda (travma ve internal derangement) sekonder olarak oluşan osteoartrit Temporomandibular eklemde en sık görülen artrit tipidir. Temporomandibular eklem patolojileri, kıkırdak dokunun histolojik yapısı gereği iyileşme potansiyeli düşük olduğu için klinik tedavileri sınırlıdır. Bu çalışmada farklı hücrelere dönüşme potansiyeli olan yağ doku kaynaklı kök hücrelerin, birçok büyüme faktörü barındıran Trombositten Zengin Fibrin ile uyarılarak, hasar oluşturulan Temporomandibular eklem kıkırdağı üzerindeki kombine etkilerinin araştırılması amaçlandı. Çalışmada tavşanların her iki Temporomandibular eklem eklem disk laterallerinde 3 mm'lik defektler oluşturuldu. Sağ tarafa ense-sırt bölgesinden alınan yağ grefti ve tavşanın kendi kanından hazırlanan Trombositten Zengin Fibrin yerleştirildi, sol tarafı sham grubu olarak planlanıp başka işlem yapılmadan kapatıldı. 8 hafta sonunda eklem kondil ve diskleri çıkarıldı, Hematoksilen&Eozin ve Safranin O-Fast Green metodları ile boyanarak incelendi. Her iki grup için eklem yüzey yapısı, kondrosit yoğunluğu, hücre kümelenmesi ve Safranin O-Fast Green boyanma kaybı histopatolojik olarak skorlandırıldı. Değerlendirilen parametreler arasında anlamlı fark saptanmadığı için, deneysel Temporomandibular eklem defekt modelinin onarımında yağ grefti ve Trombositten Zengin Fibrin kombinasyonu etkili bulunmadı.

***Anahtar Kelimeler*:** Temporomandibular Eklem, Yağ Grefti, Trombositten Zengin Fibrin

Combined Effect of Fat Graft and Platelet-Rich Fibrin on Perineural Adhesion and Scar Formation in a Rat Model

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Abstract

One of the most important factors that adversely affect the outcome of peripheral nerve surgery is the formation of perineural scar tissue after surgery. Many methods, pharmacological agents and chemicals have been tried to prevent perineural scar tissue formation. Recently, platelet-rich fibrin applications have been investigated in damaged tissue regeneration. It has been reported that positive results have been obtained in the studies of the application of local fat grafts to the damaged nerve region by making use of the potential of transformation of mesenchymal stem cells derived from fat tissue into neurogenic cells. In addition, stem cells derived from adipose tissue have been shown to be activated more quickly when platelet-derived growth factors are present. The aim of this study was, making use of platelets' adipose tissue-derived stem cell proliferating and tissue remodelling effects, to investigate the combined effect of fat graft and platelet-rich fibrin, that were wrapped around the impaired sciatic nerve, on the formation of epineural scar tissue and nerve regeneration in the adult rat model. Twenty-four Sprague-Dawley adult female rats were used. The right and left sciatic nerves were dissected. 0.5 cm circumferential epineurectomy was performed on both nerves. The right sciatic nerve was wrapped with fat graft and platelet-rich fibrin mixture and used as experimental group. The left sciatic nerve was determined as the sham group. Twelve randomly selected rats were sacrificed in the 4th week for examination of early epineural scar formation and the other twelve rats were sacrificed in the 8th week for late results. Tissues taken were stained with haematoxylin-eosin and Masson's trichrome before histological examinations. Compared to sham groups, in sciatic nerves in both early and late experimental groups; fibrotic scar tissue, inflammation, myelin vacuolization were determined to be lesser. Neovascularization and nerve regeneration were determined to have developed more. The

differences between sham groups and experimental groups were found statistically significant. As a result, it is concluded that combination of fat graft + platelet-rich fibrin decreased postoperative scar tissue formation in the nerves subjected to epineurectomy in both early and late periods and contributed to nerve regeneration.

***Keywords*:** Perineural scar tissue, fat graft, PRF, nerve regeneration

Sıçan Modelinde Yağ Grefti ve Trombositten Zengin Fibrin'in Perinöral Adezyon ve Skar Dokusu Oluşumuna Kombine Etkisi

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Özet

Periferik sinir cerrahisinin sonucunu olumsuz etkileyen en önemli faktörlerden biri, cerrahi sonrası meydana gelen perinöral skar dokusu oluşumudur. Perinöral skar dokusu oluşumunu engellemeye yönelik birçok yöntem, farmakolojik ajan ve kimyasal madde denenmiştir. Hasarlı doku rejenerasyonunda son zamanlarda özellikle trombositten zengin fibrin uygulamaları araştırılmaktadır. Yağ dokusu kaynaklı mezenkimal kök hücrelerin nörojenik hücrelere dönüşüm potansiyelinden yararlanılarak hasarlı sinir bölgesine lokal yağ grefti uygulanması araştırmalarında da olumlu sonuçlar elde edildiği bildirilmektedir. Ayrıca yağ doku kaynaklı kök hücrelerin, ortamda trombosit kaynaklı büyüme faktörleri olması durumunda daha hızlı aktive oldukları gösterilmiştir. Bu çalışmada; trombositlerin yağ doku kaynaklı kök hücreleri uyarıcı ve doku onarımını artırıcı etkisinden yararlanarak, erişkin sıçan modelinde hasar oluşturulan sıçan siyatik sinirinin etrafına sarılan yağ grefti ve trombositten zengin fibrinin epinöral skar dokusu oluşumu ve sinir rejenerasyonu üzerine kombine etkisinin araştırılması amaçlanmıştır. Çalışmada, 24 adet Sprague-Dawley cinsi erişkin dişi sıçan kullanıldı. Cilt insizyonlarından sonra sağ ve sol siyatik sinir, dikkatli bir şekilde diseke edilip gluteal kaslardan uzaklaştırıldı. Her iki sinire de 0.5 cm çevresel epinörektomi uygulandı. Sağ siyatik sinirler yağ grefti ve trombositten zengin fibrin karışımı ile sarıldı ve deney grubu olarak kullanıldı. Sol siyatik sinirler ise sham grubu olarak belirlendi. Bu sıçanlardan rastgele seçilen 12 tanesi erken dönem epinöral skar dokusu oluşumunun incelenmesi açısından 4. haftada, diğer 12 tanesi ise geç dönem sonuçlar için 8. haftada sakrifiye edidi. Alınan dokular, Hematoksilen-Eozin ve Masson trikromu boyamaları sonrası histolojik olarak değerlendirildi. Sham gruplarına göre hem erken hem geç dönem deney gruplarındaki siyatik sinirlerde; fibrotik skar dokusunun daha az olduğu, inflamasyonun daha düşük düzeylerde izlendiği, myelin vakuolizasyonunun daha az olduğu, neovaskülarizasyonun daha fazla geliştiği ve sinir rejenerasyonunun daha ileri düzeylere ulaştığı belirlendi. Sham grupları ile deney grupları arasındaki farklılıklar,

değerlendirilen tüm kriterler açısından istatistiksel olarak anlamlı bulundu. Erken ve geç dönem deney grupları arasında ise, rejenerasyon ve inflamasyon açısından geç dönem lehine istatistiksel olarak anlamlılık saptandı. Yağ grefti + trombositten zengin fibrin kombinasyonunun, epinörektomi yapılan sinirlerde cerrahi sonrası oluşan skar dokusu oluşumunu hem erken hem de geç dönemde azalttığı ve sinir rejenerasyonunu olumlu etkilediği sonucuna varıldı.

***Anahtar kelimeler*:** Perinöral skar dokusu, yağ grefti, PRF, sinir rejenerasyonu

Human Cancers Challenge a Pet Model: The Case of Canine Oral Melanoma

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Abstract

Human Mucosal Melanoma (hMM) is a rare aggressive neoplasm of neuroectodermal origin characterized by large chromosomal aberrations and a low burden of single-nucleotide polymorphisms (SNPs). Given the scarcity of available cases, pet dogs have been proposed as a reliable animal model. In pet dogs, Canine Oral Melanoma (COM) is the most common malignant tumor of the oral cavity, sharing clinical, histological, and genomic characteristics with hMM. However, in the last few years, together with the deepening of the genome-wide characterization of COMs, doubts raised from some authors on the reliability of the canine spontaneous model. To clarify these points, we thoroughly reviewed the recent literature and performed our analyses on both single genes and the whole genome. The goal was to overcome the considerations limited to the classic gene comparison and discover the common biologic properties that are altered in melanoma cancer cells, to reveal the complex path of events that ensures the survival and growth of the neoplastic population. The genome-wide analysis of the cohort revealed a profile of gained and lost chromosomal regions confirming most of the results available in the literature, while no consistent point mutations were detected in the target genes analyzed. COM, therefore, confirms to be a simpler neoplastic entity than hMM when considering the mutational burden, being instead a suitable model for the complex aberration profile, which activates or inhibits biological processes related to cancer survival and proliferation. Statistically significant dysregulated pathways, such as cellular proliferation, telomere maintenance, Tyrosine-, MAP-, PI3- kinases, and melanocytes homeostasis, are shared between COM and hMM. These similarities, however, are visible only when comparison is not limited to single aberrated driver genes and robust software-based analysis is performed. Furthermore, new evidence suggests that angiogenetic pathways could be of great relevance to the advancement of targeted therapies, which need further exploration in the next future. Altogether, the results of the scientific community confirm the closeness of genomic aberrations and pathways activation of COM and hMM. Further evidences could come from the developing epigenetic analyses and the *in vitro* and *in vivo* trial of new targeted therapies.

***Keywords*:** canine oral melanoma, human melanoma, genomic aberrations, pathways, spontaneous model.

Infectious Disease of Parrots with Special Emphasis on Patho(Physio)Logical Changes

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Abstract

This lecture will present a summary of the most common psittacine infectious diseases presented according to the classical ranking as infectious diseases showing clinical and post-mortem findings, and additional specific laboratory techniques leading to a final diagnosis. I will, being a pathologist, connect pathological changes to clinical and laboratory findings and vice versa. In clinical practice many materials are sampled like blood and faeces, but many other materials can be sampled and examined from the patient and can give valuable information that can lead to a diagnosis. These include, but are not limited to, abdominocentesis, tissue and bone marrow biopsies, crop wash, nasal flush, sinus aspiration, tracheal wash, air-sac wash, and even urine collection. Basic tests performed on most of these materials are: macroscopic evaluation (opacity, colour, smell), microscopic examination of wet mounts (0.9% saline!!) and stained smears (before and after spinning), and in some cases (e.g. biopsies) a histopathological examination. After evaluation of the stained smear, microbiology can be done making the right choice of a culture medium. The most common infectious diseases in psittacines will be presented with a short disease history, the pathological characteristics of the disease, and additional specific diagnostic tests. The viral infection that will be discussed are Pacheco's disease virus (psittacine herpesvirus infection), polyomavirus, psittacines circovirus infection (incl. PBFD), paramyxovirus type 3, and avian bornavirus infection (Proventricular dilatation disease (PDD)). The most diagnosed bacterial problems are psittacosis, mycobacteriosis, and other bacterial infections like colibacillosis, salmonellosis, and Yersinia pseudotuberculosis. Fungal infection that are a common problem in psittacines are aspergillosis, candidiasis and in some species Macrorhabdus ornithogaster can be found.

The most common metazoic parasitic infections in psittacines are nematodes (Ascaridia platyceri, Ascaridia hermaphrodita, and Capillaria sp in crop or duodenum), cestodes (Raillietina sp.), arthropods including Knemidocoptes pilae and feather mites. Protozoal

infections are very rare in psittacines and include trichomoniasis and giardiasis. Coccidia are not a problem in psittacines. Understanding the pathological changes using pathophysiologal principles will at least be a good start to explain the clinical findings. Understanding the pathological findings and selecting the right samples and diagnostic tools, including simple exam of the intestines contents (wet mount) and cytology, histology, plus more sophisticated culture and especially molecular techniques will often lead to an etiological diagnosis.

Keywords: Infectious diseases, parrot

Microbiological and Pathomorphological Examination of *Scopulariopsis Brevicaulis* Infection in Lungs and Beaks in a Canary Herd

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Abstract

Scopulariopsis brevicaulis, a soil saprophyte, is known as the most common etiological factor of dermatomycotic molds. It is a deep fungal infection. Ten canaries, that died at various times from a herd of 200 canaries, were brought to the department of pathology, Ankara University, Faculty of Veterinary Medicine. After necropsy, the samples were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, and sectioned at 5μm thickness. The sections were stained with Hematoxylin-Eosin (H&E), Gram, Periodic Acid Schiff (PAS), Ziehl-Neelsen, and Giemsa stainings. Microbiologically, Sabouraud Dextrose Agar (SDA), Blood agar and MacConkey agar were used for isolation and cultivation. *Scopulariopsis brevicaulis*, characterized by flat, velvety or powdery, white, tan, dark brown, gray, or black colonies, was isolated in the lung and beak on the 5th day in SDA. In microscopic morphology, hyphae were hyaline and septate. It was seen that scopulariopsis have finger-like conidiophores on which annellides produce chains of conidia. Histopathologically, multiple irregular red thin hyphae were seen in the lungs. Scopulariopsis may be involved in human onychomycosis or pulmonary mycosis, and have been associated with invasive human infection. However, there is no study on this infection in canaries. To the best of our knowledge, this research is the first study for *Scopulariopsis brevicaulis* in the lungs and beaks of canaries.

***Keywords*:** canary, histopathology, microbiology, *Scopulariopsis brevicaulis*

Kanarya Sürüsünde Akciğer ve Gagalarda *Scopulariopsis Brevicaulis* Enfeksiyonunun Mikrobiyolojik ve Patomorfolojik İncelenmesi

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Özet

Bir toprak saprofiti olan *Scopulariopsis brevicaulis*, dermatomikotik küflerin en yaygın etiyolojik faktörü olarak bilinir. Derin bir mantar enfeksiyonudur. İki yüz adet kanaryadan çeşitli zamanlarda ölmüş 10 adet kanarya Ankara Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na getirildi. Kanaryaların nekropsileri yapılarak alınan örnekler %10 tamponlu formalinde tespit edildi, rutin olarak işlendi, parafine gömüldü ve 5µm kalınlığında kesitler alındı. Kesitler Hematoksilen-Eozin (H&E), Gram, Periyodik Asit Schiff (PAS), Ziehl-Neelsen ve Giemsa ile boyandı. Mikrobiyolojik olarak, izolasyon ve kültivasyon için Sabouraud Dextrose Agar (SDA), kanlı agar ve MacConkey agar kullanıldı. Düz, kadifemsi veya pudra, beyaz, ten rengi, koyu kahverengi, gri veya siyah kolonilerle karakterize olan *Scopulariopsis brevicaulis*, SDA'da 5. günde akciğerden ve gagadan izole edildi. Mikroskobik morfolojisinde, hifler hiyalinize ve bölmeliydi. Scopulariopsis'in parmak benzeri konidioforlara sahip olduğu ve üzerinde annellidlerin conidia zincirleri ürettiği görüldü. Histopatolojik olarak akciğerlerde çok sayıda düzensiz kırmızı ince hifalar görüldü. Scopulariopsis, insan onikomikozisinde veya akciğer mikozisinde rol oynayabilir ve invaziv insan enfeksiyonu ile ilişkilendirilebilir. Ancak, kanaryalarda bu enfeksiyonla ilgili bir çalışma bulunmamaktadır. Yapılan literatür taramalarında bu araştırma ile ilişkili herhangi bir makaleye rastlanılmamış olup, kanaryaların akciğerlerinde ve gagalarında *Scopulariopsis brevicaulis* için yapılan ilk çalışmadır.

***Anahtar Kelimeler*:** kanarya, histopatoloji, mikrobiyoloji, *Scopulariopsis brevicaulis*

Gastrointestinal Stromal Tumor in a Female Terrier Dog

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Abstract

In this case report, two intestinal masses compatible with gastrointestinal stromal tumor, is described in a nine-year-old female Terrier dog. The dog was presented with complaints of anorexia and vomiting following ingestion of food. Abdominal ultrasonography revealed two masses in the ventral aspect of stomach and duodenum. The masses were 6.1×3.7×2.3 cm and 3.2×2×1.7 cm in size and cut surfaces of the masses were grayish white-yellow and had a solid appearance. The greater mass invaded the intestinal serosa and mucosa. Cut surfaces were grayish white – yellow and had a solid appearance. Following routine histopathological processing, masses were sectioned at 4 μm and stained with H&E and immunohistochemically with anti-c-KIT (CD117, Anti-rabbit RB-9038-R1, Freemont, CA*)* antibody. Microscopically, tumor was composed of cells with oval vesicular nucleus, spindle-shaped eosinophilic cytoplasm and a prominent nucleolus. Mitotic activity was significantly increased (5-6 mitoses at ×400 magnification) and occasionally binucleated cells were observed. Large necrotic areas surrounded by inflammatory cells were noticed. Immunohistochemically, neoplastic cells displayed positive reaction with anti-c-KIT. Gastrointestinal stromal tumor is a neoplasm which originates from intestinal Cajal cells and the tumor is mostly confused with leiomyoma and leiomyosarcoma; immunohistochemical demonstration of c-KIT is performed for differential diagnosis.

***Keywords*:** c-KIT, gastrointestinal stromal tumor, GIST, dog

Terrier Irkı Dişi Bir Köpekte Gastrointestinal Stromal Tümör

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Özet

Bu vakada, 9 yaşında Terrier ırkı dişi bir köpekten alınan intestinal kitle makroskobik, mikroskobik ve immunohistokimyasal incelemeler sonucunda gastrointestinal stromal tümör ile uyumlu bulunmuştur. İştahsızlık ve gıda alımını takiben şekillenen kusma şikayeti ile kliniğe getirilen köpeğin, batın ultrasonunda midenin ventralinde duodenum içerisinde kitle varlığı dikkati çekti. Bağırsaktan alınan kitleler 6,1x3,7x2,3 cm ve 3,2x2x1,7 cm boyutlarındaydı ve kitlelere kesit yapıldığında kesit yüzlerinin boz beyaz-sarı renge ve solid bir görünüme sahip olduğu gözlendi. Kitleler rutin patolojik işlemlerden geçirildikten sonra 4 µm kalınlığında kesilerek H.E ve immunohistokimyasal olarak *c-KIT* (CD117, Anti-rabbit RB-9038-R1, Freemont, CA*)* ile boyandı. Yapılan mikroskobik incelemede kitlenin oval, veziküler yapıda çekirdeğe; iğ şeklinde eozinofilik sitoplazmaya ve belirgin çekirdekçiğe sahip olan neoplastik hücrelerden oluştuğu görüldü. Mitotik aktivitenin oldukça artmış olduğu (x400'lük büyütmede 5-6 adet mitoz) ve nadiren çift çekirdekçiğe sahip hücrelerin de bulunduğu gözlendi. Yer yer yangısal hücreler ile çevrili geniş nekrotik sahalar bulunduğu dikkati çekti. İmmunohistokimyasal boyamada *c-KIT* ile pozitif reaksiyon gözlendi. Gastrointestinal stromal tümör, sindirim sistemindeki intestinal Kajal hücrelerinden köken alan bir tümördür ve en çok leiomyom ve leiomyosarkom tümörleri ile karıştırıldığından ayırıcı tanısı immunohistokimyasal olarak *c-KIT*'in gösterilmesiyle yapılmaktadır.

***Anahtar Kelimeler*:** c-KIT, gastrointestinal stromal tümör, GİST, köpek

Nervous System and Skull Malformations on Pomeranian Dog: Anencephaly, Bifid Tongue, And Cleft Palate

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Abstract

Anencephaly is a congenital disease manifesting with the absence of the brain due to the failure of the cranial part of the neural tube to close in the embryonic stage. The disease may be accompanied by other anomalies and usually results in premature death. A stillborn puppy of a two-years-old female Pomeranian dog is examined in this case. The lack of brain tissue and accompanying abnormal skull formation was noted macroscopically. The eyes were in an outward bulging position (protrusion) and bifid tongue together with a secondary cleft palate was present. On serial sections stained with Haematoxylin-Eosin, only the medulla spinalis among the structures of the central nervous system could be inspected microscopically. NeuN immunoreactivity of neurons in the medulla spinalis and spinal ganglions and of ganglion cells in the retina and GFAP immunoreactivity of astrocytic glial cells were detected in immunohistochemistry staining. The case was concluded to be coherent with skull and nervous system congenital malformations rarely observed in dogs.

***Keywords*:** anencephaly, neural tube defect, bifid tongue, cleft palate, dog

Bir Pomeranya Köpeğinde Sinir Sistemi ve Kafatasının Malformasyonları: Anensefali, Bifid Dil ve Yarık Damak

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Özet

Embriyonal dönemde nöral tüpün kranial kısmının kapanmaması sonucu meydana gelen beyin yokluğu olarak da bilinen anensefali, konjenital bir hastalıktır. Hastalığa diğer anomaliler de eşlik edebilir ve genellikle doğumdan önce ölümle sonuçlanır. Bu vakada, iki yaşında Pomeranya ırkı dişi bir anne köpeğin bir adet ölü doğan yavrusu incelendi. Yavruda makroskobik olarak beyin dokusunun bulunmadığı, bununla birlikte anormal kafatası oluşumu dikkati çekmekteydi. Gözlerin dışarıya doğru çıkık pozisyonda olduğu (protrüzyon), bifid dil ve beraberinde sekonder damak yarığı varlığı saptandı. Mikroskobik düzeyde Hematoksilen-Eozin ile boyanan seri kesitlerde, merkezi sinir sistemine ait yalnızca medulla spinalisin varlığı gözlendi. İmmunohistokimyasal boyamalarda ise medulla spinalis ve spinal ganglionlardaki nöronlarda ve retinada ganglion hücrelerinde NeuN immünreaktivitesi, astrositik glialarda ise GFAP boyamalarında pozitivite saptandı. Olgu, köpeklerde nadiren gözlenen kafatası ve sinir sistemindeki konjenital malformasyonlar ile uyumlu bulundu.

***Anahtar Kelimeler*:** Anensefali, nöral tüp defekti, bifid dil, damak yarığı, köpek

Mandibular Changes in Rats with Postmenopausal (type I) Osteoporosis

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Abstract

The aim of the present study is to investigate the effects of postmenopausal (type I) osteoporosis and various treatment methods on bone and bone marrow. For this purpose, 32 female Wistar rats were used and divided into four groups. No treatment was applied to the control group (n = 8). The ovariectomy group (n = 8) was subjected to ovariectomy procedure and the subjects were fed with calsium deficient diet. D-Ca group (n = 8) was fed postmenopausal osteoporosis with calsium deficient diet for 12 weeks following being subjected to ovariectomy and then subjects were treated with vitamin D and calcium. The last group, the estrogen group (n = 8), were subjected to ovariectomy procedure and subjects were treated with estrogen following a 12-week calcium deficient diet. Following the euthanasia procedure, the mandibular bones removed from the cadavers were fixed in %10 buffered formaldehyde, then the bones were taken into decalcification solution, and afterwards they were subjected to washing and routine process, and 4.5 μm-thick sections were stained with hematoxylin and eosin and examined under a light microscope. As a result of the study, histopathologically necrotic and degenerative changes (decrease in trabecular connections, increase in adipose tissue in bone marrow, enlargement of lacunae in bone, etc.) were observed in the teeth, bone and bone marrow in the ovariectomy group, and less or no change were obtained in the D-Ca and estrogen and control groups.

Keywords: Estrogen, calcium, mandibula, postmenopausal osteoporosis.

Postmenopozal (Tip I) Osteoporozis'li Sıçanlarda Mandibular Değişimler

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Özet

Mevcut çalışmanın amacı tip I Postmenopozal Osteoporozis ve çeşitli tedavi yöntemlerinin kemik ve kemik iliği üzerine etkilerini araştırmaktır. Bu amaçla 32 adet dişi Wistar ırkı sıçan dört gruba ayrıldı. Bu gruplardan kontrol grubuna (n=8) hiçbir işlem yapılmadı. Overektomi grubuna (n=8) overektomi işlemi uygulanmış ve deney sonuna kadar kalsiyumdan fakir diyetle beslendi. D-Ca grubuna (n=8) overektomiişleminden sonra 12 hafta boyunca kalsiyumdan fakir diyetle beslendi ardından D vitamini ve kalsiyum verildi. Son grup olan östrojen grubuna (n=8) ise overektomi uygulandı ve 12 hafta kalsiyumdan fakir diyetle beslendikten sonra östrojen verildi. Ötenazi işlemi ve nekropsi uygulamasını takiben kadavralardan çıkartılan mandibulalar %10'luk tamponlu formaldehit solüsyonu içerisinde tespit edildi. Sonrasında kemikler dekalsifikasyon solüsyonuna alındı ve rutin doku takip basamaklarından geçirilerek, alınan 4,5 µm kalınlığındaki kesitler hematoksilen–eozin ile boyanarak ışık mikroskobunda incelendi. Çalışma sonucunda histopatolojik olarak yalnızca overektomi uygulanan grupta diş, kemik ve kemik iliğinde nekrotik ve dejeneratif değişimler (trabeküler bağlantılarda azalma, kemik iliğinde adipöz doku artışı, kemikte lakunalarda genişleme vb.) gözlenirken, diğer gruplarda söz konusu değişiklikler ya daha hafif şekilde gözlenmiş ya da gözlenmemiştir.

Anahtar Kelimeler: Kalsiyum, mandibula, östrojen, post menopozal osteoporozis.

Methods of Inducing Allergic Contact Dermatitis with 2,4-dinitrofluorobenzene in BALB/c Mice

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Abstract

Atopic dermatitis is a chronic inflammatory skin disease manifested by a rash and severe itching. Although the pathophysiological mechanism of the disease is not fully understood, overactivation of immune cells, including macrophages, mast cells and T lymphocytes, is considered to be the main cause. Contact dermatitis is an inflammatory skin disease with both proinflammatory and antigenic properties induced by the skin's exposure to low molecular weight chemicals. Allergic contact dermatitis is the result of an increased T cell response to haptens reaching the skin. The haptens that come into contact with the skin are taken up by immature skin dendritic cells and migrate to the paracortical area of the lymph nodes. Here, they prepare hapten-specific T cells as a result of the presentation of hapten-protein complexes on major histocompatibility complex molecules. Studies have shown that 2,4-dinitrofluorobenzene, a powerful hapten in mice, is mediated by CD8 + cytotoxic T cells in allergic contact dermatitis and is downregulated by CD4 + T cells. Repeated topical application of 2,4-dinitroflurobenzene at a concentration of 0.15% dissolved in acetone in mice has been reported to induce marked infiltration of neutrophils and eosinophils, and eczematous changes of the skin, including hypertrophy of the epidermis, causing persistent itching. The use of mouse models in atopic dermatitis is a guide for a better understanding of the pathogenesis. The purpose of this review is to bring together the various methods in the literature for inducing allergic contact dermatitis in BALB/c mice with 2,4-dinitrofluorobenzene.

***Keywords*:** Atopic dermatitis, allergic contact dermatitis, 2,4-dinitrofluorobenzene, BALB/c.

BALB/c Farelerinde 2,4-dinitroflorobenzen ile Alerjik Kontakt Dermatit Oluşturma Yöntemleri

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Özet

Atopik dermatit, döküntü ve şiddetli kaşıntı ile kendini gösteren iltihaplı kronik bir deri hastalığıdır. Hastalığın patofizyolojik mekanizması tam olarak anlaşılmasa da makrofajlar, mast hücreleri ve T lenfositler dahil olmak üzere bağışıklık hücrelerinin aşırı aktivasyonu ana neden olarak kabul edilmektedir. Kontakt dermatit, cildin düşük moleküler ağırlıklı kimyasallara maruz kalmasıyla indüklenen, hem proinflamatuar hem de antijenik özelliklere sahip, yangısal bir deri hastalığıdır. Alerjik kontakt dermatit, cilde ulaşan haptenlere karşı artan T hücresi tepkisinin sonucudur. Deriyle teması olan haptenler, olgunlaşmamış deri dendritik hücreleri tarafından alınırlar ve lenf düğümlerinin parakortikal alanlarına göç ederler. Burada hapten-protein komplekslerinin majör histo-uyumluluk kompleks molekülleri üzerinde sunumu sonucu hapten-spesifik T hücrelerini hazırlanmaktadırlar. Yapılan çalışmalarda, farelerde güçlü bir hapten olan 2,4-dinitroflorobenzen, alerjik kontakt dermatitte CD8 + sitotoksik T hücrelerinin aracılık ettiğini ve CD4 + T hücreleri tarafından down-regülasyon gösterilmiştir. Farelerde, aseton içinde çözdürülmüş %0,15 konsantrasyonda 2,4-dinitroflorobenzenin tekrarlanan topikal uygulamasının, nötrofil ve eozinofillerde belirgin infiltrasyona ve epidermisin hipertrofisi dahil olmak üzere deride egzematöz değişiklikleri indüklediğini ve kalıcı kaşınmaya neden olduğu bildirilmiştir. Atopik dermatitte fare modellerinin kullanılması patogenezin daha iyi anlaşılması için yol gösterici niteliktedir. Bu derlemenin amacı 2,4-dinitroflorobenzen ile BALB/c farelerinde alerjik kontakt dermatit oluşturulmasının literatürdeki çeşitli yöntemlerini bir araya getirmektir.

***Anahtar Kelimeler*:** Atopik dermatit, alerjik kontakt dermatit, 2,4-dinitroflorobenzen, BALB/c.

The Role of Chemokine (TARC / CCL17) Regulated by Thymus Activation in Atopic Dermatitis

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Abstract

Atopic dermatitis (AD), also known as atopic eczema, is a chronic and inflammatory disease of the skin. It affects 10-20% of children and 3% of adults. Atopic dermatitis is the most common allergic skin disease. The pathogenesis of AD is complex and still not fully elucidated. Cytokines are small proteins secreted by cells. It has a special effect on the interaction and communication between cells. Cytokines can act on cells that secrete them (autocrine effect), nearby cells (paracrine effect) or in some cases distant cells (endocrine effect). Cytokines include lymphokines, monokines, interleukins, interferons, growth factors, and chemokines. Chemokines (chemotactic cytokines) are low molecular weight proteins that regulate the migration of leukocytes. Infiltration of inflammatory cells into tissues is regulated by chemokines. Many chemokine and chemokine receptors play a role in the pathogenesis of AD. The complex chemokine network affects the formation of inflammatory infiltrates and cell trafficking in the skin, including T lymphocytes, dendritic cells, eosinophils, histiocytes and mast cells. Chemokines and their receptors play an important role in AD by regulating the initiation and exacerbation of inflammation in response to allergens. The chemokine (TARC) / CCL17 regulated by thymus activation is constitutively expressed in the thymus and produced by dendritic cells, endothelial cells, keratinocytes and fibroblasts. The thymus activation-regulated chemokine (TARC / CCL17) is a member of the CC chemokine family and a potent and selective chemoattractant for Th2 cells via the CC chemokine receptor 4 (CCR4). Th2-type cells are thought to play an important role in the pathogenesis of atopic dermatitis, especially in the acute phase. Initially, immunity is controlled by Th2 cells and the release of cytokines such as IL-4, IL-5, IL-6, IL-13 and IL-31 are stimulated. Th2 cytokines support humoral immunity and IgE production, which are characteristic of atopic diseases. In addition, acute itching and inflammation are provided by cytokines produced by Th2 lymphocytes and cytokines thought to mediate activation of Th2 lymphocytes. TARC is overproduced in the skin of patients with atopic dermatitis and is thought to attract circulating Th2 cells to the skin. In patients with AD, the stratum corneum TARC (scTARC) was determined using the stripping

method and immunostaining technique, and scTARC was found to be associated with the severity of local skin lesions. ScTARC shows that it can be used as an indicator of the status of local skin lesions in patients with AD, especially in the acute phase of inflammation. The purpose of this review is to discuss the immunological and diagnostic aspects of TARC / CCL17, which is a reliable biomarker in atopic dermatitis.

***Keywords*:** Atopic dermatitis, chemokine, TARC / CCL17).

Atopik Dermatitte Timus Aktivasyonu ile Düzenlenen Kemokinin (TARC/CCL17) Rolü

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Özet

Atopik egzama olarak da bilinen atopik dermatit (AD), cildin kronik ve iltihaplı bir hastalığıdır. Çocukların %10-20'sini ve yetişkinlerin %3'ünü etkilemektedir. Atopik dermatit, en yaygın alerjik deri hastalığıdır. AD'nin patogenezi karmaşıktır ve hala tam olarak aydınlatılamamıştır. Sitokinler, hücreler tarafından salgılanan küçük proteinlerdir. Hücreler arasındaki etkileşim ve iletişimde özel bir etkiye sahiptir. Sitokinler, onları salgılayan hücrelere (otokrin etkisi), yakındaki hücrelere (parakrin etkisi) veya bazı durumlarda uzak hücrelere (endokrin etkisi) etki edebilmektedir. Sitokinler arasında lenfokinler, monokinler, interlökinler, interferonlar, büyüme faktörleri ve kemokinler bulunmaktadır. Kemokinler (kemotaktik sitokinler), lökositlerin göçünü düzenleyen, düşük moleküler ağırlıklı proteinlerdir. İnflamatuar hücrelerin dokulara sızması kemokinler tarafından düzenlenmektedir. AD'nin patogenezinde birçok kemokin ve kemokin reseptörü rol oynamaktadır. Karmaşık kemokin ağı, T lenfositleri, dendritik hücreleri, eozinofilleri, histiyositleri ve mast hücrelerini içeren enflamatuar infiltratın oluşumunu ve deride ki hücre trafiğini etkilemektedir. Kemokinler ve reseptörleri, alerjenlere yanıt olarak inflamasyonun başlamasını ve şiddetlenmesini düzenleyerek AD'de önemli rol oynamaktadır. Timus aktivasyonu ile düzenlenen kemokin (TARC)/CCL17, timusta yapısal olarak eksprese edilmekte ve dendritik hücreler, endotelyal hücreler, keratinositler ve fibroblastlar tarafından üretilmektedir. Timus aktivasyonu ile düzenlenen kemokin (TARC/CCL17), CC kemokin ailesinin bir üyesi ve CC kemokin reseptörü 4 (CCR4) yoluyla Th2 hücreleri için güçlü ve seçici bir kemoatraktandır. Th2 tipi hücrelerin, atopik dermatitin patogenezinde özellikle akut fazda önemli rol oynadığı düşünülmektedir. Başlangıçta bağışıklık, Th2 hücreleri tarafından kontrol edilir ve IL-4, IL-5, IL-6, IL-13 ve IL-31 gibi sitokinlerin salınımını uyarılır. Th2 sitokinleri, atopik hastalıkların karakteristiği olan humoral bağışıklığı ve IgE üretimini desteklemektedir. Ayrıca akut kaşıntı ve yangılanma, Th2 lenfositleri tarafından üretilen sitokinler ve Th2 lenfositlerinin aktivasyonuna aracılık ettiği düşünülen sitokinlerle sağlanmaktadır. TARC, atopik dermatitli hastaların derisinde aşırı

üretilmekte ve dolaşımdaki Th2 hücrelerini deriye çektiği düşünülmektedir. AD'li hastalarda stratum corneum TARC (scTARC) bant sıyırma yöntemi ile immün boyama tekniği kullanılarak belirlenmiş ve scTARC'ın lokal deri lezyonlarının ciddiyeti ile ilişkili olduğu bulunmuştur. ScTARC, AD'li hastalarda özellikle inflamasyonun akut fazında lokal deri lezyonlarının durumunun bir göstergesi olarak kullanılabileceğini göstermektedir. Bu derlemenin amacı atopik dermatitte güvenilir bir biyobelirteç olan TARC/CCL17'ın immünolojik ve tanısal yönlerini ele almaktır.

Anahtar Kelimeler: Atopik dermatit, kemokin, TARC/CCL17).

A Case of Mycobacteriosis in a Mackerel (*Scomber scombrus*)

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Abstract

Mycobacteriosis was observed a mackerel (*Scomber scombrus*) which bought from a fishery for cooking. Numerous granulomatous lesions were seen in visceral organs. Histopathological examination of the lesions revealed numerous foci characterized by caseous necrosis in the center of the lesion covered by epithelioid giant cells. At the Ziehl-Neelsen staining typical red rod shaped mycobacteries were observed. *Mycobacterium tuberculosis* positive immunoreaction was noticed at giant cells and macrophages at the immunohistochemical examination. According the gross and microscopical findings the disease diagnosed as mycobacteriosis.

***Keywords**:* Mycobacteriosis, pathology, immunohistochemistry, fish.

Bir Uskumruda (Scomber scombrus) Mikobakteriozis Olgusu

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Özet

Bir balıkçıdan pişirilmek için alınan bir uskumruda (*Scomber scombrus*) mikobakteriozis gözlendi. Viseral organlarda çok sayıda granülomatöz lezyon görüldü. Lezyonların histopatolojik incelemesinde epitelioid dev hücrelerin çevrelediği ve merkezinde kazeöz nekrozun bulunduğu çok sayıda odak saptandı. Ziehl-Neelsen boyamasında tipik kırmızı çubuk şeklinde mikobakteriler gözlendi. İmmünohistokimyasal incelemede dev hücrelerde ve makrofajlarda *Mycobacterium tuberculosis* pozitif immunoreaksiyon dikkati çekti. Makroskobik ve mikroskobik bulgulara göre hastalığa mikobateriozis tanısı konuldu.

Anahtar Kelimeler: Mikobateriozis, patoloji, immünohistokimya, balık.

A Case of Fibromyxolipoma in a Gold Fish

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Abstract

In this case report, it is aimed to report the histopathological findings of a subcutaneous soft mass localised on the head of a gold fish. The fish found dead in its aquarium was brought to our department for necropsy. It was observed that the mass localized on the head of the fish at necropsy was 1.5x1.5x1cm in size and covered with skin. The cross-section of the mass was whitish-yellow in color and in cystic appearance. It was noticeable that there was a mucinous fluid in the cystic areas. At the histopathological examination, some parts of the mass were found to have a fibromatous appearance consisting of fibrocytes and fibroblasts elongating in different directions, while some parts were composed of bluish-colored mucinous masses between loose connective tissue and cells. Lipomatous areas, characterized by the characteristic increase in adipose tissue, whose nuclei were pushed peripheral area of the cells, attracted attention. The mass was diagnosed as fibromyxolipoma based on macroscopic and histopathological findings. This is the first case of fibromyxolipoma in a Gold fish.

Keywords: Gold fish, tumor, fibromyxolipoma, pathology

Bir Altın Balıkta Fibromiksolipom Olgusu

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Özet

Bu olgu sunumunda bir altın balığın başında subkutan yerleşimli yumuşak kıvamlı bir kitlenin histopatolojik bulgularının rapor edilmesi amaçlanmıştır. Akvaryumunda ölü bulunan balık nekropsi için anabilim dalımıza getirildi. Nekropside balığın başında lokalize olmuş kitlenin 1.5x1.5x1cm boyutlarında üzeri deri ile kaplı olduğu gözlendi. Kitlenin kesit yüzü beyazımsı-sarı renkli ve kistik görünümdeydi, kistik alanlarda müsinöz bir sıvı bulunduğu dikkati çekti. Histopatolojik incelemelerde kitlenin bazı bölümlerinin değişik yönlere doğru uzayan fibrosit ve fibroblastlardan oluşan fibromatöz bir görünümde, bazı bölümlerinin ise gevşek bağ doku ve hücreler arasında mavimsi renkli müsinöz kitlelerden oluşmuş olduğu görüldü. Yer yer boşluklu görünümde çekirdekleri kenara itilmiş karakteristik yağ dokusu artışı ile karakterize lipomatöz alanlar dikkati çekti. Kitleye makroskobik ve histopatolojik bulgulara göre fibromiksolipom teşhisi konuldu. Bu bir balıkta saptanan ilk fibromiksolipom olgusudur.

Anahtar Kelimeler: Altın balık, tümör, fibromiksolipom, patoloji

Extracellular Vesicles: New Players in the Field of Veterinary Medicine

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Abstract

Extracellular vesicles (EVs) are a heterogeneous group of cell-derived membranous particles, principally categorized as exosomes, microvesicles, and oncosomes mainly according to their size. They are shed from every type of cell and they can be detected in every biological fluid. The importance of EVs is due to their ability to transfer their cargo, consisting of proteins, lipids, sugars and nucleic acids, influencing recipients cells. This ability makes EVs involved in many biological processes and explains the exponential scientific interest on EVs. In human medicine many studies are evaluating their role in a wide range of physiological and pathological processes such as tumorigenesis, tumour progression, viral infections, beside their possible use as cancer biomarkers or as drug delivery systems. Very recently, the EV field has been opened also in veterinary medicine and some preliminary studies have been published. This mini review aims to synthetize this initial EVs studies in veterinary medicine and to discuss their possible applications. Briefly, in small animals most studies have focused on the role of EVs in tumors. EVs have been isolated, measured, and characterized in plasma/serum of tumour-bearing dogs or in cell culture media of canine mammary cancer cell lines. In large animals, some reports have focused instead on the role of EVs in reproduction and infectious diseases, and their presence in milk has also been studied. More specifically, EVs have been isolated and characterized from porcine trophectoderm-cells and aortic endothelial cells, from porcine follicular fluid, from serum of pigs infected with African swine fever virus, from bovine embryo culture media, bovine milk and bovine oviductal fluid. Preliminary analyses have also been carried out on unconventional animals, being isolated from serum of lama and naked mole-rat. Finally, recent outcomes highlighted the possibility that EVs, reflecting environmental features, might become a tool to assess marine mammals or fishes health status. This wide set of applications and particularly their role as a carrier responsible for organism-pathogen-environment communication, makes EVs a very interesting interdisciplinary research focus easily fitting into the one health concept.

***Keywords*:** extracellular vesicles, cancer, one health, biomarkers

Histopathological and Electron Microscopic Studies on Porcine Proliferative Ileitis

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Abstract

The aim of the research was to investigate some morphological characteristics of lesions essential to porcine proliferative enteropathy (PPE) in porcine ileum, taking into consideration their implementation in diagnostics. Gross investigations of ileal lesions were performed amongst fattened pigs (n = 1068) at slaughter coming from six commercial farrow-to-finish farms located in Central South-East Bulgaria and North-East Bulgaria. Transverse sections from terminal ileum (n = 127) tissue samples were collected in a random fashion and processed routinely for histology. Further on histochemical (Warthin-Starry silver stain), immunohistochemical (IHC), (monoclonal antibody Law1DK), light and transmission electron microscopic methods were applied for specific identification of the causative agent. The matched macroscopic, microscopic histochemical and immunohistochemical observations were statistically tested. The incidence of lesions at slaughter was 5.7 % with simultaneously higher rate out of the pooled samples (48.03 %). The gross lesions were consistent with regional ileitis (47.06 %) and porcine intestinal adenomatosis (52.94 %). Histopathogically presence of distinct proliferation of immature enterocytes in the crypts of Lieberkühn, increased mitotic figures and apoptic bodies were distinguished in most samples (92.13%). The Warthin-Starry silver staining method demonstrated presence of intracellular argyrophilic microorganisms associated within 76.38 % of PPE lesions. High prevalence of IHC immunolocalization was observed (81%). Ultrastructurally intestinal crypts containing numerous proliferating immature epithelial cells accommodated copious apical intracytoplasmic vibroid, curved bacteria compatible with *L. untracellularis* were recognized. Diagnostic test correlation to IHC results, revealed greater strength of agreement in favor to gross examination, fair for HE and poor for WS. The outcome interpretation demonstrates that the application of morphological and immunohistochemical assays are reliable tools in the diagnosis and differential diagnosis of PPE in pigs, despite some

diagnostic drawbacks.

***Keywords*:** porcine proliferative ileitis, pathology

BHV- 1 Antigen Detection in Paraffinized Lung Sections of Pneumonic Sheep Lung Using Immunohistochemistry

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Abstract

Respiratory tract infections caused by some viruses with cattle origin have been demonstrated in sheep and goats. The main goal of this study was to determine Bovine Herpesvirus type 1 BHV1antigen in formalin-fixed paraffin-embedded lung tissue of pneumonic sheep, using immunohistochemistry (IHC) staining method. For this purpose, the lungs of 4079 sheep, which were raised in various farms in the Garmsar district and surrounding areas and were brought to the local abattoir for slaughtering between April and September 2016, were examined. Macroscopic pneumonia findings were detected in different lobes particularly in the apical and cardiac lobes of the lungs of 259 sheep (6.35%). The rates of mild, moderate and severe consolidations observed in the pneumonic lungs were 59.8 %, 26.3 % and 11.6 %, respectively. Pneumonias were microscopically classified in sheep as interstitial pneumonia (49.8%), suppurative bronchopneumonia (%15.7), bronchointerstitial pneumonia (11.1 %), and parasitic pneumonia (14.3%). A total of 220 pneumonic lungs, excluding parasitic pneumonia, examination with immunohistochemistry (IH) in terms of BHV 1 antigen, were considered. BHV 1 antigen was determined to be 8.63 % by the immunohistochemistry (IHC) method. In conclusion, the presence of viral antigen in lung tissues of sheep may indicate that BHV1 or possibly other species-specific herpesviruses might contribute to the development of broncho- and/or interstitial pneumonia. Moreover, it is suggested that sheep might have a role in the transmission of this virus to cattle.

Keywords: sheep, lung, BHV1, immunohistochemistry

Histopathogical Evaluation of Neuroprotective Effect of Chrysin in Cerebellar Neurotoxicity Induced by Acrylamid in Rat

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Abstract

Acrylamide (ACR) is a well-known industrial toxic chemical that produces neurotoxicity, which is characterized by progressive central and peripheral neuronal degeneration. Chrysin is a natural, biologically active flavonoid compound, which is commonly found in many plants. The antioxidant and neuroprotective properties of chrysin have been demonstrated. This study was conducted to evaluate the neuroprotective effects of chrysin against ACR-induced toxicity. For this purpose, 18 adult male Wistar rats were selected and hosed in polycarbonate boxes. Randomly assigned groups of rats (6 rats per exposure group as B and C) were exposed to ACR 50 mg/kg per day×11days i.p. and ACR 50 mg /kg per day×11days i.p. plus Chrysin 100 mg/kg respectively. The remaining 6 rats were housed in group (A) as control group. Control rats received daily i.p. injections of 0.9% saline (3ml/kg). As indices of developing neurotoxicity, weight gain, gait scores and landing hindlimb foot splay (LHF) were determined. After 11 days, rats were dissected and proper samples were collected from different areas and neuclei of cerebellum of rat for silver stain. Results did show no neurological behavior in groups A and C, whereas sever neurotoxicity was observed in groups B. In histopathological studies based on *de Olmos* technique no argyrophilic neurons or processes were observed in stained sections obtained from the cerebellum of rats belong to groups A and C, while moderate to severe argyrophilic changes were observed in different stained sections obtained from the cerebellum of rats belong to groups B. In the current study, which was based on histopathological examination using silver staining technique, chrysin exhibited neuroprotective effect against ACR – induced neurotoxicity in Wistar rats.

Keywords: Acrylamide, cerebellar neurotoxicity, chrysin, de Olmos amino-cupric silver sataining

Osteochondrosis in a New-Born Calf: A Case Report

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Abstract

Osteochondrosis is a disorder involving the enchondral ossification, in which vascular damage is associated to ischemic necrosis with disturbance of the normal bone growth. In the literature, the disease is described in several animal species, mainly pigs and horses, while is rarely reported in new-born calves. Herein we describe clinical and anatomo-histopathological aspects related to osteochondrosis in a new-born calf. The animal developed progressive difficult walking at 6 days of age, showing stiffness of the limbs and long periods of recumbency. Muscular tone was normal and higher mental functions were preserved. For the progressive worsening of its condition, the calf was humanely euthanatized 30 days after birth and submitted to anatomo-histopathological examination. Grossly, the cartilage of the scapular-humeral, humerus-radial, femoral-pelvic and femoro-tibial joints lacked the normal brightness and showed eroded appearance with irregular craters of ulceration. In some of these articulations joint spaces contained abundant clear and slightly sticky synovial fluid. Microscopically, we observed numerous necrotic cartilage canals surrounded by areas of ischemic chondronecrosis. Bacteriological and virological investigations tested negative. This case report confirms that osteochondrosis in cattle may also occur in the first month of age, thus suggesting, together with the age, a possible genetic origin.

***Keywords*:** osteochondrosis, calf, joint

Foodborne Mycotoxicoses: Risk Assessment and Underestimated Hazard for Animals or Humans (Review)

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Abstract

The molds and mycotoxins have been associated with a variety of livestock diseases, e.g.: Ergotism, known in humans as St. Anthony's fire; Equine leukoencephalomalacia; Porcine pulmonary oedema; Alimentary toxic-aleykiya; Aflatoxicosis; Mycotoxic porcine/chicken nephropathy; Stachybotryotoxicosis, and many others. The exposure of animals to various mixtures of mycotoxins presents a problem that has not been sufficiently investigated. Such mixtures of toxins often have additional or synergistic effects in farm animals. Various mycotoxicoses often have multi-mycotoxic natures being provoked by a combined effect of many mycotoxins. For example, mycotoxic nephropathy in Bulgaria and Africa has a different multi-mycotoxic origin as compared to the classical mycotoxic nephropathy, described previously in Danmark. The mycotoxins involved are; ochratoxin A (OTA), Penicillic acid (PA), Fumunisin B1 (FB1), in addition to not yet identified mycotoxin. Similarly, many other mycotoxicoses also have multimycotoxic origin. In this regard, a potent synergistic effect was found between OTA and PA, when the same were given simultaneously to pigs and chickens. Such synergy between OTA and PA or other mycotoxins such as FB1 under field conditions is likely responsible for the spontaneous nephropathy in Bulgaria, which is caused by relatively low contamination levels of OTA in food. The problem of transmitting the mixture of various contaminants from carcass further through the food chain to humans and the human health hazard from such exposure should be additionally evaluated. Some recent experiments focused attention on the immunosuppression as the first expressed toxic effect of OTA, which may become evident clinically before nephropathy and its associated biochemical changes. OTA suppression of humoral and cellular immunity, defined in principle, has been demonstrated in practice allowing development of secondary bacterial infections (e.g. salmonellosis and haemorrhagic enteritis) in pigs at only 1 ppm ochratoxin A in diet. OTA has also been described to increase the susceptibility of chickens to coccidiosis, salmonellosis and colibacillosis. Considerable aggravation of the pneumonic process in pigs infected with *M. hyopneumoniae* or *P. multocida* when treated with 20 ppm FB1 was found, as determined by macroscopic and pathomorphologic examinations and computed tomography, in addition to the premature death

in part of the pigs.

Keywords: foodborne mycotoxicoses

Diastematomyelia and Hydromyelia Case in a Goat Kid

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Abstract

In this case report, a diastematomyelia anomaly detected in the lumbar region of the medulla spinalis of a female kid was described. In the anamnesis, it was stated that the kid had difficulty standing up after birth, could not step on its hind legs and there were tremors in the hind leg muscles. When the lumbar region of the medulla spinalis of the necropsy was opened, no macroscopic finding was detected. Samples were taken from this area for histopathological examination. In histopathological examination, it was noted that the dorsal corns of the medula spinal were normal and the double horns were formed in the right ventral corn. Cell dysplasia was also detected in the left ventral cornea. In addition, a cystic structure was found in the examined lumbar region. In the light of these findings, the findings observed in goat were defined as diastematomyelia and hydromyelia. This case is the first case report about diastematomyelia and hydromyelia in our country.

***Keywords*:** Diastematomyelia, Hydromyelia, Pathology, Kids, Histopathology

Bir Oğlakta Diastematomyeli ve Hidromyeli Olgusu

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ÖZET

Bu olgu sunumunda 1 günlük, dişi oğlağa ait medulla spinalisin lumbar bölgesinde saptanan diastematomyelia anomalisi tanımlandı. Anamnez bilgide doğumdan sonra yavrunun ayağa kalkmakta güçlük çektiği, arka ayaklarına basamadığı ve arka bacak kaslarda kasılmaların olduğu ifade edildi. Nekropsisi yapılan oğlağın medulla spinalisin lumbar bölgesi açıldığında makroskobik bir bulgu saptanmadı. Bu bölgeden histopatolojik inceleme için numune alındı. Histopatolojik incelemede medula spinalin dorsal kornularının normal olduğu sağ ventral kornuda ise çift kornunun şekillendiği dikkati çekti. Sol ventral kornuda da hücre displazisi saptandı. Ayrıca incelenen lumbar bölgede kistik yapıya da rastlandı. Bu bulgular ışığında oğlakta gözlene bulgular diastematomyelia ve hidromyelia olarak tanımlandı. Bu olgu ülkemizde diastematomyelia ve hidromyelia hakkındaki ilk vaka sunumudur.

***Anahtar Kelimeler*:** Diastematomyeli, hidromyeli, patoloji, oğlak, histopatoloji

Investigation of P53, Perp and E-Cadherin Expressions In Bovine Skin Papilloma

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Abstract

Skin papilloma is a common disease in cattle. Despite the benign character of the masses, occasional multiple watching and frequent recurrence cause difficulties in terms of treatment and life comfort. Although many studies have been done in the diagnosis, pathogenesis and treatment of the disease, it is important to find new markers and methods with the developing science and technology, to review what is known about this disease and to reveal the unknown aspects. The material of the study consisted of paraffin blocks containing 25 bovine skins, 5 of which were normal with 20 papillomas. 5 µm sections were taken from the paraffin blocks, and anti-P53, Anti-PERP and Anti-E-cadherin markers and Avidin biotin peroxidase method were applied on slides with routine hematoxylin & eosin and poly-l-lysin. The presence of mutant (M) type P53 protein in skin papilloma in cattle and the role of E-cadherin and PERP proteins in tumor progression were investigated with the help of the findings obtained. In the evaluations made, it was determined that the anti-P53 intensely positive stained degenerated regions were also stained with anti-E-cadherin and anti-PERP positive, and it was concluded that the limited and in-situ characteristics of the papillomas were provided by the supportive properties of E-cadherin and PERP proteins.

***Keywords*:** Skin papilloma, P53, PERP, E-cadherin, cattle

Sığır Deri Papillomunda P53, Perp ve E-Cadherin Ekspresyonlarının Araştırılması

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Özet

Sığırlarda deri papillomu sıkça görülen bir hastalıktır. Kitlelerin benign karakterde olmasına karşın zaman zaman multiple seyretmesi ve sık sık nüks etmesi tedavi ve hayat konforu açısından zorluklara neden olmaktadır. Hastalığın teşhisi, patogenez ve tedavisinde oldukça fazla çalışma yapılmasına rağmen gelişen bilim ve teknoloji ile yeni belirteç ve yöntemlerin bulunması, bu hastalık hakkında bilinenlerin yeniden gözden geçirilmesi ve bilinmeyen yönlerin açığa çıkartılması açısından önemlidir. Çalışmanın materyalini 20 adet papillomlu 5 adet normal olmak üzere 25 sığır derisi içeren parafin bloklardan oluşturmuştur. Parafin bloklardan 5 µm kesitler alınarak rutin hematoksilen&eozin ve poly-l-lysin'li lamlara alınarak Anti- P53, Anti-PERP ve Anti-E-cadherin markerleri ile Avidin biyotin peroksidaz metodu uygulanmıştır. Elde edilen bulgular yardımıyla sığırlarda deri papillomunda mutant (M) tip P53 proteininin varlığı ile E-cadherin ve PERP proteinlerinin tümör progresyonundaki rolü araştırılmıştır. Yapılan değerlendirmelerde Anti-P53 yoğun pozitif boyanan dejenere bölgelerin aynı zamanda anti-E-cadherin ve anti-PERP pozitif boyandığı tespit edilmiş, papillomların sınırlı ve in-situ özelliklerinin E-cadherin ve PERP proteinlerinin birbirilerini destekleyici özellikleri sayesinde sağlandığı sonucuna varılmıştır.

***Anahtar Kelimeler*:** Deri papillomu, P53, PERP, E-cadherin, sığır

Ethylene Glycol Toxicity in Calves

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Abstract

Burdur Mehmet Akif Ersoy University Veterinary Faculty Animal Hospital was brought from livestock to 3 calves aged between 15 and 20 days with complaints of weakness, dehydration, inability to get up and nervous findings. Two of them, who did not respond to the treatment, were euthanized and sent to the pathology department. Both animals were macroscopically dehydrated, cachectic, and hair loss in some parts of the body. There was 1-2 liters of white light colored clear fluid in the abdominal cavity. Many organs and serous membranes in the abdominal cavity were hyperemic. In histopathological examination, haemorrhage, edema and degenerative changes were detected in many parenchymatous organs. Calcium oxalate crystals were found in many tubular lumen, especially proximal convolute tubules in kidneys. In the light of clinical, macroscopic and histopathological findings, the case was diagnosed with ethylene glycol toxicity. Ethylene glycol toxicity is often seen in cats and dogs living near industrial or auto car repair shops, and animals that accidentally drink antifreeze wastes. In this case report, ethylene glycol toxicity was reported for the first time in calves.

***Keywords*:** Ethylene glycol, toxicity, pathology, calf

Buzağılarda Etilen Glikol Toksikasyonu

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Özet

Burdur Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Hayvan hastanesine bir sığır işletmesinden halzislik, dehidrasyon, yerden kalkamama ve sinirsel bulgular şikayeti bulunan yaşları 15 ile 20 gün arasında değişen 3 adet buzağı tedavi için getirildi. Yapılan tedaviye cevap vermeyen 2 tanesi ötenazi uygulanarak patoloji anabilim dalı'na gönderildi. Her iki hayvanda da makroskobik olarak dehidre, kaşektik ve vücudun bazı bölgelerinde kıllarda dökülme tespit edildi. Karın boşluğunda 1-2 litre kadar beyaz açık renkli berrak bir sıvı mevcuttu. Karın boşluğundaki birçok organ ve seröz membranlar hiperemikti. Histopatolojik incelemede birçok parankimatöz organda kanama, ödem ve dejeneratif değişiklikler tespit edildi. Böbreklerde proksimal konvolut tubuluslar başta olmak üzere birçok tubul lümeninde kalsiyum okzalat kristallerine rastlandı. Klinik, makroskobik ve histopatolojik bulgular ışığı altında olguya etilen glikol toksikasyonu tanısı konuldu. Etilen glikol toksikasyonu genellikle sanayi ya da oto tamirhanesi civarında yaşayan kedi ve köpeklerde antifiriz atıklarını yanlışlıkla içen hayvanlarda görülmektedir. Bu olgu sunumunda ilk defa buzağılarda etilen glikol toksikasyonu bildirilmiştir.

***Anahtar kelimeler*:** Etilen glikol, toksikasyon, patoloji, buzağı

Investigation of Neuroprotective Effect of Kefir in Experimental Spinal Cord Injury

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Abstract

In this study, the antioxidant, anti-inflammatory and neuroprotective effects of kefir were investigated in spinal cord injury that was experimentally created on rats with compression trauma model. A total of 56 Wistar-Albino male rats were used in the study. Rats were divided into seven groups with 8 rats in each group. The animals were given freshly prepared kefir 18 ml/kg/day orally for 7 days before the trauma and during the trauma. Spinal cord injury was created according to the weight drop method. While animals were under general anesthesia on the 1st and 7th days before euthanasia, intracardiac blood was collected for analysis and then they were sacrificed. After sacrification, tissue sections were taken from the damaged spinal cord segment for tissue analysis. The samples were examined biochemically, immunohistochemically and histopathologically. When compared to the sham groups, kefir had a positive effect in preconditioning and treatment groups by decreasing spinal cord bleeding, edema, myelin sheath damage, liquefactive necrosis, neuronal necrosis, selectivity of canalis centralis and gitter cell levels significantly. When compared to the sham groups, kefir was found to have a positive effect in treatment groups by decreasing the neuron specific enolase (NSE), ionized calcium binding adapter molecule 1 (IBA-1), inducible nitric oxide synthase (INOS), cyclooxygenase 2 (COX-2) and myelin basic protein (MBP) levels significantly on the 1st and 7th days, and by increasing the glial fibrillary acidic protein (GFAP) level significantly.As a result, it was demonstrated that kefir had a protective and therapeutic effect on spinal cord injury.

***Keywords*:** Kefir, neuroprotective agents, spinal cord injuries

Deneysel Spinal Kord Hasarında Kefirin Nöroprotektif Etkisinin Araştırılması

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Özet

Bu çalışmada ratlarda deneysel olarak kompresyon travma modeliyle oluşturulan spinal kord hasarında kefirin antioksidan, antiinflamatuvar ve nöroprotektif etkileri araştırıldı. Çalışmada toplam 56 adet Wistar-Albino ırkı erkek rat kullanıldı. Ratlar her grupta 8 adet olmak üzere yedi gruba ayrıldı. Hayvanlara travma öncesi 7 gün süreyle ve travma süresince taze olarak hazırlanmış 18 ml/kg/gün oranında kefir oral yolla verildi. Spinal kord hasarı ağırlık düşürme metoduna göre oluşturuldu. Hayvanlar ötenazi öncesi 1. ve 7. günlerde genel anestezi altında iken analizler için intrakardiyak kanları alındı ve sonra sakrifiye edildi. Sakrifikasyon işleminden sonra, doku analizleri için hasarlı spinal kord segmentinden doku kesitleri alındı. Alınan numuneler biyokimyasal, immunohistokimyasal ve histopatolojik olarak incelendi. Kefir sham grubları ile karşılaştırıldığında, ön koşullama ve tedavi gruplarında spinal kordda kanama, ödem, miyelin kılıf hasarı, likefaksiyon nekrozu, nöronlarda nekroz, kanalis sentralisin seçilebilirliği, gitter hücre düzeylerini anlamlı düzeyde azaltarak olumlu etki gösterdi. Kefir sham grupları ile karşılaştırıldığında, tedavi gruplarında spinal kordda 1. ve 7. günde NSE, IBA, INOS, COX2 ve MBP düzeylerini anlamlı düzeyde azaltarak, GFAP düzeyini ise arttırarak olumlu etki gösterdiği tespit edilmiştir. Sonuç olarak; kefirin spinal kord hasarında koruyucu ve tedavi edici etkisi ortaya konmuştur.

***Anahtar kelimeler*:** Kefir, nöroprotektif etki, spinal kord hasarı

The First Case of Fibrosarcoma Detected in a Sultan's Parrot

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Abstract

In this study, we aimed to evaluate cell proliferation and metastatic capacity, PCNA and MMP-9 expressions in an immunohistochemical perspective at fibrosarcoma case which we detected in a heavy bleeding mass which is 20x23x7 mm in diameter, at present in the right radius distal of a three- year-old male cockatiel (*Nymphicus hollandicus*) for about 3-4 months. Biopsy samples that were taken after the surgical operation was detected in 100% formaldehyde solution. 5 um thick sections were taken from paraffin-embedded blocks that had been prepared after routine tissue tracing processes and sections were stained with hematoxylin and eosin so that histopathological changes could be detected. In the immunochemical staining process, the avidin-biotin-peroxidase method was used. During the histopathological examination of the mass, reticular tumoral cells, eddy structures, multinucleated giant cells and mitotic figures were found. In immunohistochemical staining, PCNA positive reactions were especially detected in the nucleus of the tumoral cells while MMP-9 expressions were encountered in the cytoplasm and nucleus of these cells. As a result of the literature reviews, it was found that this fibrosarcoma case is the first to be detected in cockatiel, and from this aspect it is thought that this case will contribute to the literature.

***Keywords*:** Fibrosarcoma, MMP-9, PCNA, sultan parrot

Sultan Papağanında Tespit Edilen İlk Fibrosarkom Olgusu

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Özet

Bu çalışmamızda 3 yaşındaki erkek sultan papağanında (*Nymphicus hollandicus*) sağ radiusun distalinde yaklaşık olarak 3-4 aydır bulunan, 20*23*7 mm çaplarında ve yoğun kanamalı bir kitlede tespit ettiğimiz fibrosarkom olgusunda hücre proliferasyonu ve metastaz kapasitesini PCNA ve MMP-9 ekspresyonlarını immunohistokimyasal olarak değerlendirmeyi amaçladık. Cerrahi operasyon sonrası alınan biyopsi örnekleri tamponlu %10'luk formaldehit solüsyonunda tespit edildi. Rutin doku takip işlemleri sonrası hazırlanan parafin bloklardan 5 um kalınlığında kesitler alındı ve histopatolojik değişikliklerin tespit edilebilmesi amacıyla kesitlere Hematoksilen & Eozin boyaması yapıldı. İmmunohistokimyasal boyamalarda Avidin Biotin Peroksidaz yöntemi uygulandı. Kitlenin histopatolojik muayenesinde ağsı şekilli tümoral hücrelere, girdap yapılarına, çok çekirdekli dev hücrelerine ve mitotik figürlere rastlandı. İmmunohistokimyasal boyamalarda ise PCNA pozitif reaksiyonlar özellikle tümoral hücrelerin çekirdeğinde tespit edilirken, MMP-9 ekspresyonlarına ise bu hücrelerin sitoplazmasında ve çekirdeğinde rastlanıldı. Yapılan literatür taramaları sonucunda bu fibrosarkom olgusunun sultan papağanlarında tespit edilen ilk vaka olduğu ve bu yönüyle literatüre katkı sunacağı düşünülmektedir.

***Anahtar Kelimeler*:** Fibrosarkom, MMP-9, PCNA, sultan papağanı

Investigation of Antiproliferative Effects of Grape Vinegar on Myeloma Cells

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Abstract

Vinegar is a natural product obtained from the fermentation process of apples, dates, grapes, figs and many other food products rich in carbohydrates. Vinegar has been used as a flavoring and preservative for over 2000 years. Previous studies have shown that vinegar has beneficial effects in the treatment of obesity, diabetes, cardiovascular disorders, cancer, wound healing and infections. *In vitro* and *in vivo* studies have revealed that antioxidant substances present in grapes have antineoplastic activity. The aim of this study is to examine the antiproliferative effect of home-made grape vinegar obtained from grapes by traditional method on mouse myeloma cells. Pure vinegar and neutral vinegar which is neutralized with NaOH, were applied to myeloma cells in various concentrations (50%, 25%, 12.5% 25% 3.12% 1.56% 0.78 and 0.39%) for 24 hours. After 24 hours of exposure, the viability of cells was 1.28%, 1.15%, 0.10%, 0.02%, 35.04%, 54.24%, 90.68% and 94.41% in pure vinegar, respectively and 0.03%, 0.03%, 25.66%, 67.79%, 68.01%, 78.08%, 88.74% and 93.69% in neutralized vinegar, respectively. The IC_{50} values of pure vinegar and neutralized vinegar were found to be 2.21% and 8.16% for 24 hours, respectively. The results of the present study showed that vinegar had an antiproliferative effect depending on the concentration. Low pH could be the reason as to why pure vinegar has higher antiproliferative effect than neutralized vinegar.

***Keywords*:** Antiproliferative Effect, Grape, MTT, Vinegar,

Üzüm Sirkesinin Myeloma Hücrelerinde Antiproliferatif Aktivitesinin Araştırılması

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Özet

Sirke, elma, hurma, üzüm, incir ve karbonhidrat açısından zengin birçok gıda ürününden, fermantasyon işlemi sonucu elde edilen doğal bir üründür. Sirke, 2000 yılı aşkın süredir aroma verici ve koruyucu olarak kullanılmaktadır. Yapılan çalışmalarda sirkenin obezite, diyabet, kardiyovasküler bozukluklar, kanser, yara iyileşmesi ve enfeksiyonların tedavisinde faydalı etkilerinin olduğu görülmüştür. *In vitro* ve *in vivo* yapılan çalışmalar, üzümde bulunan antioksidan maddelerin antikanser etkinliğe sahip olduğunu ortaya koymuştur. Bu çalışmanın amacı geleneksel yöntem ile üzümden elde edilen ev yapımı üzüm sirkesinin fare miyeloma hücreleri üzerine antiproliferatif etkisini incelemektir. Saf ve NaOH ile pH'si nötralize edilmiş üzüm sirkeleri çeşitli konsantrasyonlarda (%50, %25, %12,5 %25 %3,12 %1,56 %0,78ve %0,39) miyeloma hücrelerine 24 saat süre ile uygulandı. 24 saatlik maruziyetinden sonra hücrelerin canlılığı saf sirkede sırasıyla %1,28, %1,15, %0,10, %0,02, %35,04, %54,24, %90,68 ve %94,41 ve nötralize sirkede %0,03, %0,03, %25,66, %67,79, %68,01, %78,08, %88,74 ve %93,69 oranlarında bulundu. Saf ve nötralize sirkenin IC_{50} değerleri 24 saat için sırasıyla %2,21 ve %8,16 olarak bulundu. Sirkeler doza bağlı antiproliferatif etkiye sahip olduğu gözlendi. Saf sirkenin antiproliferatif etkinliğinin nötralize sirkeden yüksek olmasının sebebi düşük pH olabilir.

***Anahtar kelimeler*:** Antiproliferatif etki, MTT, Sirke, Üzüm

A Case of Monkey Pox in a Baby Monkey

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Abstract:

A monkey pox was detected in a 1 month old, female, baby monkey born in Antalya Zoo and died with skin lesions. It was reported that the baby's mother was not cared for and caregivers take care of the cub and skin lesions on the hands were reported to the caregivers. At necropsy, crusted and papullary lesions were observed especially on the face, armpit, abdomen, chest and extremities, being more prominent. It was observed that the skin was covered with highly hyperemic plaque-like lesions. No macroscopic findings were observed in internal organs except pneumonic lungs. Histopathological examinations showed severe hyperemia of the skin vessels. It was noteworthy that the epidermis of the skin was thickened densely and the crusts formed as necrotic masses on the epidermis. Inflammatory cell infiltrates were seen, more prominent in the dermis. Inclusion bodies were detected in some keratinocytes in the epidermis. Hyperemia of the vessels in the lungs and thickening of the alveolar septal tissue and inflammatory cell infiltrations were observed.

Inoculation of embryonated chicken egg (11 days old) by air sac to chorioallantoic membrane (CAM) after proper preparation of homogenized skin samples. When examined after incubation, it was thickening and pox formation in the CAM. Typical pox virus particles were observed on electron microscopic examination.

Keywords: Monkey, monkey pox, histopathology, electron microscopy, pox virus.

Bir Maymun Yavrusunda Maymun Çiçeği Olgusu

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Özet

Antalya Hayvanat Bahçesinde doğan ve 1 aylık iken deri lezyonları ile ölen, dişi bir maymun yavrusunda maymun çiçeği saptandı. Yavrunun annesi tarafından bakılmadığı, bakıcılar tarafından beslendiği ve ilgilenen bakıcılarda da ellerde deri lezyonları şekillendiği bildirildi. Nekropside özellikle yüz, koltuk altı, karın ve göğüs ile ekstremitelerde daha belirgin olmak üzere tüm deride kabuklu ve papuller lezyonlar gözlendi. Derinin yer yer oldukça hiperemik plak şeklinde lezyonlarla kaplı olduğu görüldü. Pnömonik akciğerler dışında iç organlarda makroskobik bulgu gözlenmedi. Histopatolojik incelemelerde derideki damarlarda şiddetli hiperemi gözlendi. Derinin epidermisinin yoğun şekilde kalınlaştığı, epidermis üzerinde yer yer nekrotik kitleler halinde kabukların şekillendiği dikkati çekti. Dermiste daha belirgin olmak üzere yangısal hücre infiltrasyonları görüldü. Epidermisteki bazı keratinositlerde inklüzyon cisimcikleri saptandı. Akciğerlerde damarlarda hiperemi ve özellikle alveoler septal dokuda kalınlaşma ve yangısal hücre infiltrasyonları gözlendi.

Deriden alınan homojenize örneklerin usulüne uygun hazırlanmasından sonra embriyolu tavuk yumurtasına (11 günlük) hava kesesi tarafından korioallantoik membran (CAM)'a ekim yapıldı. İnkübasyon sonrası yapılan incelemelerde, CAM'da kalınlaşma ve poks oluşumları görüldü. Elektron mikroskobik incelemede tipik poks virüs partikülleri dikkati çekti.

Anahtar kelimeler: Maymun, çiçek hastalığı, histopatoloji, elektron mikroskobi, poks virüs.

A Case of Renal Adenocarcinoma in a Male Rat

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Abstract

Occasional hematuria was observed in an eight-month-old male wistar albino rat. The severity of hematuria gradually increased, and symptoms of stagnation and loss of appetite in the animal accompanied the findings. The animal was euthanized due to the deterioration of his condition. At necropsy the abdominal region was swollen and tight. During the abdominal cavity was opened, there was a 3x4x2 cm whitish, hard mass with hemorrhage in the central area in the left kidney were observed. The outer surface of the mass was surrounded by a capsule and kidney structures were removed. Histopathological examination did not reveal any renal structures except for a small number of glomeruli and cystic tubules belonging to the renal cortex in some small areas. The mass consisted of many round acinar structures. The acinar structures contained a scarce stroma between them were noticed. Pink protein rich content was found in the lumens of some acinar structures. Solid masses were also detected in some areas. The masses were composed cubic-shaped cells with a vesicular nucleus. Slight increase in mitotic activity, anaplasia and pleomorphism were noted in some cells. Vascularization increased in the mass and hemorrhage was found in some areas. In the examination of cytological preparations made from the mass, numerous epithelial cells groups with large nuclei and abundant cytoplasm with anaplastic pleomorphic appearance were observed. A positive reaction was observed in the cytokeratin immunostaining applied to the tissue. According to histopathological and immunohistochemical findings, the mass was diagnosed as renal adenocarcinoma.

***Keywords*:** Kidney adenocarcinoma, rat, pathology

Erkek Bir Ratta Böbrek Adenokarsinomu Olgusu

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Özet

Sekiz aylık, wistar albino ırkı erkek bir ratta zaman zaman şekillenen hematüri gözlendi. Hematürinin şiddetinin gitgide arttığı, hayvanda durgunluk ve iştahsızlık bulgularının da tabloya eşlik ettiği görüldü. Durumunun kötüye gitmesi sebebiyle hayvana ötenazi uygulandı. Nekropside karın bölgesinin şişkin ve gergin olduğu gözlendi. Karın boşluğu açıldığında sol böbrekte 3x4x2 cm boyutlarında beyazımsı renkli, merkezi bölgesi ise kanamalı sert kıvamlı bir kitle bulunduğu görüldü. Böbreğe ait tüm yapıların gözden silindiği kitlenin dış yüzeyi bir kapsül ile çevriliydi. Histopatolojik incelemede bazı küçük alanlarda böbrek korteksine ait az sayıda glomerulus ve kistik tubullerin bulunduğu alanlar dışında böbreğe ait yapılar saptanmadı. Kitlenin çok sayıda yuvarlak şekilli asiner yapılardan oluştuğu gözlendi. Asiner yapıların aralarında kıt bir stromanın bulunduğu dikkati çekti. Bazı asiner yapıların lümenlerinde pembe renkli proteinden zengin bir içerik bulunduğu görüldü. Yer yer solid kitleler de saptandı. Kitleler kübik şekilli ve veziküler çekirdekli hücrelerden oluşmuştu. Bazı hücrelerde mitotik aktivitenin arttığı gözlendi, anaplazi ve pleomorfizm dikkati çekti. Kitlede damarlaşmanın arttığı ve yer yer kanamaların bulunduğu saptandı. Kitleden yapılan sitolojik preparatların incelemesinde anaplazik pleomorfik özelliklerde çok sayıda ve gruplar halinde büyük çekirdekli ve bol sitoplazmalı epitel hücreleri gözlendi. Dokuya uygulanan sitokeratin boyamasında pozitif reaksiyon saptandı. Histopatolojik ve immunohistokimyasal bulgulara göre kitleye böbrek adenokarsinomu teşhisi konuldu.

Anahtar kelimeler: Böbrek adenokarsinomu, rat, patoloji

Pathological Investigations in Tench (*Tinca tinca* (L., 1758)) Naturally Infected with *Ligula intestinalis* Plerocercoids

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Abstract

The aim of this study was to examine the pathological findings of 30 naturally infected tench (*Tinca tinca*) with *Ligula intestinalis* plerocercoids defined according to their taxonomic characteristics from Golhisar Lake (Burdur). Clinically and macroscopically, poor and irregular swimming, hemorrhages at the bases of fins and around the anus, the presence of the plerocercoids in the viscera together with perivisceral hemorrhages and distension of abdomen were generally observed in the diseased fish. Microscopically, hemorrhages, necrosis and mononuclear cell infiltrations were seen in the viscera invaded by plerocercoids. As a result, the cestode *Ligula intestinalis* plerocercoids'pathological findings on the tench were widely evaluated for the first time in Turkey.

Keywords: *Ligula intestinalis*, plerocercoid, tench, pathology.

Ligula Intestinalis Pleroserkoidleri ile Doğal Enfekte Kadife Balıklarında ((*Tinca tinca* (L., 1758)) Patolojik Bulguların İncelenmesi

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Özet

Bu çalışmanın amacı, Burdur Gölhisar Gölü'nde taksonomik özelliklerine göre tanımlanmış *Ligula intestinalis* pleroserkoidleri ile doğal enfekte 30 adet kadife balığında (*Tinca tinca*) patolojik bulguların incelenmesidir. Etkilenmiş balıklarda klinik ve makroskobik olarak zayıf ve düzensiz yüzme, yüzgeç tabanlarında ve anüs çevresinde kanamalar, iç organlarda pleroserkoidlerin varlığı ile periviseral kanamalar ve karın şişkinliği genel olarak dikkati çekti. Mikroskobik olarak, pleroserkoidlerin istila ettiği iç organlarda kanamalar, nekroz ve mononükleer hücre infiltrasyonları görüldü. Sonuç olarak, Türkiye'de ilk defa Ligula intestinalis pleroserkoidlerinin kadife balığı üzerindeki patolojik etkileri yaygın olarak değerlendirilmiştir.

Anahtar kelimeler: *Ligula intestinalis, pleroserkoid,* kadife balığı, patoloji

The Immunohistochemical Detection of Toll Like Receptors in Mycoplasma Pneumonia

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Abstract

Mycoplasma pneumonia is a highly contagious disease that causes serious economic losses in sheep, goats and cattle worldwide. The aim of this study was to investigate pathomorphologically and immunohistochemically Toll-Like Receptors (TLRs) involved in the pathogenesis of mycoplasma pneumonia (n:40). Histopathologically, purulent bronchopneumonia (n:13), fibrinous bronchopneumonia (n:14), interstitial pneumonia (n:6), granulomatous pneumonia (n:1) and non-specific lesions (n:6) were observed. The same tissue sections were immunohistochemically stained with *Mycoplasma pneumoniae*, TLR2, TLR4, and TLR6 antibodies. The TLRs showed positive reactions of varying intensity in the bronchial, bronchiolar and alveolar epithelial cells and/or inflammatory cells. While, TLR2 was predominant in purulent inflammation, in case of fibrinous type inflammation, the dominant responsive Toll-like receptor appeared to be TLR4. It was concluded that TLRs has been observed to play a major role in the pathogenesis of mycoplasma pneumonia.

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***Keywords*:** Immunohistochemistry, *Mycoplasma* spp., pathomorphology, Toll-like receptor

Mikoplazma Pnömonilerinde Toll Benzeri Reseptörlerin İmmunohistokimyasal Yöntemlerle Saptanması

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Özet

Mikoplazma pnömonileri dünyada koyun, keçi ve sığırlarda ciddi ekonomik kayıplara sebep olan oldukça bulaşıcı bir hastalıktır. Çalışmada Mikoplazma pnömonilerinin patogenezinde rol oynayan Toll benzeri reseptörlerin (Toll-Like Receptor - TLR) patomorfolojik ve immunohistokimyasal yöntemlerle araştırılması amaçlanmıştır (n:40). Histopatolojik olarak purulent bronkopnömoni (n:13), fibrinli bronkopnömoni (n:14), interstisyel pnömoni (n:6), granülomatöz pnömoni (n:1) ve non spesifik lezyonlar (n:6) gözlendi. Aynı doku kesitleri immunohistokimyasal olarak *Mycoplasma pneumoniae*, TLR2, TLR4 ve TLR6 antikorları ile boyandı. Bu boyamalarda değişen şiddetlerde bronş, bronşiyol, alveol epitel hücrelerinde ve/veya yangı hücrelerinde TLR'lerin pozitifliği saptandı. TLR2 purulent bronkopnömonilerde baskın iken, fibrinli bronkopnömonilerde baskın yanıt veren Toll benzeri reseptör TLR4 olarak gözlendi. TLR'lerin mikoplazma pnömonilerinin patogenezinde önemli bir rol oynadığı sonucuna varıldı.

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***Anahtar Kelimeler*:** İmmunohistokimya, *Mycoplasma* spp., patomorfoloji, Toll benzeri reseptör

Cardioprotective Effects of Fetal Kidney Derived Mesenchymal Stem Cells on Doxorubicin-induced Cardiotoxicity in Rats

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Abstract

Cardiotoxicity is one of the most common side effects of doxorubicin, a chemotherapy drug that is used in the treatment of many carcinomas. In recent years, stem-cell therapies have been successfully applied in prevention of cardiotoxicity. In this study, the cardioprotective efficacy of intraperitoneal administration of fetal kidney-derived mesenchymal stem cells in the prevention of DOX-induced cardiotoxicity in rats was investigated. For this purpose, thirty rats were randomly divided into three groups as Control, Doxorubicin and Mesenchymal Stem Cell groups. Adriamycin was applied as a single dose via the tail vein to Doxorubicin and Mesenchymal Stem Cell groups in order to effectuate cardiotoxicity. Stem Cell was applied to Mesenchymal Stem Cell group via intraperitoneal route after cardiotoxicity has been established. Then rats were euthanized and routine histological procedures were performed on hearts. H&E and Masson's stain were used for histopathology. Cardiac Troponin-T and I, Caspase-3, BCL-XL, VEGF and TGF-β1 antibodies were used for immunohistochemistry. Histopathologically, vacuole, edema, degeneration and necrosis were observed mostly in Doxorubicin group. The lesions in Control and Mesenchymal Stem Cell groups were less severe. Fibrosis was observed milder in Control and Mesenchymal Stem Cell groups. Cardiac Troponin T and I immunopositive staining were most commonly seen in Control followed by Mesenchymal Stem Cell groups. Immunohistochemical staining by Caspase-3, BCL-XL and TGF-β1 showed that expressions in Mesenchymal Stem Cell group were statistically similar to Control group. VEGF expressions were severe than both Doxorubicin and the Control group.

Accordingly, it was concluded that intraperitoneal Mesenchymal Stem Cell administrations contributed positively to histopathological findings, fibrosis, immunohistochemistry, especially apoptosis, neovascularization, anti-apoptotic development, whereas troponin levels were not found to be therapeutic.

***Keywords*:** Doxorubicin, immunohistochemistry, cardiotoxicity, mesenchymal Stem Cell, troponin

Sıçanlarda Doksorubisinle İndüklenmiş Kardiyotoksisitede Fötal Böbrek Kaynaklı Mezenkimal Kök Hücrelerin Kalp Koruyucu Etkileri

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Özet

Kardiyotoksisite, birçok karsinomun tedavisinde kullanılan bir kemoterapi ilacı olan doksorubisinin en yaygın yan etkilerinden birisidir. Son yıllarda kardiyotoksisitenin önlenmesinde kök hücre tedavileri başarıyla uygulanmaktadır. Bu çalışmada, sıçanlarda doksorubisin ile indüklenen kardiyotoksisitenin önlenmesinde fötal böbrek kaynaklı mezenkimal kök hücrelerin intraperitoneal uygulamasının kardiyoprotektif etkinliği histopatolojik ve immunohistokimyasal yöntemlerle araştırılmıştır. Bu amaçla otuz adet sıçan, Kontrol, Doksorubisin ve Mezenkimal Kök Hücre grupları olarak rastgele üç gruba ayrıldı. Kardiyotoksisite oluşturulması için adriamisin, Doksorubisin ve Mezenkimal Kök Hücre gruplarına kuyruk veninden tek doz olarak uygulandı. Kök hücreler, kardiyotoksisite oluşturulduktan sonra Mezenkimal Kök Hücre grubuna intraperitoneal yolla uygulandı. Daha sonra farelere ötenazi yapıldı ve kalpler üzerinde rutin histolojik prosedürler uygulandı. Histopatoloji için Hematoksilen-Eozin ile Masson Trikrom boyası kullanıldı. İmmünohistokimya için kardiyak Troponin-T ve I, Caspase-3, BCL-XL, VEGF ve TGF-β1 antikorları kullanıldı. Histopatolojik olarak en çok Doksorubisin grubunda vakuol, ödem, dejenerasyon ve nekroz görüldü. Kontrol ve Mezenkimal Kök Hücre gruplarındaki lezyonlar daha az şiddetliydi. Fibrozis, Kontrol ve Mezenkimal Kök Hücre gruplarında daha hafif gözlendi. Troponin T ve I immünopozitif boyama en yaygın olarak Kontrol ve ardından Mezenkimal Kök Hücre gruplarında görüldü. Caspase-3, BCL-XL ve TGF-β1 ile immünohistokimyasal boyamalar sonucunda, Mezenkimal Kök Hücre grubundaki ekspresyonların Kontrol grubuna istatistiksel olarak benzer olduğu gözlendi. VEGF

ekspresyonları hem Doksorubisin hem de Kontrol grubundan şiddetliydi. Buna göre intraperitoneal mezenkimal kök hücre uygulamalarının histopatolojik bulgulara, fibrozis, immünohistokimya, özellikle apoptoz, neovaskülarizasyon, anti-apoptotik gelişime olumlu katkı sağladığı, troponin seviyelerinin ise tedavi edici düzeyde bulunmadığı sonucuna ulaşıldı.

***Anahtar Kelimeler*:** Doksorubisin, immunohistokimya, kardiyotoksisite, mezenkimal kök hücre, troponin.

Immunohistochemically Investigation of Oxidative Stress-Induced DNA Damage in Papillomas and Fibropapillomas

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Abstract

In this study, we aimed to evaluate the expression of 8-oHdG immunohistochemically in order to determine the oxidative stress-induced DNA damage in a total of 40 papilloma samples taken from cattle, horses and dogs at the Department of Pathology at Kafkas University Faculty of Veterinary Medicine between 2013-2020. Biopsy samples taken after surgery were fixed in buffered 10% formaldehyde solution. Sections of 5 μm thickness were taken from the paraffin blocks prepared after routine tissue follow-up procedures and Hematoxylin & Eosin staining was applied to the sections in order to detect histopathological changes. Avidin Biotin Peroxidase method was used for immunohistochemical staining. In papilloma cases, severe hyperkeratosis, retepects extending from the epidermis to the dermis, spongiosis and balloon-like degeneration in squamous epithelial cells, basophilic inclusion bodies in granular cells, ulcerations in the epidermis layer, hemorrhagic areas and an increase in kerato hyaline granules were observed. In addition to these findings, dense connective tissue increases were detected in fibropapilloma cases. In immunohistochemical evaluations, 8-oHdG positive reactions were detected in the cytoplasm and nucleus of the epidermal cells in papilloma cases, whereas in cases of fibropapilloma, reactions in these cells were observed in the cytoplasm of fibrocytes and fibroblasts in the dermis. As a result of the literature reviews, we have not found any study data in which DNA damage due to oxidative stress was detected through 8-oHdG expression in cases of papilloma and fibropapilloma belonging to three different species, and in this respect, the findings obtained from our study are thought to contribute to the literature. Additionally, we believe that oxidative stress plays an important role in the pathogenesis of this tumor.

***Keywords*:** 8-oHdG, DNA damage, oxidative stress, papilloma

Papillom ve Fibropapillomlarda Oksidatif Stres Kaynaklı DNA Hasarının Immunohistokimyasal Olarak Araştırılması

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Özet

Bu çalışmamızda 2013-2020 yılları arasında Kafkas Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalına sığır, at ve köpeklerden alınan toplamda 40 adet papillom örneğinde oksidatif stres kaynaklı DNA hasarını belirlemek amacıyla immunohistokimyasal olarak 8-oHdG ekspresyonunu değerlendirmeyi amaçladık. Cerrahi operasyon sonrası alınan biyopsi örnekleri tamponlu %10'luk formaldehit solüsyonunda fikze edildi. Rutin doku takip işlemleri sonrası hazırlanan parafin bloklardan 5 um kalınlığında kesitler alındı ve histopatolojik değişikliklerin saptanabilmesi amacıyla kesitlere Hematoksilen & Eozin boyaması uygulandı. İmmunohistokimyasal boyamalarda Avidin Biotin Peroksidaz metodu uygulandı. Papillom vakalarında şiddetli hiperkeratoz, epidermisten dermise doğru uzanan retepektler, skuamöz epitel hücrelerinde spongiyozis ve balonumsu dejenerasyon, granüler hücrelerde bazofilik inklüzyon cisimcikleri, epidermis katmanında ülserasyonlar ve kanama alanları ile kerato hiyalin granüllerinde artış gözlendi. Fibropapillom vakalarında ise bu bulgulara ek olarak girdap tarzında bağ doku artışları tespit edildi. İmmunohistokimyasal değerlendirmelerde ise 8-oHdG pozitif reaksiyonlar papillom vakalarında epidermal hücrelerin sitoplazmasında ve çekirdeğinde saptanırken, fibropapillom vakalarında ise bu hücrelerdeki reaksiyonlara ek olarak dermisteki fibrosit ve fibroblastların sitoplazmasında rastlanıldı. Yapılan literatür taramaları sonucunda üç farklı türe ait papillom ve fibropapillom vakalarında oksidatif strese bağlı DNA hasarının 8-oHdG ekspresyonu vasıtasıyla tespit edildiği herhangi bir çalışma verisine rastlanmamış olup ve bu yönüyle çalışmamızdan elden edilen bulguların literatüre katkı sunacağı düşünülmektedir. Bunlara ek olarak oksidatif stresin bu tümörün patogenezinde önemli bir rol oynadığı kanaatindeyiz.

Anahtar Kelimeler: 8-oHdG, DNA hasarı, oksidatif stres, papillom

Mammary Squamous Cell Carcinoma in a Dog

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Abstract

Mammary neoplasms are the most common neoplasms in female dogs (1). In this case report, it is aimed to describe mammary intraductal primary squamous cell carcinoma with severe hydropic degeneration in a dog. Mammary primary squamous cell carcinoma is a rare tumor in humans and dogs (2,3). The pathogenesis of this tumor is not known exactly, and it is accepted that it is formed after squamous cell metaplasia due to cyst, abscess or chronic inflammation (3,4). 8 years old, female, 30 kg weighted Rottweiler breed dogs who was brought to Hatay Mustafa Kemal University Veterinary Health Practice and Research Hospital with the complaint of mass and discharge in the breast is the material of the presented case. Via anamnesis it was found that she had never given birth before, had not undergone ovariohysterectomy, and that no hormone therapy was administered to suppress oestrus. It was learned that a mass was noticed in the second mammary gland of the left mammary chain 20 days ago, the mass grew rapidly within 10 days and there was no general condition disorder affecting the appetite in this process. Thoracic radiographs did not show any metastatic mass in the lungs. In the clinical examination it was observed that whole mammary glands had thick and smelly discharge flow. Unilateral total mastectomy was performed to the patient after antibiotic therapy. Macroscopically, the mass was 13x10x10 in diameter and covered with ulcerated skin. The mass was elastic in consistency with whitish cut sections. Histopathological examination revealed that the epithelium of the teat ducts undergone squamous metaplasia and neoplastic transformation. At the periphery of the neoplastic lobules the atypical cells are often smaller with a more basophilic cytoplasm. It was noticed the severe hydropic degeneration of these cells towards the center. Keratin pearls (cancer pearls) without lamellation were observed at the center of the lobules. Pleomorphism and few mitotic figures were observed in neoplastic epithelial cells. In addition, large necrosis foci, inflammatory cell infiltration and calcification were detected. It is thought that metaplasia and neoplastic transformation are shaped by chronic

inflammation in the present case as mentioned in previous studies (3-5). Squamous cell carcinoma, usually seen on the skin in dogs, was described in the mammary with this study.

Keywords: Dog, Histopathology, mammary, mastectomy, squamous cell carcinoma

Bir Köpekte Memede Yassı Hücreli Karsinom

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Özet

Meme tümörleri dişi köpeklerde gözlenen en yaygın tümörlerdir (1). Bu olgu sunumunda bir köpeğin memesinde şiddetli hidropik dejenerasyonla seyreden intraduktal primer yassı hücreli karsinomun tanımlanması amaçlanmıştır. Memede bu tümör insanlarda ve köpeklerde nadir görülen bir tümördür (2,3). Bu tümörün patogenezi tam bilinmemekte olup memedeki kist, apse veya kronik yangıya bağlı şekillenen yassı hücre metaplazisi sonrası şekillendiği kabul edilmektedir (3,4). Sunulan olgunun materyalini, Hatay Mustafa Kemal Üniversitesi Veteriner Sağlık Uygulama ve Araştırma Hastanesi'ne memede kitle ve akıntı şikâyetiyle getirilen; 30 kg ağırlığında 8 yaşlı dişi bir Rottweiler ırkı köpek oluşturdu. Alınan anamnez sonucunda; daha önce hiç doğum yapmadığı, ovariohisterektomi operasyonu geçirmediği, östrusların baskılanması amacıyla herhangi bir hormon uygulamasının yapılmadığı bilgilerine ulaşıldı. 20 gün önce sol meme zinciri 2. meme bezinde bir kitle fark edildiği ve kitlenin 10 gün içinde hızla büyüdüğü, süreç içinde iştahı etkileyen genel durum bozukluğunun olmadığı öğrenildi. Alınan toraks radyografisinde akciğerlerde herhangi bir metastatik kitleye rastlanmadı. Yapılan klinik muayenede tüm meme bezlerinden koyu kıvamlı ve kokulu akıntı geldiği görüldü. Antibiyotik uyugulamalarından sonra hastaya unilatetal total mastektomi uygulandı. Makroskobik olarak, üzerinde ülserli deri bulunan kitle, 13x10x10 çapındaydı. Elastik kıvamda olan kitlenin kesit yüzü beyazımsı renkteydi. Histopatolojik incelemede, süt kanalları epitelinin skuamöz metaplaziye ve neoplastik dönüşüme uğradığı gözlendi. Neoplastik lobullerin periferinde atipik epitel hücreleri daha küçük ve bazofilik sitoplazmalıydı. Merkeze doğru bu hücrelerde şiddetli hidropik dejenerasyon oluştuğu dikkat çekti. İntraduktal epitelin çoğalarak ortasında lamellasyon göstermeyen keratin bulunan kanser incileri şekillendirdiği dikkat çekti. Neoplastik epitel hücrelerinde pleomorfizm ve az sayıda mitotik figür gözlendi. Ayrıca geniş nekroz odakları, yangısal hücre infiltrasyonu ve kalsifikasyon mevcuttu. Bu vakada daha önceki çalışmalarda belirtildiği gibi metaplazinin ve neoplastik dönüşümün kronik yangıya bağlı

şekillendiği düşünülmektedir (3-5). Bu çalışma ile köpeklerde genellikle deride görülen yassı hücreli karsinom meme bezinde tanımlanmıştır.

Anahtar kelimeler: Histopatoloji, köpek, mastektomi, meme, yassı hücreli karsinom

Avocado (*Persea americana*) Toxicity in Six Rabbits

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Abstract

Avocado (*Persea americana*) is the one of the most nutrient-rich fruit in human dietary, but it can also be highly toxic to particular animal species. Consumption of persin, which is a toxic fatty acid found in avocado leaves, barks and seeds, can be extremely harmful and leads to death in rabbits. This case report describes clinical, macroscopic and histopathological findings of avocado poisoning in 2 month old six rabbits. In history, two rabbits found death and others showed depression, weakness, anorexia, and dyspnea (in one rabbit) within 24 hours following avocado consumption and submitted to a veterinary clinic. Rabbits received emergency care and symptomatic treatment for suggested toxicosis include gastroprokinetic agent, fluid therapy and activated carbon. But two of them died shortly after therapy. At necropsies of four rabbits, livers were enlarged and congested, multifocal pale areas in myocardium were evident and one rabbit had hydropericardium. Gastric and small intestine lumens of all animals were filled with yellowish-green watery content and avocado leaves were found in gastric lumens. Histopathological findings included severe diffuse vacuolar degeneration in hepatocytes and loss of normal hepatic chord architecture, excessive zenker's degeneration and necrosis in myocytes, mononuclear cell infiltrations and multifocal hemorrhages in myocardium. In addition, accidental cartilaginous foci was observed in one right fibrous trigone. Clinical and pathological findings were found consistent with previously reported avocado poisoning cases in rabbits.

Keywords: Avocado, persin, rabbit, toxicity

Altı Tavşanda Avokado (*Persea americana*) Zehirlenmesi

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Özet

Avokado (*Persea americana*) insan beslenmesindeki besin değeri en yüksek olan meyvelerden biridir, ancak bazı hayvan türleri için son derece toksik olabilir. Avokadonun yapraklarında, kabuğunda ve çekirdeğinde bulunan Persin isimli toksik yağ asidinin tüketilmesi tavşanlar için oldukça zararlıdır ve ölümle sonuçlanabilir. Bu vaka takdimi 2 aylık altı tavşanda gözlenen avokado zehirlenmesinin klinik, makroskobik ve mikroskobik bulgularını tanımlamaktadır. Anamnezde, avokado tüketimini takiben 24 saat içinde iki tavşanın ölü olarak bulunduğu, diğer tavşanlar için depresyon, güçsüzlük, anoreksi ve dispne (bir tavşanda) şikayetleri bildirilmiştir. Kliniğine başvurulduğunda tavşanlara acil bakım ve gastroprokinetik ajan, sıvı tedavisi ve aktif karbonu içeren toksikasyon şüphesi ile semptomatik tedavi görmelerine rağmen, etkilenenlerden iki tanesi tedaviyi takiben kısa bir süre içerisinde ölmüştür. Nekropsisi yapılan dört tavşanın karaciğerlerinin genişlemiş ve konjestif olduğu, miyokardiyumlarında multifokal açık renkli alanların bulunduğu ve bir tavşanda hidroperikardiyum varlığı dikkati çekti. Tüm tavşanların mide ve ince bağırsak lümenleri yeşilimsi-sarı renkli sulu bir içerik ile doluydu ve mide lümeninde avokado yapraklarının varlığı gözlendi. Mikroskobik incelemede, hepatositlerde şiddetli diffuz vakuoler dejenerasyon, sinüzoidlerde dissosiyasyon, kalpte myokardiyumda multifokal kanamalar, myositlerde şiddetli zenker dejenerasyonu, nekroz ve mononükleer hücre infiltrasyonları görüldü. Ayrıca incelenen hayvanların birinde sağ fibröz trigonda rastlantısal olarak kartilaginöz bir yapı dikkati çekti. Klinik ve patolojik bulgular, daha önce belirtilen avokado zehirlenmeleri ile uyumlu bulunmuştur.

Anahtar kelimeler: Avokado, persin, tavşan, toksisite

Practical Rodent Pathology

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Abstract

Complete and systematic necropsy procedures are as necessary for research as they are for diagnostic pathology. Protocols should be practical, reproducible (replicable), ensure complete and systematic gross examination, as well as be adaptable to particular needs of different projects. Reporting should include relevant detail to replicate the procedures, between groups separated by months or years, as well as for publication. This protocol for mice evaluates more than 40 tissues on about 10 slides, and will be illustrated with examples of features and common findings in mice. Applications and modifications for other species are discussed briefly. Please download the manual from **http://mcp.bs.jhmi.edu/me680712-phenotyping-functional-genetics**. The manual includes information on clinical examination and clinical pathology of mice, as well as descriptions of the necropsy examination, collections and trimming, and perfusion, and a summary table of examples of common findings in tissues in order of our slide numbers.

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GORENI https://www.goreni.org/ online reference for nomenclature and diagnostic criteria for the global toxicologic pathology initiative "**INHAND**" - the International Harmonization of Nomenclature and Diagnostic criteria.

NNLA https://ntp.niehs.nih.gov/nnl/ NTP Non Neoplastic Lesion Atlas (rodents)

RITA https://reni.item.fraunhofer.de/reni/public/rita/index.php (Registry of Industrial Toxicology Animal-data) centralized collection of historical control data on rodent carcinogenicity studies from different laboratories in a comprehensive database

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Histoprotective Effect of Resveratrol and Avocado Oil Against Acetaminophen Related Acute Nephrotoxicity in Rats

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Abstract

Acetaminophen has an acceptable safety profile when used in prescribed doses, however, it can damage the kidneys when administered as an overdose. This study was designed to investigate the tissue repair effects of Resveratrol and Avocado oil on acetamiophen-induced acute nephrotoxicity in rats. 30 male Wistar rats approximately 500 gr were used randomly divided into 5 groups, each containing 6 rats: Group I = Control; Group II = Acetaminophen (600 mg/kg i.p;single dose); Group III = Acetaminophen+Resveratrol (10 mg/kg/po Resveratrol, for 4 days); Group IV = Acetaminophen+Avocado Oil (200mg/kg/po Avocado Oil, for 4 days); Group V = Acetaminophen + Resveratrol + Avocado Oil (combination treatment with Resveratrol and Avocado Oil doses). At the end of the experiment, renal tissue samples were collected for histopathological examinations. Kidney tissues were stained with hematoxylin and eosin for histological assessment. Significant histopathological changes including tubular vacuoler degeneration, tubular dilatation, cortical and medullar haemorrhage, mononuclear cell infiltration were observed in the Group II. Renal histopathological examinations showed that Resveratrol administration significantly reduced Acetaminophen induced renal damage. However, Avocado Oil is ineffective. In conclusion, it is thought that the use of Resveratrol as a prophylactic agent against renal damage may be beneficial.

Keywords: Acetaminophen, resveratrol, avocado oil, nephrotoxicity

Septicemia in a Salamander

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Abstract

In this report, a case of septicemia is described in a baby male salamander who died with symptoms of loss of appetite and lethargy. At necropsy, a slightly turbid fluid was observed in the abdominal cavity. Liver was enlarged and pale in color. In addition, many pale colored foci were noted on the surface and cross section of the liver. Intestines were full fill with gas and swollen. Hemorrhagic foci were observed in the large intestines. At the histopathological examination, many necrotic foci were noted in the spleen, liver and intestines. Intense clusters of bacteria were found in the center of many of the foci. Severe hyperemia in all lesional organs and prominent melanomacrophages in the liver were noted. In the Gram stain applied to the tissues, Gram positive cocci were observed in the lesions. The cause of death of the animal was evaluated as septicemia. Since the case was a salamander, it was found worth for presenting.

Keywords: Salamander, septicemia, pathology

Bir Semenderde Septisemi Olgusu

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Özet

Bu raporda iştahsızlık ve uyuşukluk semptomları ile ölen yavru erkek bir semender de septisemi olgusu tanımlandı. Nekropside karın boşluğunda hafif bulanık bir sıvının bulunduğu görüldü. Karaciğerin büyümüş ve soluk renkli olduğu gözlendi. Ayrıca karaciğerin yüzeyi ve kesit yüzünde çok sayıda soluk renkli odaklar dikkati çekti. Bağırsaklar gazlı ve şişkin görünümdeydi. Kalın bağırsaklarda yer yer kanama odakları gözlendi. Histopatolojik incelemede dalak, karaciğer ve bağırsaklarda çok sayıda nekrotik odaklar dikkati çekti. Odakların birçoğunun merkezinde yoğun şekilde bakteri kümelerine rastlandı. Tüm lezyonlu organlarda şiddetli hiperemi ve karaciğerde belirgin şekilde melanomakrofajlar dikkati çekti. Dokulara uygulanan Gram boyamasında etkenlerin Gram pozitif koklar şeklinde olduğu gözlendi. Hayvanın ölüm sebebi septisemi olarak değerlendirildi. Olgu bir semenderde olduğu için sunulmaya değer bulundu.

Anahtar kelimeler: Semender, septisemi, patoloji

Pulmonary Aspergillosis in a 20 Day Old Suckling Lamb

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Abstract

In this study, a case of pulmonary aspergillosis was described in a 20-day-old dairy lamb. Lamb suffering from high fever, anorexia, weakness and balance problems was brought to the Department of Pathology at Kafkas University Faculty of Veterinary Medicine for necropsy. According to the anamnesis obtained from the owner of the animal, it was recorded that the animal was given an intense and random drug treatment. Systemic necropsy of the animal was then performed. Various tissue samples were taken from the animal for histopathological and molecular examinations. On macroscopical examination, a yellowish-white colored focus 2 * 2 mm in diameter was detected in only one area of the animal's lung. Sabouraud Dextrose Agara (SDA) was cultivated from tissue samples taken for microbiological analysis of fungal agents, and the media were left to incubate at 24˚C for 4 days in aerobic conditions. The agent identification was performed according to typical macroscopic and microscopic fungal morphologies and reproductive characteristics. In histopathological examinations, granuloma structure in the lung was detected. In the center of this granuloma, in addition to severe necrosis, neutrophils and fungal hyphae were detected. It was noted that these hyphae gave a positive reaction in the PAS staining of the lung tissue. As a result of histopathological and molecular examinations, this case, which is rarely seen in dairy lambs, was diagnosed with pulmonary aspergillosis.

***Keywords*:** Aspergillus fumigatus, pulmonary aspergillosis, milk lamb

20 Günlük Bir Süt Kuzusunda Pulmoner Aspergillozis Vakası

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Özet

Bu çalışmada 20 günlük bir süt kuzusunda pulmoner aspergillozis vakası tanımlanmıştır. Yüksek ateş, iştahsızlık, halsizlik ve denge problemleri yaşayan kuzu nekropsisi yapılmak üzere Kafkas Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na getirildi. Hayvan sahibinden alınan anamnez bilgilerine göre hayvana yoğun ve gelişigüzel bir şekilde ilaç tedavisi yapıldığı kaydedildi. Ardından hayvanın sistemik nekropsisi yapıldı. Histopatolojik ve moleküler incelemeler için hayvandan çeşitli doku örnekleri alındı. Makroskobik muayenede hayvanın akciğerinde sadece bir alanda 2*2 mm çapında sarımtrak beyaz renkli bir odak tespit edildi. Fungal etkenlerin mikrobiyolojik analizi amacıyla alınan doku örneklerinden Sabouraud Dekstroz Agara (SDA) ekim yapılarak besiyerleri aerobik koşullara 24˚C'de 4 gün süreyle inkübasyona bırakıldı. Etken identifikasyonu tipik makroskobik ve mikroskobik fungal morfolojilerine ve üreme özelliklerine göre gerçekleştirildi. Histopatolojik incelemelerde, akciğerde granulom yapısı saptandı. Bu granulomun merkezinde ise şiddetli nekrozun yanı sıra nötrofiller ve mantar hifalarının varlığı tespit edildi. Akciğer dokusunun PAS boyamasında bu hifaların pozitif reaksiyon verdiği ve boya aldığı kaydedildi. Histopatolojik ve moleküler incelemeler sonucunda süt kuzularında oldukça nadir olarak görülen bu vakaya pulmoner aspergillozis tanısı konuldu.

***Anahtar kelimeler*:** *Aspergillus fumigatus*, pulmoner aspergillozis, süt kuzusu

A Case of Enzootic Nasal Adenocarcinoma (ENA) in a Honamli Goat

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Abstract

A five-year-old Honamli breed goat was brought in with symptoms of a nasal discharge, difficulty breathing, and softening of the nasal bones. The owner of the animal reported that in 250 goat herds some animals had a clear nasal discharge that persisted for more than a year, and more than 20 goats died in this period in the herd. It was stated that the disease occurred after a new male goat was taken into the herd, and the findings were formed in animals from time to time. Clinical examination revealed significant respiratory distress, depression and anorexia in the goat. Mucoid nasal discharge covered around the nose and mouth. The animal was euthanized due to poor prognosis. At necropsy, when the skin removed, softening and deformities were observed in the nasal bones on the right side. When the nasal cavity was opened, unilaterally located tumoral masses were detected on the right side The masses that completely filled the nasal cavity were soft, pink in color and covered with a mucoid exudate, and invasion into the nasal sinuses was observed. The tumor mass located in the right nasal cavity was 5x4.5x3 cm in size. It drew attention that the masses were deforming in the conchae. Lungs were markedly emphysematous. Fibrosis was seen on the epicardium of the heart. At the cytological examination of the mass, a large number of cubic shaped tumoral cells were found individually or in groups. Histopathological examination revealed that the mass consisted of abundant well-differentiated cubic or columnar shaped, cells with eosinophilic cytoplasm and round or oval nuclei. The tumor was diagnosed as Enzootic Nasal Adenocarcinoma according to the clinical and pathological findings. This is the first report enzootic nasal adenocarcinoma detected in a Honamli goat Turkey.

Keywords: Enzootic Nasal Adenocarcinoma (ENA), Honamli goat, Pathology.

Honamlı Irkı Bir Keçide Enzootik Nazal Adenokarsinom (ENA) Olgusu

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Özet

Beş yaşlı Honamlı ırkı bir keçi, burun akıntısı, solunum güçlüğü ve nazal kemiklerde yumuşama semptomları ile getirildi. Hayvan sahibi 250 başlık sürüde 1 yıldan daha fazla devam eden, şeffaf bir burun akıntısı olduğu ve sürüde bu süre içinde 20'den fazla keçinin öldüğünü bildirdi. Hastalığın sürüye yeni bir teke alındıktan sonra şekillendiği ve zaman zaman hayvanlarda bulguların şekillendiği ifade edildi. Klinik muayenede keçide belirgin bir solunum güçlüğü, depresyon ve iştahsızlık gözlendi. Mukoid burun akıntısı burun ve ağız çevresine bulaşmıştı. Prognozun kötü olması sebebiyle hayvana ötenazi yapıldı. Nekropside, kafa derisi yüzüldüğünde sağ tarafta burun kemiklerinde erime ve deformiteler gözlendi. Burun boşluğu açıldığında sağ tarafta unilateral yerleşimli tümöral kitleler saptandı. Burun boşluğunu tamamen dolduran kitleler yumuşak, pembe renkli ve üzeri mukoid bir eksudatla kaplı görünümdeydi ve nazal sinuslara invazyon gözlendi. Sağ burun boşluğuna lokalize olan tümör kitlesi 5x4,5x3 cm boyutlarındaydı. Kitlelerin konhaları deforme ettikleri dikkati çekti. Akciğerler belirgin şekilde amfizemliydi. Kalpte epikard üzerinde fibrozis görüldü. Kitlenin sitolojik incelemesinde kübik şekilli çok sayıda tümöral hücrelere tek tek veya gruplar halinde rastlandı. Histopatolojik incelemede kitlenin iyi diferensiye kübik veya kolumnar şekilli, bol, eozinofilik sitoplazmalı ve yuvarlak veya oval çekirdekli hücrelerden oluştuğu gözlendi. Klinik ve patolojik bulgulara göre tümör Enzootik Nasal Adenokarsinom olarak teşhis edildi. Bu Türkiye'de Honamlı ırkı bir keçide saptanan ilk Enzootik Nasal Adenokarsinom raporudur.

***Anahtar Kelimeler*:** Enzootik Nasal Adenokarsinom (ENA), Honamlı keçisi, Patoloji.

Investigation of the Presence of Coccidiosis by Histopathological Methods in Geese in the Kars Region

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Abstract

In this study, we aimed to detect the presence of renal coccidiosis by histopathological methods in 139 goose specimens brought dead to the Department of Pathology, Faculty of Veterinary Medicine, Kars Kafkas University between 2013-2020. Various clinical signs such as fever, respiratory distress, weakness, anorexia, tremors, inability to get up from the ground, balance disorders, rotational movement, diarrhea, wheezing were detected in geese. In systemic necropsies of geese, large and small white nodular structures were detected in the kidney. Tissue samples taken were fixed in 10% buffered formaldehyde solution. 5 µm-thick sections were taken from the paraffin blocks prepared after routine tissue follow-up procedures. Hematoxylin & Eosin staining was applied to the sections in order to detect histopathological changes. Sections were examined and photographed under a light microscope. In histopathological examinations, coccidiosis agents (E. turuncata) were found in the tubular epithelium of the kidney. Pressure necrosis, mononuclear cell infiltration, cellular hypertrophy were observed in the tubules due to the presence of E. turuncata. In addition, edema and hyperemia in the lungs, multifocal necrosis in the liver, cell infiltration in the portal spaces and enteritis were other important histopathological findings. In one case, aspergillosis was detected together with renal coccidiosis. As a result, the presence of renal coccidiyosis brought to our department in the last 8 years in Kars, which is an important goose breeding region, was revealed as 12.23% (17/139) and we concluded that this disease played an important role in goose deaths.

***Keywords*:** Histopathology, goose, renal coccidiyosis

Kars Bölgesindeki Kazlarda Koksidiyozis Varlığının Histopatolojik Yöntemlerle Araştırılması

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ÖZET

Bu çalışmada 2013-2020 yılları arasında Kars Kafkas Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na ölü olarak getirilen 139 adet kaz örneğinde renal koksidiyozis varlığının histopatolojik yöntemlerle tespit edilmesi amaçlandı. Kazlarda ateş, solunum güçlüğü, halsizlik, iştahsızlık, titreme, yerden kalkamama, denge bozuklukları, dönme hareketi, ishal, hırıltılı solunum gibi çeşitli klinik bulgular tespit edildi. Kazların sistemik nekropsilerinde böbrekte irili ufaklı beyaz nodüler yapılara rastlandı. Alınan doku örnekleri %10'luk tamponlu formaldehit solüsyonunda tespit edildi. Rutin doku takip işlemlerinden sonra hazırlanan parafin bloklardan 5 um kalınlığında kesitler alındı. Histopatolojik değişikliklerin tespit edilmesi amacıyla kesitlere Hematoksilen & Eozin boyaması yapıldı. Kesitler ışık mikroskobu altında incelendi ve fotoğraflandı. Yapılan histopatolojik incelemelerde böbrekte tubul epitellerinde yoğun bir şekilde koksidiyozis etkenlerine (*E. turuncata*) rastlanıldı. E. turuncata varlığına bağlı tubullarda basınç nekrozu, mononükleer hücre infiltrasyonu, hücresel hipertrofi gözlendi. Bunun yanı sıra akciğerlerde ödem ve hiperemi, karaciğerde multifokal nekroz, portal aralıklarda hücre infiltrasyonu ve enterit diğer önemli histopatolojik bulgulardan idi. Bir vakada ise renal koksidiyozisle beraber aspergillozis varlığı tespit edildi. Sonuç olarak önemli kaz yetiştiriciliği bölgesi olan Kars'ta son 8 yıl içerisinde Anabilim Dalımıza getirilen renal koksidiyozis varlığı % 12.23 (17/139) olarak ortaya konuldu ve bu hastalığın kaz ölümlerinde önemli rol oynadığı kanaatine varıldı.

Anahtar kelimeler: Histopatoloji, kaz, renal koksidiyozis

A Case of Spinal Nephroblastoma in a Dog

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Abstract

In this report, it is aimed to present the clinical and pathological aspects of a nephroblastoma case observed in the spinal cord of a two years old, male, German shorthaired pointer dog. The dog was brought to MAKÜ Animal Hospital Surgery Clinic with the complaint of limping in the right hind leg. On the clinical examination, a proprioceptive deficit was determined in the right hind foot. Loss of the flexor reflex in the tail and the same proprioceptive deficit in the left hind foot were detected one day later. It was observed that the patient was paraparetic and non-ambulatory. Neurological examination was also suspected of a lesion in the thoracolumbar region. No finding was observed on normal radiography. MRI of the suspected area was performed with 1.5 T magnetic resonance imaging (MRI). T1 and T2 weighted images were obtained. Isointense lesion on T1-weighted imaging and hypointense lesion on T2-weighted imaging with an irregular border of 1.5 cm between T13-L1 was observed. The lesion was determined to be intradural and extramedullary location in the T2 weighted transversal section. A dorsal laminectomy was performed first in the T13-L1 region in the operation with a prediagnosis of a tumoral mass. After dorsal laminectomy, the spinal cord was exposed. After this stage, the operation was continued with the operating microscope. The area was reached after the duratomy. After removing the tumoral mass, the dura was sutured and the muscles, subcutaneous and skin in the area were routinely closed. In the microscopic examination of the mass removed after the operation, it was observed that the mass showed dense cellularity and consisted of

uniform cell populations, these cells formed numerous rosettes, tubules and acinus formations. There was a small amount of fibrovascular stroma. It was noted that multifocal tumor cells form glomerular formations similar to fetal glomeruli. It was observed that the tumor cells were cubic-cylindrical in shape, their cytoplasmic boundaries were unclear, the nuclei were often basally localized and contained 1-2 nuclei with vesicular chromatin structure. A small amount of proteinous eosinophilic material was observed in some tubular lumens. With histopathological findings, the mass was diagnosed as nephroblastoma. After surgery no any chemotherapy was performed. The patient is now 4 years old and alive, and thanks to the incredible care of his owner, he has been limping since 3 months after surgery.

***Keywords*:** Surgery, Dog, Medulla spinalis, Nephroblastoma, Histopathology

Bir Köpekte Spinal Nefroblastom Olgusu

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Özet

Bu bildiride 2 yaşlı, erkek, Alman kısa tüylü pointer ırkı bir köpeğin omuriliğinde gözlenen nefroblastom olgusunun klinik ve patolojik yönden sunulması amaçlanmıştır. MAKÜ Hayvan Hastanesi Cerrahi Kliniğine 2 yaşlı, erkek, Alman kısa tüylü pointer ırkı köpek sağ arka ayakta topallama şikayeti ile getirildi. Yapılan muayane de sağ arka ayakta propiyoseptif defisit olduğu belirlendi. Daha sonra kuyrukta fleksor refleks kaybı ve bundan 1 gün sonra da sol arka ayakta da aynı propriyoseptif defisit belirlendi. Hastanın paraparetik ve non-ambulatorik olduğu gözlendi. Yapılan nörolojik muayene de torakolomber bölgedeki bir lezyondan şüphelenildi. Normal radyografide herhangi bir bulgu gözlemlenemedi. 1,5 T manyetik rezonans görüntüleme (MRG) ile şüphelenilen bölgenin MRG'si gerçekleştirildi. T1 ve T2 ağırlıklı görüntüler elde edildi. T1 ağırlıklı görüntüleme de lezyon izointens olarak T2 ağırlıklı görüntülemede hipointens, kenarları düzensiz T13-L1 arasında 1,5 cm çapında bir lezyon olarak görüntülendi. T2 ağırlıklı transversal kesitte lezyonun intradural ve extramedüller yerleşimli olduğu belirlendi. Tümöral kitle ön tanısıyla operasyonda ilk önce T13-L1 bölgesine dorsal laminektomi yapıldı. Dorsal laminektomiden sonra spinal kord açığa çıkarıldı bu aşamadan sonra operasyona operasyon mikroskopu ile devam edildi. Duratomi yapıldıktan sonra bölgeye ulaşıldı. Tümöral kitle uzaklaştırıldıktan sonra duramater dikilerek bölgedeki kaslar, deri altı ve deri rutin şekilde kapatıldı. Operasyondan sonra uzaklaştırılan kitlenin mikroskobik incelemesinde kitlenin yoğun hücresellik gösterdiği ve uniform özellikteki hücre popülasyonlarından oluştuğu, bu hücrelerin çok sayıda rozet, tubul ve asinus formasyonları oluşturduğu ve az miktarda fibrovasküler stromanın bulunduğu gözlendi. Multifokal olarak

tümör hücrelerinin fetal glomeruluslara benzer şekilde glomerul formasyonları oluşturdukları dikkati çekti. Tümör hücrelerinin kübik-silindirik şekilli, sitoplazmik sınırları belirsiz olduğu, çekirdeklerin sıklıkla bazal yerleşimli olduğu ve veziküler kromatin yapısıyla birlikte 1-2 adet çekirdekçik içerdikleri görüldü. Yer yer bazı tubul lümenlerinde az miktarda proteinöz eozinofilik materyal bulunduğu gözlendi. Histopatolojik bulgular eşliğinde kitleye nefroblastom tanısı konuldu. Cerrahi sonrası herhangi bir kemoterapi ilacı kullanılmadı. Hasta şu anda 4 yaşında ve yaşıyor ve sahibinin inanılmaz bakımı sayesinde ameliyattan 3 ay sonrasından beri topallayarak yürüyebiliyor.

Anahtar kelimeler: Cerrahi, Köpek, Medulla spinalis, Nefroblastom, Histopatoloji

Experimental Animal Model Concept and Specific Experimental Models

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Abstract

In this report, it is aimed to convey the experiences and pathological methodology of the specific animal experiments, that we performed in the Kırıkkale University Hüseyin Aytemiz Laboratory Animal Facility and to bring a clear point of view to the veterinary specialists who have just started their academic career. Experimental animal model term defines the controlled and planned studies that are implemented in laboratory animals held in laboratory conditions and it is aimed to highlight a physiologic process and/or pathogenetic mechanisms of a disease. While, the scientific knowledge and data achieved from these animal experimentations are irreplaceable; in reality prior usage of alternative methods such as cell culture, organoids, and digital simulations have reduced the number of animals used further in vivo experiments. In induced experimental animal models, investigator applies an external stimulus or effect (e.g. surgical operation, trauma, infectious agent, chemical carcinogen) to produce a previously validated experimental disease model in terms of detailed procedures and study design. This experimentally induced disease model is called as "*face validity*" because of its similarity to a specific animal or human disease. The "*Predictive validity*" term defines the reversal and normalization of clinical and cellular changes produced in this disease model by the administration of different treatment protocols. For example; the findings of decreased movement, difficulty in learning, and decreased enter to the open arm of elevated plus maze in reserpine induced depression-like model is face validity of the induced disease. The criteria used for confirmation successfully induced face validity in this depression model can be extended by decreased levels of DOPA and serotonin in the hippocampus and prefrontal cortex neurons of the anxious mice brains. Otherwise, the reversal of the described depression-like findings by using serotonin reuptake inhibitor drugs, e.g. fluoxetine, is predictive validity. In almost all experimental models used laboratory animals, pathological examinations have been

used a unique evaluation tool and current advanced pathological techniques such as digital pathology, histomorphometry, immunohistochemistry, tissue microarray and molecular pathology make both quantitative and representative contributions.

Keywords: Experimental model, laboratory animal, mouse, pathology, rat

Deney Hayvanı Modeli Kavramı ve Spesifik Deneysel Modeller

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Özet

Bu sunumda, Kırıkkale Üniversitesi Hüseyin Aytemiz Deney Hayvanları Laboratuvarlarında gerçekleştirdiğimiz spesifik hayvan deney modelleri ve sonrasında yapılan patolojik incelemelerin genel bir tecrübe eşliğinde aktarılması ve veteriner patoloji uzmanlığına yeni başlayan bilim insanlarına yeni bir bakış açısı kazandırılması amaçlanmaktadır. Deney hayvanı modelleri, fizyolojik bir süreç ya da hastalığın oluş mekanizmasının aydınlatılması, tedavi seçeneklerinin planlı ve kontrollü bir şekilde araştırılması için laboratuvar ortamında yetiştirilen hayvanlarda yapılan çalışmaları tanımlar. Çoğu zaman, bu çalışmalardan elde edilen bilimsel sonuçların, başka şekilde elde edilmeleri mümkün olmamakla birlikte; hücre kültürü, organoid ve dijital modellemeler ile yapılan ön çalışmaların, deneylerde kullanılan hayvan sayılarının azaltılmasına katkı sağladığı da bir gerçektir. İndüklenmiş deney hayvanı çalışmalarında, araştırıcı plan dahilinde bir dış uyaranı (cerrahi operasyon, travma, enfeksiyöz etken, kanserojen kimyasal vb.) daha önceden detaylı olarak tanımlanan bir metodoloji çerçevesinde uygulayarak hastalık modeli oluşturur. Oluşturulan benzer hastalık, gerçekte bir insan ve hayvan hastalığına benzerlik gösterdiğinden "Görsel Gerçeklik, (Face Validity)" adıyla da isimlendirilir. Bu model hastalıkta, hayvanın gösterdiği klinik ve hücresel belirtilerin farklı tedavi planlamalar ile normale döndürülmesi ise "Öngörülen Gerçeklik, (Predictive Validity)" konseptidir. Örneğin; farelere rezerpin uygulanarak oluşturulan depresyon benzeri hastalık modelinde, farelerin daha az hareket etmeleri, öğrenme zorluğu, yükseltilmiş artı labirentte açık kola girme sayısının azalması gibi bulgular hastalığın görsel gerçekliğidir. Bu anksiyete gösteren farelerin hipokampus, prefrontal korteks dokularındaki DOPA ve serotonin seviyelerinin, sağlıklı olanlara göre belirgin şekilde düşmesi görsel gerçekliktir. Anksiyete oluşturulan farelere, serotonin gerialımını inhibe eden ilaçlar (fluoksetin vb) verildiğinde, tanımlanan bulguların normale dönmesi ise öngörülen gerçekliktir. Deney hayvanlarının

kullanıldığı hemen tüm deneysel modellerde, patolojik incelemeler eşssiz bir değerlendirme aracı olarak kullanılmakta, dijital patoloji, histomorfometri, immunohistokimya, doku mikro array ve moleküler patoloji gibi gelişen teknikler ile de deney grupları arasındaki farklar hem sayısal, hem de görsel verilerle desteklenebilmektedir.

Anahtar Kelimeler: Deney hayvanı, deneysel model, fare, patoloji, rat

Hepatic Encephalopathy in Van Cats: Clinical, Hematological and Pathological Findings

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Abstract

There were two male Van cats, suffered from neurological symptoms such as ataxia, head pressing, propulsive circling, amaurosis and clinical findings with weakness, depression and lethargy in Van Cat House. They were monitorized both behavioural and locomotoric activities for about a mounth. Peripheral blood smear, blood biochemistry, rapid test kits for detection *Feline Immunodeficiency Virus (FIV)* and *Feline Leukemia Virus (FeLV)*, semi- quantitative immunocomb test (Biogal, Israel) for the diagnosis of *Feline Coronavirus- Feline Infectious Peritonitis (FIP)* (Biogal, Israel) and Sabin-Feldman Test for the diagnosis of *Toxoplasma gondii* were performed. The cats were euthanasied when their locomotor and sensitive activities were impaired. All of the tests performed for infectious diseases were negative. Blood biochemistry for both cats, respectively, aspertate aminotransferase and alanine aminotransferase 148U / l, 98U / l (Reference value 7-38 U / l) and 498 U / l, 295 U / l (Reference value 25-97 U / l) l), however urea creatinine values were normal. The blood ammonia level was measured as 334 µmol / l and 308 µmol / l, respectively (reference value 90-150 µmol / l), thus hyperammoniemia was detected. Macroscopically; both cats had enlarged liver and had pale foci. While the same pale foci were noted in the kidney of the first cat, other organs, especially the brain, were in normal appearance. Histopathologically; Alzheimer type-2 cells characterized by swelling and double nuclei in astrocytes were found in whole brain regions especially, cortical layers, basal ganglia. Also there were vacuolar changes in myelin layer (intramyelinic edema) in white matter. In the liver, vacuolar degeneration in hepatocyte cytoplasm characterized by round vacuoles was noted. The diagnosis of hepatic encephalopathy

was made in both cats with the clinical appearance, blood results and histopathological results. To the author's knowledge, the first definition of hepatic encephalopathy in Van cats in Turkey. Macroscopically, macro portosystemic shunt was not found in both cats. Therefore, a more detailed investigation of the etiopathogenesis of the disease and whether there is a genetic predisposition in Van cats should be evaluated.

***Key words*:** Hepatic encephalopathy, Van cats, Alzheimer type-2 cells, histopathology, hyperammonemia

Van Kedilerinde Hepatik Ensefalopati: Klinik, Hematolojik ve Patolojik Bulgular

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Özet

Van Kedi Evi' nde ataksi, inkoordinasyon, kendi etrafında dönme, vokalizasyon ve geçici körlük gibi sinirsel; durgunluk, yemek yememe ve laterji gibi klinik belirtiler gösteren iki erkek Van Kedisi yaklaşık 1 ay süreyle hem davranışsal hem de lokomotor aktiviteler yönünden takip edildi. Periferal kan yayması, kan biyokimyası, *Kedi İmmunyetmezlik Virusu (FIV)* ve *Kedi Lökemi Virus (FeLV)* teşhisi için hızlı test kitleri (Biogal, İsrail) *Kedi Coronavirus- Kedi Enfeksiyöz Peritonitis (FIP)* teşhisi için semikantitatif immunocomb test (Biogal, İsrail) ve *Toxoplasma gondii* teşhisi için serolojik Sabin-Feldman Test yapıldı. Kedilerde hem lokomotor hem de sensitif aktivitelerin daha da bozulduğu gözlemlenmesi üzerine ötenazileri gerçekleştirildi. Enfeksiyöz hastalıklar için yapılan testlerin tamamı negatif sonuçlandı. Kan biyokimyası her iki kedi için de sırasıyla aspertat aminotransferaz ve alanin aminotransferaz 148U/l, 98U/l (Referans değ. 7-38 U/l) ve 498 U/l, 295 U/l (Referans değ. 25-97 U/l) olarak saptandı, bununla birlikte üre kreatinin değerleri normaldi. Kan amonyak düzeyi sırasıyla 334umol/l ve 308umol/l (Referans değ. 90-150 umol/l) olarak ölçüldü ve böylece hiperamonyemi saptandı. Makroskobik olarak; iki kedide de karaciğer büyümüş, şişkin ve solgun görünümdeydi. Benzer solgun odaklar birinci kedinin böbreğinde de dikkat çekerken diğer organlar özellikle de beyin normal görünümdeydi. Histopatolojik olarak; kortikal bölegede ve bazal gangliyonlar başta olmak üzere beyinin tamamındaki astrositlerde şişkinlik ve çift nükleuslu tipik Alzheimer tip-2 hücrelerine rastlandı. Beyaz maddede daha yoğun olmak üzere myelin katında yoğun vakuoler değişiklikler (intramyelinik ödem) dikkati çekti. Karaciğerde ise hepatosit stoplazmalarında yuvarlak vakuollerle karakterize vakuoler dejenerasyon dikkat çekti. Elde edilen klinik görünüm, kan sonuçları ve histopatolojik sonuçlar ile her iki kediye de hepatik ensefalopati tanısı konuldu. Yazarın bilgisi dahilinde, Türkiye'de Van kedilerinde rapor edilen ilk hepatik ensefalopati vakalarıdır. Makroskobik olarak her iki

kedide de makro portosistemik şanta rastlanmamıştır bu nedenle hastalığın etiyopatogenezinin daha detaylı araştırılması ve özellikle Van kedilerinde genetik yatkınlık olup olmadığı değerlendirilmelidir.

***Anahtar Kelimeler*:** Hepatik ensefalopati, Van kedisi, Alzheimer tip-2 hücreleri, histopatoloji, hiperamonyemi

Investigation of Vascular Endothelial Growth Factor (Vegf) Activity in Ascites Syndrome of Meat Type Chicken

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Abstract

Broilers are susceptible to ascites syndrome, a condition associated with pulmonary hypertension, right ventricular failure, increased central venous pressure, passive congestion of the liver, and accumulation of serous fluids in the abdominal cavity. Vascular Endothelial Growth Factor is a powerful mediator that increases vascular permeability, which has a key role in physiological and pathological angiogenesis and edema formation. This study aimed to investigate the possible role of VEGF in acute and chronic ascites in broilers induced by hypoxia in a hypobaric chamber at the University of Arkansas Poultry Sciences Research Farm, Fayetteville, AR, USA. Immunohistochemistry (IHC) staining showed positive staining in the bronchial epithelium, vascular endothelium, and adventitia. The connective tissue around blood vessels also stained positively in the lungs of a limited number of animals. There was strong positivity of IHC staining in the hepatocytes. Myocardial cells and elastic fibers were stained positive in hearts of almost all animals. Tubular epithelium and glomeruli were stained positively in kidney sections and neurons were stained positively in brain tissue almost all animals. Using semi-quantitative IHC scoring of VEGF was more intense in the acute form of ascites compared to the control group; stainings were more intense by 3,28 times in the heart, 1.61 times in the lungs, 1.54 times in the kidneys, and 1.39 times in the brain. Compared with the acute phase chronic ascites, showed more intense staining in the kidney by 2.27 times, in the heart 2.02 times, in the lungs 1.81 times, and in the brain 2.41 times. Immunofluorescence (IF) staining showed similar results with IHC. ELISA of tissue lysates and bodily fluids showed that average VEGF values of lung tissues from acute forms of the disease and control were similar at (114,9872 and 114,8209 pg/ml), respectively, VEGF values of lungs for chronic forms was higher at 118,2605pg/ml.

Accordingly, VEGF appears to be secreted more from somatic cells during ascites and may play a role in the pathogenesis of the disease and edema formation.

***Keywords*:** Ascites, VEGF, chicken, pulmonary hypertension, poultry

Et Tipi Tavuklarda Asites Sendromunun Oluşumunda Vascular Endothelial Growth Factor (Vegf) Aktivitesinin İncelenmesi

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Özet

Asites sendromu, et tipi tavuklarda pulmoner hipertansiyon sonucunda sağ kalp yetmezliğini tetikleyen venöz sistemde konjesyona sebep olarak vücut boşluklarında seröz sıvı birikimine neden olur. Vascular Endothelial Growth Factor (VEGF), fizyolojik ve patolojik anjiogenezisinde ve ödem oluşumunda anahtar bir role sahip olan damarsal geçirgenliği arttıran oldukça güçlü bir mediyatördür. Bu çalışma, Arkansas Üniversitesi, Fayetteville, Kanatlı Bilimleri Araştırma Uygulama Çiftlik ve Laboratuvarında bulunan hipobarik odalarda, hipobarik hipoksi oluşturularak, hayvanlarda asites sırasında akut ve kronik dönemde VEGF'ün hastalıktaki olası rolünü immünohistokimya (İHK), immünofloresans (IF) ve ELİSA yöntemleri kullanarak incelemeyi amaçlamıştır. İHK boyamalarda; akciğerde bronş epitelleri, damar endotellerinin ve adventisyasının pozitif boyandığı, damar etrafındaki bağ doku hücrelerinin ise az sayıdaki hayvanda ve sınırlı olarak boyandığı; karaciğerde hepatositlerin çok kuvvetli boyandığı; kalp boyamalarında hayvanların tamamına yakınında miyokart hücrelerinde ve elastik fiberlerde pozitif boyandığı; böbreğe ait kesitlerde özellikle tubul epitellerinin ve glomerulusların pozitif olarak boyandığı, beyin dokusu boyamalarında hayvanların tamamına yakınında nöronların pozitif boyandığı gözlendi. Semikantitatif İHK skorlamasında kontrole kıyasla akut dönemdeki asitesli hayvanlarda kalpte (3.28), böbrekte (1.54), akciğerde (1,61) ve beyinde (1.39) kat VEGF'ün daha yoğun boyandığı dikkati çekmiştir. Kronik dönemde asites gösteren hayvanlar akut dönem ile kıyaslandığında böbrekte (2.27), kalpte (2.02), akciğerde (1.81) ve beyinde (2.41) kat daha yoğun boyamalara rastlanmıştır. İmmünofloresans boyamalarda; immünohistokimyasal boyanmalara benzerlik göstermiştir. ELİSA testinde akut dönemdeki asitesli hayvanlar ile kontrol grubu hayvanların akciğer dokularının ortalama değerleri sırasıyla (114,9872; 114,8209 pg/ml) olduğu

gözlendi. Kronik dönemdeki asitesli hayvanların akciğerlerinin ortalama değerinin (118,2605 pg/ml) kontrol ve akut döneme göre daha yüksek olduğu gözlendi. Bu çalışmaya göre, VEGF'ün somatik hücrelerden asites sırasında daha fazla salgılanmakta olduğu ve hastalığın patogenezisinde rol alıp, ödem oluşumunda rol oynayabileceği düşünülmektedir.

Anahtar Kelimeler: Asites, VEGF, tavuk, pulmoner hipertansiyon, kanatlı

Captive Visceral Cavernous Hemangiosarcoma with Common Metastasis in a Graywolf (*Canis Lupus*): A Case Report

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Abstract

A cavernous type visceral hemangiosarcoma originating from the spleen was reported primarily in a 13 -year- old male gray wolf (Canis lupus) sent to Bursa Uludag University Faculty of Veterinary Medicine, Department of Pathology. Tissues were fixed in 10% neutral buffered formaldehyde and then embedded in paraffin by passing through graduated alcohols. Sections, 3-5 micrometer thickness, were taken from the prepared blocks of and routinely stained with Hematoxylin - Eosin and immunohistochemically with primary antibodies CD31 (JC70, Roche, Germany) and CD34 (QBEND-10, Thermofisher, USA) by streptavidin-biotin-peroxidase method. In the gross examination, bleeding areas were observed of the oral cavity on the gingiva and the upper palate. When the abdominal cavity was opened, icterus in the internal organs was noticed, approximately 1.5 liters in volume. Diffuse, multifocal localized, dark-colored metastatic areas with a diameter of about 1 cm in diameter were found on the omentum and liver. The presence of a cavernous structure with a diameter of 5-6 cm in the spleen of the animal attracted attention. Besides, common anthracosis was seen in the lungs. Microscopically large cavernous structures were found in the sections prepared from the liver and omentum. The cavernous areas, full-filled erythrocytes, were insisted on, swollen, elliptically shaped capillaries with a hyperchromatic nucleus, and homogeneous eosinophilic cytoplasm. Notably, mitotic activity is increased in neoplastic cells. Besides, iron-laden macrophages were found in liver sections. It was observed that the erythrocyte-filled cavernous areas, which are bordered by splenic parenchymatous structure. In immunochemical staining from the spleen, both CD31 and CD34 primary antibodies were observed to be positively stained with endothelial cells in which is around the vascular cavities. In this case, a 13 -year- old male was diagnosed as cavernous type visceral hemangiosarcoma microscopically and immunohistochemically, characterized by a hemorrhagic exudate in the abdominal cavity.

Keywords: graywolf, wildlife, hemangiosarcoma, neoplasia, immunohistochemistry

Tutsak Bir Bozkurtta (*Canis lupus*) Yaygın Metastazlı Visseral Kavernöz Hemangiosarkom: Bir vaka raporu

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Özet

Bursa Uludağ Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalına hayvanat bahçesinden gönderilen 13 yaşlı erkek bir bozkurtta (*Canis lupus*) primer olarak dalaktan köken alan kavernöz tipte visseral hemangiosarkoma rapor edildi. Alınan dokular % 10'luk nötral tamponlu formaldehit içerisinde tespit edildikten sonra dereceli alkollerden geçirilerek parafine gömüldü. Hazırlanan bloklardan 3-5 mikrometre kalınlığında kesitler alınarak rutin olarak Hematoksilen-Eozin ve immunohistokimyasal olarak CD31 (JC70, Roche, Germany) ve CD34 (QBEND-10, Termofisher, USA) primer antikorları ile streptavidin-biotin-peroksidaz yöntemi ile boyandı. Ağız boşluğunun muayenesinde gingiva üzerinde ve üst damakta kanama alanları gözlendi. Karın boşluğu açıldığında yaklaşık 1,5 litre hacminde, pıhtılaşmamış kanlı bir sıvının varlığı ile birlikte iç organlarda sarılık dikkati çekti. Omentum ve karaciğer üzerinde çapları yaklaşık 1 cm boyutunda olan diffuz, multifokal yerleşim gösteren, koyu kırmızı renkte metastatik alanlara rastlandı. Hayvanın dalağında yaklaşık 5-6 cm çapında kavernöz bir yapının varlığı dikkati çekti. Ayrıca akciğerlerde yaygın antrakozis görüldü. Karaciğer ve omentumdan hazırlanan kesitlerde mikroskobik olarak büyük kavernöz yapılara rastlandı. Kavernöz alanlar; iperkromatik çekirdekli, sitoplazmaları homojen pembe renkte olan şişkin, elips şeklinde ve yer yer içlerinde eritrosit bulunan kapillar yapılar meydana getirmişti. Neoplastik hücrelerde mitotik aktivitenin arttığı dikkati çekti. Ayrıca karaciğer kesitlerinde demir yüklü makrofajlara rastlandı. Dalaktan trabeküler çatıların sınırladığı içleri eritrosit dolu kavernöz alanlar dikkati çekti. Dalaktan yapılan immunokimyasal boyamalarda ise hem CD31 hem de CD34 primer antikorları ile damarsal boşluklar etrafındaki endotellerin pozitif olarak boyandığı gözlendi. Bu vakada 13 yaşlı erkek bir bozkurtta karın boşluğunda hemorajik eksudat ile karakterize; mikroskobik ve immunohistokimyasal olarak kavernöz tipte visseral hemangiosarkoma tanısı konmuştur.

***Anahtar kelimeler*:** bozkurt, yaban hayatı, hemangiosarkoma, neoplazi, immunohistokimya

Effects of Nanoparticle Ozone Solutions to Laparatomy Incision Line Injection in Rats: An Experimental Research

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Abstract

In this research, the effects of nanoparticle ozone solutions on wound healing in the experimental laparotomic incision line in rats were investigated. The research was carried out with 72 Sprague-Dawley female rats. Rats were randomly divided into 9 groups. Saline solution to the 1st group, 2% lidocaine to the 2nd group, ozone-free solution to 3rd group, 500 ppm ozone solution to 4th group, 1000 ppm ozone solution to 5th group, 2000 ppm ozone solution to 6th group, 1000 ppm ozone solution to 7th group and 2% lidocaine as 50% mixture, to 8th group 2000 ppm ozone solution and 2% lidocaine as 50% mixture, to 9th group 4000 ppm ozone solution and 2% lidocaine as 50% mixture. The abdominal cavity was sutured by incision in all layers, including the peritoneum. Local wound line healing was examined histopathologically in terms of connective tissue, vascular formation, inflammation, edema, collagen, and hair follicle increase. Epidermis-dermis ratio as%, blood vessels were counted in 40X objective and other results were evaluated as 0: None, 1:Mild, 2:Moderate, 3:Severe. Results; the control group was evaluated in 3 separate groups as ozone group and ozone-lidocaine group. In terms of connective tissue formation, ozone-lidocaine groups (2.79) and ozonized groups (2.41) were observed to be increased compared to non-ozone solution groups (1.99) ($p \leq 0.0004$). In terms of vascularization ozone-lidocaine groups (2.30) and ozone groups (2.02) were found to be increased compared to the control group (1.93) ($p \leq 0.17$). In terms of inflammation, it was observed that fire (2.10) was higher in ozone-lidocaine groups and (1.86) and control group (1.62) in ozone groups ($p \leq 0.0105$). In terms of edema; It was noticed that ozone groups (1.29) and ozone-lidocaine groups (1.18) were higher than the control group (0.54) ($p \leq 0.016$). In terms of bleeding, ozone-lidocaine groups (0.72) were higher than control

groups (0.43) and ozone groups (0.45) ($p \leq 0.47$). In terms of the epidermis/dermis ratio, it was observed that ozone groups (36 times) and ozone-lidocaine groups (32 times) were higher than the control group (27 times) ($p \leq 0.09$). While the number of hair follicles was observed to be 48 in the ozone-treated group, a decrease was observed in the ozone-lidocaine group (36) compared to the control (41) ($p \leq 0.19$). As a result, it has been observed that the application of nanoparticle ozone solution alone or with lidocaine contributes to wound healing by increasing connective tissue, hair follicle number, epidermis-dermis ratio, and vascular formation in the wound line.

Keywords: Wound healing, nanoparticle ozone solutions, connective tissue, vascularization, ozone therapy

Nanopartikül Ozon Solüsyonunun Laparatomi Ensizyon Hattına Enjeksiyonunun Doku Üzerine Etkisi: Deneysel Çalışma

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Özet

Bu çalışmada nanopartikül ozon solüsyonlarının ratlarda deneysel laparatomik ensizyon hattında yara iyileşmesi üzerine etkileri araştırıldı. Uygulamada yağlı sıvı nanopartikül içeren ozon solüsyonu kullanılmıştır. Sprague-Dawley ırkı dişi 72 adet rat ile çalışma yapıldı. Ratlar rastgele 9 ayrı gruba ayrıldı. 1. gruba serum fizyolojik, 2. gruba % 2 lidocaine, 3. gruba taşıyıcı madde, 4. gruba 500 ppm ozon solüsyonu, 5. gruba 1000 ppm ozon solüsyonu, 6. gruba 2000 ppm ozon solüsyonu, 7. gruba 1000 ppm ozon solüsyonu ve % 2 lidokain %50 karışım, 8. gruba 2000 ppm ozon solüsyonu ve % 2 lidokain % 50 karışım, 9. gruba 4000 ppm ozon solüsyon ve %2 lidokain %50 karışım verildi. Karın boşluğu periton dahil olmak üzere tüm katlara ensizyon yapılarak dikildi. Bölgesel yara hattı iyileşmesi histopatolojik olarak bağdoku, damar oluşumu, yangı, ödem, kollajen, kıl folikülü artışı yönünden incelendi. Epidermis-dermis oranı % olarak, kan damarları 40'lık objektifte sayılarak diğer sonuçlar ise 0:Yok, 1:Hafif, 2:Orta, 3:Şiddetli olarak değerlendirildi. Sonuçlar; kontrol grubu, ozon grubu ve ozon-lidokain grubu olarak 3 ayrı grupta değerlendirildi. Bağ doku oluşumu yönünden ozon-lidokain gruplarının (2,79) ve ozonlu grupların (2,41) ozonsuz solüsyon gruplarına göre (1,99) artmış olduğu gözlendi ($p\leq0,0004$). Damar oluşumu yönünden ozon-lidokain gruplarının (2,30) ve ozon gruplarının (2,02) kontrol grubuna göre (1,93) arttığı görüldü ($p\leq0,17$). Yangı yönünden ozon gruplarında yangının (2,10) ozon-lidokain grupları ve (1,86) ve kontrol grubuna göre daha yüksek olduğu (1,62) gözlendi($p\leq0.0105$). Ödem yönünden; ozon gruplarının (1,29) ve ozon-lidokain gruplarının (1,18) kontrol grubuna göre (0,54) daha fazla olduğu dikkati çekti ($p\leq0,016$). Kanama yönünden, ozon- lidokain grupları (0,72) kontrol grupları (0,43) ve ozon gruplarına (0,45) göre yüksek bulundu ($p\leq0,47$). Epidermis/Dermis oranının yönünden ozon gruplarında

(36 kat) ve ozon-lidokain gruplarında (32 kat) kontrol grubuna göre (27 kat) yüksek bulunduğu gözlendi ($p \leq 0.09$). Kıl folikül sayısının ozon uygulanan grupta 48 adet olduğu gözlenirken, ozon-lidokain grubunda (36 adet) kontrole göre (41 adet) bir azalma olduğu dikkati çekti ($p \leq 0.19$). Sonuç olarak nanopartikül ozon solüsyonunun tek başına veya lidokain ile uygulanmasının yara hattında bağ doku, kıl folikülü sayısı, epidermis-dermis oranı ve damar oluşumunu artırarak yara iyileşmesine katkıda bulunduğu gözlenmiştir.

Anahtar kelimeler: Yara iyileşmesi, nano-partikül ozon solüsyonu, bağdoku, damarlaşma, ozon tedavisi

A Case of Granulosa Cell Tumor in a Female Iguana

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Abstract

At necropsy of an adult female iguana, who died with signs of abdominal distention, constipation, loss of appetite and weight loss, a 3x4.5x5cm in size, brownish and firm mass was detected in the left ovary. The cross-section of the mass was dark colored and in a lobular structure. No invasion or metastasis was observed in the surrounding tissues. At the histopathological examination, a lobular mass consisting of granulosa cells surrounded by a thick connective tissue was observed. In addition, anaplasia and pleomorphism were observed in the cells. The nucleus / cytoplasm ratio was increased in the tumoral cells. A small number of mitotic figures also detected. Infiltrations consisting of heterophile and mononuclear cells were seen in some areas. Necrotic areas were observed, especially in the central parts of the mass. Eosinophilic, round shaped materials were noted in the cytoplasm of some tumoral cells. The connective tissue bundles between the cell clusters gave the mass a lobular appearance. Except the numerous melanomacrophages in the liver, no findings were observed in the visceral organs. At the immunohistochemical examination of the tissue, tumor cells showed significant expression for cytokeratin, EpCam, estrogen receptor, slight expression for progesterone receptor, angiogenin and calretin, while negative reaction with myelin basic protein and neuroflament. According to gross and microscopic findings, the tumor was diagnosed as a granulosa cell tumor. Since there was no such report in our country before, the case was found worth presenting.

Keywords: Iguana, granulosa cell tumor, pathology

Dişi Bir İguanada Granulosa Hücre Tümörü Olgusu

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Özet

Karında şişkinlik, konstipasyon, iştahsızlık ve kilo kaybı bulguları ile ölen ergin dişi iguananın nekropsisinde sol ovaryumda 3x4.5x5cm boyutlarında, kahverengimsi ve sert kıvamlı bir kitle tespit edildi. Kitlenin kesit yüzü koyu renkli ve lobuler yapıdaydı. Çevre dokulara invazyon veya metastaz gözlenmedi. Kitlenin histopatolojik incelemesinde etrafı kalın bir bağ doku ile çevrili granulosa hücrelerinden oluşan lobuler yapıda bir kitle dikkati çekti. Hücrelerde anaplazi ve pleomorfizmin belirgin olduğu gözlendi. Hücrelerde çekirdek/sitoplazma oranı artmıştı. Az sayıda mitotik figürler de dikkati çekti. Bazı alanlarda heterofil ve mononükleer hücrelerden oluşan infiltrasyonlar görüldü. Özellikle kitlenin merkezi kısımlarında daha belirgin olmak üzere nekrotik alanlar gözlendi. Bazı tümöral hücrelerin sitoplazmalarında eozinofilik yapıda yuvarlak şekilli materyaller dikkati çekti. Hücre kümelerinin arasında bulunan bağdoku demetleri kitleye lobuler bir görünüm kazandırmıştı. Karaciğerdeki yoğun melanomakrofajlar dışında iç organlarda herhangi bir bulgu gözlenmedi. Dokunun immunohistokimyasal incelemesinde tümör hücrelerinin belirgin sitokeratin, EpCam, östrojen reseptör, hafif progesteron reseptör, angiogenin ve calretinin ekspresyonu gösterirken, myelin basik protein ve nöroflament ile negatif reaksiyon gösterdiği dikkati çekti. Makroskobik ve mikroskobik bulgulara göre tümöre granuloza hücre tümörü teşhisi konuldu. Ülkemizde daha önce böyle bir rapor bulunmaması sebebiyle olgunun sunulmaya değer bulundu.

Anahtar kelimeler: Iguana, granuloza hücre tümörü, patoloji

Trials of Histochemical Methods on Epon-Embedded Semithin Sections

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Abstract

Staining and light microscobic observation of epon-embedded semithin sections are important steps before examination on electron microscopy. Plastic embedding media, however, limit the staining agents' penetration into tissues. Monochromatic stainings (e.g. Toluidine blue) are usually used in routine electron microscopy techniques. Polichromatic stainings and histochemical methods cannot be routinely used on semithin sections. In this study, several histochemical staining protocols proposed for paraffin-embedded sections were compared with routine Toluidine blue semithin section staining. Following double fixation and tissue processing, semithin sections were obtained from liver, skin, and aorta tissues embedded in epon blocks. Sections were stained with Altmann's method (for mitochondria), Verhoeff's method (for elastic fibres), Gordon and Sweet's silver impregnation method (for Type III collagen/reticular fibres), light green (for Type I collagen), and Toluidine blue and light green double staining combination. Sections obtained from all these tissue types are also stained with the routine Toluidine blue method, and then compared with the special stainings and evaluated. Mitochondria in the hepatocytes were perfectly visible in fuchsinophilic colour in liver sections that were stained with Altmann's method, while this distinction could not be observed with Toluidine blue staining. Elastic lamellar structures were identified easily in black colour in aorta sections stained with Verhoeff's method. Elastic fibers in aorta sections stained with Toluidine blue were distinguished, but without adequate contrast due to interspace staining as well. Type III collagen/reticular fibers were perfectly visible in liver stained with Gordon and Sweet's method, while this detail was not observed in Toluidine blue staining. Elaborate observation was not possible in skin sections stained only with light green used for Type I collagen or routine Toluidine blue staining. Toluidine blue and light green double staining combination, however, yielded successfull results. Several histochemical staining protocols and a double staining combination that had not been applied to epon-embedded semithin sections before, were tested in this study for the first time. Satisfactory results were obtained in the identification

of specific tissue components studied. These findings led to the conclusion that the targeted cellular/intercellular component can be easily localized in the proceeding thin section studies.

***Keywords*:** semithin sections, histochemistry

Yarı İnce Epon Kesitlerde Histokimya Denemeleri

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Özet

Elektron mikroskobik gözlem öncesi, yarı ince epon kesitlerin boyanarak ışık mikroskobunda incelenmesi önemli bir adımdır. Ancak plastik gömme ortamları, boya ajanlarının dokuya geçişini sınırlandırır. Bu nedenle boyama yöntemlerinin çoğu epon kesitlere ya uygulanamamakta ya da istenilen kalitede sonuç elde edilememektedir. Bu sorunu gidermek amacıyla dokuları gömme öncesi boyama (blok boyama) veya boyama öncesi plastiği kesitlerden uzaklaştırma (etching) olmak üzere iki yöntem önerilmektedir. Rutin elektron mikroskobi tekniklerinde genellikle monokromatik boyamalar (örn; Toluidine blue) kullanılmaktadır. Kesitlerin boyama sırasında dökülmesi/kırışması, kesitler üzerinde presipitat oluşumu, birbirine benzer hücrelerin zayıf differansiyasyonları gibi problemler nedeniyle polikromatik boyamalar ve histokimyasal metodlar rutinde yaygın olarak kullanılamamaktadır. Bu çalışmada parafin kesitler için önerilen birkaç histokimyasal boyama protokolünün, rutin yarı ince epon kesit boyaması (Toluidine blue) ile karşılaştırılması amaçlanmıştır. Çalışmada ikili fiksasyon ve takip aşamalarından sonra epon blok haline getirilen dokulardan (karaciğer, deri, aort) 2 mikron kalınlığında yarı ince kesitler alındı. Sodyum hidroksit solüsyonu ile etching uygulandıktan sonra kesitlere; Altmann metodu (mitokondri), Verhoeff metodu (elastik lif), Gordon-Sweet gümüşleme metodu (Tip III kollajen/retiküler lif) ve Light green boyaması (Tip I kollajen), Toluidine blue+Light green ikili kombinasyon denemesi uygulandı. Tüm doku tiplerine ait kesitler ayrıca rutin Toluidine blue metodu ile de boyandı ve özel boyamalar ile karşılaştırılarak değerlendirildi. Altmann metodu uygulanan karaciğer dokusuna ait epon kesitlerde, hepatositlerdeki mitokondrionlar fuksinofilik renkte kolaylıkla görüldü. Toluidine blue boyamasında bu ayrım yapılamadı. Verhoeff ile boyanan aort epon kesitlerinde, elastik lamellar yapılar siyah renkte çok net olarak ayırdedildi. Toluidine blue ile boyanan aort kesitlerinde elastik lifler seçilmekle birlikte, ara alanlar da boyandığı için yeterli kontrast izlenmedi. Gordon-Sweet gümüşleme metodu uygulanan karaciğer epon kesitlerde Tip III kollajen/retiküler lifler kolaylıkla görülebilirken, toludine blue boyamalarında bu detay görülmedi. Deri epon kesitlerinde Tip I kollajen için uygulanan monokromatik light green

boyaması ve rutin Toludine blue boyaması detay vermedi. Ancak Toluidine blue+Light green ikili kombinasyonu oldukça başarılı sonuç verdi. Daha önce yarı ince epon kesitlerde denenmemiş histokimyasal birkaç boyama protokolü ve kombinasyon ilk kez bu çalışmada denenmiştir. Çalışılan özel doku komponentlerinin identifikasyonunda olumlu sonuçlar elde edilmiştir. Bu sayede ince kesit düzeyinde çalışmanın devamı durumunda hedeflenen sellüler/intersellüler komponentin lokalizasyonunun kolayca belirlenebileceği düşünülmüştür.

Anahtar kelimeler: yarı ince kesit, histokimya

Confocal Microscope and Its Usage in Pathology

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Abstract

Confocal microscopy is a more detailed and spesific image acqusition technique by passing a light source through thin holes instead of wide angle light sources to eliminate low resolution and low contrast problems in viewed images. Nowadays, in pathology laboratories light microscopes are used to examine cytological, histochemical and immunohistochemical stains. Each staining method has its own spesific features and purposes. Especially, dual or triple immunohistochemical staining has been used frequently in recent years. Essential point of multiple staining is based on localization of markers in different areas of cell (nucleus, cytoplasm or cell membrane). In confocal microscopic examinations, multiple staining is possible in thicker sections contrary to 5 micrometer sections prepared for light microscope even if markers are localized in same region of cell (nucleus-nucleus, cytoplasm-cytoplasm, cell membrane-cell membrane) which contributes to more detail about pathogenesis. Moreover, the results can be obtained in a short period of time such as 2 hours by using methods suitable to confocal microscopy, while results are obtained in 20 hours with routine methods used in pathology laboratories (process of fixing tissue in formalin and embedding in paraffin block). In this presentation, we aimed to share advantages and disadvantages of confocal microscopy with experience gained by usage of this method at one stage of our thesis study that we carried out in our department.

Keywords: Confocal microscope, immunohistochemical staining.

Konfokal Mikroskop ve Patolojide Kullanımı

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Özet

Konfokal mikroskopi, geniş açılı ışık kaynaklarıyla alınan görüntülerdeki düşük çözünürlük ve konstrast azlığı gibi sorunları ortadan kaldırmak için ince deliklerden aydınlatma kaynağı geçirilerek daha detaylı ve spesifik görüntü alma tekniğidir. Günümüzde patoloji laboratuvarlarında, sitolojik, histokimyasal ve immunohistokimyasal boyamalar yapılmakta ve bunların değerlendirmeleri ışık mikroskobunda yapılmaktadır. Her bir boyama yöntemini kendine özel spesifik özelliği ve amaçları mevcuttur. Bunlardan özellikle ikili-üçlü immunohistokimyasal boyamalarda son yıllarda sıklıkla kullanılmaktadır. Bu boyamalarda esas olan nokta birden fazla belirtecin, hücrenin farklı alanlarına lokalize olması (çekirdek, sitoplazma ya da hücre duvarı) esasına dayanmaktadır. Konfokal mikroskobik incelemelerde de ışık mikroskobu için alınan doku kesitlerin (5 mikron), çok daha fazla kalınlıkta olmasının yanısıra hücrelerde, birden fazla aynı bölge lokalizasyonları olsa dahi (çekirdek-çekirdek, sitoplazma-sitoplazma, hücre duvarı-hücre duvarı) birden fazla antikorun kullanılması mümkündür ki bu durum patogenezis hakkında çok daha detaylı olarak katkı sağlamaktadır. Diğer taraftan patolojide kullanılan rutin yöntemlerle (formalinde tespit edilip parafin bloğa gömülme süreci) 20 saat gibi bir sürede sonuç alınabilirken, konfokal mikroskopa uygun yöntemlerin kullanılmasıyla 2 saat gibi kısa bir zaman aralığında sonuç alınabilmektedir. Bu sunumda Anabilim Dalımız'da gerçekleştirdiğimiz tez çalışmamızın bir aşamasında bizzat kullandığımız konfokal mikroskop uygulamalarında elde edilen deneyimin avantaj ve dezavantajların paylaşılması amaçlanmıştır.

***Anahtar Kelimeler*:** Konfokal mikroskop, immunohistokimyasal boyamalar.

Imaging Sublingual Microcirculation in Horses Anaesthetized for Elective Surgery and Colic Surgery: A Pilot Study

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Abstract

This study aimed to compare the sublingual microcirculatory parameters between anaesthetized healthy and colic horses, and determine the relationship between microcirculation and arterial pressure. Seventeen horses were divided into Elective (n = 9) and Colic groups (n = 8). Invasive arterial pressure was recorded continuously. Sublingual microcirculation was assessed using the Sidestream Dark Field (SDF) imaging. Videos were captured at 3 time-points at 45 minutes intervals during anaesthesia. SDF indices included PVD (perfused vessels density), PVD-S (perfused vessels density - small vessels), PPV (proportion of perfused vessels), PPV-S (proportion of perfused vessels - small vessels), MFI (microvascular flow index) and HI (heterogeneity index). Hypotension and normotension episodes (MAP < and ≥ 60 mmHg, respectively) were identified. During normotension, horses in elective group presented better microcirculatory parameters than Colic group (PPV = 71% *vs* 52%, De Backer score = 9.5 *vs* 9, [95% CI], respectively, $p < 0.007$). PPV in both groups decreased during surgery (-35% and -24% [95% CI] in Elective and Colic group respectively, $p < 0.04$). It improved in the elective group before the end of surgery (+53% for PPV [95% CI], $p < 0.001$). No relationship was found between microcirculation and MAP. During hypotension, PVD-S was better preserved in Colic compared to Elective group (11.1 *vs* 3.8 mm/sq.mm [95% CI], respectively, $p = 0.016$).

In conclusion, sublingual microcirculation appeared better preserved in anaesthetized healthy compared to colic horses despite resuscitation manoeuvres. These results are in accordance with uncoupling of macrocirculation and microcirculation in critical horses.

***Keywords*:** SDF, colic, microcirculation, MAP.

Place and Importance of Micro-RNAs in Scientific Studies

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Abstract

miRNA (micro RNA) are endogenously synthesized small RNAs with a length of about 22 nucleotides. There are thousands of miRNAs encoded in the human genome. These miRNAs have been reported to have regulatory roles in events such as cell development, differentiation, proliferation and apoptosis. In terms of diseases, the role of miRNA in cancers was mainly studied. In terms of diseases, the roles of miRNAs mainly on cancers have been examined. In these studies; It has been determined that miRNA levels are changed in breast cancer, Burkitt's lymphoma, malignant brain tumors, thyroid cancer, lung cancer, prostate cancer and hepatocellular carcinoma. In addition to cancer, miRNA roles have been reported in immune-related diseases, neurodegenerative diseases, bacterial diseases and viral diseases. Most miRNA studies are on human-specific diseases, and these studies have been conducted in laboratory animals or in cell culture. MiRNAs studied in terms of animal diseases appear to be at a more limited level than human diseases. MiRNA profiling studies of tissue and organ development, immune response, metabolic events were conducted in cattle from farm animals. There are also miRNA studies showing phenotypic differences, yield characteristics, and susceptibility to infections. Studies on sheep and goats focused on the development of fleece, skeletal muscle and reproductive organs. There are similar studies in animals such as horses and chickens. However, studies on the relationship between infectious diseases and miRNAs in animals are limited. The roles of miRNAs in infectious diseases are important for animal diseases. Therefore, the place, importance and potential of miRNAs in previous scientific studies in Veterinary Medicine will be expressed in the presentation.

Keywords: miRNA

Mikro RNA'ların Bilimsel Çalışmalarda Yeri ve Önemi

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Özet

miRNA (Mikro RNA)'lar yaklaşık 22 nükleotid uzunluğunda endojen olarak sentezlenen küçük RNA'lardır. İnsan genomunda kodlanan binlerce miRNA vardır ve bu miRNA'ların hücre gelişimi, farklılaşması, proliferasyonu ve apoptozis gibi olaylarda düzenleyici rollerinin olduğu bildirilmiştir. Hastalıklar açısından ise miRNA' ların ağırlıklı olarak insanlarda kanserler üzerindeki rolleri incelenmiştir. Yapılan bu çalışmalarda; meme kanserinde, Burkitt's lenfomada, malign beyin tümörlerinde, tiroid kanserinde, akciğer kanserinde, prostat kanserinde ve hepatosellüler karsinomda miRNA seviyelerinin değiştiği belirlenmiştir. Kanserin yanında bağışıklık ile ilişkili hastalıklarda nörodejeneratif hastalıklarda, bakteriyel hastalıklarda ve viral hastalıklarda da miRNA rollerinin olduğu bildirilmiştir. Yapılan miRNA çalışmalarının çoğu insana spesifik hastalıklar üzerine olup, bu çalışmalar laboratuvar hayvanlarında ya da hücre kültüründe yürütülmüştür. Hayvan hastalıkları açısından incelenen miRNA'ların ise, insan hastalıklarına göre daha kısıtlı düzeyde olduğu görülmektedir. Çiftlik hayvanlarından sığırlarda; doku ve organların gelişimi, immun yanıt, metabolik olaylar gibi durumlara ait miRNA profilleme çalışmaları yapılmıştır. Ayrıca fenotipik farklılıklar, verim özellikleri ve enfeksiyonlara duyarlılıkla ilişkili olduğunu gösteren miRNA çalışmaları da mevcuttur. Koyun ve keçilerde yapılan çalışmalarda ise, yapağı, iskelet kası ve üreme organlarının gelişimi üzerinde yoğunlaşılmıştır. At, tavuk gibi hayvanlarda da benzer çalışmalar bulunmaktadır. Fakat hayvanlardaki enfeksiyöz hastalıklarla miRNA'lar arasındaki ilişki üzerine yapılmış çalışmalar kısıtlı düzeydedir. miRNA'ların enfeksiyöz hastalıklardaki rollerinin hayvan hastalıkları açısından önemlidir. Bu nedenle sunumda miRNA'ların Veteriner Hekimlik'teki daha önce yapılmış bilimsel çalışmalardaki yeri, önemi ve potansiyeli ifade edilecektir.

Anahtar kelimeler: miRNA

Caseous Lymphadenitis in a Llama

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Abstract

Caseous lymphadenitis (CLA) caused by Gram (+) *Corynebacterium pseudotuberculosis* is the important infection in sheep, goat, camelids and equids. We describe herein caseous lymphadenitis with pathomorphological findings in a 13 year-old, female llama. After necropsy has been performed, mesenteric lymph nodes, lung, liver, spleen were evaluated by using standard Hematoxylin&Eosin, Ziehl-Neelsen and Gram staining methods on formalin fixed paraffin embedded sections. Macroscopically, enlarged mesenteric lymph nodes were noticed. Whitish-grey, opaque area on the lung and white foci on the section of this area were also observed. Besides similar white foci were present in the liver, spleen, pericardium and thorax. Histologically, typical caseous granulomas and irregular multicentric granulomas were seen in all examined tissues. These granulomas were composed of a necrotic center, with/without calcification, abundant neutrophils with/without pyknotic nuclei, surrounded by a band of connective tissue. Acid – fast bacilli were not detected in any tissues with Ziehl-Neelsen staining. According to these findings and literature, this is the first reported case of a caseous lymphadenitis in an adult llama in Turkey.

Keywords: Caseous lymphadenitis, llama, pathology.

The Effect of Sepsis on the Lung Tissue; the Role of Astaxantin

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Abstract

Objective: In this study, the effects of astaxanthin in the lungs in the sepsis model related to lipopolysaccharide were investigated. Method: 28 female Wistar Albino rats were randomly divided into 3 groups. Groups; control (n = 8), lipopolysaccharide (LPS) (n = 10) and LPS + Astaxanthin (AST) (n = 10). The control unit was used to supply saline equivalent to AST and LPS. LPS group (5 mg / kg LPS, i.p.) was given single dose and LPS + AST devices [(5mg / kg LPS, i.p.) + (50 mg / kg Astaxanthin, oral)] single dose. Animals were taken for histopathological and immunohistochemical examinations in the sacrified lung 6 hours after LPS administration. Results: In histopathological analysis, hyperemia, septal wall thickening and mild inflammation and alveolar macrophage increased significantly in the lung tissue compared to the LPS unit control computer ($p < 0.05$) (Figure 1). In the LPS + AST group, it was determined that the histopathological LPS significantly decreased compared to the person ($p < 0.05$). In the immunohistochemical examination, Cas-8, IL-1β, IL-3, OPN and iNOS reactivity increased significantly in the LPS group compared to the control ($p < 0.001$). Immuno-expressions were observed in inflammatory cells in both lung epithelium. Expressions of markers in the LPS + AST group were significantly decreased compared to LPS devices ($p < 0.001$) (Figure 2). Conclusion: Astaxanthin reduced inflammatory, apoptotoic and oxidative reactions in the sepsis model induced by LPS. The appearance of sepsis The use of astaxanthin may be an additional treatment option.

Keywords: Sepsis, Astaxanthin, Lung

Lipopolisakkaritle Oluşturulan Sepsis Modelinde Akciğer Dokusu ve Astaksantinin Rolü

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Özet

Amaç**:** Çalışmada, lipopolisakkarit ile oluşturulan sepsis modelinde astaksantinin akciğer dokusu üzerindeki etkileri araştırıldı. Yöntem**:** 28 adet dişi Wistar Albino sıçan rastgele 3 gruba ayrıldı. Gruplar; kontrol (n=8), lipopolisakkarit (LPS) (n=10) ve LPS+Astaksantin (AST) (n=10) olarak oluşturuldu. Kontrol grubuna, AST ve LPS'ye eşdeğer hacimde salin solüsyonu uygulandı. LPS grubuna (5 mg/kg LPS, i.p.) tek ve LPS+AST grubuna [(5mg/kg LPS, i.p.) +(50 mg/kg Astaksantin, oral)] tek doz verildi. Hayvanlar LPS uygulamasından 6 saat sonra sakrifiye edilerek akciğer dokusu histopatolojik ve immünohistokimyasal incelemeler için alındı. Bulgular**:** Histopatolojik analizde, LPS grubunda kontrol grubuna göre akciğer dokusunda hiperemi, septal duvar kalınlaşması ve ve hafif inflamasyon ve alveoler makrofaj anlamlı olarak arttı ($p<0.05$) (Şekil 1). LPS+AST grubunda histopatolojik bulguların LPS grubuna göre anlamlı olarak azaldığı tespit edildi ($p<0.05$). İmmünohistokimyasal incelemede LPS grubunda, Cas-8, IL-1β, IL-3, OPN ve iNOS reaktiviteleri kontrol grubuna göre anlamlı olarak arttı ($p<0.001$). İmmüno-ekspresyonlar hem akciğer epitel hücreleri hem de enflamatuar hücrelerde gözlendi. LPS+AST grubunda belirteçlerin ekspresyonları LPS grubuna göre anlamlı olarak azaldı ($p<0.001$) (Şekil 2). Sonuç**:** LPS ile oluştrulan sepsis modelinde Astaksantin, enflamatuvar, apoptotoik ve oksidatif reaksiyonları azalttı. Sepsis olan hastalarda Astaksantin kullanımı ek bir tedavi seçeneği olabilir.

Anahtar kelimeler**:** Sepsis, Astaksantin, Akciğer

Investigation of the Protective and Therapeutic Effects of Thiamine in Thioacetamide-Induced Liver Injury

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Abstract

Liver damage; is a series of diseases that, including viral diseases, alcoholic hepatitis, cirrhosis, and lubrication such as non-alcoholic steatosis and non-alcoholic steatohepatitis and progress to cancer. There are many factors that affect the incidence of liver damage. These include sex, age, alcohol, aflatoxin, endogenous and exogenous hormones, diet, viral factors such as hepatitis B and hepatitis C, and parasitic infestations such as schistosomiasis; activities from the host such as obesity, immune function, hereditary diseases, diabetes mellitus, cirrhosis, non-alcoholic fatty liver. Experimentally used thioacetamide (TAA) is an important hepatotoxic substance. Thiamine (vitamin B1) is one of the important antioxidant substances. Tiamine protected against to damage of many tissues and organs particularly nervous system cells. The aim of this study is to examine therapeutic and prophylactic effects of TAA on thiamine induced liver damage. In this study, 50 male CD-1 mice, weighing 25-40 grams were used and mice were divided into 5 groups consisting of 10 each. Group 1 (Control group); they were fed with normal water for 2 months as adlibitum. Group 2 (Thiamine group); it was given as thiamine adlibitum in 50 mg / 100 ml water daily for 2 months. Group 3 (Prophylaxis group) was given adlibitum in 50 mg / 100 ml thiamine water for 1 month and at the end of this period, intraperitoneally, 200 mg / kg of thioacetamide (TAA) was given twice a week. Group 4 (Treatment group) was administered intraperitoneally twice weekly 200 mg / kg thioacetamide (TAA) for 1 month at the beginning of the study and adlibitum in 50 mg / 100 ml thiamine water after 1 month. Group 5 (TAA group) was intraperitoneally administered 200 mg / kg TAA twice a week for 2 months. At the end of the study, the mice were decapitated under anesthesia and liver tissue samples were taken for histopathological examination and blood samples for biochemical parameters. In serum samples, liver panel including bilirubin (BILEA), total bilirubin (TBIL), total protein (TP), alkaline phosphotase (ALP), aspartate aminotransferase (AST), alenin transaminase (ALT), albumin (ALB) tests were examined. During necropsy, it was observed that the liver in the TAA group became pale and the edges

were swolen. Liver samples were evaluated histopathologically and immunohistochemically for caspase-3 expression. It was noticed that all the data examined in the liver panel in serum samples increased significantly in the TAA group. TAA caused severe degeneration and necrosis in the livers. When thiamine was administered after TAA application, it improved the biochemical, pathological and immunohistochemical findings. The results of this study showed that thiamine has little prophylactic effect, but a significant therapeutic effect in thioacetamide induced liver injury.

Keywords: İmmunohistochemistry, Pathology, Prophylaxis, Treatment, Tiyoasteamid, Thiamine.

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Tiyoasetamid ile Oluşturulan Karaciğer Hasarinda Tiyaminin Koruyucu ve Tedavi Edici Etkilerinin İncelenmesi

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Özet

Karaciğer hasarı; viral hastalıklar, alkolik hepatit ve siroz gibi karaciğer hastalıklarını ve non alkolik steatoz ve non alkolik steatohepatitis gibi karaciğer yağlanmalarını içeren kansere kadar ilerleyen bir seri hastalık dizisidir. Karaciğer hasarının insidansını etkileyen birçok faktör vardır. Bunlar cinsiyet, yaş, alkol, aflatoksin, endojen ve ekzojen hormonlar, diyet, hepatit B ve hepatit C gibi viral etkenler ile şistozomiazis gibi paraziter enfestayonları içeren çevresel faktörler; obezite, immun fonksiyon, kalıtsal hastalıklar, Diyabetes mellitus, siroz, alkolik olmayan yağlı karaciğer, gibi konakçıdan kaynaklanan faktörler olarak sıralanmaktadır. Deneysel olarak kullanılan tiyoasetamid (TAA) de önemli bir hepatotoksik maddedir. Tiyamin (B1 vitamini) önemli antioksidan maddelerden birisidir. Birçok doku ve organı özellikle sinir sistemi hücrelerini zedelenmelere karşı korur. Bu çalışmanın amacı TAA ile oluşturulan karaciğer hasarında tiyaminin koruyucu ve tedavi edici etkilerinin incelenmesidir. Çalışmada 25-40 gram ağırlığında CD-1 ırkı toplam 50 erkek fare kullanıldı ve fareler 10 ar hayvandan oluşan 5 gruba ayrıldı. Grup 1 (Kontrol grubu); normal su ile 2 ay süre ile adlibitum olarak beslendi. Grup 2 (Tiyamin grubu); günlük 50 mg/100 ml su içinde tiyamin adlibitum olarak 2 ay boyunca verildi. Grup 3 (Profilaksi grubu) 50 mg/100 ml tiyamin su içinde adlibitum 1 ay verildi ve bu süre sonunda intraperitoneal olarak haftada iki kere tioacetamid (TAA) 200 mg/kg olarak verildi. Grup 4 (Tedavi grubu) çalışma başlangıcında intraperitoneal olarak haftada iki kere 200 mg/kg tioacetamid (TAA) 1 ay süre ile uygulandı ve 1 ay sonra 50 mg/100 ml tiyamin su içinde adlibitum verildi. Grup 5 (TAA grubu) intraperitoneal olarak haftada iki kere 200 mg/kg TAA 2 ay süre ile uygulandı. Çalışma sonunda fareler anestezi altında dekapite edilerek histopatolojik inceleme için karaciğer dokusu örnekleri, biyokimyasal parametreler için de kan örnekleri alındı. Serum örneklerinde bilirubin (BILEA), total bilirubin (TBIL), total protein (TP), alkalen fosfotaz (ALP), aspartat aminotransferaz (AST), alenin transaminaz (ALT), albümin (ALB) testlerini içeren karaciğer paneli incelendi. Nekropsi sırasında TAA grubudaki

farelerde karaciğerin soluklaştığı ve kenarlarının kütleştiği gözlendi. Karaciğer örnekleri ise histopatolojik ve kaspaz-3 açsından immunohistokimyasal olarak değerlendirildi. Serum örneklerinde karaciğer panelinde incelene tüm verilerin TAA grubunda önemli düzeyde arttığı dikkati çekti. TAA karaciğerlerde şiddetli dejenerasyon ve nekrozlara sebep olduğu gözlendi. Tiyamin TAA uygulaması ardından verildiğinde biyokimyasal, patolojik ve immunohistokimyasal bulguların düzelmesini sağladı. Bu çalışmanın sonuçları tiyaminin tiyoasetamid ile oluşturulan karaciğer hasarında sınırlı profilaktik etkisinin ancak önemli ölçüde tedavi edici etkisi olduğunu gösterdi.

***Anahtar kelimeler*:** İmmunohistokimya, Patoloji, Proflaksi, Tedavi, Tiyamin, Tiyoasteamid.

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Exocrine Pancreatic Atrophy in a Dog

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Abstract

The present case aims to report about exocrine pancreatic atrophy in a 5.5-year-old male Dogo Argentino for scientific community and veterinary small animal's practitioners. He was presented to the clinic with chronic severe diarrhoea and cachexia and diagnosed as Inflammatory Bowel Disease. At the necropsy, the pancreas was found to be mostly atrophic. The secretoric ductules were visibly clear with almost no pancreatic tissue visible surrounding them. Tissue samples were collected, routinely processed, and stained with Hematoxylin & Eozin; and immunohistochemistry was performed with AE1, CK8, P53, Insulin, Glucagon, and Vimentin antibodies used. Histologically, the pancreas has lost almost all its exocrine tissue. Acinar cell apoptosis and zymogen granule loss were also included. Interlobular/intralobular ductules were seen markedly. Immunohistochemistry revealed that the acini were disappeared while the secretoric ductules remained. Although the aetiopathogenesis of exocrine pancreatic atrophy has been long remained obscure, its occurrence nowadays depends on chronic pancreatitis. Atrophied parenchyma with no infiltrative inflammatory cells or fibrosis led us to think the case was idiopathic. The rarely seen exocrine pancreatic atrophy has been reported in the veterinary literature in a Dogo Argentino for the first time.

***Keywords*:** Pancreas, atrophy, immunohistochemistry, dog

Bir Köpekte Ekzokrin Pankreas Atrofisi

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Özet

Vaka, 5,5 yaşlı, Dogo Argentino ırkı erkek bir köpekte görülen pankreas atrofisini bilimsel topluluğa ve küçük hayvan hekimlerine sunmayı amaçlamaktadır. Hasta kliniğe şiddetli kronik ishal ve kaşeksi şikayetleriyle getirildi ve Yangısal Bağırsak Hastalığı teşhisi kondu. Nekropside pankreasın yüksek oranda atrofik olduğu gözlendi. Sekretorik kanallar gözle görülebilecek şekilde belirgin iken etraflarını saran pankreas dokusunun neredeyse hiç gözlenmediği dikkati çekti. Toplanan doku örnekleri rutin takibe alınarak Hematoksilen & Eozin ile boyandı; bunun yanı sıra AE1, CK8, P53, İnsülin, Glukagon ve Vimentin antikorları kullanılarak immunohistokimysal boyama yapıldı. İmmunohistokimyasal olarak, asinus yapılar kaybolurken sekretorik kanalların kaldığı ortaya kondu. Ekzokrin pankreas atrofisinin etiyopatogenezi uzun süredir tam olarak bilinmese de günümüzde varlığı kronik pankreatitis olgularıyla ilişikilendirilmektedir. Yangısal hücre infiltrasyonu ve fibrozis ile alakası olmayan atrofik parenşim, bizlere bu vakanın idiyopatik olduğunu düşündürmektedir. Veteriner hekimlikte nadir olarak görülen ekzokrin pankreas atrofisi bu vaka ile Dogo Argentino ırkı bir köpekte ilk defa bildirilmiştir.

***Anahtar Kelimeler*:** Pankreas, atrofi, immunohistokimya, köpek

Effects of Subcutaneous Injection of Nano Particle Ozone Solution on Tissue in Rats: An Experimental Study

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Abstract

In this study, it was aimed to investigate the effects of subcutaneous injection of ozone nanoparticle solutions used in different doses in rats. Sprague-Dawley race, female, 56 rats were randomly divided into 7 groups as 6 different (10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm, 5000 ppm) nanoparticle ozone solution and saline control group. Ozone injection was applied to the back of the rats once a week for 3 weeks. In order to see the local effects of the applied dose, biopsy was performed at the injection site on the 2nd, 3rd, 4th, 5th and 30th days, and the leukocyte count, hair follicle number, vascularization and epidermis/dermis ratio were examined in all groups. The two groups in the study were obtained by comparing the average values of biopsy samples on the first 4 days (2nd, 3rd, 4th and 5th days) and the value of biopsy samples on the 30th day. It was observed that the epidermis/dermis ratio increased at doses other than the 5000 ppm ozone application. In all groups, it was observed that there was an increase in hair follicle compared to the group in which saline control group was applied, as well as the increase in the number of hair follicles in the 500 ppm dose group compared to the saline water group was found to be 229% ($p < 0.0017$). In evaluating the number of vessels in the dermis depending on the dose, an increase was observed in vascularization on the 30th day when compared with the number of vessels at the end of the 30th day of the average of the first 4 days at doses of 10 ppm and 5000 ppm. No increase was observed at doses of 50 ppm and 500 ppm, however, a decrease was observed in other doses (100 and 1000 ppm). Due to the fact that nano-particle ozone solutions are in liquid form, contain oil and are slow-release, it is thought that they may damage the tissue and cause an inflammatory reaction, therefore, no significant difference was detected in terms of leukocytosis in the biopsies taken from the injection site in the first 4 consecutive days, and no necrosis was found in the dermis. As a result, it was noted that the use of nanoparticle ozone solutions, such as gas ozone, caused

subcutaneous injection of certain effects / responses in the tissue depending on the doses and caused different responses under the skin.

***Keywords*:** Nano particle ozone solution, Rat, Vascularization, Epidermis

Nano Partikül Ozon Solüsyonunun Sıçanlarda Deri Altı Enjeksiyonunun Doku Üzerindeki Etkileri: Deneysel Bir Çalışma

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Özet

Bu çalışmada, farklı dozlarda kullanılan ozon nano partikül solüsyonlarının ratlarda deri altı enjeksiyonunun etkilerinin araştırılması amaçlanmıştır. Sprague-Dawley ırkı, dişi, 56 adet rat, rastgele 6 farklı şekilde nano partikül ozon solüsyonu içeren (10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm, 5000 ppm'lik) ve serum fizyolojik kontrol grubu olmak üzere toplam 7 gruba ayrıldı. Ratların sırt bölgesine haftada 1 kez olacak şekilde 3 hafta boyunca ozon enjeksiyonu uygulandı. Uygulanan dozunun lokal etkilerini görmek amacıyla 2., 3., 4., 5. ve 30. günlerde enjeksiyon yerinden biyopsisi yapılarak tüm gruplarda lökosit sayısı, kıl folikülü sayısı, damarlanma ve epidermis/dermis oranı incelendi. Çalışmada ilk 4 günün (2.3.4. ve 5. gün) biyopsi örneklerinin ortalama değerleri ile 30. gün biyopsi örneklerinin değeri kıyaslandı. 5000 ppm'lik ozon uygulaması dışındaki uygulanan tüm dozlarda epidermis/dermis oranının arttığı gözlendi. Bütün gruplarda, serum fizyolojik tuzlu su uygulanan gruba göre, kıl folikülü artışının olduğu gözlendi, bunun yanı sıra, 500 ppm'lik doz grubunda serum fizyolojik tuzlu su grubuna oranla kıl folikülü sayısındaki artışın %229 oranında olduğu tespit edildi($p<0,0017$). Dermisteki damar sayısının doza bağlı olarak değerlendirilmesinde 10 ppm ve 5000 ppm'lik dozlarda ilk 4 gün ortalamasının 30. gün sonundaki damar sayısı ile kıyaslandığında, 30. günde damarlaşmada artış görüldü. 50 ppm ve 500 ppm'lik dozlarda ise herhangi bir artışa rastlanmadığı, bununla birlikte diğer dozlarda (100 ve 1000 ppm) azalma gözlendiği dikkati çekti. Nano partikül ozon solüsyonlarının sıvı formda olması, yağ içermesi ve yavaş salınımlı olması nedeniyle dokuda hasar yapabileceği, yangısal reaksiyon yaratabileceği düşünüldüğü, bu nedenle birbirini takip eden ilk 4 günde enjeksiyon bölgesinden alınan biyopsilerin lökositoz yönünden anlamlı bir farklılık tespit edilmeyerek, dermiste herhangi bir nekroz oluşumuna rastlanmadığı gözlendi. Sonuç olarak nano partikül ozon solüsyonların kullanımının, gaz ozon gibi, doku altına enjeksiyonunun dokuda uygulanan doza bağımlı olarak belirli etkilere yol açtığı dikkati çekti.

***Anahtar sözcükler*:** Nano partikül ozon solüsyonu, rat, vaskülarizasyon, epidermis.

A Case of Calcinosis Circumscripta in a Belgian Malinois Dog

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Abstract

In this case, a subcutaneous mass located between right os metacarpale quintum and os metacarpale quartum of 3-year-old, male, custom trained Belgian Malinois dog was diagnosed as calcinosis circumscripta after macroscopic and microscopic evaluations. Calcinosis circumscripta is an ectopic mineralization in dermal and subcutaneous tissues resulting with the formation of nodular, slow growing tumor-like masses due idiopathic, dystrophic, metastatic and iatrogenic accumulation of calcium salts. It is less frequently seen in other soft tissues. Although the etiology of the disease is not fully understood, it can be occured idiopathically or as a result of trauma. In the presented case, an ulcerative cutaneous mass, 3.5x3 cm in sizes, with fibroelastic consistency was noticed. Numerous, whitish nodules with fibroelastic consistency, were noticed on the cut section of the mass. In the microscopic examination of Hematoxylin&Eosin stained slides, presence of numerous purple colored amorphous accumulations surrounded by fibrous tissue was observed. Additionally, mononuclear inflammatory cell infiltrations were encountered in an area. Alizarine Red S, Von Kossa and Masson's Trichrome stainings were performed to reveal the nature of amorphous material, and accumulations displayed orange, brown-black and gray colorations, respectively. Clinical and hematological parameters of the dog were in physiological limits, no abnormality was detected referring to calcium deposition. It was thought that the possible traumas that the animal may receive during the customs controls may be important in the etiology of dystrophic calcium depositions in that specific body region.

***Keywords*:** Calcinosis circumscripta, Belgian Malinois dog, Von Kossa, Alizarine Red S, Masson's Trichrome.

Belçika Malinois Irkı Bir Köpekte Kalsinozis Sirkumskripta Olgusu

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ÖZET

Bu olguda Gümrük muhafazada kullanılan Belçika Malinois ırkı, 3 yaşlı, erkek köpeğin sağ ön ayağın os metacarpale quintum ve os metacarpale quartum arasındaki bölgede karşılaşılan kitle makroskobik ve mikroskobik incelemeler sonrasında kalsinozis sirkumskripta olarak teşhis edilmiştir. Kalsinozis sirkumskripta, dermis veya subkutis'te ve daha az sıklıkla diğer yumuşak dokularda tümör benzeri ancak yavaş büyüme gösteren kalsiyum tuzlarının idiopatik, distrofik, metastatik ve iatrojenik birikimi ile karakterize, orta sert, tek veya çoklu kitleler halinde kutanöz nodüller şeklinde karşımıza çıkan bir ektopik mineralizasyon şeklidir. Patogenezi tam olarak anlaşılmamış olmasına rağmen olgular idiopatik veya doku travmasının bir sonucu olarak şekillenebilir. Sunulan olguda, üzeri deri ile kaplı, 3,5x3 cm boyutlarında, fibroelastik bir kıvama sahip olan kitlenin üst yüzünde sınırlı ülser alanı mevcuttu. Kesit yüzünde multifokal dağılım gösteren beyaz renkli, sert kıvamda lobüler yapılar dikkati çekti. Hematoksilen Eozin ile boyanmış kesitlerinin mikroskobik incelemesinde subkutan yerleşim gösteren etrafı bağ doku ile çevrelenmiş, geniş, mor renkli multifokal amorf birikimler ve bazı alanlarda nekroz varlığı görüldü. Ayrıca dermiste bir alanda mononükleer yangı hücresi infiltrasyonlarına da rastlandı. Rutin Hematoksilen-Eozin boyamalara ilave olarak, kalsiyum birikimini tespit etmek için Alizarine Red S ve Von Kossa boyaması yapıldı. Amorf yapılar halinde dikkati çeken birikimlerin boyanma yöntemine göre sırasıyla turuncu ve kahverengi-siyah renk sergiledikleri dolayısıyla kalsiyum birikimi doğrulandı. Ayrıca Masson's Trichrome boyamada, mavi bir bağ doku ile çevrelenmiş olan kalsiyum birikimlerinin gri renkte boyanmış oldukları gözlendi. Klinik ve hematolojik olarak herhangi bir probleme sahip olmayan bu olguda etiyolojik olarak hayvanın gümrük muhafaza köpeği olarak kullanılması ve görev esnasında arama yaparken olası travmaların distrofik kalsifikasyonun şekillenmesinde rol alabileceği şeklinde yorumlandı.

***Anahtar sözcükler*:** Kalsinozis sirkumskripta, Belçika Malinois köpeği, Von Kossa, Alizarine Red S, Masson's trichrome.

Investigation of the harmful effects of Electric Field (50 Hz) on Female Rat Ovaries, Uterus and Vagina and the role of Astaxanthin.

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Abstract

In our study, it was aimed to investigate the effect of the 50-Hz electrical field (EA) on female genital tissue and to investigate the role of Astaxanthin (AXA), a strong antioxidant. In our study, 11-12 months old, 30 female Wistar-Albino female rats (350-500 gr) were used. The control group (Group 1, n = 10) was given 7-9 cc of pure olive oil daily for 30 days by gavage. EA group (Group 2, n = 10) was exposed to a 50-Hz electric field between parallel plates for 23 hours a day and 7-9 cc of olive oil was given daily by gavage. EA + AXA group (Group 3, n = 10) was exposed to a 50-Hz electrical field for 23 hours daily. AXA (75mg / kg/day) dissolved in 7-9 cc olive oil daily was administered by gavage. The tissues obtained with the sacrification of the rats were evaluated by histopathological examination and immunohistochemically. Besides, TAS-TOS determinations, Hormone analyzes (GnRH, FSH, LH, Estrogen, AMH) were also performed. Results: While there was no significant difference between the 3 groups in the histopathological evaluation of tissues except for hyperemia was observed in group 2. In immunohistochemical examinations, it was found that Caspase 3 and 9 were activated in Group 2 ($p < 0.001$) and this expression decreased significantly with AXA ($p < 0.01$). Similarly, estrogen receptors in the ovary, uterus, and vagina increased with EA ($p < 0.001$) and decreased with AXA; It was observed that interleukin 1-β and IL-6 values, which are indicators of oxidative stress and inflammation, increased with EA in all 3 tissues ($p < 0.001$), but AXA administration reversed these damages. Looking at the TAS-TOS-OSI values in tissues, OSI value in ovarian tissue increased significantly in group 2 ($p = 0.023$) and

decreased significantly in group 3 (p = 0.0015). In uterus, while OSI values increased significantly in group 2 (p = 0.032), it decreased significantly in group 3 (p = 0.049). Looking at the hormonal results, it was observed that the tendency of EA to increase GnRH was significantly suppressed by AXA. Lastly, AMH levels increased in group 2 (p = 0.001). There are not enough studies in the literature regarding the effect of EA on the female urogenital system. The results of our study showed that oxidative stress and apoptosis increased in tissues studied with EA and these effects were reversed with AXA.

Keywords: Electric field, female genital system, oxidative stress, Astaxanthin, Caspase 3-9.

Elektrik Alanının (50 Hz) Dişi Sıçan Yumurtalıkları, Rahim ve Vajina üzerindeki zararlı etkilerinin ve Astaksantinin rolü'nün araştırılması.

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Özet

Çalışmamızda 50-Hz elektriksel alanın (EA) dişi genital doku üzerine etkisinin araştırılması ve güçlü bir antioksidan olan Astaksantin (AXA)'in rolünün araştırılması amaçlandı. Çalışmamızda, 11-12 aylık, 30 adet dişi Wistar-Albino dişi sıçanlar (350-500 gr) kullanıldı. Kontrol grubuna (Grup 1, n=10), 30 gün boyunca günlük 7-9 cc zeytinyağı gavaj yoluyla verildi. EA grubu (Grup 2, n=10), günde 23 saat boyunca birbirine paralel plaklar arasında 50-Hz elektrik alana maruz bırakıldı ve günlük 7-9 cc zeytinyağı gavaj yoluyla verildi. EA+AXA grubu (Grup 3, n=10) ise günlük 23 saat süreyle 50-Hz elektriksel alana maruz bırakıldı. Günlük 7-9 cc zeytinyağında çözdürülmüş AXA (75mg/kg/gün) gavaj yoluyla verildi. Sıçanların öldürülmesi ile elde edilen dokular histopatolojik inceleme ve immünohistokimyasal olarak değerlendirildi. TAS-TOS tayinleri, Hormon analizleri (GnRH, FSH, LH, Östrojen, AMH) yapıldı. Dokuların histopatolojik değerlendirilmesinde 3 grup arasında belirgin fark görülmezken grup 2'de hiperemi göze çarptı. İmmunohistokimyasal incelemelerde ise Grup 2'lerde Kaspaz 3 ve 9'un aktive olduğu ($p<0.001$) ve AXA ile bu ekspresyonun anlamlı azaldığı saptandı ($p<0.01$). Benzer şekilde over, uterus ve vajina'da östrojen reseptörlerinin EA ile arttığı ($p<0.001$), AXA ile azaldığı; Oksidatif stres ve inflamasyon belirteci olan interlökin 1-β ve IL-6 değerlerinin her 3 dokuda da EA ile yükseldiği ($p<0.001$) ancak AXA uygulamasının bu hasarları geriye döndürdüğü gözlemlendi. Dokulardaki TAS-TOS-OSI değerlerine bakıldığında ise over dokusunda OSİ değeri grup 2'de anlamlı artmış ($p=0.023$), grup 3'de anlamlı olarak azalmıştır ($p=0,0015$). Uterus'da ise OSI değerleri grup 2'de anlamlı artarken

(p=0.032) grup 3'de anlamlı azaldı (p=0.049). Hormonal sonuçlara bakılacak olursa EA'nın GnRH'ı arttırma eğiliminin AXA tarafından anlamlı olarak süprese edildiği gözlendi. AMH düzeyi grup 2'de arttı (p=0.001). Dişi ürogenital sistemine EA etkisiyle ilgili literatürde yeterince çalışma bulunmamaktadır. Çalışmamızın sonuçları, EA ile çalışılan dokularda oksidatif stres ve apopitozisin arttığını AXA ile bu etkilerin geriye döndürüldüğünü saptadık.

Anahtar Kelimeler: Elektrik alan, kadın genital sistemi, oksidatif stres, Astaksantin, Kaspaz 3-9.

Bu tez çalışması Süleyman Demirel Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından TTU-2018-6728 proje numarası ile desteklenmiştir

Evaluation of Some Immunohistochemical Markers in the Diagnosis of Canine Cutaneous and Subcutaneous Round Cell and Soft Tissue Tumors

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Abstract

In this study, we aimed to evaluate some immunohistochemical tumor markers in diagnosis of various round cell tumors and soft tissue tumors encountered in the skin and subcutaneous tissues in dogs. In the study, 17 round cell tumors and 10 soft tissue tumors were evaluated. For round cell tumors, we used CD3, CD18, CD20, CD204, E-cadherin, tryptase, and vimentin antibodies. For soft tissue tumors CD31, desmin, melan-A, neurofilament, S100, cytokeratin, smooth muscle actin (SMA), sarcomeric actin, and vimentin antibodies were used as diagnostic markers. In round cell tumors, CD3 and CD18 positivity in a T-cell lymphoma case; tryptase positivity in six mastocytoma cases; CD18 positivity in four cutaneous histiocytoma cases; and only E-cadherin positivity in one cutaneous histiocytoma case; CD18, CD204, E-cadherin and vimentin positivity in three histiocytic sarcoma cases; CD204 and vimentin positivity in a malignant fibrous histiocytoma case; and positivity of CD18, CD204, E-cadherin, and vimentin in a malignant histiocytosis case were observed. In soft tissue tumors, CD31 positivity in a hemangioma case; positivity of S100, SMA and vimentin in a case of rhabdomyoma; positivity of only vimentin in a mesenchymal benign tumor case; positivity of S100 and vimentin in a myxoid peripheral nerve sheath tumor case; positivity of S100 and vimentin in a perineuroma case were observed. In one case of hemangiopericytoma SMA, sarcomeric actin, and vimentin positivity; and S100 and vimentin positivity in another case of hemangiopericytoma were also observed. Only vimentin positivity was observed in three cases of undifferentiated sarcomas. Immunohistochemical markers are essential in the diagnosis of round cell and soft tissue tumors in dogs. However, our results, when compared with the literature information, suggest that there may be some differences in immunopositivity of these tumors and that further studies are needed.

***Keywords*:** Dog, immunohistochemistry, round cell tumor, soft tissue tumor

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Köpeklerde Deri ve Deri Altı Yerleşimli Yuvarlak Hücreli Tümörler ve Yumuşak Doku Tümörlerin Tanısında Bazı İmmunohistokimyasal Belirteçlerin Değerlendirilmesi

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Özet

Bu çalışmada köpeklerde deri ve deri altı dokularda şekillenmiş olan yuvarlak hücreli tümörler ve yumuşak doku tümörlerinin immunohistokimyasal tanısında çeşitli tümör belirteçlerinin değerlendirilmesi amaçlandı. Çalışmada 17 adet yuvarlak hücreli tümör ve 10 adet yumuşak doku tümörü değerlendirildi. İmmunohistokimyasal belirteçler olarak yuvarlak hücreli tümörler için CD3, CD18, CD20, CD204, E-kaderin, triptaz ve vimentin; yumuşak doku tümörleri için ise CD31, desmin, melan-A, nörofilament, S100, sitokeratin, düz kas aktini (SMA), sarkomerik aktin ve vimentin belirteçleri kullanıldı. Yuvarlak hücreli tümörlerde bir T lenfoma olgusunda CD3 ve CD18 pozitifliği; altı mastositom olgusunda triptaz pozitifliği; dört kutanöz histiyositom olgusunda CD18 pozitifliği; ve bir kutanöz histiyositomda sadece E-kaderin pozitifliği; üç histiyositik sarkom olgusunda ise CD18, CD204, E-kaderin ve vimentin pozitifliği; bir malign fibröz histiyositom olgusunda CD204 ve vimentin pozitifliği; ve bir malignant histiyositozis olgusunda CD18, CD204, E-kaderin ve vimentin pozitifliği gözlendi. Yumuşak doku tümörlerinde ise bir hemangiom olgusunda CD31 pozitifliği; bir rabdomiyom olgusunda S100, SMA ve vimentin pozitifliği; bir mezenkimal benign tümör olgusunda yalnızca vimentin pozitifliği; bir mikzoid periferal sinir kılıf tümörü olgusunda S100 ve vimentin pozitifliği; bir perinöroma olgusunda S100 ve vimentin pozitifliği; iki hemangioperisitom olgusundan birinde SMA, sarkomerik aktin ve vimentin pozitifliği; diğerinde ise S100 ve vimentin pozitifliği; üç adet indiferensiye sarkom olgusunda ise yalnızca vimentin pozitifliği gözlendi. Köpeklerde yuvarlak hücreli tümörler ve yumuşak doku tümörlerinin tanısında immunohistokimyasal belirteçlerin oldukça gerekli olduğu aşikârdır. Bununla beraber literatür bilgileri ile karşılaştırıldığında çalışmamızda elde ettiğimiz sonuçlar, bu tümörlerin immunohistokimyasal boyanma açısından çeşitlilik gösterebileceğini ve bu konuda yapılacak daha fazla çalışmaya ihtiyaç duyulduğunu göstermektedir.

***Anahtar Kelimeler*:** İmmunohistokimya, köpek, yuvarlak hücreli tümör, yumuşak doku tümörü

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A Case of Transitional Carcinoma of the Nasal Cavity in a Golden Retriever Dog

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Abstract

Transitional carcinoma of the respiratory epithelium is one of the very rare tumors observed in dogs. At necropsy, a hard mass that filled the nasal cavity and caused deformity in the bones was detected in a 10-year-old, male, Golden Retriever dog, who died with complaints of deformity at the nasal area, difficulty in breathing, and occasionally epistaxis. The mass was pale colored and lobular in appearance. At the histopathological examination, the tumor cells consist of prismatic-cuboidal cell groups with a fibrovascular septal tissue between them were observed. Neoplastic cells had indeterminate cell borders, scarce and pale eosinophilic cytoplasm, oval-round-shaped granular nuclei, and prominent and usually single nuclei. Necrotic changes were observed in some cells. Mononuclear cell infiltrations were found in the central regions of some cell clusters. The mass invaded the bones and it was well vascularized and areas of hemorrhages detected. Hyperemia, anthracosis, emphysema and atelectasis were observed in the lungs. Marked fat accumulation was observed in the myocardium of the heart. Severe hyperemia and fatty degeneration were detected in the liver. Symptoms of glomerular lipidosis in the kidneys and proteinaceous materials in the tubular lumen were observed. Siderocytes and sporadic megakaryocytes noted in the spleen. Tumoral cells reacted negatively for vimentin and neuroflament. In the light of all findings, the tumor was evaluated as transitional carcinoma. This is the first case of respiratory transitional cell tumor detected in a dog in our country.

Keywords: Transitional carcinoma, dog, nasal tumor, histopathology

Golden Retriever Irkı Bir Köpekte Burunda Transisyonel Karsinom Olgusu

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Özet

Respiratuvar epitelin transisyonel karsinomu köpeklerde çok nadir gözlenen tümörlerdendir. Burun bölgesinde deformite, soluk alma güçlüğü, düşkünlük ve zaman zaman burun kanaması şikayetleri ile ölen ve nekropsisi yapılan 10 yaşlı, erkek, Golden Retriewer ırkı bir köpekte burun boşluğunu dolduran ve kemiklerde deformiteye yol açan sert kıvamlı bir kitle saptandı. Kitle açık renkli ve lobüler görünümdeydi. Histopatolojik incelemede tümör hücrelerinin prizmatik-kübik şekilli ve aralarında fibrovasküler bir septal dokunun bulunduğu hücre gruplarından oluştuğu dikkati çekti. Neoplastik hücrelerin belirsiz hücre sınırları, kıt ve soluk eozinofilik boyalı sitoplazmaları bulunan, oval-yuvarlak şekilli granüler çekirdekleri ve belirgin ve genellikle tek çekirdekleri olan hücrelerden oluştuğu görüldü. Bazı hücrelerde nekrotik değişiklikler gözlendi. Bazı hücre kümelerinin merkezi bölgelerinde mononükleer hücre infiltrasyonlarına rastlandı. Kitlenin kemiklere invaze olduğu ve damarlaşmanın arttığı yer yer kanamalı alanların bulunduğu dikkati çekti. Akciğerlerde hiperemi, antrakozis, amfizem ve atelektazik alanlar gözlendi. Kalpte myokatta yaygın şekilde yağlanma görüldü. Karaciğerde yaygın şekilde hiperemi ve yağ dejenerasyonu saptandı. Böbreklerde glomeruler lipidozis bulguları ile tubul lümenlerinde proteinöz materyaller görüldü. Dalakta yoğun şekilde siderositler ve tek tük megakaryositler dikkati çekti. Tümöral hücreler vimentin ve nöyro flament açısından negatif reaksiyon gösterdi. Tüm bulgular ışığında tümör transisyonel karsinom olarak değerlendirildi. Bu ülkemizde bir köpekte saptanan ilk respiratorik transisyonel hücre tümörü olgusudur.

Anahtar kelimeler: Transisyonel karsinom, köpek, burun tümörü, histopatoloji

Tricuspid Valve Endocardiosis in a Puppy with Distemper

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Abstract

Valvular endocardiosis is a disease that caused by the thickening of endocardial layer of the heart due to nonenfectious causes. The disease is more common in dogs. It is a disease that causes thickening of the atrioventricular valve and as a result dilatation in the left ventricle. One of the prominent findings of endocardiosis is severe congestive heart failure. The cause of the disease is related to valvular collagen degeneration. While endocardiosis is seen at a rate of 5% in animals younger than 1 year old, this rate increases up to 75% as the animal ages. Considering the lesions that occur in the heart valves, 57% of the cases in the mitral valve, 27% in the the mitral and tricuspidal valve, and 7.5% in the tricuspidal valve, and 6.5% in the other valves were observed. In this case; signs of congestive heart failure were detected during necropsy in an 11-month-old Kangal breed male dog who was brought to Burdur Mehmet Akif Ersoy University Clinics with complaints of melena and epistaxis and died here. There were appearances of passive congestion in parenchymatous organs and seroses of luminal organs. Severe meningeal and cortical congestion was detected in the brain. Pericardial fluid increasing was observed. When the lumen of the heart was opened, shortening and nodular thickening of the bicuspital and tricuspidal valves were seen. The changes were more severe in the tricuspital valve. There was foamy fluid in trachea and edematous appearance in the lungs. Microscopic examination revealed fibroblastic proliferations with myxomatous changes in bicuspital and tricuspital valves. In addition, there was severe demyelination in the white matter in the brain and cerebellum. Intranuclear eosinophilic inclusion bodies were found in some astrocytes and some small neurons in the granular layer of the cerebellum. A diagnosis of distemper was made according to histopathological findings. Valvular endocardiosis is observed more frequently in older animals and on the mitral valve according to the literature. Our case is very rare because of the animal was young with the lesion more severe in the tricuspid valve and furthermore distemper diagnosis was accompanied.

Keywords: Distemper, dog, tricuspid valve endocardiosis

Distemperlı Genç Bir Köpekte Triküspit Kapak Endokardiyozisi

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Özet

Valvuler endokardiyozis kalbin endokard katmanının nonenfeksiyöz nedenlerle kalınlaşmasıyla oluşan, sıklıkla köpeklerde görülen, atrioventriküler kapakta kalınlaşma ile dikkati çeken ve bunun sonucu olarak sol ventrikülde dilatasyon ile devam eden bir hastalıktır. Sol ventrikülün dilatasyonunun devamında sol kalp yetersizliği ve total kalp yetersizliği meydana gelir. Endokardiyozisin belirgin bulgularından biri ileri derecede konjestif kalp yetmezliği oluşturmasıdır. Hastalığın nedeni valvuler kollagende meydana gelen dejenerasyonla ilgilidir. Endokardiyozis, 1 yaşından küçük hayvanlarda %5 oranında görülmekteyken, bu oran hayvan yaşlandıkça % 75'lere kadar yükselmektedir. Kalp kapakçıklarında oluşan lezyonlarına göre bakıldığında, vakaların % 57'sinde sadece mitral kapakta, % 27'sinde mitral ve triküspidal kapakçıkta, % 7,5'unda sadece triküspidal kapakçıkta, % 6,5'unda ise diğer kapakçıklarda lezyon oluşmaktadır. Bu olguda; melena ve epistaksis şikâyetleriyle Burdur Mehmet Akif Ersoy Üniversitesi Klinikleri'ne getirilen ve burada ex olan 11 aylık kangal ırkı erkek köpekte, nekropsi sırasında konjestif kalp yetmezliği bulguları tespit edildi. Parankimatöz organlarda ve lümenli organların serozalarında pasif konjesyona ilişkin görünümler vardı. Beyinde şiddetli meningeal ve kortikal konjesyon tespit edildi. Perikardda sıvı artışı dikkati çekti. Kalbin lümeni açıldığında biküspital ve triküspidal kapakçıklarda kısalma ve noduler kalınlaşmalar görüldü. Değişiklikler triküspital kapakçıkta daha şiddetliydi. Tracheada köpüklü sıvı ve akciğerlerde ödemli görünüm mevcuttu. Mikroskobik incelemede biküspital ve triküspital kapakçıklarda mikzomatöz değişikliklerle birlikte fibroblastik proliferasyonlar dikkati çekti. Ayrıca beyin ve beyincikte ak maddede şiddetli demyelinasyon mevcuttu. Beyinciğin granüler katmanında bazı küçük nöronlarda ve bazı astrositlerde intranükleer eozinofilik inklüzyon cisimciklerine rastlandı. Histopatolojik bulgular eşliğinde distemper tanısı konuldu. Valvuler endokardiyozis literatür bilgilerine göre yaşlı hayvanlarda ve mitral kapakta daha sıklıkla gözlenmektedir. Vakamız ise distemper tanısıyla birlikte hayvanın genç olması ve lezyonun daha şiddetli olarak triküspit kapakta şekillenmesi yönüyle nadir bir olgudur.

***Anahtar Kelimeler*:** Distemper, köpek, triküspit kapak endokardiyozisi

The Effect of Experimental Sepsis Model Created with Lipopolysaccharide on Nasal and Oral Mucosa and the Role of Astaxanthin

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Abstract

In this study, the effects of astaxanthin (AX) on nasal and oral mucosa tissue were investigated in an experimental sepsis model created with lipopolysaccharide. Wistar Albino female rats (n = 28) were randomly divided into 3 groups. Groups; control (n = 8), lipopolysaccharide (LPS) (n = 10) and LPS + Astaxanthin (AX) (n = 10). Saline solution equivalent to AX and LPS was applied to the control group. LPS group (5 mg / kg LPS, i.p.) was given single dose and LPS + AST group [(5mg / kg LPS, i.p.) + (50 mg / kg Astaxanthin, oral)] was given single dose. Animals were sacrificed 6 hours after LPS application, and nasal and oral mucosa tissue was examined histopathologically and immunohistochemically. In histopathological analysis, it was found that hyperemia in the nasal mucosa tissue significantly increased in the LPS group compared to the control group ($p < 0.05$), and histopathological findings were significantly reduced in the LPS + AX group compared to the LPS group ($p < 0.05$). In the immunohistochemical examination, Cas-8, IL-1β, IL-3, iNOS and OPN expressions increased significantly in the nasal mucosa LPS group compared to the control group ($p < 0.001$). The expression of markers in the LPS + AX group was significantly decreased compared to the LPS group ($p < 0.001$). Expressions of IL-3, iNOS and OPN increased significantly in the oral mucosa compared to the control group ($p < 0.001$). The expression of markers in the LPS + AX group was significantly decreased compared to the LPS group ($p < 0.001$). AX reduced inflammatory, apoptotoic and oxidative reactions in the inflammation of nasal and oral mucosa in sepsis model created with LPS. The use of Astaxanthin may be an additional treatment option in patients with sepsis and upper respiratory tract inflammation.

Key words: Sepsis Model, Astaxanthin, Nasal Mucosa, Oral Mucosa.

Lipopolisakkaritle Oluşturulan Deneysel Sepsis Modelinin Nazal ve Oral Mukozaya Etkisi ve Astaksantinin Rolü

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Özet

Çalışmada, lipopolisakkarit ile oluşturulan deneysel sepsis modelinde astaksantinin (AX) nazal ve oral mukoza dokusu üzerindeki etkileri araştırıldı. Wistar Albino dişi sıçan (n=28) rastgele 3 gruba ayrıldı. Gruplar; kontrol (n=8), lipopolisakkarit (LPS) (n=10) ve LPS+Astaksantin (AX) (n=10) olarak oluşturuldu. Kontrol grubuna, AX ve LPS'ye eşdeğer hacimde salin solüsyonu uygulandı. LPS grubuna (5 mg/kg LPS, i.p.) tek ve LPS+AST grubuna [(5mg/kg LPS, i.p.) +(50 mg/kg Astaksantin, oral)] tek doz verildi. Hayvanlar LPS uygulamasından 6 saat sonra sakrifiye edilerek nazal ve oral mukoza dokusu histopatolojik ve immünohistokimyasal olarak incelendi. Histopatolojik analizde, LPS grubunda kontrol grubuna göre nazal mukoza dokusunda hiperemi anlamlı olarak arttı ($p<0.05$) ve LPS+AX grubunda histopatolojik bulguların LPS grubuna göre anlamlı olarak azaldığı tespit edildi ($p<0.05$). İmmünohistokimyasal incelemede nazal mukozada LPS grubunda, Cas-8, IL-1β, IL-3, iNOS ve OPN ekspresyonları kontrol grubuna göre anlamlı olarak arttı ($p<0.001$). LPS+AX grubunda belirteçlerin ekspresyonları LPS grubuna göre anlamlı olarak azaldı ($p<0.001$). Oral mukozada, IL-3, iNOS ve OPN ekspresyonları kontrol grubuna göre anlamlı olarak arttı ($p<0.001$). LPS+AX grubunda belirteçlerin ekspresyonları LPS grubuna göre anlamlı olarak azaldı ($p<0.001$). LPS ile oluşturulan sepsis modelinde nazal ve oral mukozada oluşan enflamasyonda AX, enflamatuvar, apoptotoik ve oksidatif reaksiyonları azalttı. Sepsis ve üst solunum yolu enflamasyonu olan hastalarda Astaksantin kullanımı tedaviye ek bir seçenek olabilir.

***Anahtar Sözcükler*:** Sepsis Modeli, Astaksantin, Nazal Mukoza, Oral Mukoza.

A Case of Generalized Tuberculosis in a Domestic Pigeon

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Abstract

In this case, a case of generalized tuberculosis diagnosed in a 3-4 aged, female, domestic pigeon brought to Burdur Mehmet Akif Ersoy University Veterinary Faculty Pathology Department for necropsy was described. In the anamnesis taken from the owner of the animal, signs of weakening, anorexia, puffy and disorganized feathers that started 3-4 months ago were determined. At necropsy, the animal was excessively cachectic, the feathers were fluffy, mixed and the hair around the anus was contaminated with feces. Internal examination revealed numerous yellowish-grey colored nodules, some of which were combined with each other, ranging in size from 0,5 to 1,5 cm localized internal organs serosal surface such as lung, liver, pericardium and intestines. Tissue samples with lesions were examined histopathologically after routine follow-up. Microscopical examination revealed typical tubercles with prominent caseification necrosis in the centers of the foci, numerous mononuclear inflammatory giant cells around them and surrounded by connective tissue. A large number of *Mycobacterium* agents were observed as pink-red stained bacilli on sections stained with the Ziehl-Neelsen method. Diagnosis of tuberculosis was made in the light of gross and histopathological findings.

***Keywords**:* Pigeon, tuberculosis, necropsy, histopathology, Ziehl-Neelsen method.

Evcil Bir Güvercinde Generalize Tüberküloz Olgusu

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Özet

Bu olguda Burdur Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalına nekropsi için getirilen 3-4 yaşlı, dişi, evcil bir güvercinde saptanan generalize tüberküloz olgusu tanımlandı. Hayvan sahibinden alınanan amnezde 3-4 ay önceden başlayan halsizlik, iştahsızlık, durgunluk, tüylerde kabarma ve zayıflık bulguları belirtilmiştir. Nekropside hayvanın aşırı kaşektik, olup tüylerin kabarık ve karışık, anüs çevresindeki tüylerin ise dışkı ile bulaşık olduğu gözlendi. İç bakıda akciğer, karaciğer, perikart ve bağırsak serozalarında yoğun olmak üzere tüm iç organlar da büyüklükleri 0,5-1,5cm arasında değişen bazıları birbirleri ile birleşmiş şekilde çok sayıda sarımsı-gri renkli nodüler yapılar saptandı. Lezyonlu doku örnekleri rutin takip işleminden geçirilerek histopatolojik olarak incelendi. Mikrosobik incelemede odakların merkezlerinde belirgin kazeifikasyon nekrozu, çevrelerinde çok sayıda mononüklear yangı ve dev hücrelerinin bulunduğu ve etraflarının bağ doku ile çevrili olduğu tipik granülomlar saptandı. Ziehl-Neelsen metoduna göre boyanan kesitlerde kırmızı basiller şeklinde çok sayıda *Mycobacterium* etkenleri gözlendi. Makroskobik ve histopatolojik bulgular ışığında olguya tüberküloz teşhisi konuldu.

***Anahtar kelimeler**:* Güvercin, tüberkülozis, nekropsi, histopatoloji, Ziehl-Neelsen metodu.

The Relationship Between Histopathological Evaluation and Prognosis in Canine Cutaneous Mast Cell Tumors

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Abstract

In dogs, Canine Cutaneous Mast Cell Tumors (CCMCT) are a common clinical condition that demonstrates various biological behaviors. A variety of histological grading systems are used in the evaluation of these tumors. Aim of this study is to determine and propose a histopathological and prognostic evaluation approach that can be used routinely in our country by evaluating the CCMCTs based on mitotic index (MI) and most commonly used histopathologic grading system namely, Patnaik's grading system and recently proposed Kiupel's two-tier grading system, especially in cases where techniques such as immunohistochemistry are not possible. In this study, 54 tumor masses from dogs were studied after recording their morphological characteristics, fixing in 10% formalin solution, and diagnosing as CCMT in histopathological examinations. The histopathological structures of the cases were determined according to both Patnaik and Kiupel grading systems, while there was no correlation between grades and recurrence, it was found that there was a significant difference between the groups in terms of survival ($p = 0.043$ and $p = 0.01$, respectively). In addition, these grading systems have been examined in terms of mitotic index (MI). A significant relationship was determined between MI and both histopathological grading systems in terms of survival after diagnosis, and significant differences in terms of prognostic determinants such as survival and mortality were observed among cases grouped according to the cutoff value of 5 for MI. As a result of the study, especially if techniques such as immunohistochemistry is not possible, it has been observed that the assessment of each tumor by using standardized MI values and histopathological gradings by using both Patnaik and Kiupel grading systems over the hematoxylin eosin preparations is the most effective, easy, fast and affordable method, and this evaluation will provide the healthiest data to the clinician in

terms of treatment recommendations.

Keywords: Canine Cutaneous Mast Cell Tumors, histopathology, prognosis.

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Köpek Deri Mast Hücre Tümörlerinde Histopatolojik Değerlendirmenin Prognoz ile İlişkisi

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Özet

Köpeklerde Deri Mast Hücre Tümörleri (Canine Cutaneous Mast Cell Tumors, CCMCT) klinikte sık rastlanan ve çok farklı biyolojik davranışlar gösteren bir hastalıktır. Bu tümörlerin değerlendirilmesinde farklı histolojik derecelendirme sistemleri kullanılmaktadır. Bu çalışmanın amacı, özellikle immunohistokimya gibi tekniklerin mümkün olmadığı durumlarda CCMCT'leri mitotik indeks (Mİ) ve derecelendirme sistemlerini, en çok kullanılan Patnaik sistemi ve son dönemde önerilmiş olan Kiupel'in iki dereceli sistemi özelinde değerlendirerek ülkemizde rutinde kullanabilecek bir histopatolojik ve prognostik değerlendirme yaklaşımı belirlemek ve önermektir. Çalışmada, köpeklerden alınan morfolojik özellikleri kaydedilip %10 formalin solüsyonu içerisinde tespit edilmiş ve histopatolojik incelemelerde CCMT olarak tespit edilmiş 54 tümör kitlesi üzerinde çalışılmıştır. Olguların histopatolojik yapıları hem Patnaik hem Kiupel derecelendirme sistemine göre belirlenmiş, nüks ile aralarında bir ilişki görülmezken sağ kalım konusunda gruplar arasında önemli fark olduğu (sırasıyla p= 0,043 ve p=0,01) saptanmıştır. Ayrıca bu derecelendirme sistemleri, mitotik indeks (Mİ) açısından da incelenmiştir. Mİ ile her iki histopatolojik derecelendirme sistemi arasında, teşhis sonrası sağ kalım süresi açısından önemli bir ilişki olduğu saptanmış, Mİ için 5 eşik değerine göre gruplanan olgular arasında sağ kalım ve mortalite gibi prognoz belirleyiciler açısından önemli farklılıklar gözlenmiştir. Çalışma sonucunda, özellikle immunohistokimya gibi teknikler mümkün değilse, hematoksilen eosin preparatlar üzerinden Patnaik ve Kiupel derecelendirme sistemleri ile standardize edilmiş Mİ değerlerinin her bir tümör için değerlendirilmesinin en etkin, kolay, hızlı ve az maliyetli yöntem olduğu ve bu değerlendirmenin klinisyene tedavi önerme açısından en sağlıklı verileri vereceği görülmüştür. Bu çalışma, İstanbul Üniversitesi-Cerrahpaşa Bilimsel Araştırma Projeleri Birimi tarafından desteklenmiştir. Proje No: TDK-2016-21181.

Anahtar Kelimeler: Kanin Kutanöz Mast Hücre Tümörü, histopatoloji, prognoz.

Examination of Ruminant Cases with Botulismus Suspected by Immunohistochemical Method

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Abstract

Botulism is a disease characterized in humans and animals by generalized and progressive paralysis caused by the neurotoxins (*BoNT)* of *Clostridium botulinum* (*C. botulinum*). Seven different antigenic types of botulinum neurotoxins A to G have been identified. Type C and D are widely considered to be *BoNTs* that induce botulism in livestock. The most important problem in botulism in animals in our country is the difficulty in diagnosing the disease. For the diagnosis of the disease, clinical and pathological observations are not adequate. Nowadays, mouse assays from blood serum or some tissues are used as reference methods to determine the presence of toxin for the definitive diagnosis of the disease. However, new diagnostic approaches need to be implemented rapidly, accurately and without ethical concerns for the diagnosis of the disease. In this study, liver, kidney, spleen, heart, brain, and intestinal tissues of 30 ruminants (20 cattle, 7 sheep and 3 goats) which were assessed as suspected botulismus with their clinical and pathological findings were examined for the first time immunohistochemically for *BoNT*-C and *BoNT*-D. In this context, *BoNT* was detected as IHC in 16 (53.33%) of the 30 ruminants. *BoNT* causes flaccid paralysis by proteolysis of SNARE proteins (SNAP-25, syntaxin and synaptobrevin) which are responsible for acetylcholine release in peripheral cholinergic neurons. In the present study, it was found that *BoNT*-C specifically proteolyzes SNAP-25 in the ruminant brain and cerebellum, and *BoNT*-D specifically proteolyzes sinaptobrevin and SNAP-25 by IHC staining. In conclusion, it was evaluated that the presence of toxin in the tissue for the first time immunohistochemically by using the antibodies specific to the toxoid form of *BoNT* C and D as well as the determination of the proteolysis status of SNAP-25 and synaptobrevin proteins that these toxins specifically affect may be useful in establishing the etiological diagnosis.

Keywords: Botulismus, SNAP-25, synaptobrevin, IHC, histopathology

Botulismus Şüpheli Ruminant Olgularının İmmunohistokimyasal Yöntemle İncelenmesi

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Özet

Botulizm insan ve hayvanlarda Clostridium botulinum (*C. botulinum*) nörotoksinlerinin (*BoNT*) neden olduğu, generalize ve ilerleyici paraliz ile karakterize bir hastalıktır. Botulinum nörotoksinlerinin A dan G ye kadar 7 farklı antijenik tipi tanımlanmıştır. Çiftlik hayvanlarında botulizme neden olan BoNT 'ların büyük oranda tip C ve D olduğu kabul edilmektedir. Ülkemizde hayvanlarda gelişen botulizm olgularındaki en önemli problem hastalığın teşhisindeki zorluklardır. Klinik ve patolojik bulgular hastalığın teşhisi için yeterli olamamaktadır. Günümüzde hastalığın kesin teşhisi için toksin varlığını ortaya konulması amacıyla kan serumu veya bazı dokulardan yapılan fare deneyleri referans metot olarak kabul edilmektedir. Ancak hastalığın teşhisine yönelik, hızlı, güvenilir ve etik problemlere yol açmayan yeni teşhis metotlarının geliştirilmesi gerekmektedir. Yapılan bu çalışma ile klinik ve patolojik bulguları ile botulismus şüpheli olarak değerlendirilen 30 adet ruminanta (20 adet sığır, 7 adet koyun ve 3 adet keçi) ait karaciğer, böbrek, dalak, kalp, beyin ve bağırsak dokuları *BoNT-C* ve *BoNT*-D yönünden immunohistokimyasal (İHK) yöntemle ilk defa incelenmiştir. Bu kapsamda 30 ruminantın 16 (%53,33)'sında İHK olarak *BoNT* tespit edilmiştir. *BoNT* periferik kolinerjik nöronlardaki asetilkolin salınımından sorumlu olan SNARE's proteinlerini (SNAP-25, sintaksin ve sinaptobrevin) proteolize ederek gevşek paralize neden olur. Yapılan çalışmada İHK boyaması ile *BoNT-C*'nin ruminant beyin ve beyinciklerinde spesifik olarak SNAP-25'i, *BoNT-D*'nin ise spesifik olarak sinaptobrevini kısmi olarak da SNAP-25'i proteolize ettiği saptanmıştır. Sonuç olarak bu çalışma ile, *BoNT*'un toksoid formuna spesifik antikorlar kullanılarak dokuda toksin varlığının İHK olarak ilk kez ortaya konulmasının yanı sıra, bu toksinlerin spesifik olarak etkiledikleri SNAP-25 ve sinaptobrevin proteinlerinin proteoliz durumlarının belirlenmesinin etiyolojik tanının konulmasında faydalı olabileceği değerlendirilmiştir.

***Anahtar Kelimeler*:** Botulismus, SNAP-25, sinaptobrevin, İHK, histopatoloji

Investigation of the Therapeutic Effect of Poppy Oil in Bovine Dermatophytosis by Pathological Methods

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Abstract

In this study, the efficacy of poppy oil and salicylic acid applications on the skin surface in bovine dermatophytosis was evaluated comparatively with pathological methods. The study material was obtained from 14 cattle (experimental group) and 7 cattle (control group), whose ages were between 1 month and 3 years old, with fungal infection on their skin, and similar care, feeding and sheltering conditions in cattle enterprises in the province of Burdur. In 7 of the experimental group animals, poppy oil was applied to the lesioned skin surface and salicylic acid was applied to the others for 14 days. Biopsy samples were taken from the animals on days 0.,7. and 14. The tissue samples were fixed in 10% buffered formaldehyde and embedded in paraffin blocks after routine tissue follow-up. Sections of 5µm thickness taken from paraffin blocks were placed on normal slides and stained with routine hematoxylin eosin and Grocott methenamine silver method. Standard avidin-biotin peroxidase method was applied to the sections taken on poly-l-lysine slides by using Anti-cytokeratin 16 primary antibodies. In histopathological examination, it was determined that the inflammatory reaction started on the 7th day in the squamous epithelium in the applications of poppy oil and pomade salicylic, and a recovery close to the control group was observed on the 14th day. It was concluded that there was no significant difference between poppy oil and pomade salicylic applications. Scientific studies on the therapeutic properties of poppy oil are limited. No studies have been found on the skin, particularly on the healing of infected or uninfected skin wounds.

***Keywords*:** Mycotic dermatitis, Poppy oil, Cow, Pathology, Skin

Sığır Dermatofitozisinde Haşhaş Yağının Tedavi Edici Etkisinin Patolojik Yöntemlerle Araştırılması

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Özet

Bu çalışmada sığır dermatofitozisinde deri yüzeyine haşhaş yağı ve salisilik asit uygulamalarının tedavideki etkinlikleri patolojik yöntemlerle karşılaştırmalı olarak değerlendirildi. Çalışma materyali olarak Burdur ilindeki sığır işletmelerinde bulunan, yaşları 1 ay ile 3 yaş arasında değişen, derilerinde mantar enfeksiyonu tespit edilmiş, bakım, beslenme ve barınma şartları birbirine benzeyen, 14 adet sığır (deneme grubu) ile 7 adet sığırdan (kontrol grubu) alınan deri biyopsisi örnekleri oluşturdu. Deneme grubu hayvanlardan 7 tanesinde lezyonlu deri yüzeyine haşhaş yağı, diğerlerine salisilik asit içeren pomad 14 gün boyunca uygulandı. Hayvanlardan 0,7 ve 14. günlerde biyopsi örnekleri alındı. Alınan dokular %10'luk tamponlu formaldehit içerisinde tespit edilerek rutin doku takibi sonrası parafin bloklara gömüldü. Parafin bloklardan 5µm kalınlığında alınan kesitler normal lamlara alındıktan sonra rutin hematoksilen eozin, grocott methenamine silver methodu ile boyandı. Poly-l-lysinli lamlara alınan kesitlere ise Anti-cytokeratine 16 primer antikoru kullanılarak standart avidin-biyotin peroksidaz yöntemi uygulandı. Histopatolojik incelemede haşhaş yağı ve pomad salisilik uygulamalarında çok katlı yassı epitelde 7. günde yangısal reaksiyonun başladığı 14. Günde ise kontrol grubuna yakın bir iyileşme gözlendiği belirlendi. Haşhaş yağı ve pomad salisilik uygulamaları arasında belirgin bir farklılık bulunmadığı sonucuna varıldı. Haşhaş yağının tedavi edici özellikleri ile ilgili yapılan bilimsel çalışmalar sınırlıdır. Deri üzerinde ve özellikle enfeksiyona uğramış ya da uğramamış deri yaralarının iyileşmesi üzerine herhangi bir çalışmaya rastlanılmamıştır.

***Anahtar Kelimeler*:** Mikotik dermatitis, Haşhaş yağı (Papaverus somnus), Sığır, Patoloji, Deri

The Role of Chronic Exposure to Amoxicillin/Clavulanic Acid on the Developmental Enamel Defects in Mice

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Abstract

Amoxicillin used in early childhood may be associated with enamel hypomineralization. Our aim was to assess disturbances of amelogenesis in mice lower incisors induced by chronic administration of amoxicillin/clavulanic acid (AMC). Twenty-eight C57BL/6 male mice, of similar age, randomly divided into a control and 3 treatment groups (n 1⁄4 7) received subcutaneous injection, once per day, for 60 days: 50, 100, and 150 mg/kg BW of AMC. Scanning electron microscopy/energy dispersive X-ray spectroscopy analysis in AMC treatment groups showed higher content in F and a decrease in P and Ca. Morphology changes ranged from scratched patterns, and small isolated pits-like enamel loss, to generalized demineralized enamel surface, giving a rough, foamy, scaly, or even cracked eggshell appearance to the affected areas. Histological analysis showed disturbances of maturation ameloblasts, which were less organized, with increased amounts of clear vacuoles in the cytoplasm and slightly more elongated and less condensed nucleus. Additionally, they were often detached from the enamel matrix. Transitional ameloblasts formed underlying the cysts of varied sizes. In conclusion, AMC dose-dependently affect ameloblast functions especially in the maturation phase, causing hypomineralized enamel formation with quantitative and/or qualitative defects.

Keywords: Scanning electron microscopy, amelogenesis, molar incisor hypomineralization, histology, morphology, hypoplastic enamel

Immunohistochemical Evaluation of Hepatic Progenitor Cells in Different Types of Feline Liver Diseases

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Abstract

Hepatic progenitor cells are periportally resident cells able to differentiate into mature hepatocytes or cholangiocytes to ensure hepatic regeneration. The reaction is defined as ductular reaction. In the present study, regenerative response of the feline liver to different hepatic diseases was investigated immunohistochemically. Regeneration of the liver through hepatocellular replication and through the proliferation of progenitor cell compartment were comparatively evaluated. Histological and immunohistochemical stainings were conducted on feline liver samples (n=30) representing various hepatobiliary diseases. CK7 and CK19 as biliary antibodies and PCNA and Ki67 as proliferation markers were used. The presence of progenitor cells in feline livers was proved, both as passive cells in normal liver and as active cells (ductular reaction) in hepatic lesions. CK7 was found to be a suitable antibody to identify feline progenitor cells. In acute events, regeneration was predominantly shaped by the division of hepatocytes. In chronic events and severe acute events (such as fulminant hepatitis), hepatocytes lost their ability to divide and regeneration occurred mainly through progenitor cells. The location of the ductular reaction varied between different feline hepatic diseases. Parenchymal ductular reaction was detected in fulminant hepatitis, chronic hepatitis, hepatocellular lipidosis and metastatic lymphoma. Septal ductular reaction was detected only in chronic hepatitis and metastatic lymphoma. This study may demonstrate the major role of hepatic progenitor cells in regeneration of the feline liver and shows how the activation pattern of ductular reaction varies according to the feline hepatobiliary disease type.

Keywords: Ductular reaction, feline, hepatic progenitor cells, immunohistochemistry, regeneration.

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POSTER PRESENTATIONS

Radiotherapy and Zoledronic Acid Treatment in a Cat with Peripheral Giant Cell Granuloma: A Case Report

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Abstract

Peripheral giant cell granuloma, formerly known as giant cell epulis, is considered as a tumor like lesion and is a very rare condition encountered in cats and dogs. In cats, these lesions display an aggressive clinical course and are reported to recur after surgery. The aim of this case report is to present this rare oral pathology in cats with clinical and microscopical findings, and the recovering process of the patient by using radiotherapy and zoledronic acid treatment. A 10-year-old, mixed, male cat was presented to the clinic with oral mass on mandible, emaciation due to feeding difficulty and salivation. On physical examination, a superficially ulcerated oral mass of 1 X 0.6 X 0.5 cm, white -yellow in color with elastic consistency was observed on the rostral mandible. The mass was completely removed by surgical operation and was sent to pathology department for histopathological examination. The tissue sample was fixed in 10% formaldehyde solution, routinely proceeded and embedded in paraffin blocks. Tissue sections of 4 - 5 μm thickness were cut and stained with Hematoxylin & Eosin. Histopathological examination revealed large numbers of multinucleated giant cells with fibrous stroma and densely packed collagen deposits. The case was diagnosed as peripheral giant cell granuloma. Thereafter, the patient received treatment with radiotherapy for 48 days and then treatment continued to be pursued with zoledronic acid once a month. As a result of these treatments, marked remission was observed for the patient. In conclusion, in comparison with the tumor like lesions, the peripheral giant cell granuloma observed in this case showed very aggressive clinical behavior. Combined radiotherapy and chemotherapy treatments applied to the patient revealed quite effective remission in the size of the lesion.

Keywords: Peripheral giant cell granuloma, radiotherapy, zoledronic acid

Lymphoblastic Lymphoma in a Dog: A Case Report

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Abstract

A two-year-old male Shiloh weighing 35 kg was presented with a 14-day history of possible infection and multiple masses on different part of the body including thorax, back of neck and scapula. Fine Needle Aspiration (FNA) was performed and based on cytology test, malignant neoplastic lesions were reported. The dog was treated using Cefixime and Co-trimoxazole. Histopathological examination after necropsy revealed a high-grade lymphoma and Immunohistochemistry (IHC) detected lymphoblastic lymphoma, based on positive TDT marker and negative CD20 and CD3 markers. Lymphoma is a general term used to describe cancers that arise from the lymphocytes and in general, Lymphoblastic lymphoma is an aggressive malignant disease. There are more than thirty different histologic subtypes of lymphoma described in the dog. B-cell lymphoblastic lymphoma is less common than T-cell lymphoblastic lymphoma in dogs. Lymphoblastic lymphomas are very aggressive, and widespread disease is common. All specimens were processed routinely, paraffin-embedded, sectioned, and stained with hematoxylin-eosin after necropsy. Arriving at a diagnosis of lymphoma is typically fairly straightforward. Fine needle aspirate (FNA) of an enlarged lymph node often provides the initial or presumptive diagnosis of the most common lymphomas. Histopathology of a lymph node biopsy is required to definitively diagnosis the grade of a lymphoma. Further staining with Immunohistochemistry (IHC) was performed. According to histopathological examination, lymphoma was diagnosed and further tests including Immunohistochemistry (IHC) with CD20, CD30 and TDT markers confirmed a lymphoblastic lymphoma in this Shianloo dog.

***Keywords**: Lymphoblastic Lymphoma, Shiloh, Pathology, Immunohistochemistry, Mashad*

A Case of Meibomian Adenoma in a Dog

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Abstract:

Meibomian adenomas are benign neoplasms that arise from meibomian glands located on the inside of the eyelid and show slow growth. The aim of the study is presenting a case of meibomian adenoma in a dog, The material of the study; the 9 years old Beagle breed formed masses taken from the upper right eyelid. As a result of histopathological examinations of the masses sent to Afyon Kocatepe University Veterinary Faculty Pathology Laboratory, it was determined that there was a meibomian adenoma.

***Keywords*:** Meibomian adenoma, dog, meibomian gland tumors.

Bir Köpekte Gözlenen Meibomian Adenoma Olgusu

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Özet

Meibomian adenomalar, göz kapağının iç tarafında bulunan meibomian bezlerden köken alan ve yavaş büyüme gösteren benign neoplazmalardır. Bu çalışmada bir köpekte gözlenen meibomian adenoma olgusunun sunulması amaçlanmıştır. Çalışmanın materyalini; 9 yaşında Beagle ırkı bir erkek köpeğin sağ üst göz kapağından alınan kitleler oluşturdu. Afyon Kocatepe Üniversitesi Veteriner Fakültesi Patoloji Laboratuvarına gönderilen parçaların histopatolojik incelemeleri sonucunda, meibomian adenoma olduğu tespit edildi.

***Anahtar Kelimeler*:** Meibomian adenoma, köpek, meibomian bez tümörleri.

Extracutaneous Uterine Mast Cell Tumor in a Dog: A Case Report

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Abstract

Mast cell tumors constitute 6% of all tumors in dogs and 13% of skin tumors. Although they are included in the classification of skin tumors, primary extracutaneous cases are reported to be rarely encountered.

There are very few publications on mast cell tumors in Turkey and among them, very limited information on extracutaneous mast cell tumors can be accessed. The purpose of this case report is to present the histopathological findings of a rare extra cutaneous mast cell tumor that diagnosed in the uterus, which has not yet been reported as localization.

An 11-year-old, mixed, male dog was presented to the clinic with hemorrhagic vaginal discharge, after the ultrasonography, it was taken to ovariohysterectomy operation and both corns and ovaries were removed and sent to the laboratory for histopathological examination. The tissue sample was examined macroscopically and fixed in 10% formaldehyde solution, routinely processed, and embedded in paraffin blocks. Tissue sections of 4 - 5 μm thickness were cut and stained with Hematoxylin & Eosin. Histopathological examination revealed mast cells with monomorphic morphology, round prominent nuclei, and large cytoplasm within the uterine layers in the uterine tissue. Eosinophil and neutrophil leukocytes were seen among the mast cells. In Giemsa staining for mast cells, it was observed that the granules of these cells were stained. These cells were infiltrated into the uterine muscle tissue. According to Patnaik 1984 Grade I; A diagnosis of well differentiated mast cell tumor was made. For the control of systemic metastasis, cytological examination of the patient's circulating blood was recommended in 1-3-6-12 months and then in annual periods.

As a result, to our knowledge a case of mast cell tumor in uterus which has not been reported before, will raise awareness clinically and histopathologically.

Keywords: Mast cell tumor, extracutaneous, uterus, dog

A Case of Vaginal Xanthoma in a Cow

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Abstract

A case of vaginal xanthoma in a 3-year-old Holstein cow is described macroscopically, microscopically and immunohistochemically. A pink-colored mass surgically excised from the vagina was hard, nodular and 328 g in weight. Macroscopically, the cross section of the 17x15x8.5 cm vaginal mass showed whitish lobular structures divided by thin septums. Histopathologically, the mass was consisted of lobular areas surrounded by a stroma and composed of numerous foamy macrophages, abundant lipid material and rarely giant cells. Immunohistochemically, the mass was positive for vimentin, proliferating cell nuclear antigen (PCNA) and CD 68, but negative for smooth muscle actin (SMA), glial fibrillary acidic protein (GFAP) and S100 protein. To the authors' knowledge, this is the first report of vaginal xanthoma in a cow.

Keywords: Vaginal xanthoma, pathology, immunohistochemistry, cow.

Bir İnekte Vaginal Ksantom Olgusu

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Özet

Bu olgu sunumunda, 3 yaşlı, Holştayn ırkı bir inekte teşhis edilen vaginal ksantom'un makroskobik, mikroskobik ve immunohistokimyasal olarak tanımlanması amaçlandı. Vaginadan pembe renkli, sert kıvamlı, 328 gr ağırlığındaki bir kitle cerrahi olarak uzaklaştırıldı. Makroskobik olarak, 17x15x8.5cm çapındaki vaginal kitlenin kesit yüzü, ince septumlarla bölünmüş beyazımsı lobüler yapılar gösteriyordu. Histopatolojik olarak, kitle stromayla sarılı lobüler alanlardan oluşuyordu ve çok sayıda köpüklü makrofajlar, yoğun lipid materyali ve az sayıda dev hücre içeriyordu. Immunohistokimyasal olarak vimentin, prolifere hücre nükleer antijen (PCNA) ve CD 68 ile pozitif; düz kas aktin (SMA), glial fibriler asidik protein (GFAP) ve S100 protein ile negatif reaksiyon saptandı.Yapılan literatür taramasına göre bu, bir inekte bildirilen ilk vaginal ksantom olgusudur.

Anahtar Kelimeler: Vaginal ksantom, patoloji, immunohistokimya, inek.

A Case of Xanthoma in a Saanen Goat

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Abstract

In this case report, a case of xanthoma detected under the skin of the sacral region in a 2-year-old Saanen goat was described. When the skin was skinned, a light brown-toned mass measuring 8.5x4x0.5cm was detected. It was noticed that this mass was gray-white-yellowish in color throughout its cross-sectional face. Histopathological examination revealed that foamy macrophages were concentrated, as well as giant cells, lipid material and cholesterol clefts. It was observed that such a collection of histopathological cells and materials formed lobules surrounded by stroma. In order to detect lipid material, frozeen sections were taken and stained with Oil-Red-O. In this staining, lipid material was detected in the cytoplasm of both extracellular and large foamy macrophages. In addition, positive results were obtained from vimentin, proliferated cell nuclear antigen and CD68 markers immunohistochemically applied to tissue sections, while negative results were obtained from smooth muscle actin, glial fibrillary acidic protein and S100 marker staining. This case report is important as it is the first report about xanthoma in a goat.

***Keywords*:** Xanthoma, Goat, Pathology, Histopathology, Immunohistochemistry

Bir Saanen Keçisinde Ksantom Olgusu

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Özet

Bu olgu sunumunda, 2 yaşlı Saanen ırkı bir keçide sakral bölgede deri altında saptanan ksantom olgusu tanımlandı. Deri yüzüldüğünde açık kahverengi tonda 8.5x4x0,5cm ebatlarında bir kitle saptandı. Bu kitlenin tüm kesit yüzü boyunca boz-beyaz-sarımtırak renkte olduğu dikkati çekti. Histopatolojik incelemede köpüklü makrofajların yoğunlukta olduğu, bunun yanı sıra dev hücreleri ile lipid materyal ve kolesterol kleftlerine rastlandı. Bu şekildeki histopatolojik hücre ve materyal topluluğunun stroma ile çevrili lobüller oluşturduğu görüldü. Lipid materyalinin tespiti amacıyla frozeen kesitler alınarak Oil-Red-O ile boyandı. Bu boyamada hem ekstrasellüler hem de büyük köpüklü makrofajların sitoplazmalarında lipid materyali saptandı. Ayrıca, doku kesitlerine immunohistokimyasal olarak yapılan vimentin, prolifere hücre çekirdek antijeni ve CD68 markırlarından pozitif sonuçlar alınırken, düz kas aktin, gliyal fibriler asidik protein ve S100 markır boyamalarından negatif sonuçlar alındı. Bu olgu sunumu bir keçideki ksantom hakkındaki ilk rapor olması açsından önem taşımaktadır.

***Anahtar Kelimeler*:** Ksantom, Keçi, Patoloji, Histopatoloji, İmmunohistokimya

Expression of ADAMTS7 in Myocardial Dystrophy Associated with White Muscle Disease in Lambs

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Abstract

The aim of the present study was to investigate the role of ADAMTS7 gene in the pathogenesis of myocardial dystrophy associated with white muscle disease in lambs. A total of 217 cardiac tissue samples of lambs with white muscle disease were used in the study. Histopathological sections of the tissue samples were stained with hematoxylin-eosin and examined using Western-blot, real-time PCR and immunohistochemistry for ADAMTS7 gene expression in cardiac tissues and the findings were statistically evaluated. Histopathological examinations revealed fibrosis associated with hyalinization, necrosis and granular calcifications in cardiomyocytes. Western blot and rt-PCR showed a statistically significant upregulation in ADAMTS7 protein. Immunohistochemical analysis showed statistically significantly strong cytoplasmic immunopositive cell densities for ADAMTS7 antibody. The study results determined that ADAMTS7 gene was significantly expressed in myocardial dystrophy associated with white muscle disease in addition to its role in the pathogenesis of this disease.

***Keywords*:** ADAMTS7, white muscle disease, heart, lamb

Kuzularda Beyaz Kas Hastalığına Bağlı Oluşan Myokardial Distrofide ADAMTS7 Salınımı

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Özet

Bu çalışmada, kuzularda beyaz kas hastalığına bağlı oluşan myokardial distrofinin patogenezinde ADAMTS7 geninin rolünün araştırılması amaçlandı. Çalışmada toplam 217 beyaz kas hastalığı tespit edilen kuzuya ait kalp dokusu, negatif kontrol amacıyla ise farklı hastalıklardan ölen ve kalp hasarı görülmeyen kuzulara ait kalp dokuları kullanıldı. Doku örneklerinden hazırlanan kesitler hematoksilen-eosin ile boyanarak histopatolojik olarak incelendi. Kalp dokularındaki ADAMTS7 ekpresyonun belirlenmesi amacıyla Western blot, real-time PCR ve immunohistokimyasal yöntemler uygulandı. Elde edilen sonuçlar istatistik olarak değerlendirildi. Yapılan histopatolojik incelemelerde kardiomysitlerde hyalinleşme, nekroz ve granuler kalsifikasyonlar ile birlikte yer yer fibrozis görüldü. Western blot ve real-time PCR analizlerinde ADAMTS7 düzeyinin istatistik olarak anlamlı oranda upregule olduğu tespit edildi. İmmunohistokimyasal boyamalarda dejeneratif hücrelerde ADAMTS7 antikoru için şiddetli sitoplazmik immunopozitif reaksiyon yoğunluğu istatistik olarak anlamlı yüksek bulundu. Çalışma sonuçları beyaz kas hastalığına bağlı oluşan myokardial distrofide ADAMTS7 genin rolünün olabileceği belirlenmiştir.

***Anahtar Kelimeler*:** ADAMTS7, beyaz kas hastalığı, kalp, kuzu

A Case of Frontal Osteoma with Pulmonary Adenomatosis, Paratuberculosis, and Systemic Parasitosis in a Sheep

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Özet

Objective: Osteomas are well-differentiated, benign tumours with mature bone tissue and firm structure. Osteomas are rare in animals. It has been reported in domestic animals such as horses, cattle, goats, cats and dogs. Osteomas are most common in the maxilla, mandibula, sinonasal bones, face and skull bones. Bone tumours and osteomas are much less common in sheep. Currently, a total of four cases; one in the femur, the other in the zygomatic arch, one in the maxillary sinus and two in the head region, have been reported. In the present report, a case of frontal osteoma occurring in a sheep is presented along with diseases with different aetiology such as pulmonary adenomatosis, paratuberculosis and systemic parasitosis which are relatively common in sheep is presented due to the rare occurrence of this tumour in sheep.

Materials and Methods: The case material was 2 years old adult Akkaraman breed sheep which was admitted to the Department of Internal Medicine due to severe anaemia and diarrhoea and died during treatment. Tissue samples collected during necropsy were fixed in 10% formalin solution. The skull was then decalcified in 10% formic acid solution. Tissues were embedded in paraffin and sectioned at 5 µm thickness. The sections were stained with hematoxylin-eosin (HxE). Additionally, sections obtained from intestines and lymph nodes were stained with Ziehl-Neelsen method to detect acid-fast bacteria, sections obtained from the decalcified head mass were stained with Masson's Trichrome staining method.

Results: In systemic necropsy, a hard mass of 7x5x4 cm was observed in the frontal region of the skull. The longitudinal section of the mass revealed that it was extending from the frontal bone to frontal sinus. In the lungs, focal, transparent and shiny masses of approximately 1x1 cm in size were detected at three different areas. The intestinal mucosa thickened and their lumen narrowed. Histopathologic examination of decalcified mass excised from the frontal region revealed multiple immature trabecular osteoid structures. Among the trabecular bones that formed the tumour, cell-poor collagen-rich fibrous tissue was detected. Histopathologic examination of the lungs showed papillary growths in the alveoli and bronchiole epithelial cells with the presence of tumoral masses in the lumens and mononuclear cell infiltrations in the alveoli and interstitial area. Diffuse

inflammatory cell infiltration, most of which consisted of mononuclear inflammatory cells was detected in the intestines, including mucosal and submucosal layers extending into the muscular layer. Multinucleated Langhans type giant cells containing nuclei arranged in a horseshoe-shaped pattern in the cell periphery were present around the Lieberkühn crypts of the duodenum accompanied by intense mononuclear inflammatory cell infiltration. Ziehl-Neelsen staining revealed clusters of phagocytosed magenta-coloured rod-shaped bacteria compatible with paratuberculosis aetiology in giant cells and some histiocytes. Giant cells and club-shaped bacteria were also found in the jejunum and ileum. Furthermore, parasitological examinations revealed; 4 *Oestrus ovis* larvae in the nasal cavity, 484 *Dicrocoelium dendriticum* in the gallbladder, Cysticercus spp. in the rumen serosa. Additionally, 850 cestodes 2350 nematodes and some protozoan species were determined in the duodenum, jejunum, and ileum. Among these species *Haemonchus contortus, Ostertagia* spp., *Trichostrongylus* spp., *Eimeria spp*. (three different types) and *Stilesia spp.* type parasites were present.

Conclusion: In the differential diagnosis of osteomas, ossified fibroma, odontomas, osteochondromas, fibrous dysplasias and exostoses should be considered. The pathogenesis of osteomas is not fully known. However, embryological developmental defects, trauma or bone infections can be considered among possible etiological factors. In the present case, no signs of trauma or infection were detected in the area where the tumour developed. Therefore, the aetiological factor could not be determined. Although it is very rare in sheep, when masses that occur in the skull during clinical examination or necropsy and affect the nasal sinuses are detected, osteomas should also be considered in the differential diagnosis.

Keywords: Sheep, Pulmonary adenomatosis, Paratuberculosis, Frontal osteoma

Bir Koyunda Pulmoner Adenomatozis, Paratuberkülozis ve Sistemik Parazitozis ile Birlikte Seyreden Frontal Osteom Olgusu

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Özet

Amaç: Osteom, iyi farklılaşmış, olgun kemik dokusu içeren, sert yapıda, iyi huylu bir tümördür. Osteomlara hayvanlarda ender olarak rastlanmaktadır. Evcil hayvanlardan at, sığır, keçi, kedi ve köpeklerde bildirilmiştir. Osteomlar en sık maksilla, mandibula, sinonazal kemikler, yüz ve kafa kemiklerinde görülmektedir. Koyunlarda ise kemik tümörleri ve osteomlar çok daha nadir görülmektedir. Şu ana kadar yapılan çalışmalarda biri femurda, diğeri zigomatik arkta, biri maksillar sinüsde ve ikisi de kafa bölgesinde olmak üzere toplam olarak dört olgu bildirilmiştir. Bu olguda, koyunlarda nispeten sık görülen pulmoner adenomatozis, paratüberkülozis ve sistemik parazitozis gibi farklı etiyolojilere sahip hastalıklar ile birlikte seyreden ve koyunlarda oldukça nadir bildirilen frontal osteom olgusu, literatürde bu tümör ile ilgili oldukça sınırlı veri olması sebebiyle sunuldu.

Materyal ve Metot: Çalışmanın materyalini şiddetli anemi ve ishal nedeniyle iç hastalıkları kliniğine getirilen ve tedavi sırasında ölen Akkaraman ırkı, yaklaşık 2 yaşlı bir koyun oluşturdu. Nekropsi esnasında alınan doku örnekleri %10'luk formalin solüsyonunda tespit edildi. Daha sonra, kafatası %10'luk formik asit solüsyonu içerisinde dekalsifiye edildi. Dokular parafine gömüldü ve 5 µm kalınlığında kesitler alındı. Alınan kesitler hematoksilen-eozin (HxE) ile boyandı. Ayrıca, asit-fast bakterilerin tespiti amacı ile bağırsak ve lenf yumrularından elde edilen kesitler Ziehl-Neelsen metoduyla, dekalsifiye olmuş kafadaki kitleden elde edilen kesitler Masson'un Trikrom boyama yöntemi ile boyandı.

Bulgular: Yapılan sistemik nekropside kafatasında, frontal bölgede 7x5x4 cm boyutlarında, düzgün sınırlı, sert bir kitle fark edildi. Uzunlamasına kesitte kitlenin frontal kemikten frontal sinüse kadar uzandığı görüldü. Akciğerlerin üzerinde, üç farklı noktada fokal, yüzeyi şeffaf ve parlak, yaklaşık 1x1 cm ebatlarında kitleler tespit edildi. Bağırsak mukozalarının kalınlaşmış ve lümenlerinin daraldığı kaydedildi. Frontal bölgeden alınan kitlenin dekalsifiye edilmesinin ardından yapılan histopatolojik incelemede multifokal yerleşimli, geniş alanlara yayılmış çok sayıda olgunlaşmamış kemik trabekülleri görüldü. Tümörü oluşturan trabeküller arasında

hücreden fakir, kollajen zengin fibröz bir dokunun bulunduğu tespit edildi. Akciğerlerde alveoller ile bronşiyol epitellerinde papiller üremeler yanında alveol lümenleri ile interstisyel bölgede mononükleer hücre infiltrasyonları görüldü. Bağırsaklarda mukoza, submukoza ve kas katmanını da içeren diffuz karakterde, çoğunluğunu mononükleer hücrelerin oluşturduğu yangı hücresi infiltrasyonları fark edildi. Duodenumda Lieberkühn kriptleri çevresinde şiddetli mononükleer yangı hücresi infiltrasyonları ve at nalı tarzı çekirdek dizilimi sergileyen eozinofilik sitoplazmalı, çok çekirdekli Langhans tipi dev hücreleri görüldü. Ziehl-Nielsen boyaması yapıldığında dev hücreleri ve bazı histiyositlerin içinde fagosite edilmiş, magenta renginde, çomak şeklinde ve paratüberkülozis etiyolojisi ile uyumlu aside-dirençli bakteri kümeleri belirlendi. Dev hücreleri ve çomak şeklinde bakteriler jejunum ve ileumda da tespit edildi. Ayrıca, parazitolojik incelemeler sonucu nazal boşlukta 4 adet *Oestrus ovis* larvası, safra kesesinde 484 tane *Dicrocoelium dendriticum*, rumen serozasında *Cysticercus* spp., duodenum, jejenum ve ileum boyunca 850 adet sestod, 2350 nematod yanında bazı protozoan türleri belirlendi, bu türler arasında *Haemonchus contortus, Ostertagia* spp., *Trichostrongylus* spp., *Eimeria* spp. (üç farklı tip) ve *Stilesia* spp. türü parazitler identifiye edildi.

Sonuç: Osteomların ayırıcı tanısında ossifiye fibroma, odontomlar, osteokondromlar, fibröz displaziler ve eksostoslar dikkate alınmalıdır. Osteomların patogenezi tam olarak bilinmemektedir. Ancak, embriyolojik gelişim hataları, travma ya da kemik enfeksiyonları muhtemel etiyolojik faktörler arasında sayılabilir. Sunulan bu olguda tümörün geliştiği bölgede travma ya da enfeksiyona ait bir bulgu tespit edilemedi. Bu nedenle etiyolojik faktör belirlenemedi. Koyunlarda, her ne kadar oldukça seyrek görülse de, klinik muayene ya da nekropsi sırasında kafatasında meydana gelen ve nazal sinüsleri de etkileyen kitleler tespit edildiğinde, ayırıcı tanıda osteomların da dikkate alınması gerekmektedir.

***Anahtar Kelimeler*:** Koyun, Pulmoner adenomatozis, Paratuberkülozis, Frontal osteom

Early and Long-term Macroscopical and Histomorphological Manifestations: Natural Encephalitizoon Infestation in Rabbits

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Abstract

Encephalitozoon is an eukaryotic and microsporidian protozoan parasite It is known as obligate and intracellular pathogens. In the study, it is aimed to reveal necropsy and histopathological findings of rabbits infested with *Encephalitizoon cuniculi* investigating at different time. The seropositive infested animals are collected from rabbit colonies licenced from Ministry of Agriculture and Livestock and laboratory animals kept in cages which laboratory animal breeding facility after minimal 5 year-period clinic observation to better understood of acute and long-term *E. cuniculi* infection. The rabbits aged up to approximately 30 months with a body weight of 1.5-2.5 kg in the colony. After serological confirmation the parasite, the infested animals were necropsied at different months and histopathologically examined suitably to routine hematoxylin-eosin staining in addition to Gram and PAS stainings. Acute and sudden manifestations at the cadavers were related to cataract, uveitis, retinitis. Subacute and chronic findings followed by the mentioned eye manifestations, encephalitis, nefritis as well as degenerative changes in neurons, kidney tubules and hepatocytes. In other organs, there were no any outstanding finding. The parasitic spores were confirmed by Gram and PAS staining. In conclusion, the main findings were observed accordingly to previous reports. However, sudden lesions were mostly encountered in the eyes although brain, kidneys and other organ (such as liver) helds were seen latter stages during the observation. It is underestood that such kind of systemic findings were covered with subclinical and persisted infection at ongoing time. It is believed that the study help to better percieved of pathogenetic mechanism in both acute and long-terms.

***Keywords*:** Encephalitizoonosis, acute and chronic infestation, necropsy and histomorphological findings, rabbit.

Erken ve Uzun Dönem Makroskopik ve Histomorfolojik Bulgular: Tavşanlarda Doğal Ensefalitizoon Enfestasyonu

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Özet

Ensefalitozoonozis, ökaryotik ve mikrosporidian bir protozoan parazittir. Zorunlu ve hücre içi patojenler olarak bilinirler. Bu çalışmada, *Encephalitizoon cuniculi* ile enfekte tavşanların farklı zamanlarda incelemeleri yapılarak, enfeksiyon seyrinde nekropsi ve histopatolojik bulgularının ortaya çıkarılması amaçlanmıştır. Seropozitif enfekte hayvanlar, akut ve uzun süreli *E. cuniculi* enfeksiyonunun daha iyi anlaşılması için en az 5 yıllık klinik gözlem sonrasında Tarım ve Hayvancılık Bakanlığı'ndan ruhsatlı tavşan sürülerinden ve hayvan yetiştirme tesisinin laboratuarında tutulan laboratuvar hayvanlarından toplanır. Tavşanlar 1.5-2.5 kg vücut ağırlığı ile yaklaşık 30 aya kadar yaştalardı. Parazitin serolojik olarak doğrulanmasından sonra, enfekte hayvanlara farklı aylarda nekropsi uygulandı ve rutin hematoksilen-eozin boyamayla birlikte yöntemine uygun şekilde Gram ve PAS boyamaları yapılarak histopatolojik olarak incelendi. Kadavralarda karşılaşılan akut ve ani gelişen belirtiler katarakt, üveitis, retinitis ile ilişkiliydi. Subakut ve kronik bulgularda bahsi geçen göz bulguları yanı sıra ensefalitis, nefritis ile nöronlarda, böbrek tübüllerinde ve hepatositlerdeki dejeneratif değişiklikler izlendi. Diğer organlarda göze çarpan bir bulgu yoktu. Parazitik sporlar, Gram ve PAS boyama ile doğrulandı. Sonuç olarak, esas bulgular önceki literatürlerle uyumlu olarak gözlendi. Ancak ani lezyonların en çok gözlerde, gözlemin ilerleyen aşamalarında ise beyin, böbrekler ve diğer organların (karaciğer gibi) tutulduğu görüldü. Bu tür sistemik bulgular, devam eden zamanda subklinik ve kalıcı enfeksiyonla örtüştüğü anlaşıldı. Çalışmanın hem akut hem de uzun dönemde patogenetik mekanizmanın daha iyi algılanmasına yardımcı olduğuna inanılmaktadır.

***Anahtar Kelimeler*:** Ensefalitizoonozis, akut ve kronik enfestasyon, nekropsi ve histomorfolojik bulgular, tavşan.

Splenic Lipoma in a Red Kangaroo

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Abstract

In this report, it is aimed to present a case of lipoma observed in the spleen with systemic infection in a red kangaroo. In the necropsy of the red kangaroo sent from the Bursa zoo, it was seen that congestion and a white nodule with a diameter of 0.5 cm were found in the liver. It was observed that the medullas of the kidneys were darker than normal. Widespread petechial hemorrhages were noted in the urinary bladder mucosa with normal color of urine. When the thorax was opened, it was observed that the lungs collapsed and the color was generally normal, but with areas of disseminated hemorrhage. There was a slight thickening of the left ventricular wall and a large amount of post mortal blood clots in the lumen. No lesion was noticed in the brain. Microscopically, it was noted that the epicardium of the heart was thickened slightly with connective tissue (fibrosis). Sarcocystis spp. was found in a cardiomyocyte in an area. A cystic structure surrounded by connective tissue and mild mononuclear cells in an area of the liver was seen. It was observed that sinusoids were filled with moderate erythrocytes. The presence of adipose tissue in two foci within spleen parenchyma was noted (lipoma). Severe congestion was observed in the renal medulla. The presence of erythrocytes (hemorrhage) in the alveolar cavities was noted multifocally in the lung. The findings were consistent with epicardial fibrosis and circulatory failure, as well as nonmyeloid neoplasia (lipoma) of the spleen, parasitic hepatitis, and sarcocytosis of the heart. To the knowledge of the authors, a case of splenic lipoma in red kangaroos has not been reported before.

***Keywords*:** Kangaroo, lipoma, spleen

Bir Kırmızı Kanguruda Dalak Lipomu

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Özet

Bu bildiride bir kırmızı kanguruda sistemik enfeksiyon ile birlikte dalakta gözlenen lipom olgusunun sunulması amaçlanmıştır. Bursa Hayvanat Bahçesi'nden gönderilen kırmızı kangurunun nekropsisinde karaciğerin konjestif olduğu ve bir odakta 0,5 cm çaplı beyaz bir nodüle rastlandı. Böbreklerin medullasının normalden daha koyu olduğu görüldü. İdrarın renginin normal olduğu, idrar kesesi mukozasında ise yaygın peteşiler dikkati çekti. Göğüs boşluğu açıldığında akciğerlerin kollabe olduğu ve renginin genel olarak normal olduğu fakat disemine hemoraji alanları ile bezeli olduğu gözlendi. Kalpte sol ventrikül duvarında hafif derecede kalınlaşma ve lümende çok miktarda postmortal kan pıhtısı görüldü. Beyinde herhangi bir lezyon dikkati çekmedi. Mikroskobik olarak kalpte epikardiyumun bağ doku ile hafif derecede kalınlaştığı dikkati çekti (fibrozis). Bir alanda kardiyomyosit içerisinde *Sarcocystis spp.*'ye rastlandı. Karaciğerde bir alanda çevresi bağ doku ve hafif şiddette mononükleer hücreler ile çevrili kistik yapı dikkati çekti. Sinuzoidlerin orta şiddette eritrositlerle dolu olduğu gözlendi. Dalak parenkimi içerisinde iki odakta yağ doku varlığı dikkati çekti (lipom). Böbrek medullasında şiddetli konjesyon gözlendi. Akciğerde multifokal olarak genellikle sınırlı görünümde, alveol boşluklarında eritrositlerin varlığı (hemoraji) dikkati çekti. Bulgular epikardiyal fibrozis ve dolaşım yetmezliği ile birlikte, dalakta nonmyeloid neoplazi (lipom), paraziter hepatitis ve kalpte sarkosistozis ile uyumlu bulunmuştur. Yazarların bilgisine göre kırmızı kangurularda dalakta lipom olgusu daha önce bildirilmemiştir.

***Anahtar Kelimeler*:** Dalak, kanguru, lipom

FULL TEXTS

Foodborne mycotoxicoses: risk assessment and underestimated hazard for animals or humans (review)

Stoycho STOEV*

Abstract:

The molds and mycotoxins have been associated with a variety of livestock diseases, e.g.: Ergotism, known in humans as St. Anthony's fire; Equine leukoencephalomalacia; Porcine pulmonary oedema; Alimentary toxic-aleykiya; Aflatoxicosis; Mycotoxic porcine/chicken nephropathy; Stachybotryotoxicosis, and many others. The exposure of animals to various mixtures of mycotoxins presents a problem that has not been sufficiently investigated. Such mixtures of toxins often have additional or synergistic effects in farm animals. Various mycotoxicoses often have multi-mycotoxic natures being provoked by a combined effect of many mycotoxins. For example mycotoxic nephropathy in Bulgaria and Africa has a different multi-mycotoxic origin as compared to the classical mycotoxic nephropathy, described previously in Danmark. The mycotoxins involved are; ochratoxin A (OTA), Penicillic acid (PA), Fumunisin B1 (FB1), in addition to not yet identified mycotoxin. Similarly, many other mycotoxicoses also have multimycotoxic origin. In this regard, a potent synergistic effect was found between OTA and PA, when the same were given simultaneously to pigs and chickens. Such synergy between OTA and PA or other mycotoxins such as FB1 under field conditions is likely responsible for the spontaneous nephropathy in Bulgaria, which is caused by relatively low contamination levels of OTA in food.

The problem of transmitting the mixture of various contaminants from carcass further through the food chain to humans and the human health hazard from such exposure should be additionally evaluated. Some recent experiments focused attention on the immunosuppression as the first expressed toxic effect of OTA, which may become evident clinically before nephropathy and its associated biochemical changes. OTA suppression of humoral and cellular immunity, defined in principle, has been demonstrated in practice allowing development of secondary bacterial infections (e.g. salmonellosis and haemorrhagic enteritis) in pigs at only 1 ppm ochratoxin A in diet. OTA has also been described to increase the susceptibility of chickens to coccidiosis, salmonellosis and colibacillosis.

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Considerable aggravation of the pneumonic process in pigs infected with *M. hyopneumoniae* or *P. multocida* when treated with 20 ppm FB1 was found, as determined by macroscopic and pathomorphologic examinations and computed tomography, in addition to the premature death in part of the pigs.

Introduction

Protecting the humans or animals from various food-born diseases becomes more and more important task of many specialists in food safety issues in the field of animal and human health in all over the world, especially with the important mission to ensure safe and healthy feeds or foods for animals and humans free from any dangerous contamination levels of mycotoxns. Various safety and quality control activities along the food supply chain are usually important to enhance the safety of various foods and feeds and to prevent the foodborn diseases, but the same activities at each level require integration into an well-coordinated and integrated system. The existence of target professionals and knowledge in various scientific fields as biochemistry, agriculture, veterinary medicine, environmental health science, food science and technology, in addition to the upgrading of the respective target knowledge and skills via professional courses are of crucial significance for implementing these safety and quality control activities in the practice. However, only integrated approach to food safety, which includes systematic identification and assessment of hazards in foods production, various means to control them and introducing of Hazard Analysis and Critical Control Point (HACCP) system, which can improve significantly the food safety management, could resolve all kind of existing problems in this field. The effective surveillance control and the respective enforcement of food safety laws and regulations is also compulsory in order to enhance the security of food and to reduce the number of food-borne diseases and the consequent social burdens of health care. Therefore, a harmonization of various national standards or an elaboration of common standards and regulations for mycotoxins contents in various food commodities or raw materials should be undertaken for better protection of the consumer in all over the world ensuring a global food safety and fair international trade (1-3).

Foodborne mycotoxicoses and the Hazard of joint mycotoxin exposure in the field

The mycotoxins are well known contaminants of food/feed and often present a serious health hazard for animals or humans around the world. In developing countries, having lower standards of food quality, mycotoxin-contaminated food or feed can induce a lot of health problems and ailments in animals or humans. Mycotoxins are secondary toxic metabolites produced by many fungi in some agricultural stuffs or ground feed, susceptible to fungal

infestation. The mold invasion in cereals is usually happen in the field or after the harvest. The mycotoxin production is sometimes unavoidable, because of some environmental factors, e.g. raining, at the time of harvest or the subsequent poor storage conditions. The multiple mycotoxin contamination of foods/feeds with known mycotoxins, in addition to some unknown mycotoxins is occurring at a high rate (4-5). Although, there are limited evidences on the definites effects of target mycotoxins or mycotoxins combinations in the course of various human/animal diseases, there are considerable amount of proves supporting the association between mycotoxins and certain animal syndromes or livestock diseases including ergotism, various kinds of fusariotoxicoses as alimentary toxic-aleykiya in humans, porcine pulmonary oedema or vulvovaginitis, equine leukoencephalomalacia or human oesophageal carcinoma, various aflatoxicoses, mycotoxic porcine/chicken nephropathy stachybotryotoxicosis and many others (Figure 1) (1-6).

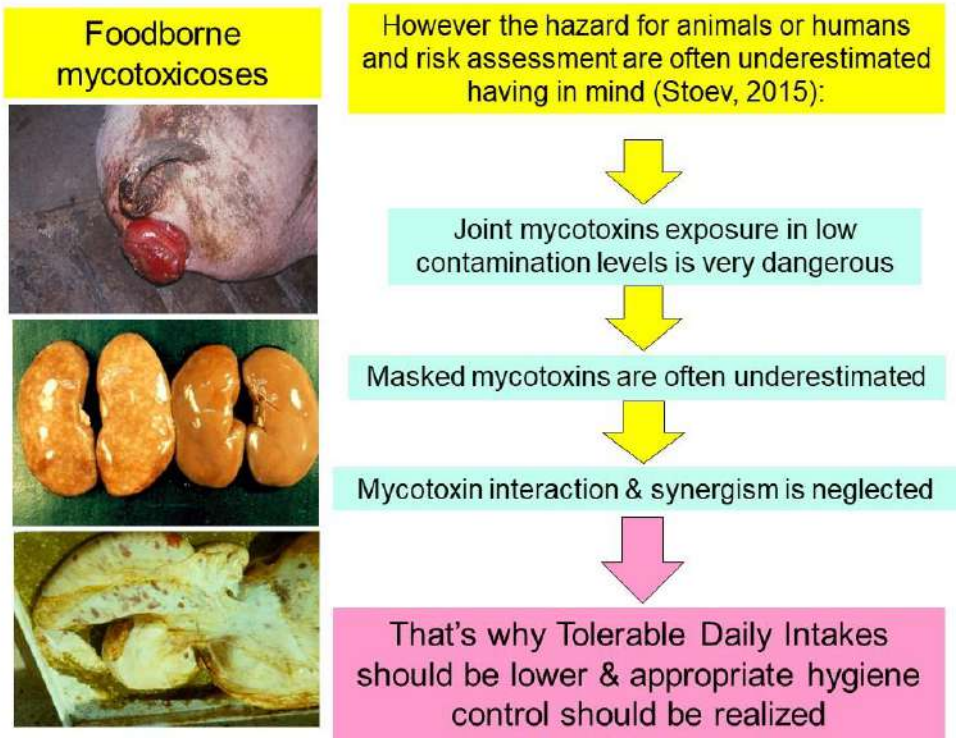

Figure 1. Foodborne mycotoxicoses: from up to down: a) Spontaneous Fusariotoxicosis in 3-5 month pigs caused by *F. sporotrichiella* or *F. poae* – many vulvovaginites accompanied by massive swelling of the vulva and vaginal or rectal prolapses due to Zearalenone (2); b) Macroscopic appearance of kidneys with mycotoxic nephropathy with enlarged and mottled appearance in pig of 6-8 month-age (left) and normal kidneys in pig of the same age (right) (3); c) Stachybotryotoxicosis in cattle with strong haemorrhagic diathesis on the serosa of the

abomasus and rumen and ulcerative-necrotic damages, which are translucent under the serosa (spontaneous case in Bulgaria) (3).

Ergotism, caused by the fungus *Claviceps purpurea*, is provoked by many different mycotoxins as ergometrine, ergine, ergotamine, ergosine, ergosecaline, ergocristine, ergocryptine and some biogene amines as histamine and acetylcholine, which are presented in the fungal sclerotia - compact mass of hyphae cemented, which resembles a large, dark wheat grain. Mycotoxins of significance for the development of ergotizm are however ergometrine, ergotamine and ergocristine. The disease is also known as St Anthony's fire, which is one of the oldest food-borne diseases in man causing hallucinatory symptoms and death of many people in France and other European countries during the Middle Ages (7). The cases of ergotism have been reported in a French village of Pont Saint Esprit in 1951 (8-9) and in Ethiopia in 1978 (10). The disease is still of public health importance, especially in the developing world (11-12). It is characterized mainly by dry gangrene of the extremities, loss of one or more limbs, in addition to some gastrointestinal symptoms. Among animals, many species are sensitive to this foodborn mycotoxicosis, but the the horses and ruminants are among the most sensitive species. The mycotoxins, responsible for ergotism, also increase the contraction of the uterus and provoke many abortions and a prolapse of uterus, in addition to the vasoconstriction of blood vessels and the subsequent ischemic necroses in peripheral body parts as tail, ears, crown of hooves, etc (11-12).

Aflatoxins were discovered due to their devastating effect on turkey poults (Turkey-X-disease) and some other chicks in 1960 in England, when more than 100 000 turkey poults died (13) as the target toxicity of these mycotoxins were found in the liver, which is usually enlarged and yellow. The so-called Turkey X disease was eventually tied to high levels of aflatoxin in Brazilian peanut meal imported as a feed ingredient. Aflatoxin contamination is most common in African, Asian, and South American countries with warm and humid climates, but also occurs in temperate areas of North America and Europe. The young animals and ducks or turkeys are usually the most sensitive specieses and haemorrhagic diathesis, liver necroses, enlarged gall bladder with oedematous wall and yellowish staining of the tissue around it, proliferative processes in the hepatic interstitium, strong inflammatory-necrotic changes in the digestive tract and mild dystrophic changes in other organs were also often found in addition to liver damages, whereas in chronic course the main damages are usually ascites, hydrothorax, icterus, cirrhosis of the liver or lung edema, but in the cattles – thickening of the skin around

the mouth and neck in addition to some papiloms on the mucosa of abomasus are also observed (3).

On the other hand, ochratoxin A was found as a major causal agent in mycotoxic nephropathy (MN) in pigs (15-16) and probably Balkan Endemic Nephropathy in humans, widely encountered in Balkan countries (17-20) (Figure 2). This nephropathy, however, was recently proved to have much complicated pathology and aetiology in some countries as Bulgaria and South Africa (4-5). In the same countries spontaneous nephropathy in pigs (16) or chicks (21) described previously, was recently found to be provoked by the combined action of several mycotoxins as ochratoxin A, penicillic acid and fumonisin B_1, in addition to not yet identified metabolite, having a strong synergistic interaction (4-5). A part of the same mycotoxins produced by sorage fungi such as ochratoxin A and penicillic acid are secondary fungal metabolites encountered in feeds/food made mainly from cereals or fibrous plants, which is kept in storehouse conditions and increased humidity, whereas the other target mycotoxin fumonisin B_1 produced by field fungi mainly contaminates maize before harvesting. Usually, the farms with such nephropathy problems had a history of unappropriate feed storage, but sometimes the problem came from certain feed plants whose grains, collected during moist and rainy days, had not been properly dried. Subsequently, all farms used these plants as their main feed source had nephropathy problems and growth depression among their pigs, but after changing the source of feed supply the problems with poor growth of pigs disappeared (16). The timely diagnosis of the same disease during the meat inspection at slaughterhouses is very important, because of the harmful effects, which can be observed in human kidneys after consuming the meat of animals with nephropathy problems and the respective mycotoxin content. Therefore, the exposure of humans to the same hazardous and relatively heat stable target mycotoxins from chicken/pigs meat should be prevented (18).

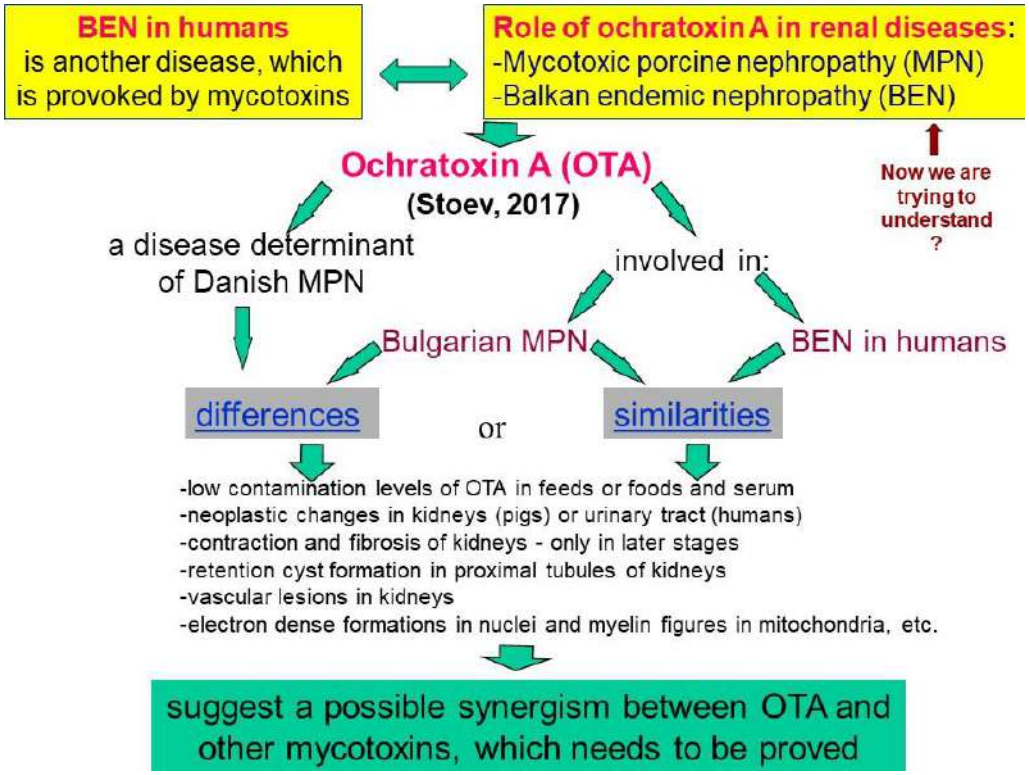

Figure 2. Similarities and differences betwee Bulgarian and Danish porcine nephropathy and Balkan Endemic Nephropathy in humans (18).

The maize moulded by various *Fusarium* fungi was suspected to be a cause of horse or pig diseases, including the death in many horses more than 150 years ago and this was subsequently associated with the presence of *Fusarium moniliforme (F. verticillioides).* However, the mycotoxins provoking the same diseases, the fumonisins, were not well known until the 1980's (22-23). We became aware about the significant hazard of these mycotoxins, especially of fumonisin B_1, after a number of outbreaks of equine leukoencephalomalacia (24-25) and porcine pulmonary oedema killing many horses or pigs, fed on diets containing fumonisin-contaminated maize in the U.S. during 1989 and 1990 (26). These damages in the horse brain and the pigs lung appeared to be due to the alteration of vascular function and endothelial cell permeability provoked by disruption of sphingolipid metabolism (27). On the other hand, fumonisin B_1 can decrease myocardial contractility in pigs, which is thought to be mediated by increased sphingosine inhibiting the L-type calcium channels in the myocardium (28) and that is why this mycotoxin can subsequently complicate the pulmonary edema via the induced left sided heart failure. Such a disease in myocardial contractility in humans was observed in 1980 by Dr. George Campbell, who practiced in rural hospitals in South Africa and observed a similar disease in different patients, which he called Idiopathic Congestive

Cardiopathy (ICC) - weakness of the heart due to influx of much blood in it. Only elderly people, whose main diet were made from home produced maize and who drank a lot of home made beer have been sicked, which clearly suggests that the main reason for this disease is probably fumonisin B_1, in addition to some other fusarium mycotoxins or provoking factors (3,29).

Zearalenone is another *Fusarium* mycotoxin, contaminated the moldy maize that has been found to possess oestrogenic activity in animals and to provoke swelling of the vulva and mammary glands, infertility, vulvovaginitis, vaginal and/or rectal prolapse in swine (3,29-32) (Figure 1). We should have in mind that hyperestrogenism (vulvovaginitis, prolapse of the rectum and vagina, etc.), due to the high content of zearalenone in feed usually occurred after a longer period of exposure to moulded feed, whereas the first sign of fusariotoxicosis caused by *Fusarium graminearum* and *Fusarium culmorum* are usually associated with cytotoxic (gastrointestinal changes and degenerative lesions in internal organs), neurotropic (paresis of hind limbs), emetic, and immunosuppressive (secondary bacterial infections) effect ofdeoxynivalenol (DON) (1-3,29).

Another fusariotoxicosis is this one caused by *F. sporotrichiella* and *F. poae*, but less attention is also given to *F. tricinctum* and *F. cladosporium.* The same fungi produce high levels of T-2 toxin and DAS (Diacetoxyscirpenol), which have highly cytotoxic, immunosuppressive and genotoxic activity, accumulate in the body and are toxic in all animals and people (1-3,29). Intoxication usually occurs after feeding on feed made from grain, and hay or straw, wintering in the open. Poisoning in humans is also known as alimentary toxic aleukia (33). Toxins have a local irritant effect and cause serous-haemorrhagic inflammation, necrosis and ulceration in the digestive tract, dystrophy in liver, kidney, heart, brain and peripheral ganglia of the vegetative nervous system leading to some spasms and tremors of some muscles, and sometimes paresis of hind limbs. Damages are seen also in the blood vessel wall and a hemorrhagic diathesis is provoked (1-3).

Stachybotryotoxicosis caused by the fungus *Stachybotrys alternans (Stachybotrys altra),* which easily develops on moist substrates (hay, straw, oats) rich in cellulose and forms a black coating, is another problematic mycotoxicosis among animals provoked by several mycotoxins as satratoxins, verrucarins and roridins. The same mycotoxins can initially provoke only a local irritation in the mouth and nasal areas or swelling of the lips and fissures of the mouth, but they usually cumulate in the body, damage the vessels (leading to hemorrhages and edematous changes) and haematopoietic tissue (leading to leukopenia and thrombocytopenia), and also provoke degenerative changes in all internal organs as well secondary (trophic) deep

symmetrical areactive necroses and ulcers in the mouth and digestive tract (1-3,34-35) (Figure 1). Fungus remains outside the substrate (soil) for long time, which explains the stationarity of this mycotoxicosis.

It is also important to emphasize that the main concern for human health in regard to aflatoxins, ochratoxin A and fumonisins in developed countries appeared to be their carcinogenic or genotoxic and teratogenic effects rather than their acute effects (36-37) (Figure 3). It is important to mention, that fumonisin B_1 was first discovered in connection with oesophageal cancer in South Africa (38) and was also shown to be a liver carcinogen in rats (39) and nephrotoxin (40-41), and recently the same mycotoxin is suspected to be involved in human and animal nephropathies in Bulgaria and South Africa) together with ochratoxin A and Penicillic acid, as recently proved (4-5).

This time there a lack of sound evidences for the definitive relationship between mycotoxins and current human diseases in developing countries, which however does not necessarily imply that dietary exposure does not represent a real risk for the men. In these countries, many individuals are also chronically exposed to high levels of mycotoxins in their diet. Various epidemiological studies revealed a correlation between the level of fumonisin B_1 in corn, the amount of corn consumed in the diet, and the rate of oesophageal cancer (38,42,43) and therefore this mycotoxin has been clearly associated with human oesophageal cancer in some countries as China and South Africa. It was now proved that highest level of oesophageal cancer in South Africa (38,42,44) and China (45) occurred among those populations consuming the largest amount of corn with the highest level of fumonisin B_1 content. It is now alarming that pregnant women who are exposed to high levels of fumonisins in their diet have a high risk to bear a child with a birth defect of the brain or spinal cord. The last studies clearly suggest that maternal ingestion of high levels of fumonisin B_1 among women during early pregnancy may increase the risk of neural tube defects (NTDs) such as brain and spinal cord defects (46-47)

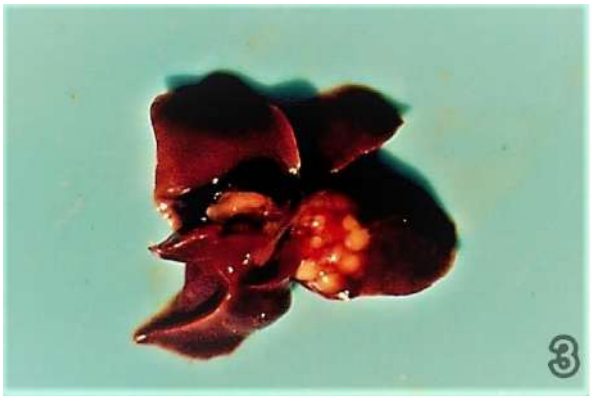

Figure 3. Carcinoma in the liver in a mouse at the end of the second year of exposure to 10 ppm OTA and 50-60 ppm PA (37)

Figure 4. Colon of pig fed on diet containing 1 ppm OTA for 47 days and sick from Dysenteria suum. Necrotic accretions on the mucosal surface) and haemorrhagic content in the lumen of colon (red arrow). (Pig experiment in BG) (54)

Lots of mycotoxins are known to have many different adverse effects as cytotoxic, genotoxic, immunotoxic, carcinogenic or teratogenic effects on animals and/or humans. Trichothecenes are reported to be immunotoxins (48). Aflatoxins are not only hepatotoxins in animals or humans (49), but they are also carcinogens, genotoxins, immunotoxins suppressing both cellular and humoral response and cause growth retardation in animals (50-52). Ochratoxins (mainly ochratoxin A) and fumonisins are proven nephrotoxins (1-5), immunotoxins (53-55), genotoxins (50,56) and carcinogens (36-37) (Figure 3).

It is important to emphasize that some experiments focused attention on the immunosuppression as the first expressed toxic effect of ochratoxin A, which may become evident clinically before nephropathy and the other biochemical changes. For the first time, a high susceptibility to natural infectious disease and/or secondary bacterial infections has been demonstrated in pigs fed on ochratoxin A contaminated diet in levels often encountered in the field (54). The reported suppression of humoral and cellular immunity provoked by this mycotoxin, defined in principle (57), has been demonstrated in practice via natural development of secondary bacterial infections in pigs at only 1 ppm ochratoxin A in diet. It seems that humoral immunity has been affected to the extent of allowing development of clinical disease in pigs at only 1 ppm ochratoxin A in diet (54) (Figure 4).

It was found that the repetitive exposures to the trichothecenes T-2 toxin can increase susceptibility to *Mycobacterium bovis, Salmonella typhimurium, Lysteria monocytogenes* and *Staphylococcus aureus* infections in animals or chickens (58-61).

Some other authors observed a much heavier progression of *Pasteurella multocida* (62) or porcine reproductive and respiratory syndrome (PRRS) virus infections in swine (63-65) compromised by feed intake of fumonisin B_1. This proinflammatory effect of fumonisin B_1 (66) is probably due to the reduction of phagocytic activity of pulmonary macrophages (62) and the increased permeability of pulmonary capillaries (67). The immunosuppressive effect of fumonisin B_1 is supposed to be due to the accumulation of free sphingoid bases, which inhibits the proliferation of lymphocytes (66). It has been reported, that fumonisin B_1 given at diet levels of 10 ppm also possesses a significant immunosuppressive effect on humoral immune response in pigs, which was manifested with lower antibody titers at days 21 and 35 after immunization against the Morbus Aujesky (66).

Ochratoxin A, which possesses a strong suppressive effect on both cellular and humoral immune response in animals/chicks, has also been found to increase the susceptibility of chickens to coccidiosis (55, 68, 69), salmonellosis (70-72) and colibacillosis (73). The increased mortality among chicks/pigs fed on moldy diet containing ochratoxin A is probably due to the increased susceptibility to secondary bacterial enteric disease (54) or to a complicated and heavy progression of some often encountered parasitic diseases (55,69) or microbial infections (58,74).

Risk assessment, hazard for animals or humans and possible hygiene control

The incidence of molds and mycotoxins occurrence can be decreased by the application of various preventative measures both before and after harvest including, for example, appropriate control measures, timely harvesting, cleanup, drying and storage practices, creating of plant cultures resistant to fungi infestation, management of insect infestation, crop rotation, and others (3).

The first limits for mycotoxins were set in the late 1960s for the aflatoxins. By the end of 2003, approximately 100 countries had developed specific limits for mycotoxins in foodstuffs and feedstuffs, and the number continues to grow. Such maximum permissible limits have been set for some mycotoxins by National or International organisations in EU or in some particular countries all over the world as World Health Organisation (WHO), Joint FAO/WHO Expert

Committee on Food Additives (JECFA), EU Scientific Committee for Food (SCF) and some others.

The European Community has introduced maximum permissible limits for aflatoxins, ochratoxin A, patulin and some other mycotoxins in specific products and is also actively involved in considering which other mycotoxins need regulation (3,75,76). It is a pity that many countries in the world have their own limits or legal regulations for the effective control of some mycotoxins in foods or feeds, but there still are not internationally recognised limits (3).

These days, in spite of all difficulties mentioned above, some efforts should be undertaken for development of internationally harmonized regulatory control measures for mycotoxins in order to protect public health and to ensure fair trade at international level. The increase of scientific reports on ochratoxin A contamination in beverages and many kinds of foods or final food products, provoked JECFA to assess all available information on the subject and to propose 112 ng/kg b.w. as a provisional tolerable weekly intake (PTWI) for ochratoxin A (77), which corresponds to about 16 ng/kg body weight per day. Subsequently, its PTWI was decreased to 100 ng/kg b.w., which corresponds to about 14 ng/kg body weight per day (78). In this particular case, JECFA bases its calculation of the PTWI mainly on the nephrotoxic effect of this mycotoxin and does not address the question of the toxin's carcenogenic effect. On the other hand, Kuiper-Goodman and Scott, takes into account that the carcinogenic effect is the most important effect and therefore base their analysis on this effect, and find that Tolerable Daily Intakes (TDIs) in such cases range from 0,2 to 4,2 ng/kg b.w, depending on the method used. With a big anxiety we have to warn the people, that the calculated average daily intakes of humans in the endemic for Balkan Endemic Nephropathy (BEN) areas in Bulgaria from 26.8 ng/kg b.w. for 1988, 36.4 ng/kg b.w. for 1989 and 34.2 ng/kg b.w. for 1990 respectively (18) exceeds strongly the TDI of 14 ng/kg b. w., proposed by JECFA (78). Moreover, it can be seen clearly that the average daily intake in humans from the same endemic areas in Bulgaria exceeds strongly the both TDIs (18) calculated on the base of cancerogenic effect (0.2 to 4.2 ng/kg b.w) or even on the base of nephrotoxic effect (14 ng/kg b. w.) of ochratoxin A (79).

When considering the ochratoxin A contamination of various animal products, we can conclude that the current measures undertaken in some European countries are also very inappropriate or irrelevant (18,20). For example in Denmark, there are regulations, according to which all “enlarged and/or mottled kidneys” are investigated for residues of ochratoxin A at slaughter time and all carcasses, whose kidneys contained ochratoxin A levels above 10 µg/kg are subsequently condemned (75). Such regulations are not very clever or safe or even they are

not satisfactory, because macrscopic changes in pig kidneys can be found only after 1-3 months ochratoxin A exposure via the feeds (15,80). Therefore, in spite of such toxicological investigations of “enlarged and/or mottled kidneys” ochratoxin A contaminated pork may enter easily the human food chain and thus represents a real public health hazard (2,14,18,20) for humans in these countries.

Having in mind the well based assumption as well as some recent proves (18,20) that mycotoxins and especially ochratoxin A are involved in aetiology of BEN in humans, the possible exposure of humans to this very hazardous toxin from pork or chicken meat (by the way "feed – pork/chicken - food") should be effectively prevented. In this regard, a much better and clever procedure for preventing any dangerous exposure of humans to this hazardous mycotoxin from meat would be a toxicological analysis of a few blood samples of pigs/chicken from risky farms having nephropathy problems or suspected of mycotoxic nephropathy only several weeks (in pigs) or several days (in chicken) before slaughter and a change in the feed source for a week (pigs) or for 2-3 days (chicken), if it is necessary. Another possible measure would be the prolongation of the period of feed deprivation of pigs/chicken before slaughter (18,20). The short half-life of ochratoxin A in pigs (72 - 120 hours) and especially in chickens (4 hours) (57) leads to a quick decrease of its concentration in blood and various tissues after changing the feed source or after prolonging the period of feed deprivation before slaughtering. In such a way, the loss of condemnation of pig/chicken production would be prevented and a much better procedure (than toxicological invesigations of "enlarged mottled kidneys" as accepted in Denmark) would be realized for preventing the human exposure to ochratoxin A from meat. The possible preventive measures in already slaughtered chicks should include condemnation and removing of the kidneys and liver, where ochratoxin A is accumulated, whereas in already slaughtered pigs only the kidneys should be condemned and/or removed (18,20).

Mycotoxin interaction and uderestimated hazard of joint mycotoxin exposure for animals and humans

With a big concern we have to pay attention to the circumstance that vrious mixtures of mycotoxins usually have at least an additive, if not synergistic toxic effect (3,18,20) and the same are often produced by the same fungus. For example, a potent synergistic effect was established between ochratoxin A and penicillic acid that are produced by the same ochratoxinogenic fungi, when the same mycotoxins were given simultaneously to pigs or chickens (80-83). It seems that the presence of multiple toxins in various foods and food

products presents a new important concern and challenge since toxicological information on the effects of simultaneous exposure is still very scarce and limited (4,5,18,20). It is expected that in a diverse human diet, exposure will be obviously to multiple mycotoxins at a low concentration on an intermittent rate over long periods of time (18,20). Unfortunately, at present time, the ultimate effect of such a continuos exposure is still unknown or very limited, although there is some evidence of strong synergistic or additive effect between some mycotoxins as ochratoxin A, penicillic acid, fumonisin B_1 and citrinin (4,5,67) found simultaneously in some feeds in farms with nephropathy problems (4,5) or between various couple of mycotoxins as ochratoxin A and Penicillic acid (80-83) or between ochratoxin A and fumonisin B_1 (67,84), etc. Now, we are almost sure that simultaneous exposure to those mycotoxins even in low contamination levels might be an important factor for development of chronic renal diseases in animals and humans, especially after long-term exposure as the same mycotoxins were recently found in high- (fumonisin B_1 and penicillic acid) or low contamination levels (ochratoxin A and citrinin) in most of the feeds originated from farms with mycotoxic porcine or avian nephropathy in Bulgaria and South Africa (4,5). Therefore, these days, it is of a particular importance to introduce new regulations and limits in regard to combined contamination of food by several mycotoxins having in mind their possible interaction and increased hazard for humans as well. Obviously, simultaneous analysis of food for co-occurence of target mycotoxins should be initiated world-wide and the real hazard of such co-occurrence for human health should be carefully assessed. Moreover, new critical limits at critical control points should be introduced by HACCP system in EU and in all over the world having in mind the interaction of target mycotoxins co-contaminated various foods and food products.

It is obvious that human exposure to mycotoxins cannot be completely prevented, because they are natural contaminants. Target mycotoxins such as aflatoxin B_1, zearalenone and ochratoxin A are much more dangerous and would represent safety hazard twice, because of their transmission in milk of lactating cows of either parent toxin or toxic metabolites as aflatoxin M_1 or α zearalenol (85) as well as because of the transmission in eggs or meat as ochratoxin A (85). It becomes more and more clear that national programmes for the monitoring, prevention and control of mycotoxin contamination based on the assessment and analysis of the situation in each separate country are not enough and helpful now. Therefore, an implementation of carefully designed surveillance studies and modern internationally recognized biomonitoring methods measuring the exposure to mycotoxins of individuals should be introduced world-wide for profound evaluation of the factors which are compromising the

quality of the products of the commodity system and leading to the production of molds and mycotoxins.

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Investigation of Neuroprotective Effect of Kefir in Experimental Spinal Cord Injury

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Abstract: In this study, the antioxidant, anti-inflammatory and neuroprotective effects of kefir were investigated in spinal cord injury that was experimentally created on rats with compression trauma model. A total of 56 Wistar-Albino male rats were used in the study. Rats were divided into seven groups with 8 rats in each group. The animals were given freshly prepared kefir 18 ml/kg/day orally for 7 days before the trauma and during the trauma. Spinal cord injury was created according to the weight drop method. While animals were under general anesthesia on the 1st and 7th days before euthanasia, intracardiac blood was collected for analysis and then they were sacrificed. After sacrification, tissue sections were taken from the damaged spinal cord segment for tissue analysis. The samples were examined biochemically, immunohistochemically and histopathologically. When compared to the sham groups, kefir had a positive effect in preconditioning and treatment groups by decreasing spinal cord bleeding, edema, myelin sheath damage, liquefactive necrosis, neuronal necrosis, selectivity of canalis centralis and gitter cell levels significantly. When compared to the sham groups, kefir was found to have a positive effect in treatment groups by decreasing the neuron specific enolase (NSE), ionized calcium binding adapter molecule 1 (IBA-1), inducible nitric oxide synthase (INOS), cyclooxygenase 2 (COX-2) and myelin basic protein (MBP) levels significantly on the 1st and 7th days, and by increasing the glial fibrillary acidic protein (GFAP) level significantly.As a result, it was demonstrated that kefir had a protective and therapeutic effect on spinal cord injury.

Keywords: Kefir, neuroprotective agents, spinal cord injuries

Deneysel Spinal Kord Hasarında Kefirin Nöroprotektif Etkisinin Araştırılması

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** *Bu çalışma»18.M.021 nolu» proje ile Hatay MKÜ BAP tarafından desteklenmiştir.*

Özet: Bu çalışmada ratlarda deneysel olarak kompresyon travma modeliyle oluşturulan spinal kord hasarında kefirin antioksidan, antiinflamatuvar ve nöroprotektif etkileri araştırıldı. Çalışmada toplam 56 adet Wistar-Albino ırkı erkek rat kullanıldı. Ratlar her grupta 8 adet olmak üzere yedi gruba ayrıldı. Hayvanlara travma öncesi 7 gün süreyle ve travma süresince taze olarak hazırlanmış 18 ml/kg/gün oranında kefir oral yolla verildi. Spinal kord hasarı ağırlık düşürme metoduna göre oluşturuldu. Hayvanlar ötenazi öncesi 1. ve 7. günlerde genel anestezi altında iken analizler için intrakardiyak kanları alındı ve sonra sakrifiye edildi. Sakrifikasyon işleminden sonra, doku analizleri için hasarlı spinal kord segmentinden doku kesitleri alındı. Alınan numuneler biyokimyasal, immunohistokimyasal ve histopatolojik olarak incelendi. Kefir sham grubları ile karşılaştırıldığında, ön koşullama ve tedavi gruplarında spinal kordda kanama, ödem, miyelin kılıf hasarı, likefaksiyon nekrozu, nöronlarda nekroz, kanalis sentralisin seçilebilirliği, gitter hücre düzeylerini anlamlı düzeyde azaltarak olumlu etki gösterdi. Kefir sham grupları ile karşılaştırıldığında, tedavi gruplarında spinal kordda 1. ve 7. günde NSE, IBA, INOS, COX2 ve MBP düzeylerini anlamlı düzeyde azaltarak, GFAP düzeyini ise arttırarak olumlu etki gösterdiği tespit edilmiştir. Sonuç olarak; kefirin spinal kord hasarında koruyucu ve tedavi edici etkisi ortaya konmuştur.

Anahtar kelimeler: Kefir, nöroprotektif etki, spinal kord hasarı

Introduction

Acute spinal injuries have increased recently with the rapid growth of the industry, transportation and construction industry [1]. Spinal cord injuries (SCI) could lead to complete and permanent loss of neurological function [2,3]. Despite the advancement of current drug and surgical techniques, there is no surgical technique or therapeutic agent that would provide a complete recovery in cases with spinal cord injury [4,5]. Therefore, researchers are still looking for new medical treatments for the treatment of spinal injuries [1]. Spinal cord injury has two mechanisms, which are primary and secondary. Primary injury refers to mechanical damage; and secondary injury refers to the progressive cell damage that occurs after the trauma [6,7]. In most previous studies, spinal cord injury mechanism was believed to be a secondary necrosis due to ischemia and cellular hypoxia [8,9]. It was demonstrated that the free oxygen radicals, lipid peroxidation and apoptosis played an important role in the development of secondary injury in the physiopathological studies on spinal cord injuries [4,10]. In their study, Hou et al. reported that primary injury was followed by excitotoxicity, edema, inflammation, demyelination and glial scar [10].

Neurotrophins are intracellular factors that are important for neural plasticity. Brain-Derived Neurotrophic Factor (BDNF) is considered as an important protein that affects brain function as well as the peripheral nervous system. It plays an important role in neuronal activity and growth, serves as a neurotransmitter modulator and participates in neural plasticity. In addition to preventing the cell deaths, BDNF affects several functions, which enable the growth of new neurons (neurogenesis) and synapses, and support the cognitive function. In addition, it demonstrates a neuroprotective effect under adverse conditions such as cerebral ischemia, hypoglycemia, neurotoxicity and glutamatergic stimulation by supporting neuronal differentiation, maturation and survival in the nervous system [11-14]. Low levels of BDNF could be directly related to Alzheimer's disease, accelerated aging, poor neural development, neurotransmitter system dysfunction, obesity, depression and even schizophrenia [15].

Kefir has a polysaccharide structure with whitish-yellowish color. It resembles cauliflower and has a sourish taste. It owes its strong antioxidant characteristic to the high amount of lactic acid bacteria it involves [16,17]. In addition, kefir is reported to have antifungal [17], anti-inflammatory [18-20], antibacterial [21], antitumoral [22], immunological [23], cholesterol-lowering [20, 24, 25] and antiapoptotic [21, 24] effects [26].

Spinal cord (medulla spinalis) injury leads to important social and economic problems and there is no clear solution regarding its definitive treatment. Today, there are medicines available for use; however, the research still continues due to the fact that their effectiveness has not been fully revealed and they have serious side effects. Many studies have been carried out with the aim of ensuring that individuals, who cannot continue their active lives and have lost their workforces as a result of the injury, return to social life again. In addition, Guven et al. emphasized that ultrastructural studies are needed to develop kefir as a promising therapeutic agent to be used in spinal cord injury [27].

This study, which was designed based on previous experimental studies on the use of this neuroprotective effect for therapeutic purposes in cases with neural tissue damage such as trauma and ischemia, was planned to investigate the antioxidant, anti-inflammatory and neuroprotective effects of kefir in spinal cord injury created in rats through the experimental trauma model. The is a preconditioned study, in which kefir was evaluated in terms of its protective effect on the injured spinal cord tissue and its therapeutic during the period after exposure to trauma.

Material and Method

Approval was obtained for this study with the decision of HMKU, Animal Experiments Local Ethics Committee (HADYEK) 2017/10-2. In this study, 56 healthy male Wistar-Albino rats that were 10-12 weeks old and weighed 300-400g were used. The animals were placed in individual cages and divided into seven equal groups with 8 animals in each group. Experimental applications were carried out in line with the conditions for the care and use of laboratory animals (12 hours light; 12 hours darkness and 24±3°C, individual cages). During the experimental applications, rats were fed on commercial rat food (pellet food) including 22.5% HP, 2750 Kkal/kg and tap water ad libitum.

The animals with spinal cord injury were given 18 ml/kg/day freshly prepared kefir orally. Kefir grains were washed with distilled water and inoculated in UHT (Ultra High Temperature) whole milk. After each preparation of the beverage, the grains were filtered by sieving the fermented milk and washed again for later use. When the grains were not used, they were maintained in milk at 4°C. Kefir was prepared by adding 5% kefir grains to sterile milk by fermentation at 25°C for 24 hours.

Animals: Group I; (Control group). Group II; (Sham-A group) trauma was created only and they were sacrificed on the 1st day. **Group III; (Sham-B group)** trauma was created only

and they were sacrificed on the 7th day. **Group IV; (Preconditioning A)** they were given 18ml/kg/day PO kefir for 7 days before the trauma, and they were sacrificed one day after the trauma was created. **Group V; (Preconditioning B)** It was the preconditioning group which was given 18ml/kg/day PO kefir for 7 days before the trauma, continued to be fed on 18ml/kg/day PO kefir for 7 days after the trauma and were sacrificed on the 7th day. **Group VI; (Treatment A)** They were given 18ml/kg/day PO kefir after the trauma and were sacrificed on the 1st day. **Group VII; (Treatment B)** They were given given 18ml/kg/day PO kefir for 7 days after the trauma and were sacrificed on the 7th day. The animals were determined to be healthy during the clinical examinations performed before the trauma. Their neurological examinations were evaluated through Modified Tarlov Scale and finger opening tests. After trauma, neurological examinations of the related groups were repeated on the 1st, 3rd and 7th days. According to the group protocols, intracardiac blood was collected for analysis under anesthesia on day 1 or after 7 days, and tissue samples were collected from the trauma region in the spinal cord after sacrifice in the preconditioning groups.

Creation of spinal cord damage: In all surgical groups, 10 mg/kg Xylazine hydrochloride intraperitoneal (ip) (Alfazyne 2% injection 50 mL, EGE-VET, Turkey) and 50 mg/kg ketamine hydrochloride (ip) (Alfamine 10% injection 50 mL EGE-VET, Turkey) were given for general anesthesia. After the anesthetized rats were identified in the sternal position, the back part was shaved and antisepsis was provided with povidone-iodine.

Paravertebral muscles were reached after crossing the skin and subcutaneous tissues with a two-cm incision at the T5-T12 level with reference to the interscapular distance. The paravertebral muscles were dissected and the vertebral laminae were reached through the spinous process. The spinal cord was exposed at the T7-T10 level by total laminectomy preserving dura mater integrity.

Spinal cord injury was created using the weight drop method by dropping a 10g metal bar with 3mm diameter from a height of 10 cm (100 g/cm). Rats were made paraplegic. Following hemostasis, paravertebral muscles and skin were sutured in accordance with their anatomical layers. While animals were under general anesthesia on the 1st and 7th days before euthanasia, intracardiac blood was collected for analysis and then they were sacrificed. After sacrification, sections were taken from the proximal and distal of the trauma for tissue analysis. The samples were examined biochemically, immunohistochemically and histopathologically.

Neurological examination

In the study, neurological examinations were performed before the sacrification in the groups 2, 4 and 6, which underwent surgical procedure and were sacrificed on the first day; and they were performed on the 1st, 3rd and 7th days in groups 1, 3, 5 and 7, which underwent control and surgical procedure and were sacrificed on the seventh day.

In the neurological examination, Modified Tarlov Scale and finger opening tests were performed.

Modified Tarlov Scale (MTS) was used for clinical motor examination [28] (Table I).

Finger opening test: The rat was lifted from its back and its back extremities were suspended. Opening of the fingers was observed and the reflexes were classified as follows: 0: fingers not opened; 1: fingers slightly opened; 2: fingers fully opened [28].

Biochemical Analyses

At the end of the experiment, intracardiac blood was collected under deep anesthesia and the animals are sacrificed. Later, plasma was extracted from the serous fluid and stored in the freezer (-24 0C). In the samples, BDNF (Brain Derived Neurotrophic Factor) levels were calculated by ELISA using commercial kits.

Histopathological and Immunohistochemical Assessments

Macroscopic changes that could occur in the spinal cord after the experimental trauma were noted and photographed. The spinal cord extracted was placed in 10% buffered formalin for pathological examination. For histopathological examinations, the tissues were exposed to the xylol series according to routine methods and then embedded in paraffin. The sections obtained with a thickness of 4 μm were stained with Hematoxylin Eosin (H&E).

Avidin-Biotin Complex Peroxidase (ABC-P) method was applied to tissue sections prepared from the same tissue blocks used in histopathological examination and taken into adhesive slides for immunohistochemical examination. For this purpose, Mouse specific HRP (ABC) (Abcam, ab128971) kit was used and the recommended procedure was applied in the kit. Endogenous peroxidase activity was eliminated by maintaining in 3% H_2O_2 methanol for 30 minutes. Depending on the type of antibodies, either Proteinase K (Abcam, ab64220) or temperature was applied as antigen retrieval (Table III). Sections were incubated with the blocking serum of the kit at 37˚C for 10 minutes to prevent non-specific antigenic binding. Ionized calcium binding adapter molecule 1 (IBA-1), inducible nitric oxide synthase (INOS),

cyclooxygenase 2 (COX-2), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE) and Myelin basic protein (MBP) antibodies were used in order to identify the lesions that could occur in the spinal cord. The brand, dilution rate, incubation time and incubation temperature for each section are presented in Table IV. This was followed by treatment steps with biotinized serum (goat serum, at 37˚C for15 min) and streptavidin peroxidase (at 37˚C for 20 min). The AEC (RED) Substrate kit (Zymed Laboratories inc., Cat. No: 00-2007) was used as the chromogene. Sections, which were stained in Harris hematoxylin for 3-5 minutes and washed in tap water, were covered with coverslip with the aid of glycerin (water-based) adhesive (Vision Mount, Lab Vision Corporation, Ref: TA-060-UG). All steps were carried out in a moist camera environment, preventing the sections from drying out. Phosphat Buffer Saline (PBS, pH 7.4) was used in the washes. After all the microscopic results obtained were examined under a light microscope, their microphotographs were taken.

Statistical Analysis

Statistical analyzes were performed using SPSS (version 17) software. Data were given as mean ± standard error (SE). The differences between the groups were analyzed with the One Way ANOVA Bonferroni Test. Statistical significance was interpreted according to $p<0.05$ level.

Results

Neurological examination findings

Modified Tarlov Test and finger opening test were performed while evaluating the neurological examination (Day 1 was evaluated as acute; days 3 and 7 were evaluated as subacute).

In terms of Modified Tarlov Scale values, significant differences were found between control groups and, the sham groups, preconditioning groups and treatment groups on the 1st day ($p<0.05$). In the neurological examination performed on the first day, the Modified Tarlov values in the preconditioning groups were higher compared to the sham and treatment groups. Modified Tarlov values on the 3rd and 7th days in the preconditioning (group 5) and treatment (group 7) groups were higher compared to the sham (group 3) value. Modified Tarlov values in the treatment group were higher compared to the preconditioning groups on the 3rd and 7th days (Table II). Looking at the Modified Tarlov Scale values, it can be argued that kefir provides clinical improvement.

In terms of finger opening tests, significant differences were found between control groups and, the sham, preconditioning and treatment groups on the 1st day ($p<0.005$). On the first day, finger opening test values in preconditioning groups were higher compared to the sham and treatment groups. The fact that the finger-opening test values in the preconditioning group were

higher compared to the sham and treatment groups on the 1st day showed that preconditioning played a protective role in preventing peracute injury. On the 7th day, finger opening test values in preconditioning (group 5) and treatment (group 7) groups were found to be higher compared to the sham (group 3) value (Table II). Looking at the finger opening test values, it can be argued that kefir provides a clinically moderate benefit in both the peracute and subacute periods.

Histopathological Results

The cells in the anterior horn of the spinal cord were examined for damage. For this, the cells that have lost their eosinophilic cytoplasm and nucleus were considered dead neurons due to ischemic damage. Cells with cytoplasmic nissl bodies, thin chromatin and prominent nucleolus were considered alive. Histopathological findings observed were classified as none (0), mild (1), moderate (2) and severe (3).

Group I; control group: The spinal cord had normal gray matter and white matter structure (Figure 1a). No morphological changes were observed. **Group II;** (Sham-A): There was prevalent edema, hemorrhage in both gray and white matter, vacuolization in myelin sheaths and neuronal necrosis; and the structure of neurons lined with ependymal cells were observed to be damaged. Few amount of microglia cells were noted (Figure 1b). **Group III;** (Sham-B): They were similar to the Group 2 with more severe morphological changes in the damage zone (Figure 1c). In addition, intensive gitter cells were noted in this group (Figure 1d). **Group IV** (Preconditioning A): There was localized edema, hemorrhage, vacuolization in myelin sheaths and neuronal necrosis; and the structure of neurons lined with ependymal cells were observed to be damaged. **Group V** (Preconditioning B): There was no hemorrhage, liquefactive necrosis and gliosis; however, there was edema, hemorrhage, damage and vacuolization in myelin sheaths and neuronal necrosis; and the damage in structure of neurons lined with ependymal cells were observed to decrease (Figure 2a and Figure 2b). **Group VI** (Treatment A): Moderate edema, hemorrhage, vacuolization in myelin sheaths and neuronal necrosis were also present in this group. It was observed that while the structure of the canal where ependymal cells were lined was preserved in some parts, it was damaged in some other parts (Figure 2c). **Group VII** (Treatment B): The edema, hemorrhage, vacuolization in myelin sheaths and neuronal necrosis were milder compared to Group VI. It was observed that while the structure of the canal where ependymal cells were lined was preserved in some parts, it was damaged in some other parts (Figure 2d).

Histopathological changes shaped in the spinal cord are summarized in Table IV.

When compared to the sham groups, it was observed that kefir had positive effects in preconditioning and treatment groups by significantly reducing spinal cord bleeding, edema, myelin sheath damage, liquefactive necrosis, neuronal necrosis, selectivity of canalis centralis and gliosis/gitter cell levels ($p<0.05$-0.005, Table IV). On the other hand, kefir demonstrated a therapeutic effect on the 7th day by reducing liquefactive necrosis and gliosis/gitter cell levels more than the first day in the preconditioning and treatment groups ($p<0.005$, Table IV).

Immunohistochemical Results

While immunoreactivity was not observed against GFAP, NSE, INOS and IBA-1 antibodies in the control group (Figure 3a); COX-2 positive staining was observed in neurons in the central nervous system that was not damaged as well as MBP positive staining in the form of cords in the myelin sheaths.

GFAP positive fibrous astrocytes attracted attention most intensely in the 5th and 7th groups, as a result of the increase in the severity of the lesion and the repair in the astrocytes in parallel with the increase in the experimental period in the groups where experimental spinal cord injury was created (Figure 3b). Weaker immunoreactivity was observed in Groups 3, 4, and 6, respectively; whereas immunosuppressive staining was not observed in Group 2.

The strongest immunoreactivity against the antibody used to demonstrate the COX-2 protein levels increasing after the spinal cord injury in astrocytes and microglia was observed in Groups 3, 2, 6, 4, 7 and 5, respectively. In addition, immunoreactivity was noted in non-damaged neuron cytoplasms (Figure 3c).

The strongest staining against the NSE antibody used to determine neuronal damage was observed in groups 3, 2, 6 and 4, respectively (Figure 3d). While there was moderate immune reactivity related to neuronal damage in group 7 compared to other groups and group 5, there was a significant decrease in the number of immune positive cells in group 5.

Immunohistochemical staining was performed for the presence of myelin basic protein (MBP) to determine the destruction of myelin sheaths. The strongest staining was observed particularly in the areas with severe damage. In the myelin sheaths with granular and irregular appearance among the healthy mylelin sheaths in the form of cords, the strongest immunoreactivity was observed in the animals that belonged to the 3rd and 2nd groups, respectively (Figure 4a). Immunoreactivity, which demonstrated the myelin sheaths in organized form or in the form of

cords as a result of minimal myelin disintegration based on decrease in the damage, was also noted in groups 5, 7, 4 and 6, respectively (Figure 4b).

IBA-1 and INOS positive cells were observed in the microglia cells around the vacuolizations in the damage area in groups 3, 2, 4, 6, 7 and 5, respectively, ranging from the minimal to mild according to the severity of immunoreactivity (Figures 4c, 4d, 4e, 4f). INOS immunoreactivity was observed in some blood vessel endothelial among these microglial cells (Groups 3, 2, 4.6, respectively).

Immunohistochemical changes in the spinal cord were graded as none (0), mild (1), moderate (2) and severe (3); and they are presented in Table V.

Compared to sham groups, it was found that kefir had positive effects in preconditioning groups by decreasing NSE, IBA-1, INOS, COX-2 and unorganized-MBP levels significantly on the 1st and 7th days, and increasing the GFAP level ($p<0.05$-0.005).

Compared to sham groups, it was found that kefir demonstrated a positive effect in treatment groups by decreasing the levels of NSE, IBA-1, INOS, COX-2 and MBP significantly on the 1st and 7th day and increasing the GFAP level ($p<0.05$-0.005, Table V). On the other hand, kefir had a therapeutic effect by decreasing the levels of NSE, IBA-1, INOS, COX-2 and MBP more than day 1 on the 7th day in the preconditioning and treatment groups ($p<0.005$, Table V).

Serum BDNF findings

When compared to the control group, Serum BDNF levels decreased significantly in Sham A-B, Preconditioning A-B and Treatment A-B groups. When compared to Sham and B groups, kefir increased BDNF levels in preconditioning and treatment groups on the 1st and 7th days. Compared to the Sham A group, kefir increased BDNF levels on the 1st day more than the 7th day in preconditioning and treatment groups ($p>0.05$, Table VI).

Discussion

Pathological injury mechanisms after SCI are mostly focused on primary and secondary injuries. Primary trauma of the spinal cord leads to irreversible primary injury [29,30]. In contrast, primary injury cascade reactions lead to reversible secondary injury with more serious levels of injury compared to primary injuries [31]. Acute spinal cord injury could result in severe central nervous system damage as well as motor and sensory dysfunction; and it has a high rate of disability [1]. Despite the importance of motor dysfunction repair in SCI patients [32], basic mechanisms have not been demonstrated yet and more basic research is required. In

the study, the values in the preconditioning group were found to be higher compared to sham and treatment groups in the neurological evaluation with the Modified Tarlov Scale. In terms of the finger opening test, the differences between Preconditioning B and Treatment A groups were significant in the neurological evaluation on the 1st day ($p<0.05$). Many studies have reported that antioxidants could delay the progression of neurodegeneration [33]. Zhang et al. applied compression from the T8 level to the spinal cord in rats and demonstrated a locomotor healing in the Tarlov Scale with combined anti-inflammatory therapy [46]. In our study, it can be argued that the Modified Tarlov Scale (MTS) values in the preconditioning group were higher than those in the other groups; and kefir contributed to the clinical improvement after trauma. Based on these findings, it can be argued that the preconditioned application of kefir provides clinical benefits. While there was a decrease in the finger opening test values after the trauma, an increase was observed in the groups that were fed on kefir. Similar to the findings of our study, Şirin et al. reported that there was a significant decrease in finger opening test values after spinal cord injury [7].

BDNF mainly promotes the survival and regeneration of neurons; and it has been defined in many brain regions including bulbus olfactorius, cortex, hippocampus, basal forebrain, mesencephalon, hypothalamus, brainstem, and spinal cord [34]. Endogenous BDNF originates from Schwann cells and muscle tissues; and it plays an important role in the remyelination of the axon. After SCI, the requirement for BDNF is increased [35,36]. Liang et al. reported that over-expression of BDNF reduced in vitro SCI inflammation by the induction of TrkB and pp-38 protein expression. In addition, inhibition of TrkB enhances the anti-inflammatory effects of BDNF on TrkB and pp-38 protein expression by inhibiting the effects of BDNF to reduce inflammation in the in vitro SCI model [37]. BDNF levels decrease in many neurodegenerative diseases such as Parkinson's disease (PD) [38], multiple sclerosis (MS) [39] and Huntington's disease [40]. Therefore, we believe that BDNF could be useful in identifying spinal cord injury and in monitoring the post-treatment process and prognosis. When compared to the control group, Serum BDNF levels decreased significantly in Sham A-B, Preconditioning A-B and Treatment A-B groups. When compared to Sham and B groups, kefir increased BDNF levels in preconditioning and treatment groups on the 1st and 7th days. When compared to the Sham A group, kefir demonstrated a therapeutic effect by increasing the BDNF levels in the preconditioning and treatment groups more on the 1st day compared to the 7th day ($p>0.05$).

Spinal cord injury always initiates an inflammatory response characterized by the infiltration of leukocytes and the synthesis of cytokines and chemokines. This excessive inflammation

induced by spinal cord injury induces degeneration of neurons and apoptosis of oligodendrocytes, causing a progressive injury. The activity of astrocytes and oligodendrocytes is observed during central nervous system repair. Astrocytes are one of the first cell populations to detect spinal cord injury; and then, they participate in modulation of inflammatory responses. Astrocytes participate in repairing damaged parts of the brain; the presence of significant increase in fibrous astrocytes during astrocytosis could be detected by strong GFAP expression in the brain tissue [41,42]. Intense astrocytosis does not occur within a short time following the spinal cord injury; and it is considered as a chronic process. Similarly, myelin damage occurs immediately at the time of the injury; however, it does not start to appear morphologically within the few days following the trauma and it becomes visible with the sustained release of the metabolites applied on the damage [43]. In the study, the GFAP and MBP expressions differed between the groups particularly in parallel with the increase in trauma and kefir application periods. However, it cannot be argued that kefir application provides full protection both in protecting myelin sheath structure and in support of astrocytosis. We attribute this to the shortness of the duration for both trauma and kefir application in order to better observe morphological lesions. In the study, spinal cord injury lesions in the application of kefir that continued for 7 days before and after the formation of damage was observed less in both neurological examinations and histopathological level compared to other groups (Figure 1-2, Table II).

Enolase protein, which is abundantly expressed in cytosol, may migrate to the cell surface as a result of the stimuli and contribute to different pathological conditions such as injury, autoimmunity, infection, inflammation and cancer. The rise of neuron-specific enolase (NSE), known to play a role in the pathogenesis of hypoxic-ischemic brain injury, has been blamed for neuronal damage following spinal cord injury. For this reason, NSE is believed to be an important marker that directly evaluates functional damage in neurons [44]. While a large number of NSE positive neurons were observed in the group that did not receive kefir after the trauma, a significant reduction in immunoreactivity was also noted despite complete protection was not provided with the inclusion of kefir ($p<0.005$).

Traumatic spinal cord injury directly causes axonal and myelin damage as well as migration of inflammatory cells to the inflamed region [43]. Miller et al. reported that focal hemorrhage and necrosis were observed at the 1st hour following the trauma, and stated that there was an increase in IBA-1 and INOS expression in macrophages and microglia in the area of damage [43]. Monocytes and microglia begin to multiply around the primary lesion after 48 hours

following the spinal cord injury [45]. Neutrophils are no longer present after 3 days. They are replaced by a large number of monocytes and microglia. In traumatic injuries, the inflammatory response, in which the activities of the leukocytes are critical in the repair of the tissue. However, the inflammatory response interrupts the healing of the wound in cases of spinal cord injury [46]. Microglia activation is a common incidence in spinal cord trauma and is claimed to cause tissue damage during elaboration of proinflammatory agents. INOS is an inducible enzyme found in macrophages and endothelial cells. INOS release is very low in the brain and often cannot be expressed. However, the production of cytokines causes INOS expression in microglia and astrocytes, resulting in continuous and high levels of nitric oxide production, which could lead to further tissue damage, especially with toxic byproducts such as peroxynitrite. Nitric oxide is also toxic to neurons and is responsible for neuronal death.

Playing a role in the rearrangement of the actin cytoskeleton, IBA-1 is a cell surface marker directly associated with microglia activation, migration and phagocytosis [47,48]. Following the spinal cord injury, INOS and IBA-1 positive staining is observed in the microglia cells and macrophages around the vacuolizations and disintegrated myelin sheaths; however, immunoreactivity was observed to decrease due to the anti-inflammatory effect of kefir.

There are two forms of the cyclooxygenase enzyme called COX-1 and COX-2. Many nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase activity; and thus, the whole prostoglandin synthesis. Therefore, they are effective in the treatment of pain and fever. In experimental acute spinal cord injury, the production of COX-2, mRNA and protein is identified to increase between 2-48 hours; and it was determined that COX-2 inhibition would contribute to the results of spinal cord injury selectively. In the central nervous system without damage, the presence of COX-2 is in the neurons was demonstrated Immunohistochemically [49]; however, it was reported that the increase in COX-2 related to the damage accelerated neuronal death and the neuroinflammatory response resulting from the production of prostaglandin E2 (PGE_2) [50,51]. It is reported that COX-2, which is normally observed in neurons, is released with the injury from astrocytes and microglia, respectively [52,53]. In this study, severe COX-2 immunoreactivity was observed in groups, in which trauma was created and kefir application was not performed. Despite complete protective effect of kefir, which is reported to have anti-inflammatory effect [18-20], was not observed, was able to provide reduction in the COX-2 release even with the application for 7 days before and after the experiment.

Conclusion

As a result, histopathological examinations concluded that kefir had a positive effect in preconditioning and treatment groups by decreasing spinal cord bleeding, edema, myelin sheath damage, liquefactive necrosis, neuronal necrosis, selectivity of canalis centralis and gitter cell levels significantly. Immunohistochemical examinations concluded that kefir had a positive effect in treatment groups by decreasing the NSE, IBA-1, INOS, COX-2 and MBP levels in the treatment groups on the 1st and 7th days and increasing the GFAP level significantly. This was supported by the increase that was observed in serum BDNF levels. In addition, no side effect or negative consequences have been reported for the consumption of kefir, which is a probiotic substance; on the contrary, it is reported to have antioxidant, anti-inflammatory, antiapoptotic, antitumoral, cholesterol-lowering and neuroprotective effects. In the present study, it can be argued that kefir reduces the negativities formed at the cellular level against the traumatic spinal cord damage; however it cannot be argued that it provides a complete protection. We believe that this may be related to the shortness of the period of kefir consumption. Therefore, there is a need for further studies, in which kefir would be applied for a longer time and in greater amounts. It is predicted that the consumption of this beverage, which is easily accessible and easy to prepare, would have protective effects in spinal cord injuries.

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Table I. Modified Tarlov Scale Score

Degree	Modified Tarlov Scale
0	Complete paralysis in the back extremities, no movement in the back extremities, no weight bearing
1	Noticeable back limb movements, no weight bearing
2	Frequent and / or strong back limb movement, pronounced posterior limb movements that do not result in weight overlay or locomotion
3	Back extremities support body weight, can take one or two steps
4	There is a slight loss in walking
5	Normal walking

Table II. Effects of Kefir on Modified Tarlov Test and Finger Opening Test in Experimental Spinal Cord Injury

Groups	**Modified Tarlov 1. day**	**Modified Tarlov 3. day**	**Modified Tarlov 7. day**	**Finger extension test 1. day**	**Finger extension test 3. day**	**Finger extension test 7. day**
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Group 1: Control A	5,00±0,00	5,00±0,00	5,00±0,00	2,00±0,00	2,00±0,00	2,00±0,00
Group 2: Sham A	1,66±0,33*	.	.	0,16±0,16*	.	.
Group 3: Sham B	1,71±0,47*	1,14±0,55*	1,42±0,57*	0,28±0,18*	0,28±0,28*	0,42±0,29*
Group 4: Preconditioning A	1,85±0,67*			0,57±0,29*		
Group 5: Preconditioning B	2,50±0,42*	1,87±0,54*	2,28±0,60*	0,87±0,22*≠	0,25±0,16*	0,85±0,34*
Group 6: Treatment A	1,25±0,55*			0,00±0,00β		
Group 7: Treatment B	2,00±0,68*	2,00±0,75*	3,14±0,55*	0,50±0,26*	0,62±0,26*	0,71±0,18*

A: Group sacrificed on day 1, B: Group sacrificed on day 7

*: When compared with the control group, the differences between the groups were found significant ($p < 0.005$).

≠: Finger extension test, on the first day, the differences between the Preconditioning Group B and the Control ($p < 0.001$) groups were found to be significant ($p < 0.001$).

β : Finger extension test, The differences between preconditioning group B and treatment group A were found to be significant on day 1 ($p<0.05$).

Table III. Reconstitution rate, incubation time, incubation temperature and antigen retrieval status of the antibodies used

Antibody	**Antigen retrieval**	**Primary antibody dilution / time / temperature**	**Chromogen**
Anti-NSE, C Terminal anibody (Sigma, SAB4500768-100UG) ®	10 minutes, Proteinöz K, 45℃	1/100 PBS 1 hour 45℃	AEC (RED) Subsrate Kit (Zymed Laboratories Inc. 00-2007)®
Anti Cox2, C-Terminal antibody (Sigma, SAB4502491-100UG)®	Sitrat tamponlu (pH 6,0, % 0,1 Tween) (3x5 dk) 700 watt'da 3x 5'er	1/200 PBS 2 hours 37℃	AEC (RED) Subsrate Kit (Zymed Laboratories Inc. 00-2007)®
Anti-GFAP antibody (Abcam, ab7260)	10 minutes, Proteinöz K, 45℃	1/100 PBS 1 hour 45℃	AEC (RED) Subsrate Kit (Zymed Laboratories Inc. 00-2007)®
Anti-Iba-1 antibody (Abcam, ab108539)	10 minutes, Proteinöz K, 45℃	1/100 PBS 15 minutes Oda sıcaklığında	AEC (RED) Subsrate Kit (Zymed Laboratories Inc. 00-2007)®
Anti-iNOS antibody (ab3523)	Sitrat tamponlu (pH 6,0, % 0,1 Tween) (3x5 dk) 700 watt'da 3x5'er dk	1/100 PBS + 4℃ 1 gece	AEC (RED) Subsrate Kit (Zymed Laboratories Inc. 00-2007)®

Table IV. Histopathological changes in the spinal cord

Groups	Haemorrhage	Edema	Myelin Sheath Damage	Liquefaction Necrosis	Necrosis in Neurons	Canalis Sentralis Not Selected	Gliosis-Gitter Cells
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
1:Control	0,00±0,00	0,00±0,00	0,12±0,12	0,00±0,00	0,00±0,00	0,12±0,12	0,00±0,00
2:Sham A	2,00±0,18	1,75±0,16	1,87±0,22	2,00±0,18	1,62±0,18	2,25±0,16	0,62±0,18
3:Sham B	2,25±0,25	2,25±0,16	2,75±0,16	2,62±0,26	2,87±0,12	2,87±0,12	2,50±0,37
4:Preconditioning A	1,75±0,16	1,75±0,25	1,50±0,26 β	1,75±0,31 ≠	0,87±0,22 *, β	2,25±0,16	0,25±0,16
5:Preconditioning B	0,25±0,16 €, β	1,00±0,00 *, β	1,00±0,00 *, β	0,37±0,18 €, β	1,00±0,00 β	1,00±0,00 €, β	0,00±0,00 β
6:Treatment A	1,50±0,26	2,00±0,18	2,25±0,16	1,87±0,12	1,75±0,16 β	1,12±0,35 €, β	0,25±0,16 β
7:Treatment B	1,12±0,22 β	1,00±0,00 *, β	1,00±0,00 *, β	0,00±0,00 €, β	0,75±0,16 €, β	0,50±0,26 €, β	0,12±0,12 β

A: First day, B: Seventh day

Compared with the Sham A group: *:$P<0.05$; € : $p<0.005$

Compared with the Sham B group: ≠: $p<0.05$, β : $p<0.005$

Compared with the Preconditioning A group: α: $p<0.05$, ¥ : $p<0.005$

Compared with the Preconditioning B group: $: $p<0.05$, &: $p<0.005$

Table V. Immunohistochemical changes in the spinal cord

Groups	**GFAP**	**NSE**	**IBA**	**iNOS**	**COX2**	**MBP organized**	**MBP unorganized**
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
1: Control	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	3,00±0,00 α	3,00±0,00	0,00±0,00
2:Sham A	0,00±0,00	2,75±0,16	1,25±0,16	1,00±0,18	2,00±0,00	2,00±0,18	2,12±0,12
3:Sham B	0,62±0,18	3,00±0,00	1,75±0,16	1,87±0,12 *	2,75±0,16 €	1,12±0,12€	1,87±0,12
4:Preconditioning A	0,62±0,18 *	1,62±0,18 €,β	0,87±0,22 ≠	0,75±0,16 β	1,62±0,18 B	0,75±0,16€	1,12±0,12 €,β
5: Preconditioning B	2,00±0,00 €,β	0,50±0,18 €,β	0,37±0,18 *, β	0,37±0,18 β	0,87±0,12 €,B	2,00±0,00β	0,50±0,18 €,β
6:Treatment A	0,50±0,18	2,00±0,00 €,β	0,62±0,26 β	0,62±0,26 β	1,75±0,16 B	0,62±0,18€	1,00±0,00 €,β
7:Treatment B	2,00±0,00 €,β	1,00±0,00 €,β	0,50±0,18 β	0,50±0,18 β	1,00±0,00 €,B	0,87±0,12€	0,87±0,12 €,β

A: First day, B: Seventh day, α : Immunoreactivity in neurons

Compared with the Sham A group: *:$P<0.05$; € : $p<0.005$

Compared with the Sham B group: ≠: $p<0.05$, β : $p<0.005$

Table VI. Effects of Kefir on Serum BDNF (ng / ml) in Experimental Spinal Cord Injury

Groups	Group 1: Control	Group 2: Sham A (1.day)	Group 3: Sham B (7.day)	Group 4: Preconditioning A (1.day)	Group 5: Preconditioning I (7.day)	Group 6: Treatment A (1.day)	Group 7: Treatment B (7.day)
Mean	63,94	12,06	31,14	30,96	33,41	26,09	36,58
SE	±19,93	± 5,51	±14,32	±11,10	±16,57	±10,77	±13,84

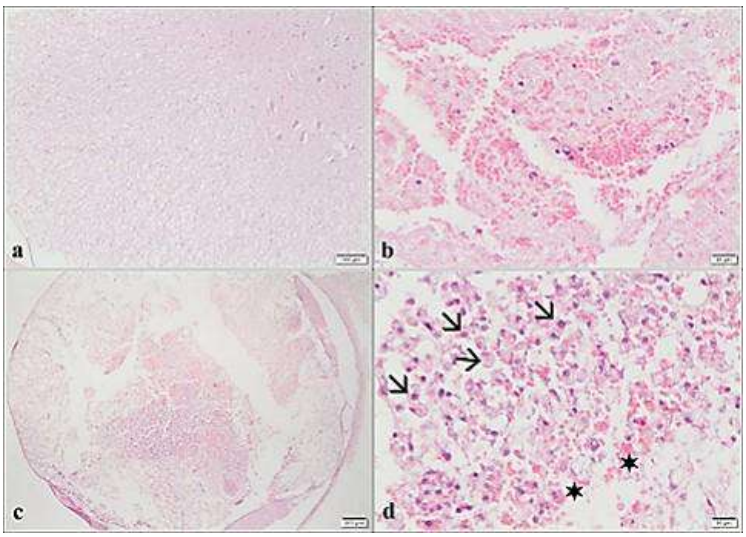

Figure 1. Histopathological changes in the medulla spinal of control and sham groups **a.** Medulla spinalis normal gray matter and white matter structure in the control group, HE. **b.** Hemorrhage and microglial cells in both gray and white matter in Sham A group, HE. **c.** In the Sham B group, large necrosis sites in the transversal section, vacuolization in myelin sheaths, and the failure to observe structure of the channel lined with ependymal cells, HE. **D.** In the group of Sham B intensely gitter cells (arrows) and hemorrhage (stars), HE.

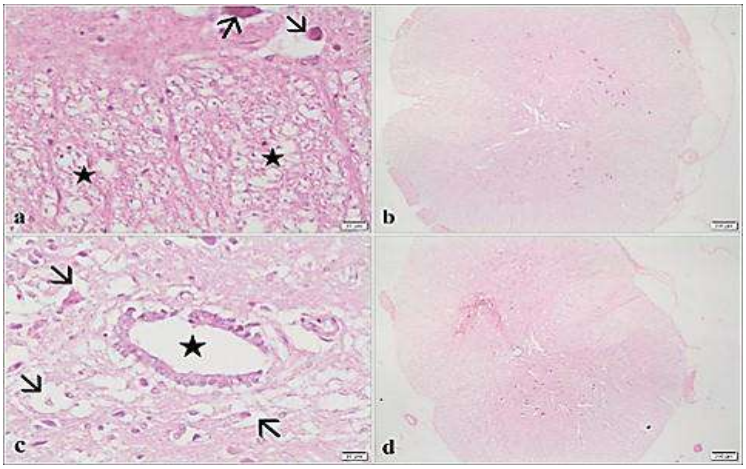

Figure 2. Histopathological changes in the medulla spinal of the preconditioning and treatment groups **a.** In Preconditioning B group, basophilic necrotic neurons (arrows) with loss of nuclei, damage and vacuolization (stars) in myelinated sheaths, HE. **b.** In Preconditioning B group, the canalis centralis structure is preserved, HE. **c.** In Treatment A group, pericular edema (arrows) and condition of the canalis centralis (star), HE. **D.** The appearance of the spinal cord in the Treatment B group, HE.

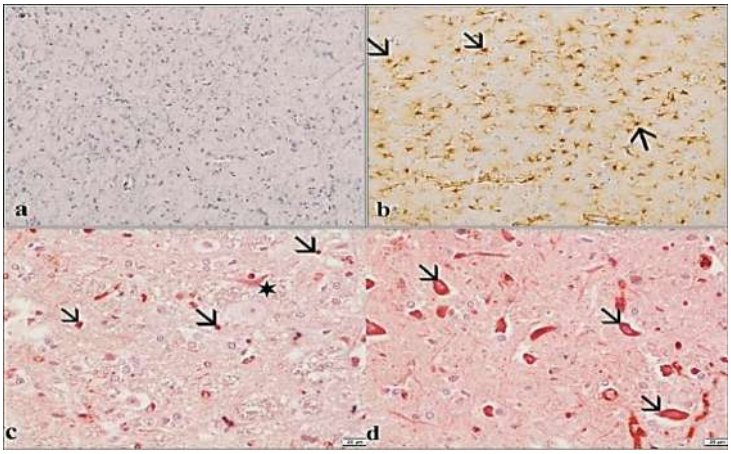

Figure 3. Immunohistochemical findings for GFAP, COX-2 and NSE antibodies **a.** Medulla spinalis GFAP negative, DAB, ABC in control group. **b.** In Preconditioning B group medulla spinalis GFAP positive astrocytes (arrows), DAB, ABC. **c.** COX-2 positive microglia (arrows) and mild positive neuron cytoplasm in medulla spinalis in Sham A group, AEC, ABC. d. In Sham A group, NSE positive neurons (arrows) in medulla spinalis, AEC, ABC.

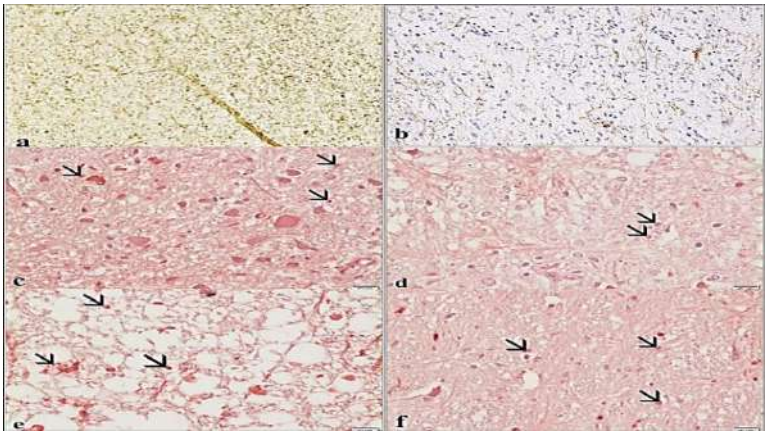

Figure 4. Immunohistochemical findings regarding MBP, IBA-1 and INOS antibodies **a.** In Sham B group, medulla spinalis MBP positive myelin sheaths, DAB, ABC. **b.** IN Preconditioning A group, MBP weak positive myelin sheaths in medulla spinalis, DAB, ABC. **c.** In Sham A group, IBA-1 positive microglia (arrows) in medulla spinalis, AEC, ABC. **d.** In treatment A group, IBA-1 weak positive microglia (arrows) in medulla spinalis, AEC, ABC. **e.** In Sham B group, medulla spinalis INOS positive microglia (arrows), AEC, ABC. **f.** In Treatment B group, medulla spinalis INOS weak positive microglia (arrows), AEC, ABC.

Methods of Inducing Allergic Contact Dermatitis with 2,4-dinitrofluorobenzene in BALB/c Mice

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Summary

Atopic dermatitis is a chronic inflammatory skin disease manifested by a rash and severe itching. Although the pathophysiological mechanism of the disease is not fully understood, overactivation of immune cells, including macrophages, mast cells and T lymphocytes, is considered to be the main cause. Contact dermatitis is an inflammatory skin disease with both proinflammatory and antigenic properties induced by the skin's exposure to low molecular weight chemicals. Allergic contact dermatitis is the result of an increased T cell response to haptens reaching the skin. The haptens that come into contact with the skin are taken up by immature skin dendritic cells and migrate to the paracortical area of the lymph nodes. Here, they prepare hapten-specific T cells as a result of the presentation of hapten-protein complexes on major histocompatibility complex molecules. Studies have shown that 2,4-dinitrofluorobenzene, a powerful hapten in mice, is mediated by CD8 + cytotoxic T cells in allergic contact dermatitis and is downregulated by CD4 + T cells. Repeated topical application of 2,4-dinitroflurobenzene at a concentration of 0.15% dissolved in acetone in mice has been reported to induce marked infiltration of neutrophils and eosinophils, and eczematous changes of the skin, including hypertrophy of the epidermis, causing persistent itching. The use of mouse models in atopic dermatitis is a guide for a better understanding of the pathogenesis. The purpose of this review is to bring together the various methods in the literature for inducing allergic contact dermatitis in BALB/c mice with 2,4-dinitrofluorobenzene.

Keywords; atopic dermatitis, allergic contact dermatitis, 2,4-dinitrofluorobenzene, BALB/c.

BALB/c Farelerinde 2,4-dinitroflorobenzen ile Alerjik Kontakt Dermatit Oluşturma Yöntemleri

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Özet

Atopik dermatit, döküntü ve şiddetli kaşıntı ile kendini gösteren iltihaplı kronik bir deri hastalığıdır. Hastalığın patofizyolojik mekanizması tam olarak anlaşılmasa da makrofajlar, mast hücreleri ve T lenfositler dahil olmak üzere bağışıklık hücrelerinin aşırı aktivasyonu ana neden olarak kabul edilmektedir. Kontakt dermatit, cildin düşük moleküler ağırlıklı kimyasallara maruz kalmasıyla indüklenen, hem proinflamatuar hem de antijenik özelliklere sahip, yangısal bir deri hastalığıdır. Alerjik kontakt dermatit, cilde ulaşan haptenlere karşı artan T hücresi tepkisinin sonucudur. Deriyle teması olan haptenler, olgunlaşmamış deri dendritik hücreleri tarafından alınırlar ve lenf düğümlerinin parakortikal alanlarına göç ederler. Burada hapten-protein komplekslerinin majör histo-uyumluluk kompleks molekülleri üzerinde sunumu sonucu hapten-spesifik T hücrelerini hazırlanmaktadırlar. Yapılan çalışmalarda, farelerde güçlü bir hapten olan 2,4-dinitroflorobenzen, alerjik kontakt dermatitte CD8 + sitotoksik T hücrelerinin aracılık ettiğini ve CD4 + T hücreleri tarafından down-regülasyon gösterilmiştir. Farelerde, aseton içinde çözdürülmüş %0,15 konsantrasyonda 2,4-dinitroflorobenzenin tekrarlanan topikal uygulamasının, nötrofil ve eozinofillerde belirgin infiltrasyona ve epidermisin hipertrofisi dahil olmak üzere deride egzematöz değişiklikleri indüklediğini ve kalıcı kaşınmaya neden olduğu bildirilmiştir. Atopik dermatitte fare modellerinin kullanılması patogenezin daha iyi anlaşılması için yol gösterici niteliktedir. Bu derlemenin amacı 2,4-dinitroflorobenzen ile BALB/c farelerinde alerjik kontakt dermatit oluşturulmasının literatürdeki çeşitli yöntemlerini bir araya getirmektir.

Anahtar Kelimeler; atopik dermatit, alerjik kontakt dermatit, 2,4-dinitroflorobenzen, BALB/c.

Atopik Dermatit

Atopik dermatit, döküntü ve şiddetli kaşıntı ile kendini gösteren kronik iltihaplı bir deri hastalığıdır. Günümüzde çocuklarda % 10-20 ve yetişkinlerde % 1-3 olan hastalığın prevalansı artmaktadır. Hastalığın patofizyolojik mekanizması tam olarak anlaşılmasa da makrofajlar, mast hücreleri ve T lenfositler dahil olmak üzere bağışıklık hücrelerinin aşırı aktivasyonu ana

neden olarak kabul edilmektedir. Hastalığın evresine bağlı olarak, hem Th2 hem de Th1 hücrelerin atopik dermatitin immünogenezine katıldığı öne sürülmüştür (1).

Kontakt Dermatit ve Alerjik Kontak Dermatit

Kontakt dermatit, cildin düşük moleküler ağırlıklı kimyasallara maruz kalmasıyla indüklenen, hem proinflamatuar hem de antijenik özelliklere sahip, yangısel bir cilt hastalığıdır. Haptenler, proinflamatuar özellikleri sayesinde tahriş edici kontakt dermatit olarak da bilinen antijene özgü olmayan bir cilt iltihabı oluşturmaktadır. Haptenlerin cilt ile temasından sonra, deri hücrelerinde aktivasyon meydana gelir ve bu da bir dizi enflamatuar sitokinlerin (IL-1b, IL-6 ve TNF-a dahil) ve kemokinlerin (CCL20 dahil) hızlı üretimine neden olmaktadır (2).

Haptenler, etkili bağışıklık için peptitlere bağlanması gereken, kimyasal yapı açısından oldukça heterojen, oldukça reaktif küçük lipofilik kimyasallardır. Alerjik kontakt dermatit (AKD), haptenlerin ciltle temas etmesi sonucunda T hücrelerinde meydana gelen artışın bağlı oluşmaktadır (3). Hassaslaştırma aşamasında, cilde nüfuz eden haptenler, spesifik T hücresi öncüllerini aktive etmek ve klonal olarak genişletmek için bölgesel lenf düğümlerine göç eden dendritik hücreler tarafından toplanmaktadır (3,2). İlgili haptene yeniden maruz kalmak, alerjik kontakt dermatitin efferent fazını ve klinik ekspresyonunu başlatmaktadır (3).

Ayrıca haptenler, alerjik kontakt dermatiti (AKD) indüklemek için kendi kendine modifiye edilmiş proteinler oluşturarak immünojenik hale gelmektedir. Temas duyarlılığı olarak da bilinen AKD, lenf düğümlerinde hazırlanan ve reaksiyonun afferent ve efferent fazı sırasında deride toplanan hapten-spesifik T hücrelerinin aracılık ettiği gecikmiş tip bir hiperduyarlılık reaksiyonu olarak ileri sürülmüştür. Deriyle teması olan haptenler, olgunlaşmamış deri dendritik hücreleri tarafından alınır ve lenf düğümlerinin parakortikal alanına göç etmektedir. Burada hapten-protein komplekslerinin majör histo-uyumluluk kompleks molekülleri üzerinde sunumu sonucu hapten-spesifik T hücrelerini hazırlamaktadırlar. Aynı hapten uygulamasının tekrarlanması, ciltte keratinosit apoptozunun indüksiyonu ile inflamasyonu başlatarak, ciltteki efektör T hücrelerinin hızlı bir şekilde toplanmasına yol açmaktadır (2).

2,4-Dinitroflorobenzen

Yapılan çalışmalarda, farelerde güçlü bir hapten olan 2,4-dinitroflorobenzene, alerjik kontakt dermatitte CD8 + sitotoksik T hücrelerinin aracılık ettiğini ve CD4 + T hücreleri tarafından aşağı doğru düzenlendiği gösterilmiştir (2). Farelerde, aseton içinde çözdürülmüş % 0.15 konsantrasyonda 2,4-dinitroflurobenzenin tekrarlanan topikal uygulamasının, nötrofil ve

eozinofillerde belirgin infiltrasyona ve epidermisin hipertrofisi dahil olmak üzere deride egzematöz değişiklikleri indüklediğini ve kalıcı kaşınmaya neden olduğu bildirilmiştir (4). DNFB uygulanmasından bir veya iki hafta sonra duyarlı hale getirilmiş farelerde IFNg, TNF-α, IL-2 gibi Th1 sitokinlerinin yükselmesinin sonucu bir Th1 yanıtı görülmektedir (5).

Fare Modelleri

Atopik dermatitte fare modellerinin kullanılması patogenezin daha iyi anlaşılması için yol gösterici niteliktedir (6).

Farelerde alerjik dermatit modeli üç gruba ayrılabilmektedir.

Spontan modeller; 8 haftadan büyük NC/Nga farelerinde normal koşullarda klinik olarak yüz, kulaklar, burun, boyun ve sırt derisinde çeşitli derecelerde dermatit gözlenmiştir. Geleneksel NC/Nga farelerinde spontane olarak şekillenen dermatitin insan AD'sine benzer klinik ve histolojik özelliklere sahip olduğu görülmüş ve muhtemelen bazı çevresel faktörler tarafından tetiklendiği gösterilmiştir (7).

Transgenik modeller; Atopik dermatit (AD) insidansı ile bağlantılı olan filaggrini (FLG) kodlayan gende bir işlev kaybı mutasyonu sonucu oluşan bariyer anormalliği, AD'nin patogenezinde önemli bir faktör olarak bildirilmiştir. Bariyer işlevinde genetik bir kusura (FLG) sahip fareler, AD modeli sağlamaktadır. Flaky tail (Flg (ft)) fareler, filaggrin eksikliği olan ve filaggrinin AD üzerindeki rolünü araştırmak için tanıtılmıştır. Bu fareler, deride artan toplam IgE ve Th17 ekspresyonu ile uyumlu egzamatöz deri lezyonu göstermiştir. Flg (ft) farelerde akar alerjeninin uygulanması, önceden bariyer bozulması olmaksızın hem klinik belirtileri hem de laboratuvar bulgularını önemli ölçüde arttırdığı görülmüştür (8).

İndüklenmiş modeller; Daha kolay erişilebilen laboratuar hayvanlarını kullanarak basit ve tekrarlanabilir bir hayvan modeli olarak geliştirilmiştir. Hapten ile maruz bırakılmış farelerde antijen spesifik IgE antikoru üretilmiş, aynı zamanda ani tip aşırı duyarlılık reaksiyonu gözlenmiştir. Bu basit ve tekrarlanabilir model, AD'nin tedavisi için potansiyel olarak terapötik ajanların değerlendirilmesinde önemli görülmüştür. Bu modeli olası tedavilerin denemelerinde diğer modellere göre kullanmanın en büyük avantajı, tekrarlanabilirliğinin yanı sıra kulak kalınlığını ölçerek nicel değerlendirmenin kolaylığıdır (9).

Yöntemler

10 haftalık dişi BALB/c farelerinin kullanıldığı bir çalışmada, 2,4-dinitroflurobenzen (DNFB), aseton ve zeytinyağı (4:1 oranında) ile hazırlanan çözeltide çözdürülmüş. Farelerin tıraş edilmiş

sırt bölgelerine 100 µl %0,5 DNFB 1 hafta boyunca topikal olarak uygulanmaktadır. Altı gün sonra, kulaklarının iç ve dış yüzeylerine 20 µl % 0.2 DNFB uygulanarak alerjik kontakt dermatit şekillendirilmiştir (10).

Yedi haftalık dişi BALB/c farelerinin kullanıldığı bir çalışmada, fareler deneyden bir hafta önce SPF koşulları altında tutulmuş. 21 ± 2 ◦C sıcaklıkta ve % 50-70 nemde 12 saat aydınlık / karanlık döngüsünde, klimalı bir hayvan odasında barındırılmıştır. 2,4-dinitroflorobenzen (DNFB) 4:1 oranında aseton ve zeytinyağı ile %0.2 ve %0.5 konsantrasyonlarda hazırlanmış. Fareler, iki gün boyunca günde bir kez 30 ul %0,5 DNFB uygulanarak duyarlı hale getirilmiştir. Duyarlılaşmadan sonraki 5. günde, farelerin sırt derisine 30 ul %0.2 DNFB yüklenmiştir. Daha sonra, farelerin sırt derisine 3 gün aralıklarla 3 kez tekrar tekrar 30 u1 %0,2 DNFB uygulanarak alerjik kontakt dermatit şekillendirilmiştir (5).

Sekiz haftalık dişi BALB/c fareleri yapılan bir çalışmada, fareler SPF koşulları altında tutulmuş. Hayvanlar, 23±2 ° C sabit sıcaklık ve % 40±5 bağıl neme sahip klimalı bir odada barındırılmıştır. Standart bir diyet ve su sağlanmıştır. DNFB, aseton ve zeytinyağı (4: 1) karışımı içinde çözdürülmüş. Duyarlılık için ilk hafta her gün farelerin tıraşlanmış sırtlarına 100 uL % 0.5 DNFB topikal uygulanmış ve AD benzeri deri lezyonlar şekillendirilmeye başlanmıştır. İlk haftadan sonra, 4 hafta daha haftada iki kez 100 uL % 0,2 DNFB uygulanmıştır. Lezyonlar 5. haftanın sonunda gelişmiş. 6. haftada iltihaplanmayı sürdürmek için DNFB bir kez uygulanmıştır (11,12).

Başka bir çalışmada sekiz haftalık dişi BALB/c fareleri patojen içermeyen koşullar altında tutulmuş. Hayvanlar, 22 ± 1 °C'de ve %55 ± %10 bağıl nemde barındırılmıştır. DNFB ile duyarlılaştırma için, aseton içinde çözündürülmüş 100 mL % 0,15 DNFB, BALB/c farelerinin tıraşlanmış abdominal derisine topikal olarak uygulanmıştır. Bir hafta sonra, tıraş edilmiş sırt derisi, her üç günde bir dört kez tekrarlanan 50 mL % 0,15 DNFB ile muamele edilmiştir. Tıraşlanmış sırt derisine aynı hacimde aseton uygulanmış ve alerjik kontakt dermatit oluşturulmuştur (13).

Altı haftalık dişi BALB/c fareleriyle yapılan başka bir çalışmada fareler, 12 saatlik aydınlık/karanlık döngüsünde ve 25 ± 2°C'de tutulmuştur. Farelerin, tıraşlanmış abdominal derisine aseton ve zeytinyağı (4: 1) içinde seyreltilmiş 50 uL %0,5 DNFB uygulanarak 0. ve 1. günlerde duyarlı hale getirilmiş. Farelerin daha sonra 5. ve 12. günlerde her iki kulağın arka tarafına 20 uL %0,15 DNFB uygulanmıştır. Farelerin kulak kalınlığı yumuşak dokunuşlu bir mikrometre ile ölçülmüştür (14).

Sonuç

Hayvan modelleri atopik dermatitin etiyopatogenezi üzerine yapılan araştırmalar için yararlı olmaktadır. Hayvan modelleri ile yapılan çalışmalar, immünolojik değişikliklerin (Th2 bağışıklık tepkileri ve artan serum IgE seviyeleri) deri lezyonları ile ilişkili olduğunu göstermektedir. NC/Nga farelerinin kendiliğinden AD benzeri lezyonlar geliştirmesi AD çalışmaları için bir yarar sağlasa da, kolay bulunmaması ve pahalı olması yararlılığını sınırlamaktadır. Daha kolay bulunan, basit ve tekrarlanabilir hayvan modelleri geliştirilmiştir. İndüklenmiş modellerin, AD'nin tedavisi için terapötik ajanların değerlendirilmesinde önemli görülmüştür. Bu modellerin üretilebilirliği ve kulak kalınlığının ölçülmesiyle nicel değerlendirme sağlaması tedavi denemelerinde diğer modellere göre daha avantajlı olduğunu göstermektedir. AD benzeri lezyonların kısa sürede şekillenmesi ise bir başka avantaj olarak düşünülmektedir (9).

Hapten veya fare türünün değiştirilmesi ile AD'nin klinik belirtilerindeki heterojenliği yansıtan çeşitli kronik inflamasyon türleri indüklenebilir. Dolayısıyla, AD'nin klinik belirtilerindeki değişkenlik, farklı haptenler ve fare türleri kullanılarak yeniden üretilebilmektedir (9).

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The Role of Chemokine (TARC / CCL17) Regulated by Thymus Activation in Atopic Dermatitis

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Summary

Atopic dermatitis (AD), also known as atopic eczema, is a chronic and inflammatory disease of the skin. It affects 10-20% of children and 3% of adults. Atopic dermatitis is the most common allergic skin disease. The pathogenesis of AD is complex and still not fully elucidated. Cytokines are small proteins secreted by cells. It has a special effect on the interaction and communication between cells. Cytokines can act on cells that secrete them (autocrine effect), nearby cells (paracrine effect) or in some cases distant cells (endocrine effect). Cytokines include lymphokines, monokines, interleukins, interferons, growth factors, and chemokines. Chemokines (chemotactic cytokines) are low molecular weight proteins that regulate the migration of leukocytes. Infiltration of inflammatory cells into tissues is regulated by chemokines. Many chemokine and chemokine receptors play a role in the pathogenesis of AD. The complex chemokine network affects the formation of inflammatory infiltrates and cell trafficking in the skin, including T lymphocytes, dendritic cells, eosinophils, histiocytes and mast cells. Chemokines and their receptors play an important role in AD by regulating the initiation and exacerbation of inflammation in response to allergens. The chemokine (TARC) / CCL17 regulated by thymus activation is constitutively expressed in the thymus and produced by dendritic cells, endothelial cells, keratinocytes and fibroblasts. The thymus activation-regulated chemokine (TARC / CCL17) is a member of the CC chemokine family and a potent and selective chemoattractant for Th2 cells via the CC chemokine receptor 4 (CCR4). Th2-type cells are thought to play an important role in the pathogenesis of atopic dermatitis, especially in the acute phase. Initially, immunity is controlled by Th2 cells and the release of cytokines such as IL-4, IL-5, IL-6, IL-13 and IL-31 are stimulated. Th2 cytokines support humoral immunity and IgE production, which are characteristic of atopic diseases. In addition, acute itching and inflammation are provided by cytokines produced by Th2 lymphocytes and cytokines thought to mediate activation of Th2 lymphocytes. TARC is overproduced in the skin of patients with atopic dermatitis and is thought to attract circulating Th2 cells to the skin. In patients with AD, the stratum corneum TARC (scTARC) was determined using the stripping

method and immunostaining technique, and scTARC was found to be associated with the severity of local skin lesions. ScTARC shows that it can be used as an indicator of the status of local skin lesions in patients with AD, especially in the acute phase of inflammation. The purpose of this review is to discuss the immunological and diagnostic aspects of TARC / CCL17, which is a reliable biomarker in atopic dermatitis.

Keywords; atopic dermatitis, chemokine, TARC / CCL17).

Atopik Dermatitte Timus Aktivasyonu ile Düzenlenen Kemokinin (TARC/CCL17) Rolü

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Özet

Atopik egzama olarak da bilinen atopik dermatit (AD), cildin kronik ve iltihaplı bir hastalığıdır. Çocukların %10-20'sini ve yetişkinlerin %3'ünü etkilemektedir. Atopik dermatit, en yaygın alerjik deri hastalığıdır. AD'nin patogenezi karmaşıktır ve hala tam olarak aydınlatılamamıştır. Sitokinler, hücreler tarafından salgılanan küçük proteinlerdir. Hücreler arasındaki etkileşim ve iletişimde özel bir etkiye sahiptir. Sitokinler, onları salgılayan hücrelere (otokrin etkisi), yakındaki hücrelere (parakrin etkisi) veya bazı durumlarda uzak hücrelere (endokrin etkisi) etki edebilmektedir. Sitokinler arasında lenfokinler, monokinler, interlökinler, interferonlar, büyüme faktörleri ve kemokinler bulunmaktadır. Kemokinler (kemotaktik sitokinler), lökositlerin göçünü düzenleyen, düşük moleküler ağırlıklı proteinlerdir. İnflamatuar hücrelerin dokulara sızması kemokinler tarafından düzenlenmektedir. AD'nin patogenezinde birçok kemokin ve kemokin reseptörü rol oynamaktadır. Karmaşık kemokin ağı, T lenfositleri, dendritik hücreleri, eozinofilleri, histiyositleri ve mast hücrelerini içeren enflamatuar infiltratın oluşumunu ve deride ki hücre trafiğini etkilemektedir. Kemokinler ve reseptörleri, alerjenlere yanıt olarak inflamasyonun başlamasını ve şiddetlenmesini düzenleyerek AD'de önemli rol oynamaktadır. Timus aktivasyonu ile düzenlenen kemokin (TARC)/CCL17, timusta yapısal olarak eksprese edilmekte ve dendritik hücreler, endotelyal hücreler, keratinositler ve fibroblastlar tarafından üretilmektedir. Timus aktivasyonu ile düzenlenen kemokin (TARC/CCL17), CC kemokin ailesinin bir üyesi ve CC kemokin reseptörü 4 (CCR4) yoluyla Th2 hücreleri için güçlü ve seçici bir kemoatraktandır. Th2 tipi hücrelerin, atopik dermatitin patogenezinde özellikle akut fazda önemli rol oynadığı düşünülmektedir. Başlangıçta

bağışıklık, Th2 hücreleri tarafından kontrol edilir ve IL-4, IL-5, IL-6, IL-13 ve IL-31 gibi sitokinlerin salınımını uyarılır. Th2 sitokinleri, atopik hastalıkların karakteristiği olan humoral bağışıklığı ve IgE üretimini desteklemektedir. Ayrıca akut kaşıntı ve yangılanma, Th2 lenfositleri tarafından üretilen sitokinler ve Th2 lenfositlerinin aktivasyonuna aracılık ettiği düşünülen sitokinlerle sağlanmaktadır. TARC, atopik dermatitli hastaların derisinde aşırı üretilmekte ve dolaşımdaki Th2 hücrelerini deriye çektiği düşünülmektedir. AD'li hastalarda stratum corneum TARC (scTARC) bant sıyırma yöntemi ile immün boyama tekniği kullanılarak belirlenmiş ve scTARC'ın lokal deri lezyonlarının ciddiyeti ile ilişkili olduğu bulunmuştur. ScTARC, AD'li hastalarda özellikle inflamasyonun akut fazında lokal deri lezyonlarının durumunun bir göstergesi olarak kullanılabileceğini göstermektedir. Bu derlemenin amacı atopik dermatitte güvenilir bir biyobelirteç olan TARC/CCL17'ın immünolojik ve tanısal yönlerini ele almaktır.

Anahtar Kelimeler; atopik dermatit, kemokin, TARC/CCL17).

Atopik Dermatit

Atopik egzama olarak da bilinen atopik dermatit (AD), cildin kronik, tekrarlayan iltihaplı bir hastalığıdır. Çocukların % 10-20'sini ve yetişkinlerin % 3'ünü etkiler. Atopik dermatit, en yaygın alerjik deri hastalığıdır. AD'nin patogenezi karmaşıktır ve hala tam olarak anlaşılamamıştır (1).

Köpeklerde Atopik Dermatit

Köpek atopik dermatiti (KAD), yaygın olarak çevresel alerjenlere yönelik IgE antikorları ile ilişkili karakteristik klinik özelliklere sahip, genetik olarak yatkınlık gösteren enflamatuar ve kaşıntılı bir alerjik deri hastalığı olarak tanımlanmıştır (2). Deri bariyer fonksiyonundaki anormallikler ile (3) deri lenfositlerin dermise infiltrasyonu sonucu (4), şiddetli pruritus ve karın ventralinde, koltuk altlarında veya aksillar bölgede eritem, tüy döküntüsü, hiperpigmentasyon ve likenifikasyon gibi deri lezyonları görülmektedir (5). Atopik dermatitin patogenezi karmaşıktır. Patogenezde deri bariyeri önemli bir rol oynamaktadır. Bütünlüğü bozulan derinin, mikrobik yapışmaya, alerjenik proteinlerin penetrasyonuna ve anormal enflamatuar ve alerjik yanıtların başlatılması izin verdiği düşünülmektedir (3).

T Yardımcı Hücreler

Atopik dermatitte köpeklerde, insanlar gibi, düzensiz bir bağışıklık yanıtına sahiptir. Başlangıçta bağışıklık, Th2 hücreleri tarafından kontrol edilir ve IL-4, IL-5, IL-6, IL-13 ve IL-31 gibi sitokinlerin salınımını uyarır, ancak kronik iltihaplanma şekillenmiş ise Th1, Th2, Th17, Th22 hücreli aracılar ile gerçekleştirilir. Bu hücre alt kümeleri, belirli efektör fonksiyonlara aracılık etmeye yardımcı olur ve antijen sunan hücrelere yanıt olarak gelişir (3). Th2 sitokinleri IL-4, IL-3, IL-6 ve IL-13, atopik hastalıkların karakteristiği olan humoral bağışıklığı ve IgE üretimini desteklemektedir (6). akut kaşıntı ve yangılanma, Th2 lenfositleri (örn., IL-4, IL-5, IL-10, IL-13 ve IL-31) tarafından üretilen sitokinler ve Th2 lenfositlerinin aktivasyonuna aracılık ettiği düşünülen sitokinlerle (örneğin, IL-25 ve IL-33) sağlanmaktadır (3).

Sitokinler

Sitokinler, hücreler tarafından salınan küçük salgılanan proteinlerdir ve hücreler arasındaki etkileşim ve iletişim üzerinde özel bir etkiye sahiptir. Sitokin genel bir isimdir; diğer isimler arasında lenfokin (lenfositler tarafından yapılan sitokinler), monokin (monositler tarafından yapılan sitokinler), kemokin (kemotaktik aktiviteye sahip sitokinler) ve interlökin (bir lökosit tarafından yapılan ve diğer lökositler üzerinde etkili olan sitokinler) bulunur. Sitokinler, onları salgılayan hücrelere (otokrin etkisi), yakındaki hücrelere (parakrin etkisi) veya bazı durumlarda uzak hücrelere (endokrin etkisi) etki edebilir (7).

Kemokinler

Kemokinler, proteinin amino terminalinin yakınında bulunan bir veya iki sistein kalıntısının konumuna göre dört alt aileye bölünmüş 8–14 KDa heparin bağlayıcı peptitlerin geniş bir ailesidir (8). Th2 hücrelerinin taşınmasında CC kemokinlerinin ve reseptörlerinin rol oynadığı düşünülmektedir (9). Karmaşık kemokin ağı, T lenfositleri, dendritik hücreleri, eozinofilleri, histiyositleri ve mast hücrelerini içeren enflamatuar infiltratın oluşumunu ve deride ki hücre trafiğini etkilemektedir. Kemokinler ve reseptörleri, alerjenlere yanıt olarak inflamasyonun başlamasını ve şiddetlenmesini düzenleyerek AD'de önemli rol oynamaktadır (1).

Timus Aktivasyonu ile Düzenlenen Kemokin (TARC/CCL17)

Timus aktivasyon regülasyon kemokini (TARC), CC kemokinlerinden biridir ve alerjik inflamasyon sırasında CC kemokin reseptör 4 (CCR4) ile bağlanarak Th2 hücrelerinin

taşınmasını sağlamaktadır (9). AD'li köpeklerin lezyon derisinde seçici olarak eksprese edildiği, ancak sağlıklı veya lezyonsuz deride olmadığı belirtilmiştir (5). Bu sonuçlar TARC'nin AD'de alerjik inflamasyonu başlatmak için önemli bir kemokin olabileceğini ve hastalık şiddetini değerlendirmek için güvenilir bir belirteç olarak kullanılabileceğini düşündürmektedir (9).

Histopatolojik Değerlendirmeler

Histopatolojik olarak AD, esas olarak perivasküler, subepidermal ve intraepidermal boşluklarda bulunan CD41 bellek T hücrelerinden oluşan bir enflamatuar infiltrat ile karakterize edilir. T hücrelerinin yanında, farklı dendritik hücre alt grupları, mast hücreleri ve eozinofiller, enflamatuar infiltrasyonu oluşturur (10).

Vestergaard ve arkadaşları, NC/Nga farelerinde atopik benzeri dermatit oluşturarak bir çalışma yapmışlardır. Bu çalışmada NC/Nga fareleri steroid merhem ile tedavi edildiğinde TARC ekspresyonunun lezyonel cildin iyileşmesiyle eşzamanlı olarak kaybolduğunu ve TARC'ın AD'nin patogenezinde önemli rol oynadığını ileri sürmüşlerdir (11).

Atopik dermatitli hastalardan alınan deri biyopsilerine immünohistokimyasal yöntemler uygulandığında, ağırlıklı olarak keratinositlerde ve daha az ölçüde endotel hücrelerde, dermal infiltrasyon hücrelerinde immünoreaktif TARC seviyelerinin arttığı gözlenmiştir (12).

Wakugawa ve arkadaşlarının, yaptıkları bir immünohistokimyasal çalışmada, akut eritematöz lezyonları ve kronik likenifiye lezyonları olan AD hastalarının cildindeki CCR4 ekspresyonunu incelemişler ve CCR4'ün, epidermisteki mononükleer hücrelerin çoğunda ve üst dermisteki damarların çevresinde eksprese edildiği gösterilmiştir (13).

Saeki ve Tamaki, atopik dermatit için bir fare modeli olarak kabul edilen NC/Nga farelerinde, TARC'ın lezyonlu derinin bazal epidermisinde yüksek oranda eksprese edildiğini, ancak lezyonsuz deride eksprese edilmediği göstermiştir (14).

Uchida ve arkadaşları, yaptıkları bir çalışmada, immünohistokimyasal teknikler kullanarak AD'li lezyonlu deride Th2-tipi kemokin (TARC) ve reseptörünün (CCR4) ekspresyonunu araştırmıştır. Lezyonel AD derisinde TARC ekspresyonu epidermisin bazal hücresinde, venüllerde ve ekrin bezinde tespit edilmiş, ancak AD'li hastaların lezyonsuz derisinde daha az immünreaktivite görülmüştür. Normal kontrol deneklerinin (atopik olmayan)

derisinde çok az görülmüştür. İmmünohistokimyasal analiz ile AD'li hastalarda TARC ve CCR4 ekspresyonu yukarı regüle edilir ve AD'nin hastalık şiddeti ile ilişkili olduğu düşünülmektedir. Kemokin reseptörlerinin ekspresyonu, sadece T hücre farklılaşmasının yararlı bir markörü olarak hizmet etmekle kalmayacağını, CCR4 ve CCR3 antagonistleri gibi farklı fonksiyonel alt kümelerin AD hastalarında immünoterapi için potansiyel adaylar olabileceğini düşünmüşlerdir (15).

Serum TARC seviyeleri AD'li hastalarda diğer inflamatuvar deri hastalıkları olanlara göre anlamlı ve seçici olarak daha yüksektir ve bu seviyeler AD'nin şiddeti ile orantılı olarak artmaktadır. Serum TARC seviyesinin belirlenmesi bu nedenle klinik ortamlarda AD'nin objektif bir göstergesi olarak kullanılır çünkü serum IgE seviyesi veya kan eozinofil sayısı gibi geleneksel laboratuvar ölçümlerinden daha hassas ve doğrudur. Atopik dermatit yönetimi, durumu değerlendirmek ve uygun tedaviyi reçete etmek için lokal deri lezyonlarının semptomlarını değerlendirmeyi gerektirir. Bununla birlikte, lokal semptomların ciddiyetini değerlendirmek için objektif ölçülerin kullanılabileceği bir yöntem belirlenmemiştir. Bu nedenle Morita ve arkadaşları, TARC'a özgü bir antikor ile numunelerin immün boyama işleminden sonra floresan tabanlı bir tespit yöntemi oluşturmuştur. AD'li hastalarda stratum corneum TARC (scTARC) bant sıyırma yöntemi ile immün boyama tekniği kullanılarak belirlenmiş ve scTARC'ın lokal deri lezyonlarının ciddiyeti ile ilişkili olduğu bulunmuştur. ScTARC, AD'li hastalarda özellikle inflamasyonun akut fazında lokal deri lezyonlarının durumunun bir göstergesi olarak kullanılabileceğini göstermektedir. ScTARC ayrıca AD'li hastalarda serum TARC seviyesi ile de korele bulunmuştur, bu da serum TARC'ın inflamatuar cilt lezyonlarında aşırı üretilen TARC'dan kaynaklandığını düşündürmektedir. Stratum corneum'da tespit edilen TARC'ın keratinosit orijinli olduğu düşünülmektedir, çünkü bir immüno boyama tekniği, immünoreaktif TARC'ın hem AD'li hastalarda hem de AD benzeri lezyonlar sergileyen NC/Nga farelerinde lezyonel derinin keratinositlerinde eksprese edildiğini açıkça göstermiştir (16).

Boer ve arkadaşlarının, yaptığı bir çalışmada, AD'li hastalar ile sağlıklı derideki lipid enzimleri β-glukoserebrosidaz (GBA) ve asit sfingomiyelinazın (ASM) lokalizasyonunu ve aktivitesini analiz etmişler ve GBA ve ASM de görülen değişiklikler ile dağılmış stratum corneum (SC) lipid bileşimi arasında bir korelasyon olduğunu bildirmişlerdir. AD biyobelirteci olan TARC lokalizasyonu da kontrol ile karşılaştırıldığında AD derisinden farklı olduğu görülmüştür. AD gibi inflamatuar hastalıklarda tetiklenen bir kemokin olan TARC'ın lokalizasyonu, AD'li

deride sadece canlı epidermis veya stratum granulosum/stratum cornem arayüzünde değil, SC'nin tamamında gözlemlenebilir. Bugüne kadar yapılan çalışmalarda TARC, AD teşhisi için en önemli biyobelirteçlerden biri olarak bildirmektedir. TARC'ın epidermiste görselleştirilmesi henüz sistemik değişikliklerin olmadığı durumda lokal değişiklikleri göstermektedir (17).

Tüm bunlar, AD şiddetindeki lokal değişiklikleri yorumlamak için, lokal epidermal ortamın görselleştirilmesinin, AD hastalarının klinik görünümleri arasındaki farklılıkları aydınlatmada faydalı olabileceğini göstermektedir (17).

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Tricuspid Valve Endocardiosis in a Puppy with Distemper

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ABSTRACT

Valvular endocardiosis is a disease that caused by the thickening of endocardial layer of the heart due to nonenfectious causes. The disease is more common in dogs. It is a disease that causes thickening of the atrioventricular valve and as a result dilatation in the left ventricle. One of the prominent findings of endocardiosis is severe congestive heart failure. The cause of the disease is related to valvular collagen degeneration. While endocardiosis is seen at a rate of 5% in animals younger than 1 year old, this rate increases up to 75% as the animal ages. Considering the lesions that occur in the heart valves, 57% of the cases in the mitral valve, 27% in the the mitral and tricuspidal valve, and 7.5% in the tricuspidal valve, and 6.5% in the other valves were observed. In this case; signs of congestive heart failure were detected during necropsy in an 11-month-old Kangal breed male dog who was brought to Burdur Mehmet Akif Ersoy University Clinics with complaints of melena and epistaxis and died here. There were appearances of passive congestion in parenchymatous organs and seroses of luminal organs. Severe meningeal and cortical congestion was detected in the brain. Pericardial fluid increasing was observed. When the lumen of the heart was opened, shortening and nodular thickening of the bicuspital and tricuspidal valves were seen. The changes were more severe in the tricuspital valve. There was foamy fluid in trachea and edematous appearance in the lungs. Microscopic examination revealed fibroblastic proliferations with myxomatous changes in bicuspital and tricuspital valves. In addition, there was severe demyelination in the white matter in the brain and cerebellum. Intranuclear eosinophilic inclusion bodies were found in some astrocytes and some small neurons in the granular layer of the cerebellum. A diagnosis of distemper was made according to histopathological findings. Valvular endocardiosis is observed more frequently in older animals and on the mitral valve according to the literature. Our case is very rare because of the animal was young with the lesion more severe in the tricuspid valve and furthermore distemper diagnosis was accompanied.

Keywords: Distemper, dog, tricuspid valve endocardiosis

Distemperlı Genç Bir Köpekte Triküspit Kapak Endokardiyozisi

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ÖZET

Valvuler endokardiyozis kalbin endokard katmanının nonenfeksiyöz nedenlerle kalınlaşmasıyla oluşan, sıklıkla köpeklerde görülen, atrioventriküler kapakta kalınlaşma ile dikkati çeken ve bunun sonucu olarak sol ventrikülde dilatasyon ile devam eden bir hastalıktır. Sol ventrikülün dilatasyonunun devamında sol kalp yetersizliği ve total kalp yetersizliği meydana gelir. Endokardiyozisin belirgin bulgularından biri ileri derecede konjestif kalp yetmezliği oluşturmasıdır. Hastalığın nedeni valvuler kollagende meydana gelen dejenerasyonla ilgilidir. Endokardiyozis, 1 yaşından küçük hayvanlarda %5 oranında görülmekteyken, bu oran hayvan yaşlandıkça % 75'lere kadar yükselmektedir. Kalp kapakçıklarında oluşan lezyonlarına göre bakıldığında, vakaların % 57'sinde sadece mitral kapakta, % 27'sinde mitral ve triküspidal kapakçıkta, % 7,5'unda sadece triküspidal kapakçıkta, % 6,5'unda ise diğer kapakçıklarda lezyon oluşmaktadır. Bu olguda; melena ve epistaksis şikâyetleriyle Burdur Mehmet Akif Ersoy Üniversitesi Klinikleri'ne getirilen ve burada ex olan 11 aylık kangal ırkı erkek köpekte, nekropsi sırasında konjestif kalp yetmezliği bulguları tespit edildi. Parankimatöz organlarda ve lümenli organların serozalarında pasif konjesyona ilişkin görünümler vardı. Beyinde şiddetli meningeal ve kortikal konjesyon tespit edildi. Perikardda sıvı artışı dikkati çekti. Kalbin lümeni açıldığında biküspital ve triküspidal kapakçıklarda kısalma ve noduler kalınlaşmalar görüldü. Değişiklikler triküspital kapakçıkta daha şiddetliydi. Tracheada köpüklü sıvı ve akciğerlerde ödemli görünüm mevcuttu. Mikroskobik incelemede biküspital ve triküspital kapakçıklarda mikzomatöz değişikliklerle birlikte fibroblastik proliferasyonlar dikkati çekti. Ayrıca beyin ve beyincikte ak maddede şiddetli demyelinasyon mevcuttu. Beyinciğin granüler katmanında bazı küçük nöronlarda ve bazı astrositlerde intranükleer eozinofilik inklüzyon cisimciklerine rastlandı. Histopatolojik bulgular eşliğinde distemper tanısı konuldu. Valvuler endokardiyozis literatür bilgilerine göre yaşlı hayvanlarda ve mitral kapakta daha sıklıkla gözlenmektedir. Vakamız ise distemper tanısıyla birlikte hayvanın genç olması ve lezyonun daha şiddetli olarak triküspit kapakta şekillenmesi yönüyle nadir bir olgudur.

Anahtar Kelimeler: Distemper, köpek, triküspit kapak endokardiyozisi

GİRİŞ

Kronik edinsel atrioventriküler kapak hastalıkları köpeklerde en yaygın kardiyak morbidite ve mortalite nedenidir ve konjestif kalp yetmezliğine neden olduğu 100 yılı aşkın zamandır bilinmektedir (Jarcho, 1975; Fox, 2012). Köpeklerde mikzomatöz mitral kapak hastalığı ilk kez 1817 yılında Delabere Blaine tarafından tanımlanmıştır (Blaine, 1817). Mikzomatöz valvüler dejenerasyon ve valvüler endokardiyozis olarak da tanımlanan bu durum genellikle Poodle, Pomerian ve Chihuahua gibi küçük ırklarda daha sıklıkla görülmekte, büyük ırk köpeklerde ise daha nadir olarak görülmektedir (Borgarelli and Buchanan, 2012; Robinson and Robinson, 2016). Lezyon erkeklerde dişilerden daha sık şekillenmekte ve prevalans 1 yaşından küçük hayvanlarda %5 oranındayken, bu oran hayvan yaşlandıkça % 75'lere kadar yükselmektedir (Robinson and Robinson, 2016). Endokardiyozis esas olarak sol atriyoventriküler kapağı etkilerken, sağ atriyoventriküler kapak daha daha az etkilenir ve lezyonun şiddeti bu bölgede daha hafiftir. Bazen de aortik ve pulmoner semilunar kapakçıklar tutulabilmektedir. Etkilenen atriyoventriküler kapakçıklarda kısalma ve kalınlaşma gözlenir (Lester, 1995). Kalp kapakçıklarında oluşan lezyonlara göre, vakaların % 57'sinde sadece mitral kapakta, % 27'sinde mitral ve triküspidal kapakçıkta, % 7,5'unda sadece triküspidal kapakçıkta, % 6,5'unda ise diğer kapakçıklarda lezyon oluştuğu bildirilmiştir (Miller and Gal, 2017). Sık görülen bir mekanizma olarak; genellikle sol atrioventriküler kapaktaki endokardiyozis, mitral yetmezliğe ve sol atriumda yetersiz kan akışına sebep olarak sol kalp yetmezliği ile sonuçlanabilmektedir (Corcoran et al., 2004).

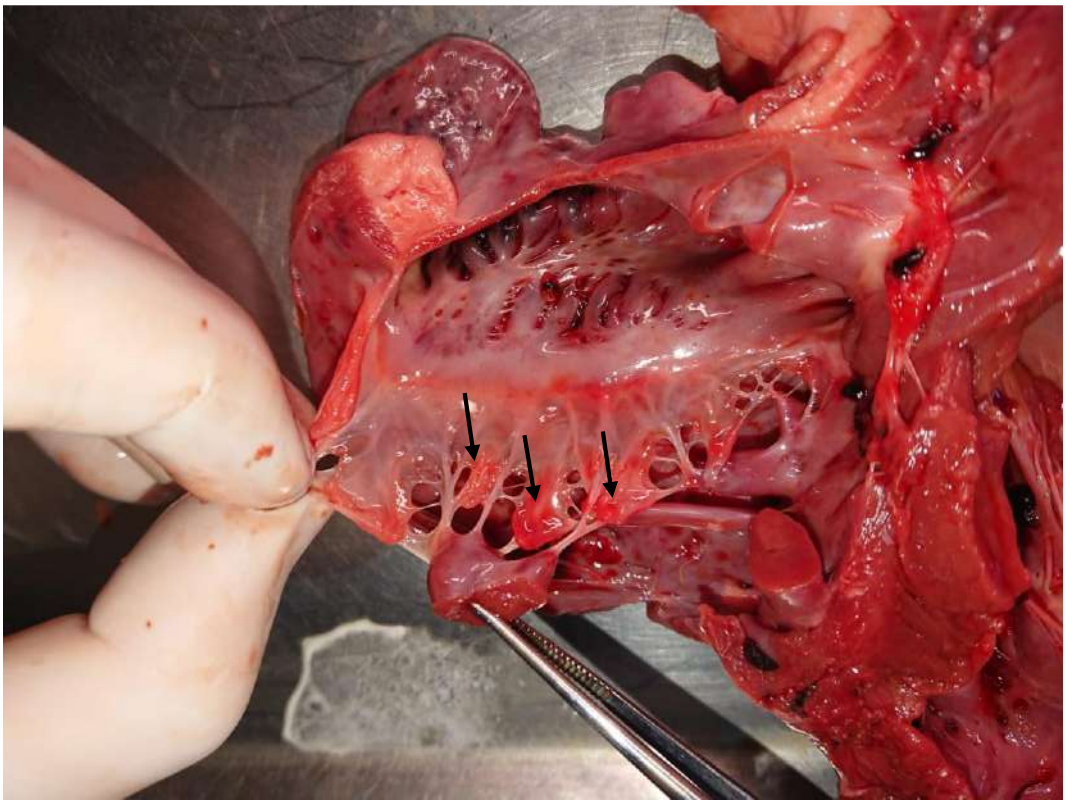
Bu olgu sunumunda 11 aylık bir Kangal ırkı köpekte distemper ile birlikte gözlenen valvüler endokardiyozisin patolojik bulgular eşliğinde tanımlanması amaçlanmıştır.

GEREÇ YÖNTEM

Burdur Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Kliniklerine ishal, halsizlik, iştahsızlık ve sinirsel semptomlar şikâyetleriyle 11 aylık, Kangal ırkı, erkek bir köpek getirildi. Hızlı test kiti kullanılarak distemper ön tanısı konulan köpeğin, uygulanan tedaviye cevap vermeyerek ölmesi üzerine Patoloji Anabilim Dalı'na nekropsi amacıyla gönderildi. Nekropsi sonrası alınan dokular %10'luk tamponlu formaldehit solüsyonuyla tespit edildi. Rutin doku takibi sonrası dokular parafin bloklara gömüldü. Parafin bloklardan 4-5 µm'lik kesitler alınarak hematoksilen & eozin ile boyandı. Kesitler ışık mikroskobunda incelendi.

BULGULAR

Makroskobik incelemede bağırsakların serozal damarları hiperemik olup, lümeninde sarı-sulu içerik görüldü. Mezenteriyel lenf düğümleri şişkin ve kesit yüzü nemli görünümdeydi. Karaciğer şişkin, kenarları kütleşmiş olup kesit yüzünden kan sızmaktaydı. Akciğerler şişkin, koyu kırmızı renkte olup, cranial ve medial loblarda subpleural peteşiyel kanamalara rastlandı. Ayrıca kesit yüzünde kanla karışık köpüklü sıvı sızmaktaydı. Perikard yüzeyinde yer yer peteşiyel kanamalar ile perkard boşluğundaki sıvı artışı dikkati çekti. Kalp büyümüş ve apexi yuvarlaklaşmış görünümdeydi. Kalp boşlukları açıldığında biküspital ve triküspidal kapakçıklarda kısalma ve noduler kalınlaşmalar görüldü (Resim 1). Değişiklikler triküspital kapakçıkta daha şiddetliydi. Kalbin sağ ventrikülünde dilatasyon mevcuttu. Bu bulgular dışında meningeal damarlarda dolgunluk fark edildi.

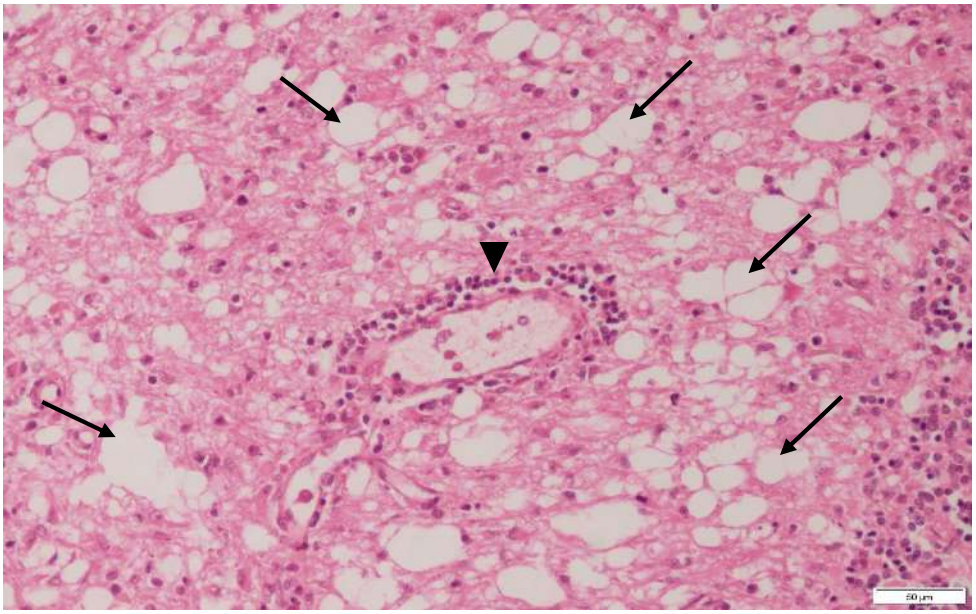


Resim-1: Triküspital kapakta mikzomatöz nodüler proliferasyonlar (oklar).

Figure-1: Myxomatous nodular proliferation on the tricuspit valve.

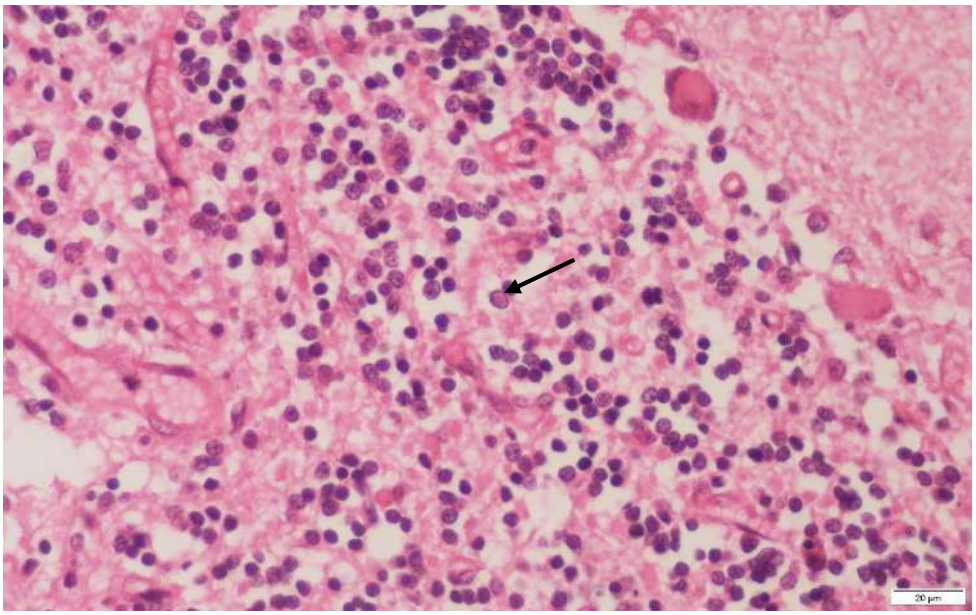
Mikroskobik olarak bağırsaklarda enteritis kataralis, mezenteriyel lenf düğümlerinde lenfadenitis nonpurulenta, karaciğerde pasif konjesyon, akciğerde kanama ve ödem mevcuttu.

Beyinde ise nonpurulent meningoensefalitis ve demyelinizasyon ile birlikte beyincikte granüler katmanda astrosit, glia ve küçük nöronlarda intranükleer inkluzyon cisimciklerine rastlandı (Resim 2 ve 3). Kalpte biküspital ve triküspital kapakçıklarda mikzomatöz değişikliklerle birlikte fibroblastik proliferasyonlar dikkati çekti (Resim 4 ve 5).



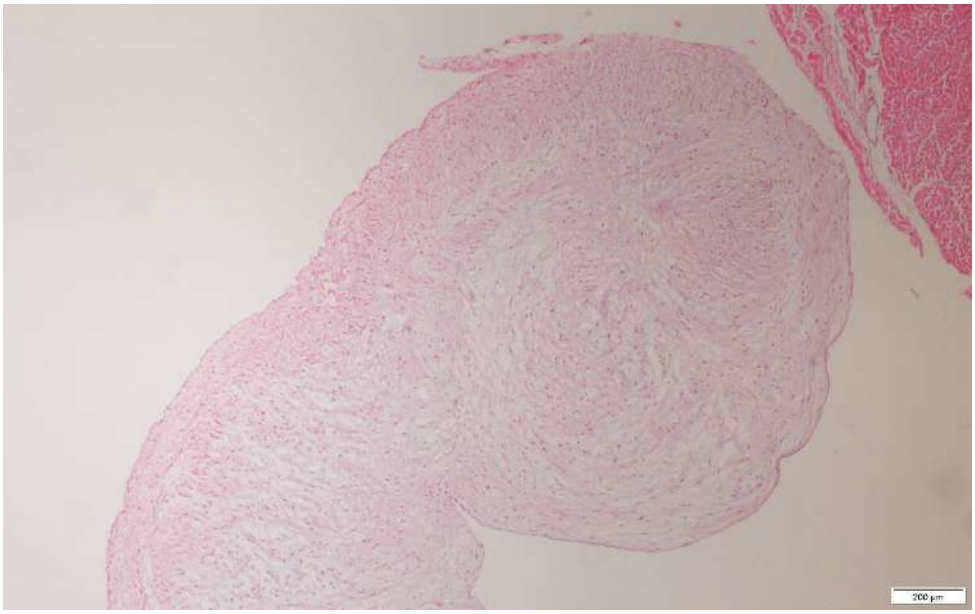
Resim-2: Beyinde ak maddede demyelinasyon (oklar) ve perivasküler yangısal hücre infiltrasyonu (ok başı). H&E. 200x.

Figure-2: Demyelination (arrows) and perivascular inflammatory cell infiltration (arrow head) in the brain. H&E. x200.



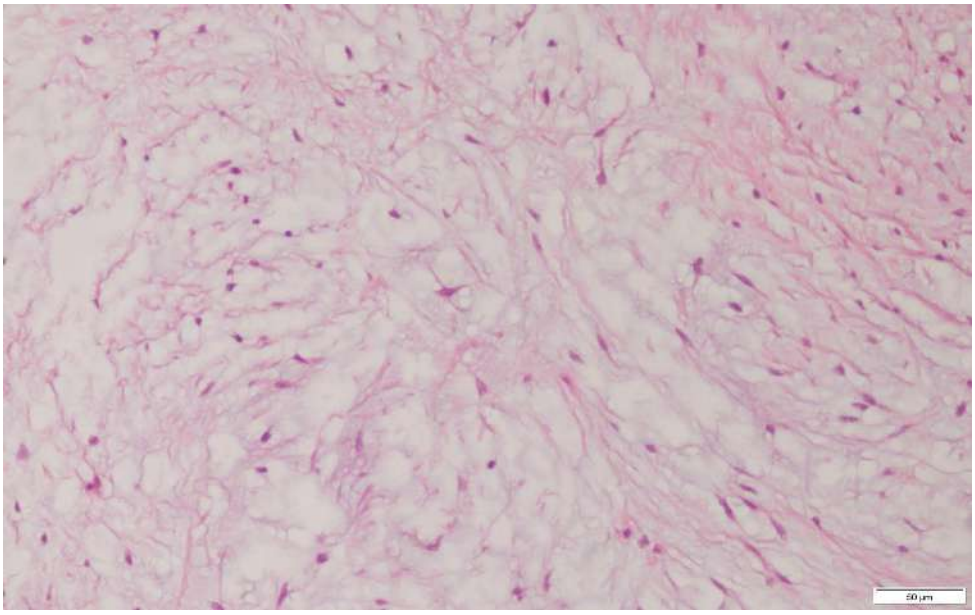
Resim-3: Beyincikte granüler katmanda intranükleer inklüzyon cisimciği (ok). H&E. 400x.

Figure-3: Intranuclear inclusion body in the granular layer of cerebellum (arrow). H&E. x400.



Resim-4: Mizomatöz nodüler proliferasyon. H&E. 40x.

Figure-4: Myxomatous nodular proliferation. H&E. x40.



Resim-5: Mikzomatöz fibroblastik proliferasyonlar. H&E. 200x.

Figure-5: Myxomatous fibroblastic proliferations. H&E. x200.

TARTIŞMA

Mikzomatöz mitral kapak hastalığı makroskobik olarak 4 grupta klasifiye edilmektedir. Tip 1'de minimal değişiklikler mevcuttur ve birkaç adet ayrık nodül gözlenir. Tip 2'de temas bölgelerinde nodüller daha büyüktür (hafif değişiklik). Tip 3'te birleşik, plak benzeri deformiteler oluşturan büyük nodüller mevcuttur (orta dereceli değişiklik). Tip 4'te ise kapakçıklarda belirgin bozulma şekillenmiştir (şiddetli değişiklik). Tip 3 ve 4'te korda tendineaların tutulumu da şekillenir (Miller and Gal, 2017). Olgumuzda gözlenen valvüler lezyonlar tip 2, 3 ve 4 ile uyumluydu. Şiddetli nodüler oluşumların bulunduğu alanlarda korda tentineaların da etkilendiği görüldü ve triküspit kapakta lezyonlar daha şiddetliydi.

Daha önce canine morbilliviruslar myokarditisli köpeklerden PCR ile ortaya konulmuştur (Molesan et al., 2019). Kim ve arkadaşları ise 4 köpekte distemper ile ilişkili myokarditis şekillendiğini, bu olguların üçünün PCR pozitif olduğunu ve tüm olgularda immunohistokimyasal olarak pozitiflik gözlediklerini bildirilmişlerdir (Kim et al., 2020). Başka bir çalışmada canine distemper virusunun kronik enfeksiyöz myokardiyal hastalıklı köpeklerde tespit edilen en yaygın etken olduğu bildirilmiştir (Santilli et al., 2019). Olgumuzda myokarditise dair bir bulguya rastlanmamış fakat her iki atrioventriküler kapakta valvüler endokardiyozis gözlenmiştir.

Endokardiyozis köpeklerde sıkça görülen bir hastalıktır ve genellikle yaşlılarda ve küçük ırklarda daha sıklıkla gözlenmektedir (Robinson and Robinson, 2016). Ayrıca lezyona triküspital kapakta %7,5 gibi düşük bir oranda karşılaşılmaktadır (Miller and Gal, 2017). Olgumuzdaki köpeğin büyük ırk ve 1 yaşından küçük olması ve ayrıca lezyonların daha şiddetli olarak triküspital kapakta şekillenmiş olması nadir olarak karşılaşılabilecek bir durumdur. Ayrıca distemper hastalığının da olguya eşlik ettiği gözlenmiştir. İnsanlarda mikzomatöz mitral kapak hastalığı ile enfeksiyöz ajanların ilişkisini ortaya koymaya yönelik olarak yapılan bir araştırmada *Mycoplasma pneumoniae* ile güçlü bir ilişkinin bulunduğu bildirilmiştir (Tiveron et al., 2017). Canine distemper virüs ve valvüler endokardiyozis arasındaki ilişkinin ortaya konulabilmesine yönelik olarak da hem immunohistokimyasal hem de moleküler teknikleri içeren daha ileri çalışmalara ihtiyaç duyulmaktadır.

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