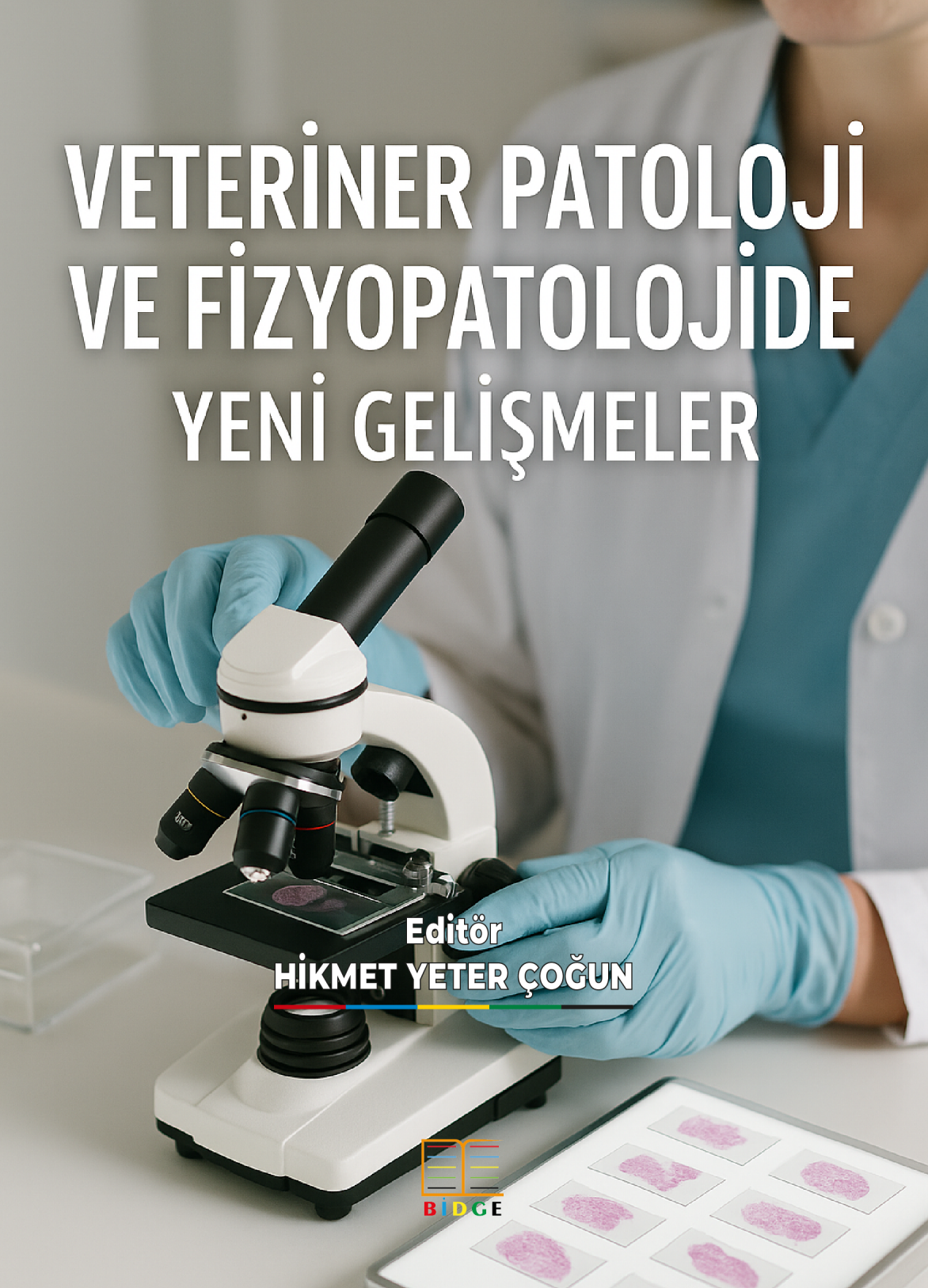
VETERİNER PATOLOJİ VE FİZYOPATOLOJİDE YENİ GELİŞMELER



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İÇİNDEKİLER

BÖLÜM 1

KAPSAİSİN'İN HİSTOLOJİK, HİSTOPATOLOJİK ARAŞTIRMALARDAKİ ROLÜ

Reşit UĞRAN[1]

Giriş

Kapsaisin, *Capsicum* türlerine özgü bir alkaloid olup, duyusal sistemde TRPV1 (Transient Receptor Potential Vanilloid 1) reseptörlerini aktive ederek acı hissini oluşturan primer moleküldür. Son yıllarda yapılan deneysel ve klinik araştırmalar, kapsaisinin yalnızca analjezik etkileriyle sınırlı kalmayıp, farklı doku ve organ sistemlerinde histolojik ve histopatolojik düzeyde çok çeşitli biyolojik tepkiler oluşturduğunu ortaya koymuştur. Bu molekülün, hücre proliferasyonu, inflamatuvar yanıtlar, oksidatif stres, apoptotik sinyalleme ve doku onarımı gibi çok sayıda fizyopatolojik süreci etkileyebildiği gösterilmiştir.

Literatürde karaciğer, gastrointestinal sistem, sinir dokusu, pankreas, böbrek ve kas dokuları üzerinde yapılan çalışmalarda kapsaisinin, düşük ve orta dozlarda antiinflamatuvar ve antioksidan etkiler gösterdiği; bu sayede hücre bütünlüğünü ve histolojik yapıyı koruyabildiği rapor edilmiştir. Örneğin, hepatositlerde lipid

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birikiminin azaldığı, inflamatuvar hücre infiltrasyonunun sınırlı kaldığı ve fibrozis gelişiminin engellendiği gözlemlenmiştir. Ancak bazı çalışmalarda, özellikle yüksek dozda ve uzun süreli uygulamalarda mukozal doku hasarı, hücre nekrozu, villus yapısında bozulma ve histolojik skorlarda belirgin artış gibi olumsuz sonuçlar da kaydedilmiştir.

Kapsaisin, dünyada en çok tüketilen acı biber olan Capsicum cinsi bitkilerden elde edilen acı biberlerin aktif maddesidir. Kapsaisin ve ilgili bileşikler, kapsaisinoidler adı verilen doğal olarak oluşan bir kimyasal grubu oluşturur. Kapsaisin, acı biberlere karakteristik keskin tadını verir ve bu kimyasalların bitki tarafından otçullara ve mantarlara karşı doğal bir savunma olarak üretildiğine inanılmaktadır. Kapsaisinin insan vücudu üzerindeki etkileri bir asırdan fazla süredir araştırılmaktadır. Acı biberler Capsicum cinsine aittir ve günlük hayatta yaygın olarak tüketilen sebzeler, çeşniler ve baharatlardır. Bu cinste yaklaşık 25 tür vardır ve bilinen beş çeşidi C. annuum, C. frutescens, C. chinense, C. baccatum ve C. pubescens'tir (Hernandez-Perez & ark., 2020: 2973). Kapsaisinoidler, acı biberlerin baharatlı lezzetlerinin ana kaynağıdır ve kapsaisin, ana keskin bileşen olarak kabul edilen kapsaisinoidlerin yaklaşık %69'unu oluşturur. Kapsaisin içeriği farklı acı biberler arasında büyük ölçüde değişir ve bu da biber meyvesinin keskinliğinde farklılıklara neden olur (Srinivasan, 2016:1490).

Şu anda, acı biber özütü ve biyoaktif bileşiklerinin antibakteriyel, antioksidan, ağrı kesici ve iltihap giderici etkiler gibi çeşitli farmakolojik etkilere sahip olduğu bildirilmektedir. Ancak, acı biberlerin ana maddesi olan kapsaisin hala tartışmalıdır (Srinivasan, 2016:1488). Son çalışmalar kapsaisinin iki ucu keskin bir kılıç olduğunu göstermiştir. Düşük konsantrasyonlarda potansiyel biyolojik aktivitelere sahiptir, ancak yüksek konsantrasyonlarda olumsuz etkilere neden olma eğilimindedir.

Gastrointestinal sistem (GIT), en büyük iç organ sistemidir ve her türlü dış girdi gıdasını içerir. Yaşam deneyimi, büyük miktarda kapsaisin yemenin mide ekşimesine, gastrointestinal ağrıya ve ishale (van Avesaat & ark., 2016:306, Hammer & Vogelsang, 2007:281) neden olabileceğini göstermiştir. Hogyes, 1878'de insan cildine uygulandığında Capsicum özütünün ürettiği yanma hissi ve hiperemiyi gözlemlemiştir (Toh & ark.1955:175). Daha sonra çok sayıda hayvan çalışması, kapsikum özütünün intravenöz enjeksiyonundan sonra kan basıncında düşüş, tükürük ve mide salgısında artış ve bağırsak aktivitesinde artış olduğunu ortaya koymuştur (de Lille & Ramirez, 1935, Nast, 1923). Sonuç olarak, kapsaisin heyecan verici bir farmakolojik ajan olmuştur ve farklı klinik koşullardaki faydası araştırılmaktadır.

Bu kitap bölümü, kapsaisin'in biyolojik sistemlerdeki histopatolojik etkilerini sistematik olarak ortaya koymayı amaçlamaktadır. Farklı organ sistemlerinde yapılan deneysel çalışmalar üzerinden; kullanılan doz aralıkları, uygulama yolları, deney süreleri ve elde edilen mikroskobik bulgular kapsamlı şekilde analiz edilecektir. Aynı zamanda histolojik değerlendirme teknikleri, boyama yöntemleri ve skorlama kriterleri de karşılaştırmalı olarak sunulacaktır. Bu doğrultuda, kapsaisinin potansiyel terapötik kullanım alanları ile toksik etkileri arasındaki denge bilimsel çerçevede değerlendirilecektir.

Kapsaisin Kimyasal, Biyolojik Ve Toksikolojik Özellikleri

Kapsaisin (8-metil-N-vanillyl-6-nonenamide), Capsicum türlerine özgü fenolik yapıdaki lipofilik bir alkaloiddir ve biberin acılığını sağlayan başlıca biyolojik bileşendir. Kimyasal olarak vanilloid grubuna ait olan kapsaisin, TRPV1 (Transient Receptor Potential Vanilloid 1) kanalına bağlanarak sinir hücrelerinde Ca^{2+} girişini tetikler ve bu sayede sıcaklık ve ağrı duyusunu ileten nörosensöryel yolları aktive eder (Reyes-Escogido & ark,

2011:1254). Bu özellik, kapsaisinin lokal analjezik olarak kullanılmasını sağlarken aynı zamanda hücresel sinyalleme, inflamatuvar süreçler ve oksidatif stres ile ilgili birçok biyolojik sistem üzerinde de etkili olmasına neden olmaktadır.

Kapsaisinin biyolojik etkileri arasında antiinflamatuvar, antioksidan, termojenik, lipolitik ve antikanserojen özellikler ön plana çıkar. Özellikle gastrointestinal sistemde mukozayı koruyucu etkiler sergileyebileceği, karaciğerde lipid birikimini azaltabileceği ve bazı kanser hücre hatlarında apoptozu indükleyebileceği belirtilmiştir (Parvez, 2017:1903). Bununla birlikte, kapsaisinin çift yönlü bir molekül olduğu unutulmamalıdır. Yüksek dozlarda veya uzun süreli maruziyette mukozal iritasyon, hücre nekrozu, DNA hasarı, inflamasyon ve potansiyel karsinojenite gibi ciddi toksikolojik etkiler rapor edilmiştir (Surh & Lee, 1995:1848). Ayrıca topikal kapsaisin uygulamalarında ciltte eritem, yanma hissi ve lokal inflamasyon gibi reaksiyonlara sık rastlanmakta; aerosol formlarında ise solunum yolu iritasyonuna yol açabilmektedir (Bley & ark., 2012:848).

Toksikolojik profili incelendiğinde, kapsaisinin güvenli kullanım sınırlarının deneysel ve klinik çalışmalarla dikkatle belirlenmesi gerektiği ortaya çıkmaktadır. Özellikle terapötik potansiyeli olan bir bileşik olarak değerlendirilirken, uygulama dozu, süresi, maruz kalma yolu ve bireysel metabolik yanıtlar gibi faktörler dikkate alınmalıdır. Bu kapsamda, kapsaisin hem farmakolojik açıdan umut vadeden hem de dikkatli kullanılmadığında toksisite riski taşıyan bir bileşik olarak değerlendirilmektedir.

Kapsaisinin Sindirim Sistemi Üzerine Etkileri

Sindirim sistemi, yalnızca besinlerin mekanik ve kimyasal olarak parçalanmasından değil, aynı zamanda bağışıklık düzenlenmesi, hormonal denge, mikrobiyota-ev sahibi etkileşimleri

ve genel metabolik sağlık üzerinde hayati roller oynayan çok yönlü bir sistemdir. Besinlerin sindirimi ve emilimi yoluyla makro ve mikro besinlerin vücuda kazandırılması, yaşamın sürdürülebilirliği açısından temel bir işlevdir. Ancak bu sistemin önemi yalnızca besin emilimi ile sınırlı değildir; bağırsak bariyerinin bütünlüğü ve burada yerleşik olan mikrobiyal flora, enfeksiyonlardan korunmada ve bağışıklık sisteminin yönlendirilmesinde kritik roller üstlenir (Aureliano & Ma, 2025). Özellikle bağırsak mikrobiyotasının düzenli yapıda olması, yalnızca sindirim sağlığını değil, aynı zamanda mental sağlık, inflamasyon düzeyi ve hatta otoimmün hastalıkların oluşum riskini de etkilemektedir (Zeng & ark., 2025). Bu nedenle, sindirim sistemi yalnızca bir "besin işleme ünitesi" değil, aynı zamanda bir bağırsak-beyin ekseni ile sinir sistemi üzerinde etkili olan bir düzenleyici merkez olarak değerlendirilmektedir (Gu & ark., 2025). Son dönem literatür, sindirim sisteminin disfonksiyonunun; diyabet, kardiyovasküler hastalıklar, obezite ve nörodejeneratif bozukluklar gibi sistemik hastalıklarla doğrudan ilişkili olabileceğini ortaya koymaktadır (Pachauri & Sharma, 2025:4).

Karaciğer enerji metabolizmasında, safra asidi salgılanmasında, ilaç metabolizmasında, detoksifikasyonda ve diğer işlevlerde hayati bir rol oynar (Luo & ark., 2022:490). Karaciğer hastalığı her yıl 2 milyon ölüme neden olur ve tüm ölümlerin %4'ünü oluşturur ve karaciğerle ilişkili ölümlerin yaklaşık üçte ikisi erkeklerde görülür (Devarbhavi & ark., 2023:517). Karaciğer hastalığının görülme sıklığı artmaya devam etmektedir (Yu & ark., 2014:3), basit steatozdan ve alkolsüz yağlı karaciğer hastalığından (NAFLD) alkolsüz steatohepatite (NASH), siroza ve karaciğer kanserine kadar uzanan durumları kapsamaktadır (Li & ark., 2019:11645). Bu nedenle karaciğer hastalıklarının ortaya çıkmasını önlemek için etkili yollar bulmak çok önemlidir. Sukmanadi & ark., (2021) yaptıkları bir çalışmada kapsaisinin aflatoksin B1

zehirlenmesi olan farelerde karaciğer histolojisini iyileştirdiğini bildirmişlerdir. Kapsaisin'in, PPAR γ ve HMG CO-A redüktaz gen ekspresyonunu azaltarak ve antifibrotik ve antiinflamatuar ajan olarak etki ederek, HFD'nin karaciğer ve safra kesesinde neden olduğu histolojik değişikliklere karşı büyük bir koruyucu etkiye sahip olduğu ileri sürülmüştür (Hehgazy & ark., 2023:248). Kapsaisin ile yapılan bir başka çalışmada ise kapsaisin'in, oksidatif stresi, apoptotik sinyalleri ve sitokin yolunu düzenleyerek Siklofosfamid (CPM) kaynaklı hepatotoksisiteyi zayıflattığı bildirilimiş olup kapsaisin'in hastaların kemoterapisi sırasında bir takviye olarak önemli bir rol oynayabileceğine de değinilmiştir (Alam & ark., 2023:911). Kapsaisinin AFB-1 indüksiyonuna karşı koruyucu bir etkiye sahip olduğu; karaciğer lezyonlarının oluşumunu önleyerek, bunun hepatoprotektif olarak potansiyel bitkisel ilaç adayı olduğu gösterilmiştir (Effendi, 2021:812). Farelerde yapılan başka bir çalışmada Kaspaisin'in, öncelikle Diethylnitrosamine (DEN) kaynaklı oksidatif karaciğer hasarını azaltarak preneoplastik lezyon gelişimini zayıflattığı ve söz konusu bulguların, kapsaisin'in hepatokarsinogenezin erken evrelerinde ve sonrasında uygulandığında umut vadeden bir kemopreventif ajan olabileceği göstermektedir (Sarmiento-Machado, & ark., 2021:820). Kapsaisin'in daha yüksek dozda ve uygun şekilde kulanıylmasıyla alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) serum seviyelerini düşürdüğü, ayrıca malondialdehit (MDA), reaktif oksijen türleri (ROS), nitrit, NF-kB, TLR4, IL-1β, TNF-α, kaspaz 3, DNA parçalanmasının hepatik seviyesini düşürdüğü ve sirtuin 1, Nrf2, süperoksit dismutaz (SOD) aktivitesini ve hem oksijenazı (HO-1) artırmıştır. Kapsaisinin bu yararlı etkileri proapoptotik Bax, antiapoptotik Bcl2, mitokondriyal ve metabolik düzenleyiciler PGC-1α, sirtuin 1 ve AMPK ve inflamasyonla ilişkili faktörler için gen ifadesinin tersine çevrilmesi ve/veya iyileştirilmesi ile ilişkilendirilmiştir. Ek olarak, kapsaisin karaciğer histopatolojik değişikliklerini azaltarak ortaya çıktığı gibi hepatoprotektif bir etki

göstermiştir. Bu bulgular, kapsaisinin septik koşullar altında oksidatif ve inflamatuvar süreçleri aşağı düzenlemesinin yanı sıra mitokondriyal disfonksiyon ve apoptozu azaltma potansiyeline sahip olması nedeniyle hepatoprotektif bir özelliğe sahip olduğunu açıkça gösterdiğine dikkat çekilmiştir (Ghorbapour & ark., 2023). Kuchařová & ark. (2021:385) kapsaisinin septik koşullarda oksidatif ve inflamatuvar süreçleri düzenlemesinin yanı sıra mitokondriyal disfonksiyon ve apoptozu azaltma potansiyeline sahip olması nedeniyle hepatoprotektif bir özelliğe sahip olduğu göstermişlerdir.

Mide, sindirim sisteminin temel yapı taşlarından biri olup, vücut için hem mekanik hem de kimyasal sindirim süreçlerinin başlangıç noktasını oluşturur. Özofagus ile duodenum arasında yer alan bu kaslı yapı, yalnızca gıdaların geçici olarak depolanmasından değil, aynı zamanda kompleks biyolojik süreçlerin yönetilmesinden sorumludur. Midenin iç yüzeyi, mukus tabakasıyla kaplı olan gastrik bezlerle donatılmıştır; bu bezler, hidroklorik asit (HCl), pepsinojen, gastrin ve çeşitli mukoproteinleri salgılayarak hem asidik ortamı düzenler hem de protein sindirimini başlatır (Smyth & Fitchett, 2023:90).

Vücudun en önemli organlarından olan mide fizyolojik olarak, ağızda başlayan sindirim sürecini hızlandırmakla kalmaz, aynı zamanda besinlerin mikrobiyal kontaminasyondan arındırılmasına katkı sağlar. Düşük pH (1.5–3.5) aralığında çalışan mide asidi, çoğu bakteriyel patojeni etkisiz hale getirerek bağışıklık sisteminin ilk savunma hattı görevini üstlenir (Martinsen & ark., 2005:96). Ayrıca burada salgılanan intrinsik faktör, B12 vitamininin ince bağırsakta emilimini mümkün kılar; dolayısıyla mide fonksiyonlarının bozulması, pernisiöz anemi gibi ciddi metabolik bozukluklara zemin hazırlayabilir (Allen, 2009:26). Mide sadece sindirime hizmet etmez, aynı zamanda nöroendokrin sistemle de sıkı ilişkidedir. Gastrin, ghrelin ve somatostatin gibi hormonlar hem sindirim süreçlerini hem de açlık-tokluk sinyallerini yöneterek enerji

metabolizmasını doğrudan etkiler. Özellikle ghrelin hormonu, hipotalamusu uyararak iştah kontrolünde belirleyici rol oynar (Kojima & Kangawa, 2005:496). Bu yönüyle mide, gastrointestinal sistemin ötesinde nörogastroenterolojik bir işlev üstlenmektedir. Mide hastalıkları, hem lokal hem de sistemik sonuçlara yol açabilir. Gastrit, ülser, H. pylori enfeksiyonu, reflü ve mide kanseri gibi yaygın rahatsızlıklar, mide fonksiyonlarındaki aksamanın insan sağlığı üzerindeki etkilerini ortaya koymaktadır. Özellikle mide mukozasının yapısal ve fonksiyonel bütünlüğü, hem sindirim sağlığı hem de sistemik homeostaz için kritik bir gerekliliktir (Correa, 1992).

İnsanlarda yapılan çalışmalarda, kapsaisinin asit salgısını engellediğini, alkali ve mukus salgılarını ve mide mukozası kan akımını uyardığını, bunların hepsinin mideden asidin atılmasına yardımcı olduğunu, böylece peptik ülserin iyileşmesini desteklediğini ortaya koymuştur (Abdel-Salam & ark.,1997:157). Gastrik dokularda makroskobik ve mikroskobik ülser skorları ve mukozal bariyer bütünlüğü değerlendirilmiş ve kapsaisinin etanol kaynaklı gastrik ülser modelinde antioksidan, anti-inflamatuar ve anti-apoptotik özellikleriyle mukozal bütünlüğü koruduğu bildirilmiştir (Keçeci & ark., 2005:99). Yapılan bir çalışmada yüksek kapsaisin içeren diyetin TRPV1 ekspresyonunu ve bağırsak mikrobiyota kompozisyonunu düzenleyerek mide kanseri metastazını teşvik etmede potansiyel risk taşıdığını vurgulamış ve mide kanseri hastaları için acı biberlerin kontrollü tüketiminin önemini gösterilmiştir (Deng, & ark., 2023).

Bağırsak, sindirim sisteminin temel bir organlarından biridir. İnce ve kalın bağırsak olmak üzere iki ana bölümden oluşur. Bu yapılar yalnızca besinlerin emiliminden değil, aynı zamanda bağışıklık düzenlemesi, hormonal denge ve sinir sistemiyle iletişim gibi çok sayıda hayati işlevden de sorumludur (Ghashghaei & ark.., 2025). İnce bağırsak, sindirilen gıdalardan makro ve mikro

besinlerin emildiği ana bölümdür. Villus ve mikrovillus yapıları sayesinde yüzey alanı artar ve maksimum düzeyde emilim sağlanır (Chaves de Jesus & ark., 2025:551). Bağırsak florası ya da mikrobiyota, yüz trilyonu aşkın mikroorganizmadan oluşur ve bağışıklık sistemini eğitmek, patojenlerle savaşmak ve inflamasyonu düzenlemek gibi görevler üstlenir. Mikrobiyal dengenin bozulması; obezite, diyabet, kanser ve nörolojik bozukluklar dahil birçok hastalıkla ilişkilendirilmiştir (Lambrou & ark., 2025:96; Yang & ark., 2025:23). Bağırsaklar ve beyin arasında çift yönlü bir iletişim vardır. Bu eksen, duygudurum düzenlemesi, stres tepkisi ve davranışsal fonksiyonlar üzerinde rol oynar. Serotoninin yaklaşık %90'ı bağırsaklarda sentezlenir (Hao & ark., 2025). Bağırsak, vücudun en büyük immün organıdır. Mukoza-associated lenfoid doku (MALT), potansiyel olarak zararlı patojenlere karşı ilk savunma hattını oluşturur (Ye & ark., 2025:1205). Yang ve ark. (2024) yaptıkları çalışmanın sonucunda kapsaisinin bağırsak bariyer fonksiyonunu onarma yeteneği, sadece inflamatuar faktörlerin kan dolaşımına taşınmasını azaltmakla kalmadığı, aynı zamanda lipid emilimini de önemli ölçüde etkilediğini bildirmişlerdir. Usman & Makiyah (2024:137) sıçanlar üzerinde yaptığı deneysel bir çalışmada, *Capsicum frutescens L.* suyunun oral verilmesi, ince bağırsak mukozasında goblet hücrelerinin sayısında belirgin azalma ile sonuçlanmıştır. Bu durum, mukoza koruyucu mukus tabakasının zayıflamasına ve gastrointestinal savunma sisteminin bozulmasına neden olabileceğine dikkat çekmişlerdir.

Kumar & ark., (2025:120) yürüttüğü bir hayvan çalışmasında, yüksek dozda capsaicin uygulamasının bağırsakta Substans P salınımını artırarak mukozal inflamasyon ve hücre ölümü oluşturduğu gösterilmiştir. Aynı çalışmada, capsaicin'in TRPV1 reseptörü aracılığıyla bu etkileri oluşturduğu ve N-acetyl-L-tryptophan (L-NAT) uygulamasının bu hasarı engelleyebileceği belirtilmiştir. Kapsaisinle oluşturulan abdominal hiperaljezi

modelinde bağırsak histolojisinde bozulma ve intestinal permeabilitenin artışı gözlemlemiştir. Bu bulgu, irritabl bağırsak sendromu benzeri durumlarda kapsaisin'in rolü olabileceğini düşündürmektedir (Yoshioka & ark., 2025:272). Song & ark., (2024) fareler üzerinde yaptığı deneysel bir çalışmada, kapsaisin'in TRPV1 reseptörünü aktive ederek intestinal epitel hücrelerinde apoptozis, artan permeabilite ve doku nekrozu ile sonuçlandığı gösterilmiştir. Bu bulgular, özellikle irritabl bağırsak sendromu ve inflamatuar hastalık modellerinde önem taşıdığını bildirmişlerdir. Başka bir araştırmada, düşük doz kapsaisin uygulamasının anti-inflamatuar etki sağladığı, ancak yüksek dozda bağırsak mukozasında pro-inflamatuar sitokinlerin (IL-6, TNF-α) arttığı ve histolojik doku bütünlüğünün bozulduğu gözlemlemişlerdir (Zhao& ark., 2021).

Sonuç

Kapsaisin, doğada yaygın olarak bulunan bir alkaloid olup, son yıllarda yalnızca gustatuvar ve nörosensöryel etkileriyle değil, aynı zamanda doku düzeyinde oluşturduğu biyolojik yanıtlarla da dikkat çeken bir araştırma konusu haline gelmiştir. Bu kitap bölümünde ele alındığı üzere, kapsaisinin gastrointestinal sistem ve hepatik dokular üzerindeki histolojik ve histopatolojik etkileri oldukça katmanlı ve doz-bağımlıdır.

Mide mukozasında yapılan deneysel çalışmalarda kapsaisinin, özellikle yüksek dozlara maruz kalındığında, epitel bütünlüğünde bozulma, yüzeysel erozyonlar ve mukus salgısında azalma gibi değişikliklere neden olabildiği görülmüştür. Bu durum, mideyi koruyan bariyerlerin zayıflamasına ve peptik ülser gibi patolojilere zemin hazırlayabileceğini göstermektedir.

Bağırsak düzeyinde ise kapsaisinin etkileri daha da belirginleşmektedir. İnce bağırsak mukozasında goblet hücresi yoğunluğunun azalması, villuslarda kısalma ve kript hiperplazisi gibi morfolojik değişiklikler; aynı zamanda intestinal

permeabilitenin artması ve inflamatuar hücre infiltrasyonu gibi histopatolojik bulgularla desteklenmiştir. Bu etkilerin TRPV1 reseptörü aracılığıyla geliştiği ve inflamatuar mediyatörlerin (örneğin, IL-6, TNF-α) salınımıyla bağlantılı olduğu bildirilmiştir. İlginç şekilde, düşük doz kapsaisin uygulamalarında bağışıklık yanıtının düzenlenmesine ve epitel bütünlüğünün korunmasına katkı sağlayan pozitif etkiler de gözlemlenmiştir.

Karaciğer üzerine olan etkiler ise daha sistemiktir ve genellikle bağırsak-liver aksı yoluyla değerlendirilir. Bazı çalışmalarda, yüksek doz kapsaisinin hepatositlerde balonlaşma, vakuolizasyon ve fokal nekroz gibi histopatolojik bulgulara yol açtığı; aynı zamanda oksidatif stres ve proinflamatuar yanıtları tetiklediği rapor edilmiştir. Bununla birlikte, antioksidan savunma sistemlerini aktive ederek hepatoprotektif etkiler de gösterebileceği yönünde bulgular mevcuttur.

Tüm bu bulgular değerlendirildiğinde, kapsaisin hem lokal hem de sistemik düzeyde çift yönlü (bifazik) etkilere sahip karmaşık bir bileşik olarak öne çıkmaktadır. Histolojik ve histopatolojik açıdan bu maddenin etkilerini anlamak, yalnızca toksikolojik riskleri değerlendirmek açısından değil, aynı zamanda terapötik potansiyelini belirlemek açısından da büyük önem taşımaktadır. Gelecekteki araştırmalarda, doz, süre, uygulama yolu ve hedef dokuya özgü etkilerin daha ayrıntılı incelenmesi; kapsaisinin klinik kullanımı açısından güvenli sınırların tanımlanmasına katkı sağlayacaktır.

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BÖLÜM 2

EVCİL HAYVANLARDA SIK KARŞILAŞILAN DERİ TÜMÖRLERİ

Nihat YUMUŞAK[1]

Giriş

Kanser veya tümör, insanlık tarihi kadar eski bir hastalıktır. Son yüz yılda özellikle sanayileşmenin neden olduğu çevresel etkiler, beslenme bozuklukları ve stresin kanser olgularında artışa neden olduğu bilinmektedir. Ayrıca yoğun kimyasal maddelere maruziyet ve bu maruziyete bağlı oluşan hormonal değişiklikler ile genetik hasarlar, egzersiz yaşam tarzı, hatalı veya yetersiz beslenmeye bağlı metabolizma bozuklukları ve kronik hastalıklar kanser oluşumunun başlıca nedenleri arasında yer alır. İnsanlarda olduğu gibi evcil hayvanlarda da yapılan birçok araştırmada kanser görülme sıklığında artış olduğu belirlenmiştir. Özellikle köpek ve kedilerde diğer türlere oranla kanser görülme oranı daha yüksektir. Bu türlerde hemen hemen her yaşta kanser olguları ile karşılaşılsa da yaşlı hayvanlarda deri ve meme kanseri gibi bazı kanser türlerinin daha yaygın olarak oluştuğu belirlenmiştir (Sharif, 2006) (Goldschmidt & Hendrick, 2002) (Gülçubuk & Gürel, 2005: 61) (Sönmez & Özmen, 1996: 69).

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Papillom

Çok katlı yassı epitelin aşırı artışı ile karakterize olan bu tümörler; her türde görülse de özellikle kedi, köpek, sığır ve atlarda daha yaygın olarak karşılaşılmaktadır. Çoğunlukla viral kökenli olan tümörler, deri üzerine yapılan sürekli irritasyonların da tümör oluşmasında etkili olduğu bildirilmektedir. Köpek ve atlarda lezyonlar solit kitleler halinde görülebilse de baş, boyun ve ağız çevresinde çok sayıda farklı büyüklüklerde şekillenebilirler. Sığırlarda penis, vulva ve memede yerleşim gösterirler. Koyun ve keçilerde ise daha çok kulakta şekillendiği görülür. Makroskobik olarak tümörler farklı büyüklüklerde, deriden taşkın ve polipoid ya da karnabahar formundadırlar. Travmalara ve enfeksiyonlara maruz kalmasıyla kanama ve ülserler şekillenebilir. Mikroskobik olarak; çok katlı yassı epitelin aşırı derecede arttığı, bazal membranın bütünlüğünü kaybetmediği görülür. Epitel katın hiperkeratotik olduğu ve hücrelerde yer yer vakuoler dejenerasyonların geliştiği dikkati çeker. Bazı olgularda ise bu yapılara yoğun bağdoku proliferasyonları eşlik eder ve fibropapillom olarak adlandırılır. Fibropapillomlar daha sık atlarda ve sığırlarda genital bölgede yerleşim gösterir (Allison, 1965) (Jones, Hunt, & King, 1997: 251) (Smith, 1996: 1417) (Hamada, 1990: 393) (Madewell & Theilen, 1987: 238) (Spradbrow & ark., 1997: 469).

Bazal Hücreli Tümör

Epidermisin bazal tabakasında yer alan basal hücrelerden köken alan proliferatif tümörlerdir. Bütün türlerde görülen bu tümörler köpek ve kedilerde en yaygın karşılaşılan deri tümörleridir. Ayrıca atlarda ve nadir olarak koyunlarda tespit edilmiştir. Genetik yatkınlık, radyasyon ve kimyasal etkenler tümör oluşumunda etkili nedenler arasında yer alır. Genellikle yetişkin (7-9 yaşlı) köpek ve kedilerde daha sık karşılaşılır. Yapılan çalışmalarda erkek

hayvanlarda dişi hayvanlara oranla daha yaygın olarak şekillendiği bildirilmiştir. Her ırk köpek ve kedide görülsede Terrier, Poodle, Huskie köpek ırkları ile Siam ve Persian ırkı kedilerde daha yaygın karşılaşılır. Daha çok baş, boyun ve bacaklarda bazende abdomen ve göğüste yerleşim göstermektedirler. Tümörler noduler tarzda, tek ya da çok sayıda olabilen, elastik kıvamlı, iyi sınırlanmış ve intradermal yerleşim gösteren kitleler olarak göze çarpar. Lezyonun bulunduğu kıllarda dökülme ve ülserasyonlar görülür. Kitlelerin kesit yüzü kahverengi-siyah, merkezinde kistik dejenerasyonlar görülebilir. Mikroskobik olarak; tümör hücreleri küçük ve orta boyutlarda dar mavi sitoplazmalı uniform yapıda, yuvarlak çekirdekli, kısmen yoğun kromatinli, belli-belirsiz çekirdekçikli, çok sayıda mitotik figürün bulunduğu hücrelerden ibarettir. Malign tümörlerde; hücreler daha büyük ve poligonal olup granüler sitoplazmaya sahiptirler. Lokal olarak yerleşim gösteren bu tümörlerin metastaz yapmazken zaman zaman cerrahi müdahalelerden sonra nüks yaptıkları bildirilmiştir (Gorham, Penny, & Bradley, 1990: 466) (Goldschmidt & ark., 1998) (Fehrer & Lin, 1986: 1469) (Dundr & ark., 2004: 70) (Barr & ark., 1993: 308) (Courtney, Hawkins, & Graziano, 1992: 122).

Yassı Hücreli Kanser

Çok kattlı yassı epitelin skuamous hücrelerinden köken alan oldukça malign karakterde tümörlerdir. Pigmentsiz ya da az kıllı derinin uzun süre ultraviole ışınlarına yada kronik irritasyonlara maruz kalmasıyla tümör şekillenebilir. Yapılan bazı çalışmalarda bu tümörlerin oluşmasında papilloma virusların rollerinin olduğu bildirilmiştir. Bütün türlerde yaygın olarak karşılaşılsa da özellikle yaşlı köpek, kedi, sığır, koyun ve atlarda görülür. Irk yatkınlığı tam olarak tespit edilmemiştir. Fakat Labrador, Dalmaçya ve Beagle ırkı köpekler ile Holstein, Hereford ve Simmental sığır ırklarında daha yaygın olarak karşılaşılmaktadır. Tümörler, kedilerde burun, kulak kepçesi ve göz kapakları, köpeklerde bacakların iç bölgeleri ile

perineumda, sığırlarda konjunktivada, atlarda ise vulva, prepisyum ve perianal bölgeye sıklıkla lokalize olur. Ayrıca birçok bölgede multiple kitleler halinde görülebilir. Makroskobik olarak tümör sert kıvamlı ve beyaz-gri renktedir. Çevrede bulunan deride ve kıllarda pigmentsizlik, ödem, kabuklanma, hiperemi, nekroz ve ülserasyon oluşur. Bakteriyellerin etkisiyle zamanla tümör yüzeyi krater manzarasına dönüşür ve yüzeyinde yoğun miktarda kanlı purulent eksudat görülür. Histolojik olarak en belirgin bulgusu bağımsız keratin adacıklarıdır. Bazı olaylarda, elastik ve kollagen demetlerde dejenerasyonlar ve dermiste kalınlaşma görülür. Ayrıca epidermal hiperplazi, hiperkeratoz, keratonosit displazisi, epidermisin bazal ve spinozum bölgelerinde görülür. Hücreler yuvarlak ve ovaldir. Lobullü nükleus, multiple, büyük çekirdekçik; tek ya da çok çekirdekli dev hücreler yaygındır. Anizositosiz, anizokaryosiz, mitotik figürler belirgin ve yaygın perinüklear vakuoller görülür. Sitoplazma tukuaz-mavidir. Yavaş yayılım gösteren bu tümörlerin invazyon yeteneği fazladır (Goldschmidt & ark., 1998) (Goldschmidt & Hendrick, 2002: 45) (Clarke, 1991: 148) (Espinosa De Los & ark., 2003: 153) (Madewell & Theilen, 1987: 238) (Levine, Earle, & Wilson, 1990: 68).

Bazoskuamöz Karsinom

Özellikle köpeklerin baş, boyun, bacak, karın ve sırtlarında derialtı yerleşim gösteren düşük maling karakterli tümörlerdir. Tümörün şekillendiği bölgelerde tüylerde dökülme ve zamanla ülserleşmeler oluşabilir. Kesit yüzlerinde subkutise doğru uzantılar veren, farklı büyüklerde, lobuler karakterde, yer yer kistik yapıların olduğu bir yapı gözlenir. Hücre adacıklarının çevresinde diferensiye olmamış basoloid hücrelerin ve bunların arasında keratinize hücrelerin görülmesi önemli histopatolojik bulgulardır. Adacıkların merkezinde atipik hücrelerden ibaret keratinizasyon şekillebilir. Atipik hücreler genellikle pleomorfik çekirdekli olup, bazen mitotik aktivasyon, diskeratinizasyon ve melanin içerebilirler (Espinosa De

Los Monteros & ark., 2003: 90) (Goldschmidt & ark., 1998) (Goldschmidt & Hendrick, 2002: 45).

Sebaseöz Bez Tümörleri

Bu tümörler epidermiste bulunan yağ bezlerinden köken alan tümörlerdir. Genellikle iyi huylu olan bu tümörler histolojik yapılarına göre hiperplazi, adenom, epitelyom ve kötü huylu karsinomlar olarak sınıflandırılır. Sebaseöz hiperplazilerinin adenomlarla ayrımının yapılması oldukça güçtür. Sebaseöz tümörler köpeklerde diğer türlere oranla daha sık karşılaşılır. Bu tümörler köpeklerde yaygın olarak görülürken kedilerin daha az bildirilmiştir. Hiperplaziler özellikle yaşlı köpeklerde multiple kitleler şeklinde yüzeyden taşkın, noduler tarzda ve ülserli bir yüzeye sahiptir. Adenomlar köpeklerde daha çok baş bölgesinde, kedilerde ise sırt bölgesinde ve kuyrukta yerleşim gösterir. Malign tümörler ise daha çok multisentrik olarak yerleşim gösterirler. Dermiside içine alan kitleler bazen subkutan dokuya invazyonlar gösterebilirler. Tümörlerin kesit yüzleri solgun sarıdan beyaza kadar değişen renkte ve kalın bağ doku ile küçük lopçuklara ayrılmıştır. Sebaseöz kanallarda dilatasyon ve keratin bulunabilir. Mikroskobik olarak; bağ doku trabekülleri ile multiple lobüllere ayrılırlar. Gelişi güzel dizilmiş hücreler ovoid ya da veziküler çekirdekli, değişen miktarlarda eozinofilik sitoplazma olup bağımsız hücrelerle sınırlıdır. Lobullerin periferinde basofilik rezerv hücreleri, hiperkromatik çekirdek ve dar sitoplazmaya sahiptir. Neoplastik hücrelerde değişik oranlarda anizositosiz ve anizokaryosiz görülürken Nukleus büyük ve hiperkromatiktir. Hücresel nekroz ve mitotik figürler değişik oranlarda görülür. Sebositlerde mitotik aktivite gözlenmez. Malign tümörlerde cerrahi müdahaleleri takiben lokal infiltrasyonlar görülmüştür (Atasever & ark., 2005: 131) (Goldschmidt & ark., 1998) (Jakab, 2003: 36) (Madewell & Theilen, 1987: 238) (Scott & Anderson, 1990: 19) (Scott & Anderson, 1991: 16).

Meibomian Tümörler

Göz kapağının iç açısında yer alan bezlerden köken alan, çevresinden iyi sınırlı, kahverengimsi-siyah veya soluk kırmızı renkte, papillomatöz üremeler şeklinde görülen bu tümörler daha çok köpeklerde bildirilmiştir. Lezyonların yüzeyinde sekonder enfeksiyonların işe karışmasıyla ülserasyonlarla sık karşılaşılmaktadır. Sıklıkla tümörlerde meydana gelen travmalar sonucu bez salgısı kronik granulomatöz yangıya neden olur (Goldschmidt & ark., 1998) (Vail & Withrow, 1996: 167).

Apokrin Tümörler

Köpek ve kedilerin patilerinde yer alan bir tür terbezi olan apokrin bezlerinden köken alan tümörlerdir. Nadir olarak karşılaşılan ve daha çok kedi ve köpeklerin patilerinde yerleşim gösteren ter bezi tümörleri ayrıca baş, boyun, karın ve göğüste şekillenebilir. Tümör kitleleri deride noduler tarzda bazen subkutanöz olarak yerleşim gösterir. Kimi olgularda hafif dışa taşkın kitlelerin yüzeylerinde; tüylerde azalmaya, hiperemi ve ülserasyonlar gözlenir. Kesit yüzleri; içlerinde beyaz-açıkkahve renkli bir içerik bulunan kistler ve nekrozdan ibarettir. Histolojik olarak; fibröz stromayla sınırlandırılış adacıklardan ibaret olup tümör hücrelerinde hiperkromatik yuvarlak çekirdek ve belirgin çekirdekçik görülür. Mitotik figürler yaygın olarak görülür. Bazı tümör stromasında plazma hücresi ve seroid içeren makrofajlar görülebilir. Bu tümörlerin yayılımı oldukça farklılık gösterse de sekonder etkenlerin karıştığı tümörler daha hızlı yayılır ve metastazlar gösterebilirler (Chintimani & ark., 2003: 253) (Kalaher, Anderson, & Scott, 1990: 400) (Chun & ark., 1997: 33) (Weiss & Fezer, 1968: 249).

Kıl Folikül Tümörleri

Trikolemmom: Evcil türlerde nadir olarak karşılaşılan ve kıl foliküllerinden köken alan iyi huylu tümörler olup köpeklerde

bildirilmiştir. Deri altında yerleşim gösteren kitleler sert kıvamlı ve kapsülle çevrilidir. Mikroskobik olarak hücre adacıklarının çevresinde yer alan basal hücrelerin sitoplazmalarında biriken glikojenin varlığı diğer kıl folikül tümörlerinden ayrımının yapılmasında önemli bir bulgudur. Mikroskobik olarak, hücre adacıklarının iyi kopsülle sınırlandırılmış ve periferdeki hücrelerin çit tarzında dizildiği, bazı olgularda ise hücrelerin trabekül veya şeritler tarzında dizilim gösterdiği ve merkezinde kistiklerin olduğu yapılar görülür. Yer yer neoplastik hücrelerde melanin pigmenti görülebilir (Walsh & Corapi, 1986: 115) (Diters & Goldschmidt, 1983: 123) (Headington & French, 2002: 430) (Murphy & Elder, 1990: 216).

Trikoblastom: Kıl foliküllerinin germinative hücrelerinden köken alan iyi huylu tümörlerdir. Kedi ve köpeklerde yaygın olarak görülen bu tümörler atlarda ise ender karşılaşılır. Deri altında yerleşim gösteren kitleler 0.5-18 cm kadar değişebilen boyutlarda olabilir. Genellikle baş, boyun ve ön bacaklarda yerleşim gösterir. Kitle yüzeyinde zamanla ülserleşme ve kıllarda dökülmeler görülebilir. Hücreler; solgun eozonofilik sitoplazmalı, kenarları belirsiz ve belirgin çekirdeğe sahiptirler. Tümör histolojik olarak ribbon, medusoid, trabekuler, spindle ve granuler hücreli tip olmak üzere beş gruba ayrılır. Ribbon tipte, hücrelerin kurdela tarzı dizildikleri; Medusoid tipte, merkezden perifere doğru akar tarzda hücre diziliminin olduğu; Trabeküler tipte, lobullerin periferindeki hücreler çit tarzında dizildiği; Granuler hücreli tip tümörlerde ise neoplastik hücrelerin oluşturduğu adacıklar mevcuttur (Kazakov, Kempf, & Michal, 2004: 304) (Kanitakis, Brutzkus, Butnaru, & Claudy, 2002: 498) (Yu, Joo, & Cho, 2005: 6) (Usmani, Rofagha, & Hessel, 2002: 358) (Tronnier, 2001: 143).

Trikoepitelyom: Daha sık köpeklerde görülen kıl foliküllerinin proliferasyonu ile karakterize iyi huylu tümörlerdir. Sırt, boyun, göğüs ve kuyruk bölgesinde yerleşim gösterirler.

Genellikle birbirinden bağımsız dermise ve subkutan dokuya yerleşmiş kitleler şeklinde görülürler. Epidermal ülserasyonlar ve allopesi görülebilir. Mikroskobik olarak merkezinde keratin birikiminin olduğu neoplastik hücrelerden oluşmuş hücre adacıkları dikkati çeker. Hücreler, hiperkromatik çekirdekli ve eozonofilik sitoplazmalı olup çekirdek veziküler yapıdadır (Goldschmidt & Hendrick, 2002: 45) (Rofagha & ark., 2001: 663) (Abramo & ark., 1999: 479).

Pilomatrikom: Daha çok köpeklerde, ender olarak da kedilerde bildirilen bu tümörler genellikle baş, boyun, göğüs ve kuyrukta sert derialtı kitleler halinde görülür. Kesit yüzü lobuler yapılı, gri-beyaz bir görümdedir. Yer yer melanin pigmentine bağlı renk değişiklikleri bulunabilir. Lobulusların merkezinde dejeneratif hücrelerle birlikte distrofik kireçlenme ve lameller farklılıklar görülebilir. Ayrıca, buralarda çok çekirdekli dev hücreleri ile fibroblastlar dikkati çeker, lobullerin merkezinde şekilsiz eozonofilik materyal birikebilir (Masserdotti & Ubbiali, 2002: 22) (Rodriguez & ark., 1995: 247) (Forbis & Helwi, 1961: 606) (Thinakaran & ark., 1998: 769) (Unger & ark., 1990: 847).

Melanositik Tümörler

Dermis ve epidermiste yer alan melanositlerden köken alırlar. Bütün türlerde görülsede özelikle köpeklerde, atlarda ve domuzlarda yaygın olarak karşılaşılır. Köpeklerin ağız, kuyruk, skrotumda ve göz kapaklarında, gri donlu atların perineum ve kuyruk bölgesine, domuzlarda ise multisentrik olarak görülebilir. Farklı boyut ve şekillerde olup, taşıdıkları pigment (melanin) miktarına göre boz-beyaz renkten siyaha kadar değişen oranlarda farklı renklere sahiptirler. Tümör hücrelerinde bulunan pigment miktarına bağlı olarak tümörler melanotik ve amelanotik melanom olmak üzere iki sınıfta değerlendirilirler. Melanotik melanomlar fazla miktarda melanin pigmenti ihtiva eder ve siyah-kahve renkli

bir görünümdedirler. Amelanotik melanomlar ise daha az oranda pigment bulundururlar bu nedenle daha açık bir renktedirler. Tümörün rengi veya melanin miktarıyla malignite arasında herhangi bir bağıntı bulunmamaktadır. Fakat genel bir kanı olarak derinin kıllı bölgelerinde meydana gelen tümörler benign, mukokutanöz dokularda meydana gelenler ise malign olarak değerlendirilmektedir. Histolojik olarak, intrasitoplazmik ve hücrelerarası bölgede serbest halde kahverengi-siyah melanin pigmenti bulunur. Özellikle amelanotik melanomlarda pigmentin hücre sitoplazmasında görülmesi zordur. Bu amaçla melaninin varlığını belirlemek için farklı boyamalardan yararlanılır. Melanositik tümörler belirgin fibrovasküler stromaya sahiptirler. Neoplastik melanositler küçük kümeler halinde epidermisin bazal bölümünde dizilim gösterirler. Bu hücreler değişik oranlarda nüklear hiperkromaziye ve pleomorfizme sahiptir. Mitotik figür gözlenir. Benign tümörler yavaş invazyonlar yaparken, malign melanomlar beyin, kalp ve dalak gibi organlara metastaz yaparak ölümlere neden olabilir. (Bolon, Calderwood Mays, & Hall, 1990: 96) (Foley, Valentine, & Kincaid, 1991: 363) (Goldschmidt, 1994: 507) (Köküuslu, Kutsal, & Özdemir, 1990: 646) (Smith, Goldschmidt, & Mcmanus, 2002: 651) (Bottger, Dowden, & Kay, 1992: 548) (Kao, Helwig, & Graham, 1992: 2942).

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BÖLÜM 3

THE IMPORTANCE OF BOTULİSM IN VETERİNARY MEDİCİNE AND CURRENT DİAGNOSTİC METHODS

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Introduction

Botulism is a fatal disease characterised by paralysis of muscles caused by neurotoxins produced by Clostridium botulinum, Clostridium baratii and Clostridium butyricum. All mammals, birds, fish and humans are affected by the disease (Jones & al., 2022). Botulism is a neuro-paralytic intoxication caused by ingestion of toxins in water or feed (Smith & al., 2019). The disease is characterised by the paralysis of muscles, which is a result of the inhibition of acetylcholine in neuromuscular connections. Clostridium botulinum is a Gram-positive, obligate anaerobic, spore-bearing bacterium that produces neurotoxins. It is found worldwide and its spores are widespread in the environment, where they are resistant to various physical and chemical factors. These spores are able to survive in the environment for more than 30 years.

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These spores have the capacity to contaminate food, feed and water, and, under favourable anaerobic conditions, they transform into vegetative bacterial forms. It is during this phase that they are able to produce one or more toxins (Peck, Stringer & Carter, 2011).In addition to the formation of neurotoxins, endospore formation is important for proteolytic and non-proteolytic C. botulinum. Endospores are highly resistant and can remain viable after heat treatment. In order to enhance the control of Clostridial botulism neurotoxin formation (BoNT), it is imperative to comprehend the mechanisms of spore resistance and the physiological processes during the transition from spore to vegetative form. C. botulinum comprises seven or eight distinct types (A, B, C (C1 and C2), D, E, F, G), contingent on the antigenic properties of the BoNT. The neurotoxins in question are responsible for the manifestation of clinical symptoms and paralysis. The following toxins have been identified as the causative agents of botulism in humans: A, B, E and F. In contrast, C, D, their mosaic toxin forms C/D and D/C, have been observed to rarely cause type A and B disease in animals. The affected animal species include wild and domestic poultry, horses, cattle and certain fish species. The severity of the reaction to botulism neurotoxins varies between different animal species. Horses are the most susceptible animal, while in cattle, botulism can manifest in either an acute or chronic form. In sheep, the disease often manifests chronically, with a relatively low mortality rate. Dogs are less susceptible to the disease, and serotypes A and B rarely cause illness. Poultry are affected by serotype C, and to a lesser extent by types A and E. Type G has been isolated from soil but has not yet been reported to cause intoxication. Furthermore, BoNTs have been identified as significant biological weapons (Arnon & al., 2001; Lindström & al., 2010; Peng Chen & al., 2012; Woudstra & al., 2012; Anniballi & al., 2013).

BoNT antigenic types, ranging from A to G, are responsible for the proteolytic processing of SNARE proteins (e.g. SNAP-25 and synaptobrevin), which are integral to the release of acetylcholine in peripheral cholinergic neurons, resulting in flaccid paralysis and death. Currently, mouse experiments are regarded as the reference standard for the definitive diagnosis of the disease. Nevertheless,

there is a necessity to develop new diagnostic methods that are rapid, reliable and do not cause ethical problems (Ateş & al., 2021).

History

The earliest documented instances of BoNT can be traced back to the 1700s (Erbguth & Naumann, 1999), with the German physician and poet Justinus Kerner providing a notable account of cases of sausage poisoning in 1817. In 1822, Justinus Kerner recognised food intoxication leading to skeletal muscle paralysis and loss of parasympathetic function and suggested that the agent could be used in the treatment of disorders such as St Vitus dance, hypersalivation and hyperhidrosis (Erbguth & Naumann, 1999). Concurrently, a Russian physician described a similar clinical picture as fish poisoning (Kopera, 2011).

In 1870, the German physician Muller coined the term 'botulism' to describe this clinical picture, deriving it from the Latin word 'botulus', meaning 'sausage', as it developed after food intoxication due to sausage consumption (Erbguth, 2008). The causative agent and toxin were first identified by Van Ermengem in the immediate aftermath of a major outbreak in Belgium in 1895, which was attributed to the consumption of infected sausages (Erbguth & Naumann, 1999). Subsequent studies by Van Ermengem in 1897 identified *C. botulinum* bacteria as the causative agent of the disease, and demonstrated that this microorganism produces a toxin capable of causing muscle weakness in animals, similar to the human form of the disease (Caya *&* al., 2004; Kopera, 2011). In the subsequent years, type B was identified as the causative agent of outbreaks of poisoning with paralysis in Europe, affecting individuals who consumed sausages and other home-prepared foods (canned food) (Erbguth & Naumann, 1999). Type A was subsequently identified in 1904 (Erbguth & Naumann, 1999).

The first pure separation of the toxin was performed by Dr Herman Sommer in 1928. The crystallised form of BoNT was obtained from *C. botulinum* culture by Dr Schantz in 1946. Dr A. S. Burgen stated in 1949 that BoNT works by inhibiting the release of acetylcholine at the neuromuscular junction. Kerner was the first to suggest that the toxin could be used for therapeutic purposes. He

claimed that low doses of the toxin would reduce over-activity of the sympathetic nervous system. He also stated that it could be used to reduce hypersecretion of body fluids, sweat or mucus, and to treat ulcers in malignant diseases. These views of Kerner's have now been realised and Kerner has taken his place among the creators of modern botulinum therapy (Erbguth & Naumann, 1999; Dorner *&* al., 2013). In 1964, Dr Drachman from the Johns Hopkins University demonstrated that the injection of BoNT into muscles resulted in the development of paralysis. Based on these observations, in 1973, the ophthalmologist Dr Scott achieved positive outcomes in monkeys injected with BoNT into the eye muscles to correct strabismus. Subsequent to this publication by Dr Scott, the utilisation of BoNT for therapeutic purposes commenced. The FDA formally approved the use of BoNT for therapeutic purposes in humans in 1977 (Ting & Freiman, 2004; Truong *&* al., 2009). The employment of BoNT as a weapon of war emerged as a subject of discourse during the Second World War, with the United States of America (USA) developing gelatin capsules containing BoNT for utilisation as biological weapons (Dembek *&* al., 2007). However, in 1969, the USA made a unilateral declaration of the destruction of all its biological weapons stocks (Dembek *&* al., 2007). Consequently, the Biological and Toxin Weapons Convention Treaty was endorsed by over 100 countries in 1972, following which the USA closed all facilities dedicated to the production of biological weapons (Koirala & Basnet, 2004).

Etiology

C. botulinum is a sub-terminal spore-forming, motile, Gram-positive, obligate anaerobe, Gram-positive, obligate anaerobe, rod-shaped bacterium of the family *Clostridiaceae,* which is widespread in nature, especially in soil, seawater and agricultural products. The bacteria are 3-20 μm long, about 1 μm wide and 4 μm in diameter. All but serotype G BoNT-producing bacteria produce haemolysing R-type colonies when grown on blood medium. They do not ferment lactose and do not produce H2S (Murray *&* al., 2007).

The vegetative forms are not resistant to the external environment and their growth is inhibited below 4°C and at pH less

than 4.5. *C. botulinum* toxins are heat-sensitive, water-soluble, acid-resistant, high-molecular-weight proteins. BoNT is highly sensitive to environmental factors; while it loses its toxic properties in air within 12 hours, it becomes inactive in sunlight within 1-3 hours. While it is destroyed in a few seconds at boiling temperature, it becomes completely inactive in 5-10 minutes at 75-80°C. When treated with 3mg/l free chlorine in water, 99.7% is inactivated within 20 minutes. Toxins do not lose their activation at low pH (3.5 - 6.5) in growing cultures, but an alkaline environment eliminates the effect of the toxin. It is also reported that the toxin is inactivated by ultraviolet irradiation at a wavelength of 254 μm (Midura, 1996; Cheng *&* al., 2008).

C. botulinum is divided into 4 groups, I, II, III, IV, which are closely related to each other by their physiological, phenotypic and genetic characteristics, but distantly related to other groups. According to this distinction, all A types and proteolytic strains of groups B and F are in group I, all E types and non-proteolytic strains of groups B and F are in group II, C and D strains are in group III, G type and a new bacterium *C. argentinense* are in group IV (Hill *&* al., 2007; Grenda, Kukier & Kwiatek, 2014).

Although the neurotoxins produced by *C. botulinum* are serologically classified into 7 groups from A to G, another serotype, serotype H, was identified in 2013 (Espelund & Klaveness, 2014). Sequence analysis of the toxin-encoding region later showed that this new serotype is a hybrid of serotype A and serotype F (Maslanka *&* al., 2015). Furthermore, these 7 serotypes are subdivided into numerous subtypes, 32 of which are associated with human intoxications. It has also been reported that there are approximately 40 different genetic variants of BoNT. (Kull *&* al. 2015; Maslanka *&* al., 2015).

BoNT serotype F subtype 7, secreted by *C. baratii*, is a type of botulism caused by food or small intestinal colonisation, although rare sequence analysis has found the ORFX gene to be present and similar to the *C. perfringens* uviAB operon gene (Dover *&* al., 2014).

BoNT serotype E subtypes 4 and 5 secreted by *C. butyricum* cause infant botulism and are isolated from soil, animal and human

faeces. Sequence analysis indicates that *C. butyricum* contains the bont/E and ORFX genes (Macdonald *&* al., 2011). Neurotoxin-producing *Clostridium* species are listed in Table 1.

Table 1: Neurotoxin-producing bacteria

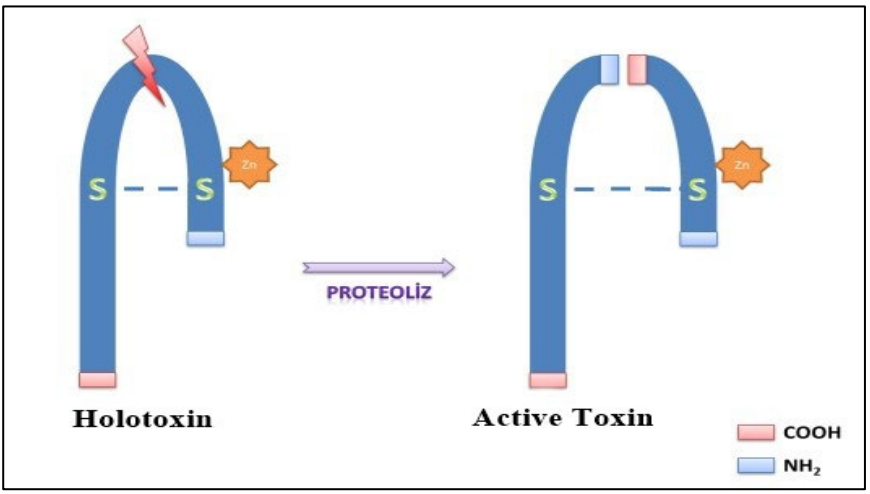
Bacteria	**Group**	**Serotype**
C. botulinum	I	A, B, F
	II	E, B, F
	III	C, D
C. argentinense	IV	G
C. baratii	V	F
C. butyricum	VI	E

Reference: (Hobbs & al., 2019)

BoNT is synthesised as a 150-kDa holotoxin and subsequently cleaved by a bacterial trypsin-like protease into two chains of 50-kDa and 100-kDa, linked by disulfide bonds (Kull *&* al., 2015).

Upon reaching the neuromuscular junction, BoNT binds to polysialo-gangliosides on the surface of motor neurons via the binding site on its heavy chain (Smith *&* al., 2022). It then interacts with protein receptors and is taken into the cell via endocytosis in vesicles (Jones *&* al., 2019). With the translocation site at the N-terminal end of the heavy chain, BoNT is transferred from vesicles to the cytosol (Brown *&* al., 2021). The structure of BoNT is shown in Figure 1.

Figure 1: Structure of BoNT



Reference: (Abdelkareem, 2015)

Cell-important SNARE proteins are responsible for the uptake and exocytosis of neurotransmitters into vesicles on the inner surface of the plasma membrane. Different BoNT serotypes cleave different neuronal substrate molecules. As a result, acetylcholine release into the synaptic cleft is inhibited and reversible muscle paralysis occurs (Jankovic, 2004; Shukla & Sharma, 2005).

Botulinum toxins are composed of a 150-kDa upright chain molecule, which consists of a 100-kDa heavy chain (responsible for membrane targeting and binding to target cells) linked by a disulfide bond to a 50-kDa light chain (responsible for toxic damage to the nerve).

The binding of the botulinum toxin molecule to the neuron occurs via receptors, with subsequent entry into the cell via endocytosis. The endocytotic vesicle is acidified, causing the 50kDa light chain to separate from the 100kDa heavy chain. The 50kDa light chain then escapes from the endosome by pH-dependent translocation and is transported into the cytoplasm of the neuron. Once in the cytoplasm of the neuron, the 50kDa light chain acts as a zinc-dependent metalloprotease, cleaving and inactivating vital intracellular docking proteins that are important for the release of acetylcholine at the neuromuscular junction. These critical docking

proteins are located on the synaptic vesicle containing the neurotransmitter acetylcholine or on the cell membrane at the neuromuscular junction. These docking proteins include synaptobrevin-2 on the synaptic vesicle and syntaxin-lA and SNAP (synaptosomal-associated protein)-25 on the neuromembrane at the neuromuscular junction. Botulinum toxins B, D, G and F cleave specific sites on synaptobrevin-2. In addition, SNAP-25 is cleaved by botulinum toxins A, C1 and E, while syntaxin lA is cleaved by botulinum toxin C1 (Lalli *&* al., 1999; Coffield, 2003).

Pathogenesis

Typically, botulism results from the ingestion of feed contaminated with a pre-formed toxin. The bacterial spores of the organism are widespread in all soils and have the potential to contaminate most plant material. An anaerobic environment is required for the bacteria to multiply and produce toxins. Ingestion of spoiled hay or silage is a common source of botulinum toxin type B. Wet, cool spring weather is thought to prevent rapid fermentation of silage, causing the pH to rise. This can lead to the growth of *C. botulinum* spores and toxin production (Notermans *&* al., 1981; Divers *&* al., 1986).

Ingestion of dead animals accidentally collected in hay and silage, or ingestion of poultry litter containing dead bird parts, are common sources of botulinum toxin type C poisoning (Galey *&* al., 2000).

Botulinum toxin type D poisoning is prevalent in areas where phosphorus is deficient, where the ingestion of contaminated bones is common. There is evidence that the toxin can be present in the bone marrow for years (Martin, 2003).

Ingestion of contaminated water from shallow, warm, nutrient-rich pools has also been identified as a source of poisoning. Wound infections can also lead to botulinum poisoning, although this is rare in ruminants and is more frequently observed in horses. In cattle, the onset of botulism occurs subsequent to the ingestion of a preformed toxin, which is absorbed by the intestinal tract and disseminated to the nervous system via the bloodstream. The toxin

enters primarily via cholinergic neurones and acts at the neuromuscular junction of skeletal muscle by inhibiting the release of the neurotransmitter acetylcholine. The resultant weakness and paralysis are the consequence of this inhibition. The outcome of botulism in cattle is typically fatal, due to paralysis of the diaphragm muscles, which results in respiratory arrest (Lalli *&* al., 1999; Hatheway, 2018).

Botulism in Veterinary Medicine

The toxins produced by *C. botulinum* have been shown to cause toxemia in humans and animals. Serotypes C and D are the types that are responsible for the majority of cases of disease in most animals. In Europe, the commercial poultry sector has become a focal point for concern regarding botulism, as the disease has been observed in both egg and meat production. The contamination of poultry with BoNT occurs through the ingestion of invertebrates, leading to the accumulation of BoNT levels in the bloodstream and subsequent manifestation of symptoms associated with paralysis (Sato *&* al., 2016). The disease has also been observed in cattle. Canines, on the other hand, are more likely to be affected by eating rotten food or infected meat (Lamoureux *&* al., 2015). However, horses are typically affected by serotype B and acquire the disease in adults by consuming pre-formed toxins in their feed, a process analogous to human foodborne botulism. The disease course is associated with overall toxin exposure, and often fatal outcomes occur before timely treatment with the correct antitoxin therapy (Johnson *&* al., 2016).

Foodborne Botulism: Foodborne botulism represents the predominant form of poisoning caused by the production of toxins in foodstuffs by *C. botulinum* prior to ingestion. The bacterium is classified as a Gram-positive anaerobic bacterium (Lindström & Korkeala, 2006). The bacterium's growth and the formation of BoNT are especially prevalent in products with reduced oxygen content, under certain temperatures of both packaging and storage, and in the presence of certain preservative factors (Thirunavukkarasu *&* al., 2018). Notable instances of this phenomenon include home-prepared products such as dried or salted meats, canned products, and

fermented fish. These poisonings typically manifest as sporadic events, affecting multiple individuals within a household, with symptoms generally emerging within 12-72 hours following toxin ingestion. In rare instances, commercially prepared foods may also be implicated (Lindström & Korkeala, 2006).

C. botulinum bacteria have a predilection for non-acidic environments (pH>4.6). Consequently, the production of BoNT is not observed in acidic foods. A product with a low pH value, BoNT formed before the onset of acidic conditions is not capable of degradation. In order to inhibit and prevent bacterial growth and BoNT production, it is necessary to alter the pH value, as well as various other parameters, such as salt concentration and storage conditions (Peck, 2006). The mortality rate from foodborne botulism in developed countries is 5-10%. In the event of a BoNT outbreak, it is imperative that pre-production product samples are collected immediately, stored correctly and sent to the laboratory for analysis. This approach facilitates rapid identification of the contamination source, expedites problem resolution, and effectively concludes the outbreak (Lindström & Korkeala, 2006).

Botulism in Cattle: Bovine botulism is predominantly caused by BoNT/C, D, C/D and D/C, although sporadic cases of type A and B have been reported. Presently, BoNT/D/C is the most prevalent type of BoNT implicated in outbreaks of bovine botulism on a global scale. The ingestion of pre-formed BoNTs present in feed, water or any other source of toxin is thought to result in the onset of bovine botulism. Animal carcasses, poultry manure and improperly stored feed provide favourable conditions for the growth of *C. botulinum* and production of BoNTs (Le Maréchal *&* al., 2019). The epidemiological cycle of *C. botulinum* in cattle is presented in figure 2.

The disease known as bovine botulism is characterised by a range of symptoms, including paralysis. In most cases, the onset of this condition is characterised by paralysis of the tail and hind legs, progressing subsequently to the head. The incubation period for the disease is variable, ranging from a few hours to 2 weeks. The disease manifests in three distinct forms: a peracute form, characterised by

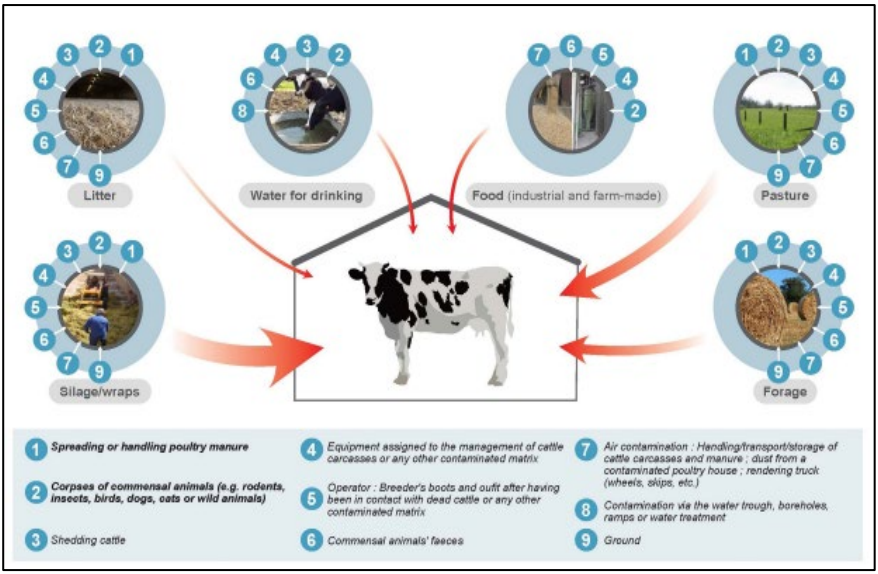
sudden side-lying and death within a few hours; an acute form, exhibiting typical clinical signs (anorexia, ataxia, apathy, weakness, dysphagia, increased salivation, paralysis, compression of the head to the kidneys and dropping of feed from the mouth), with a mortality rate of 2-3 days following the onset of symptoms; and a subacute form, presenting with attenuated clinical signs, from which animals can occasionally recover (predominantly observed in type C outbreaks). Substantial economic losses are frequently associated with bovine botulism on cattle farms. Key elements in the differential diagnosis of such cases include hypocalcaemia, hypophosphataemia, various forms of enterotoxaemia, listeriosis, paralytic rabies, and poisoning by organophosphates or lead (Kummel *&* al., 2012).

The diagnosis of the condition is based on clinical signs and epidemiological association, with a particular emphasis on the suspicion of high-risk sources, such as poultry manure, or the detection of a dead animal in feed. As with other species, the diagnosis should be confirmed by the detection of BoNT or BoNT-producing *Clostridia* in samples from animals or indoor environments. Analysis of multiple samples obtained from diverse animals may be necessary to ascertain the presence of BoNT or BoNT-producing *Clostridia*, thereby confirming the diagnosis. While there is currently no curative treatment for bovine botulism, antitoxins can be used successfully; however, their cost is not financially viable for most farms (Carolina C. Guizelini *&* al., 2019).

Vaccines that target BoNTs have been developed. A plethora of vaccines have been developed worldwide (Anniballi *&* al., 2013). In order to prevent outbreaks of bovine botulism, it is imperative to implement biosecurity measures when managing farms, producing feed and storing it. The presence of animal carcasses in feed (silage, hay, grain, etc.) and especially in feed stored in suboptimal conditions can serve as a potential source for outbreaks. It is imperative to exercise meticulous vigilance at all stages, from feed harvesting to animal distribution. The implementation of specific biosecurity measures between poultry and cattle is essential to prevent cross-contamination between the two production sites.

Equipment must undergo a thorough cleaning and disinfection process (Meurens & al., 2023).

Figure 2: Epidemiological cycle of C. botulinum in cattle



Reference: (Meurens & al., 2023)

Botulism in Poultry: Botulism is a prevalent disease among both wild and domestic avian species. On a global scale, avian botulism is considered the most significant disease affecting waterfowl. Some species, especially scavengers such as vultures, are known to be resistant (Smart & al., 1983). Experimental studies have demonstrated the susceptibility of various avian species to all types of BoNTs when administered intravenously (Miyazaki & Sakaguchi, 1978). However, differences in susceptibility have been observed among different species, and the impact of dosage also varies (Miyazaki & Sakaguchi, 1978). The epidemiological cycle of *C. botulinum* in poultry is illustrated in figure 3.

In the context of wildlife and farm environments, the only BoNTs that have been implicated in outbreaks of avian botulism are BoNT/C, D, C/D, C/D, D/C and E (Rogers & al., 2021). In contrast, BoNT/A has been observed to occur with much less frequency.

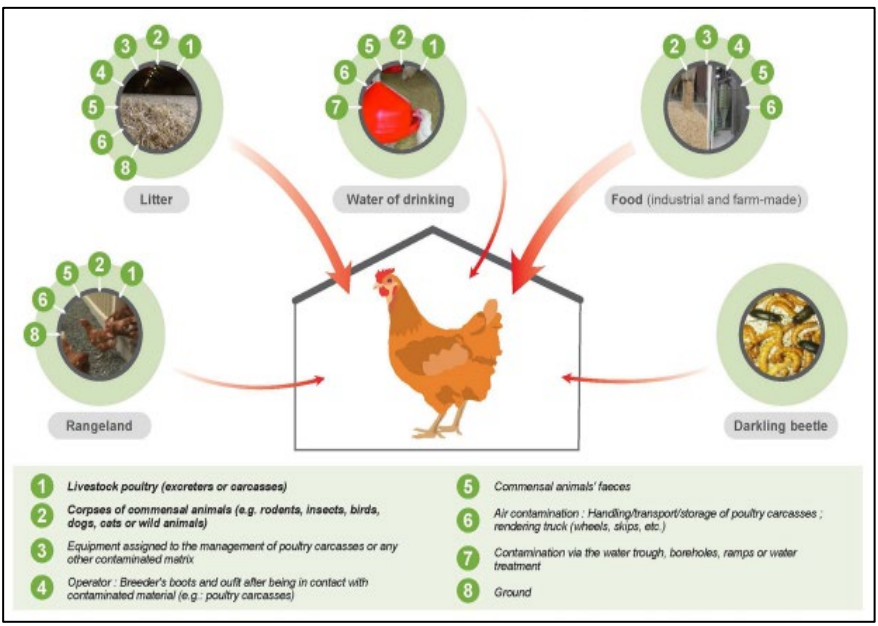
The aetiology of poultry botulism remains to be fully elucidated. The consumption of BoNTs or in situ BoNT production by *C. botulinum* has been postulated as the causative agent, with both sources of BoNTs potentially being present during an outbreak (Skarin & al., 2015). In the context of broiler chickens, it is hypothesised that BoNT is produced in situ in the secum, as a substantial amount of toxin is required for the manifestation of clinical signs in this particular bird species. It is hypothesised that the amount of BoNT typically present in the immediate vicinity of a flock is insufficient to cause botulism. Experimental studies have confirmed the importance of the secum in the development of the disease. The ingestion of spores by birds results in the subsequent development of the disease, characterised by the production of BoNT within the secum. The spores, vegetative cells and BoNTs are then excreted in faeces and reacquired by birds through coprophagy. The toxin then enters the circulatory system and reaches nerve endings, causing paralysis (Miyazaki & Sakaguchi, 1978).

The variability in the prevalence of poultry botulism can be explained by the presence of a very low level of *C. botulinum* carriage, given the low sensitivity of current methods in the samples tested. The manifestation of clinical signs in poultry species is characterised by flaccid paralysis, progressing from the legs to the nictitating membranes, and frequently resulting in respiratory failure. In the context of broiler flocks, the initial manifestation of the disease is characterised by leg paralysis, which is then followed by wing paralysis (Bano & al., 2013). A hallmark of the disease is paralysis of the animal's neck, hence the term 'flexible neck' has been coined to describe the condition (Bano & al., 2013). High mortality rates have been reported. It is noteworthy that avian botulism is recognised as the most significant disease affecting waterfowl worldwide (Meurens & al., 2023).

The differential diagnosis encompasses a range of etiologies, including ionophore poisoning, particularly in turkeys, phosphate and selenium poisoning, Marek's disease, and avian influenza. The diagnosis is made on the basis of clinical signs, epidemiologic correlation, and laboratory results. The therapeutic efficacy of beta-lactams in combating botulism outbreaks on poultry farms has been

well-documented (Anniballi & al., 2013). The implementation of biosecurity measures in poultry farms is imperative to prevent the onset and recurrence of outbreaks. These measures encompass rodent control, optimal storage conditions for feed, effective feed distribution, and regular disposal of deceased birds. Given the high resistance of spores in the environment, meticulous cleaning and disinfection with sporicides and disinfectants is imperative following a botulism outbreak, encompassing both the barn and equipment. In the context of wild birds, the most effective measure to prevent or mitigate outbreaks is the removal and proper disposal of dead birds (Meurens & al., 2023).

Figure 3: Epidemiological cycle of C. botulinum in poultry



Reference: (Meurens & al., 2023)

Botulism in Horses: Clinically, equine botulism is characterised by a gradually progressive myasthenia, initially manifested as mild dysphagia, decreased tone and strength of the tongue muscles, mild mydriasis and decreased tail tone. This condition eventually causes the animal to assume a supine position. The rate of progression of the clinical signs of botulism depends on the dose of toxin. The

consumption of large quantities of toxin can result in death within 24 hours, whereas ingestion of small doses can take 10-15 days before the onset of clinical signs. In typical field cases, signs of dysphagia and weakness are observed for 2-3 days after the first detection of clinical signs, and the animal collapses within 3-4 days. In rare cases, a definitive diagnosis may be challenging. Treatment regimens are based on the administration of botulism antitoxin in combination with supportive therapy, which requires the provision of oral fluids and nutrition. When treated with antitoxin, the prognosis for recovery is favourable if the horse can stand. Vaccination with type B botulinum toxoid has been demonstrated to provide excellent protection (Whitlock & McAdams, 2006).

Botulism in Fur Animals: Fur animal botulism outbreaks are typically attributed to type C toxins; however, instances associated with types A and E have been documented on occasion. Botulism is widely regarded as a significant hazard in the context of fur animal production. The most affected species are fur animals such as minks, foxes and ferrets. Large-scale outbreaks have also been reported. For instance, in 2002, 52,000 foxes raised in 83 production farms in Finland were affected (Le Maréchal, Woudstra & Fach, 2016).

Botulism in Fishes: Fish exhibit sensitivity to the C. botulinum type E toxin. The median lethal dose (MLD) for silver salmon is 90 MLD. BoNT E has been identified as the causative agent of visceral toxicosis in carp. This visceral toxicosis has been responsible for significant economic losses in the carp industry, with some studies reporting losses of up to 37.8% of total production in certain years. However, the prevalence of this disease in fish farms, and its impact on fish populations, remains the subject of limited data. However, type E spores are regularly consumed and digested by healthy fish (Le Maréchal, Woudstra & Fach, 2016). The following table 2 provides a detailed overview of the different types of toxins that are responsible for the onset of botulism in various animal species.

Table 2: Toxin types causing botulism in animals

Animal Species	Toxin types	Degrees
Cattle	D, D/C	++++
	C, C/D	++
	A, B	+
Bird	C, C/D	++++
	E, D	++
	A	+
Horse	C, D	+++
	B	+
	A	+
Fur animals	C, C/D	++++
	A, E	+
Fish	E	?

Reference: (Le Maréchal, Woudstra & Fach, 2016)

Diagnosis

The rapid course of the disease, the extreme toxicity of BoNTs and the lack of any treatment for the paralysing condition require a rapid BoNT detection method that is compatible with food and environmental samples. There are some applications for reliable detection of BoNT beyond clinical diagnostics (Čapek & Dickerson, 2010; Singh, Stanker & Sharma, 2013).

As mentioned earlier, BoNTs pose a significant bioterrorism threat and there is a need for detection methods that are rapid, sensitive, easy to use and potentially usable in the event of an attack (Janik & al., 2019).

Mouse Experiment: The mouse assay is one of the most widely utilised tests for the confirmation of active BoNT levels

(Thirunavukkarasu & al., 2018). The assay is performed by injecting samples of suspected toxin into the peritoneum of mice. The mice are then observed for any signs or symptoms of disease, which can include hind limb paralysis, dyspnoea and, ultimately, death by respiratory paralysis (Cheng & al., 2008). This typically manifests within 48 hours post-injection (Čapek & Dickerson, 2010). Determining the amount of BoNT in a sample is crucial, and this is achieved by measuring both the maximum sample dilution that results in mouse death and the minimum dilution that does not cause mouse death (Solomon & Lilly Jr, 2001). The serotype of the toxin is determined by the administration of serotype-specific antitoxins prior to the injection of the sample. Mice are then observed for a further 48 hours to ascertain whether the sample contains a serotype-specific antitoxin capable of conferring protection. The entire process, including the administration of antitoxins and the observation period, must be completed within a 4-6 day period. This test is regarded as highly sensitive, with the capacity to detect as little as 10 pg/mL of toxin, in addition to identifying functionally active toxins (Singh, Stanker & Sharma, 2013). However, it is important to note that the test is associated with certain disadvantages, including cost, ethical considerations related to the use of live animals, and the time required for its completion (Rasooly & Do, 2008).

Elisa: ELISA (enzyme-linked immunosorbent assay) is a biochemical process that uses antibody-conjugated enzymes to detect the presence of a specific antigen (Asensio & al., 2008). ELISA has a wide range of applications and is frequently used in the medical field to diagnose various diseases, as well as a quality control tool to test for cross-contamination in food production (Jones & al., 2019). The three most common forms of ELISA are direct, indirect and sandwich (Ng & al., 2010).

The detection limit of ELISA ranges from 2 pg/mL to 2 ng/mL, with a typical test time of 5-6 hours (Singh, Stanker & Sharma, 2013). The presence of NAP (neurotoxin-associated proteins) has been shown to hinder the detection of BoNT complexes in immunological tests. This NAP complex obstructs the antigenic sites of the toxin, rendering them inaccessible for binding with antibodies. Consequently, the development of numerous assays has

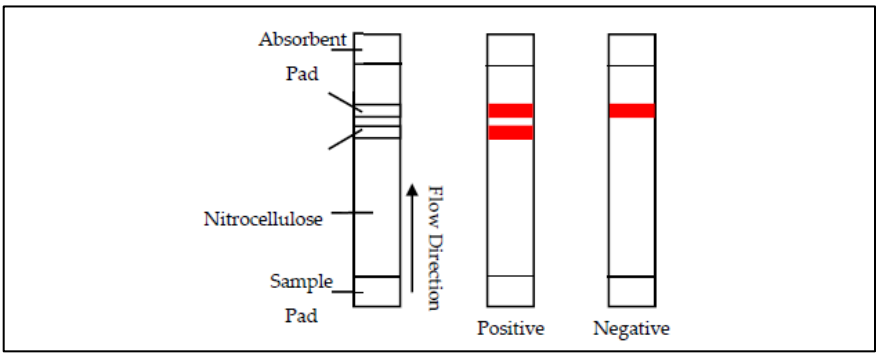
been facilitated by the utilisation of highly purified BoNT samples (Szílagyi & al., 2000). Consequently, numerous research groups have emerged, with a focus on the development, characterisation and screening of novel antibodies capable of binding to free epitopes (Stanker & al., 2008). This endeavour aims to circumvent the challenge posed by NAPs, thereby facilitating more precise and effective diagnostic methods.

In an effort to investigate bovine botulism, endeavours have been made to develop ELISAs against type C and D *C. botulinum* toxins. Partially purified type C and D toxins were utilised as antigens in the development of these ELISAs. The specificity of the ELISAs was evaluated on sera from 333 adult beef and dairy cattle in areas with no history of botulism in animals. The test was also evaluated on sera from 41 herds, including herds vaccinated against botulism, confirmed cases of botulism and herds from areas where the disease is considered endemic. The ELISAs were capable of detecting the presence of antibodies to botulinum toxins from vaccinated cattle and animals in recovery, as well as from clinically normal animals from unvaccinated herds that had experienced botulism outbreaks. Furthermore, antibodies have been identified in unvaccinated animals from herds devoid of botulism cases within areas where botulism has been diagnosed. Furthermore, sera from some unvaccinated cattle with high ELISA reactivity have shown to have a protective effect in botulinum toxin neutralisation tests in mice (Gregory & al., 1996).

Immunochromatographic Assay: Lateral flow tests (LFTs) are a type of handheld test most commonly known as pregnancy tests. In the context of detecting BoNT, these tests employ antibodies conjugated to colloidal gold, resulting in a colour response in the presence of the toxin (Ching & al., 2012). The sample is added to a sample pad where any toxin in the sample binds to the antibodies on the colloidal gold. Thereafter, the conjugate migrates along the nitrosellulose strip to capture antibodies and bind if positive, thereby producing a colour response. The excess conjugate then travels towards the control antibodies, where it binds and produces a second colour response, indicating that the sample has migrated correctly.

The operational principle of the lateral flow test is illustrated in figure 4.

Figure 4: Lateral flow test diagram



Reference: (Hobbs & al., 2019)

In comparison with alternative detection methodologies, LFA boasts a number of key advantages, including its cost-effectiveness, the minimal equipment and expertise requirements, and its extremely rapid response time. These advantages make it an ideal candidate for field use. However, it is important to note that LFA has comparatively low sensitivity levels when compared to ELISA and similar immunological methods. When employing a detection antibody conjugated with gold nanoparticles, the standard detection limit range is between 5 and 50 ng/mL (Gessler & al., 2007). Increased sensitivity was observed when mustard peroxidase or silver enhancement was used. This increased sensitivity was observed up to a concentration of 50 pg/mL (Chiao & al., 2004). The inability of LFA to discriminate between active and denatured toxins is a significant drawback, arising from the utilisation of antibodies, as observed in ELISA methods (Ching & al., 2012).

The LFA system has been the subject of studies that have demonstrated increased sensitivity. Their system was able to detect levels as low as 20pg/mL for BoNT/A using a very small sample size (1 µL). This enhancement was achieved by reconstructing lateral flow strips utilising gold nanoparticles and specific substrate peptides, integrating endopeptidase activity with the assay. It is suggested that this technology can be extended with more specific

substrate peptides designed for other BoNT serotypes. This adaptation is imperative for the method to be embraced within the diagnostic and food testing domains. However, the methodology is encumbered by two significant disadvantages: the time-consuming nature of the improved system, which requires approximately 12 hours for analysis, and the resultant increase in cost due to the integration of the endopeptidase activity assay (Liu & al., 2017).

Polimeraz Chain Reaction (PCR): Immuno-PCR is an ELISA-type immunoassay that employs PCR to amplify the ELISA signal. The detection method is based on the formation of antigen and antibody complexes. A distinctive feature of Immuno-PCR is that it utilises the formation of an antigen-antibody complex and binds with known DNA molecules instead of the enzyme format that is normally used. Following complex formation, the amplification of DNA fragments bound to the BoNT specific antibody can be readily achieved using conventional or qPCR (Real Time PCR) (Čapek & Dickerson, 2010). Immuno-PCR has been employed to detect botulinum neurotoxin serotype A, exhibiting comparable sensitivity levels to those associated with MBA. The ability to determine levels of active toxin significantly increases the potential to replace MBA in the pharmaceutical manufacturing sector (Wu & al., 2001). The employment of biotinylated modified DNA tags and streptavidin as a bridging molecule to bind antibodies has been documented to achieve a sensitivity of 1 pg/mL relative to serotype A (Chao & al., 2004).

In the liposome-PCR assay, approximately 60 copies of the reported DNA are encapsulated in a liposome. Furthermore, the outer surface of the liposome is labelled with a special molecule. This molecule is typically trisialoganglioside for BoNT binding. The modified surface of these loaded lipid-based vesicles can be bound with a complex consisting of the capture antibody and the corresponding toxin. This is followed by the degradation of the vesicles and qPCR of the released DNA. This method has been employed to detect BoNT/A in purified water, with levels as low as 20 pg/mL being detected (Mason & al., 2006). This assay demonstrates a high level of sensitivity, approximately 100,000 times lower than the mouse assay. However, it should be noted that

this particular detection method has not been used and validated in other settings, such as clinical or food samples. This may prevent it from having wide applications in the diagnostic and food testing sectors. It is noteworthy that both immuno-PCR and L-PCR tests require approximately nine hours for completion (Johnson & al., 2016).

Real-time PCR has been developed for the qualitative or quantitative detection of BoNT-producing organisms or toxin genes. These tests, which can provide critical information within 4 hours using automated extraction platforms, can be used with a variety of clinical and food matrices. The potential for rapid results and serotyping makes real-time PCR a useful screening tool in foodborne outbreaks or a bioterrorism event. Furthermore, the same assays have the potential to rapidly screen for toxin genes in situations where patients present with atypical symptoms. A multiplex real-time PCR assay has been developed that distinguishes *Clostridium* groups I and II, which are mainly associated with human botulism, as well as genes associated with toxin production (Centurioni & al., 2022).

Biosensors: Biosensor technologies encompass a broad spectrum of BoNT detection methodologies, with the predominant platforms comprising SPR (surface plasmon resonance), refractometry, fluorescence, and chemical luminescence. Evidence suggests that evanescent wave technology is most commonly used for biosensors that rely on fluorescence. In this method, fluorophores are attached to the molecules, which, upon exposure to the evanescent fields of the biosensor, produce a signal when bound to the surface. The immuno-sandwich assay is frequently employed as the assay of choice in this context, comprising the analyte of interest, two antibodies for immobilisation and quantification, and all of these components are then located on the sensor surface. Utilising this system, the sensitivities of the sensors tested range from 150 pg/mL to 200 ng/mL for botulinum neurotoxin serotypes E and B, respectively. It is important to note that these results are typically obtained from analyte samples in simplified buffer systems, which limits their wide acceptance in diagnostic and food testing fields. However, it has been reported that serotype A detection can reach a

LoD of approximately 50 ng/mL even in more complex matrices such as food samples (Rowe-Taitt & al., 2000; Sapsford & al., 2005).

The capacity of aptamer oligonucleotide fragments to furnish protein-specific binding confers a number of advantages over the utilisation of antibodies, including a more straightforward screening method, enhanced stability, and sustainable use. This technique combines an electrochemical approach that integrates enzymatic amplification with an aptamer probe and uses an aptamer probe, typically 70-80 bp in length. This undergoes a structural change from its standard conformation upon binding with the toxin present in the sample. The aptamer is then labelled with both biotin and fluorescence. Upon binding to the toxin, the structural change of the aptamer elicits a conformational unfolding, leading to the unfolding of the fluorescent reporting tag, which in turn causes an electrical response to be generated. Consequently, only a specific toxin can generate an augmented current response. The LOD of this aptamer-based system has been measured at 40 pg/mL (BoNT/A), a figure comparable to the sensitivities seen in conventional ELISA, yet it is unable to determine levels of active toxin, a potential hindrance to its application in pharmaceutical manufacturing tests (Wei & Ho, 2009).

Typically, biosensor-based platforms are completed within 20 minutes and, depending on the sensor design, are capable of detecting multiple analytes simultaneously. The rapidity with which results are obtained renders biosensors among the most expeditious available platforms (Eivazzadeh-Keihan & al., 2018). Increased sensitivity has been demonstrated with the recently developed Newton Photonics SPR biosensor. This biosensor has been shown to have a LOD of 6.76 pg/mL (BoNT/A light chain), thus enabling active toxin measurement in the pharmaceutical manufacturing sector. The SPR method has a detection time of less than 20 minutes (Patel & al., 2017).

Fluorescence Resonance Energy Transfer Test (Fret): FRET is a technique used to measure the proximity between molecules and their interactions. This methodology is advantageous in the study of numerous biological phenomena, including protein-protein

interactions, nucleic acid hybridisation, and protein conformation changes. The method offers a wide range of research areas, which are used to determine the position, quantity and interactions of a molecule. In the context of enzyme endopeptidase activity detection, the observable change in fluorescence of a substrate is frequently utilised. In FRET experiments, an oligopeptide that mimics a native substrate is utilised, and the cleavage site of this substrate bears two labels on either side. One of these is known as a 'fluorescence quencher', whilst the other is a 'fluorescence donor'. The occurrence of FRET is contingent upon the proximity of the two tags to each other, thereby facilitating the transfer of fluorescence energy from the donor to the quencher (Guo & al., 2014).

Subsequent to the cleavage of the substrate by the toxin, the labels are detached, thereby obstructing energy transfer (Singh, Stanker & Sharma, 2013). The decline in FRET is directly proportional to the toxin concentration. The LOD of FRET-based platforms is contingent upon the substrate incorporated into the assay, though it typically ranges from 40 ng to 60 pg/mL (Čapek & Dickerson, 2010).The analysis speed through FRET is approximately three hours on average, although Guo & al. (2014) have documented a more efficient analysis time of two hours with BoNT/B. The assay's sensitivity is approximately 100,000 times more sensitive than MBA and can detect down to 1 fg/mL. The assay's workflow involves initial toxin capture using toxin-specific antibodies bound to microbeads during the immunoseparation step. These microbeads are then resuspended in endopeptidase reaction buffer, which contains the synthetic FRET substrate. This allows for the determination of active toxin levels. This approach is critical for determining active toxin levels, which should be prioritized over MBA in the pharmaceutical manufacturing field (Bagramyan & al., 2008).

Flow Cytometry: Flow cytometry is a technique that can be used to analyse and quantify toxins using fluorescent assays. Through the application of fluorescent magnetic beads, a multiple assay was developed to test BoNT/A and B, as well as ricin, abrin and *Staphylococcus* enterotoxin B in various food matrices. The assay was able to detect toxin levels of 21 pg/mL and 73 pg/mL,

respectively (Pauly & al., 2009). This assay was improved by automating the sampling process. The process commences with the placement of magnetic beads that have been coupled with a capture antibody within the flow chamber. In this chamber, the toxin is initially captured from the test matrix, followed by a washing step to remove any unbound toxins. Subsequently, the captured toxins are then bound by the antibodies. The final stage of the process involves analysis by flow cytometry. The sensitivity limit of the method is set at 50 pg/mL in relation to the heavy chain fragment of BoNT/A. This enables the quantification of the active toxin levels, with an analysis time of 4 hours. This feature of the method is a key component of the ability of a backup method to be used in the pharmaceutical industry (Ozanich Jr & al., 2009).

Flow cytometry tests boast a number of advantages over ELISA. The capacity to detect multiple toxins or botulinum serotypes in a single sample is of critical importance for the widespread use of a detection technique in diagnostics and food testing, and they are more amenable to automation. Immobilising the toxin on beads has advantages over a microtiter plate, such as better capture kinetics and analyte concentration. This detection method has been tested in other media and shown to be suitable for BoNT detection in a wide range of product matrices. However, it should be noted that flow cytometry does require instrumentation that is more expensive than ELISA or MBA (Ozanich Jr & al., 2009; Pauly & al., 2009).

Fluorescent Endopeptidase Assay: The fluorescent endopeptidase assay is a laboratory test that is used to measure the activity of a specific enzyme in a sample. This assay involves the use of a fluorescent substrate, which is cleaved by the enzyme under test, resulting in products that become fluorescent. The degree of fluorescence is proportional to the activity of the enzyme, and the assay can be used to detect even small quantities of enzyme. Botulinum toxin is an endopeptidase enzyme. The assay involves the mixture of a sample of botulinum toxin with a fluorescent substrate, resulting in the cleavage of the substrate by the enzyme and the subsequent emission of fluorescent products. The intensity of the resulting fluorescence is directly proportional to the enzyme activity.

The assay's primary application is in the detection of botulinum toxin.

The investigation of fluorescent endopeptidase tests against various BoNT serotypes has revealed the potential of these assays. One notable example involves the formulation of peptides labelled with a fluorescent tag, which are serotype-specific and immobilised on a solid substrate. This method's ability to recognise multiple serotypes is of particular importance in the diagnostic and food testing sectors (Schmidt & al., 2001). This assay has also been developed in a semi-automated microfluidic format using digestion of the same fluorescently labelled peptide (Frisk & al., 2009).

Centrifugal Microfluidic Technology: Centrifugal microfluidic technology is a technique that facilitates the separation of components through the utilisation of rotating liquids within small samples at elevated speeds. This technology is frequently employed in the separation of bacteria and other microorganisms in biological samples. The centrifugal microfluidic assay offered by SpinDx represents a suitable option for both laboratory and field detection of BoNTs (Koh & al., 2015).

The suspected sample is then mixed with a detection solution comprising BoNT-specific antibodies, surface-functionalised capture beads and fluorescence-labelled detection antibodies. These antibodies immobilise to the functionalised beads upon exposure to the equivalent antigen. This process is conducted at room temperature; however, a recent instrument modification allows for temperature variations due to the incorporation of a heating element.The mixture is then applied onto a preloaded density medium, which is contained within a channel located in the disc-based system. Centrifugation of the mixture then results in the microparticles diffusing through the density medium, leading to the formation of a sphere at the end of the line. The level of BoNT is then quantified through the measurement of fluorescence.A key advantage of the system is its ability to distinguish the active toxin, a feature enabled by a recent modification.The entire test takes less than 30 minutes, with the sample being loaded onto the disc and placed in the test reader. In comparison with MBA, the sensitivity of

SpinDx was determined to be 90 ng/mL, which is 100 times more sensitive than MBA (Koh & al., 2015). This finding indicates that active toxin detection is a significant component in the process of replacing MBA in the pharmaceutical industry.

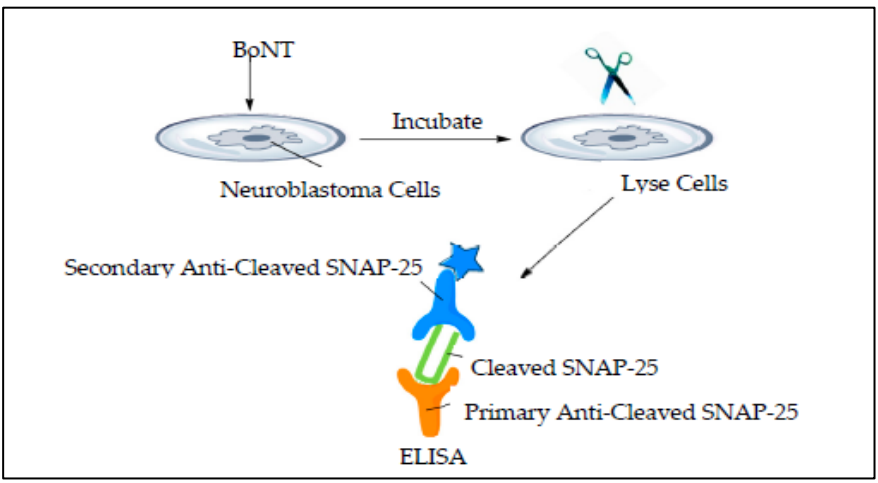
Cyclic Voltammetry and Electrochemical Impedance Spectroscopy: Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) are two electrochemical methods that have been employed in the diagnosis of botulism. CV is an electrochemical technique that provides information about the rate and mechanism of electrochemical reactions on an electrode surface. Botulinum toxin gains and gives electrons on the electrode surface through a redox reaction, and CV can be used to detect the presence of botulinum toxin by analysing the electrochemical behaviour of the toxin. Electrochemical Impedance Spectroscopy (EIS) is a technique that involves the measurement of impedance values of electrochemical interactions that occur on an electrode surface. It has been demonstrated that changes in these values can be used as an indication of the presence of botulinum toxin. The popularity of electrochemical techniques is increasing due to their use in the development of sensor platforms. Their primary advantage is that they allow for high sensitivity to surface interactions and changes to be achieved. These methodologies are utilised in a variety of areas, including sensors for various biomarkers, toxins and pathogens. The utilisation of both CV and EIS has been employed in the development of BoNT sensors (Ye & al., 2013; Bertok & al., 2019).

Endopeptidase Mass Spectrometry: Endopeptidase mass spectrometry is a method employed in the analysis of proteins, with the purpose of determining the sequences of the peptides that are unique to the proteins in question. In order to carry out this process, it is first necessary to digest the protein sample with an endopeptidase enzyme, which will cut peptide bonds in the protein chain at a certain point, thus causing the peptides to break into smaller fragments. The peptides that have been cut by the endopeptidase are then analysed with a mass spectrometry instrument. The mass spectrometer measures the masses of the peptides and thus obtains the unique mass spectrum of each peptide. These spectra are then processed through specialised software and

the unique sequences of the peptides are determined. Endopeptidase mass spectrometry is frequently used in proteomics research and biomedical research. This method is particularly useful when mixtures of proteins need to be analysed quickly. Furthermore, it has been demonstrated that this method can be utilised for the identification of post-translational modifications of proteins (Rosen & al., 2017).

Cell Based Assays: Cell-based tests have been shown to replicate certain aspects of botulism in a living organism. Various assays have been developed that utilise continuous cell lines or primary neurons derived from avian or rodent spinal cord cells (see Figure 5 for a schematic representation of a typical cell-based platform employed for BoNT detection). While assays based on continuous cell lines did not demonstrate optimal sensitivity in the detection of BoNTs in complex matrices, a sensitivity comparable to MBA was observed in some primary cell line assays (Dong & al., 2004).

Figure 5: Schematic representation of the cell-based test



Reference: (Hobbs & al., 2019)

Cell-based assays have been employed as a model for BoNT detection, finding application in several areas of interest, including cell surface receptor binding, endocytosis, internalisation, membrane translocation and SNARE cleavage (Hakami & al., 2010). This combination suggests that cell-based assays are an ideal

candidate for BoNT inhibitor screens. However, there are some disadvantages associated with their use in the food or diagnostic sectors. Firstly, the required level of speed is technically challenging and requires expertise and facilities to maintain cell lines. Furthermore, the sensitivity of these assays ranges from 1-10 ng/mL (Hakami & al., 2010).

Immunohistochemistry (IHC): The system is designed to facilitate the detection of an antibody developed against a macromolecule for analysis. The protein to be detected in the tissue is referred to as the antigen, and the protein employed for detection is known as the antibody. This method has proven to be advantageous in demonstrating the location of the protein to be labelled within the tissue. Two categories of antibodies are utilised: polyclonal and monoclonal. Polyclonal antibodies are antibodies produced by immunising an animal with an antigen, and following injection of an antigen from an animal, such as a rat, into another animal, the antigen is recognised as a foreign substance and antibodies are produced against the antigen given by B lymphocytes. These polyclonal antibodies are then isolated from the blood and purified, and the antibody can bind and mark the protein in rat tissues or cells. Monoclonal antibodies are antibodies produced by a single group of B lymphocytes. These cell columns develop from multifaceted myeloma, a tumour that develops from plasma cells that produce a single antibody. There are commercially developed antibodies against some toxin types for the detection of BoNTs in organs and tissues obtained after necropsy. This method can contribute to the postmortal laboratory diagnosis of BoNTs in veterinary medicine (Smith & al., 2022).

Conclusion

A fundamental objective in botulinum neurotoxin (BoNT) diagnostics is to minimise the utilisation of experimental animals, irrespective of the sector in which the test is required. This objective necessitates a reduction in the use of the mouse assay, the prevailing gold standard for BoNT detection, which employs an estimated 600,000 mice each year worldwide. Alternative methods offer

several advantages, including enhanced sensitivity, rapid sample analysis and reduced cost.

Given the potential severity of botulism, prompt diagnosis is paramount, necessitating ongoing research and development in diagnostic methods. The development of in vitro tests for the sensitive and rapid detection of all botulinum toxin types is imperative, with validation required against diverse matrices. Concomitantly, the utilisation of molecular-biological detection and diagnostic tools for screening and surveillance purposes is recommended, with a view to reducing reliance on the mouse assay. While the diagnosis of botulism disease is based on the detection of toxin in the patient, the investigation of botulism outbreaks and the causative organism is also necessary. The laboratory diagnosis of each case of botulism should include every effort to isolate *C. botulinum* from the patient as well as from the source. In addition to the identification of the type of toxin produced, these isolates should be typed to reveal the physiological group of *C. botulinum*. Genetic characterisation of isolates should be included in routine diagnostic and epidemiological investigations.

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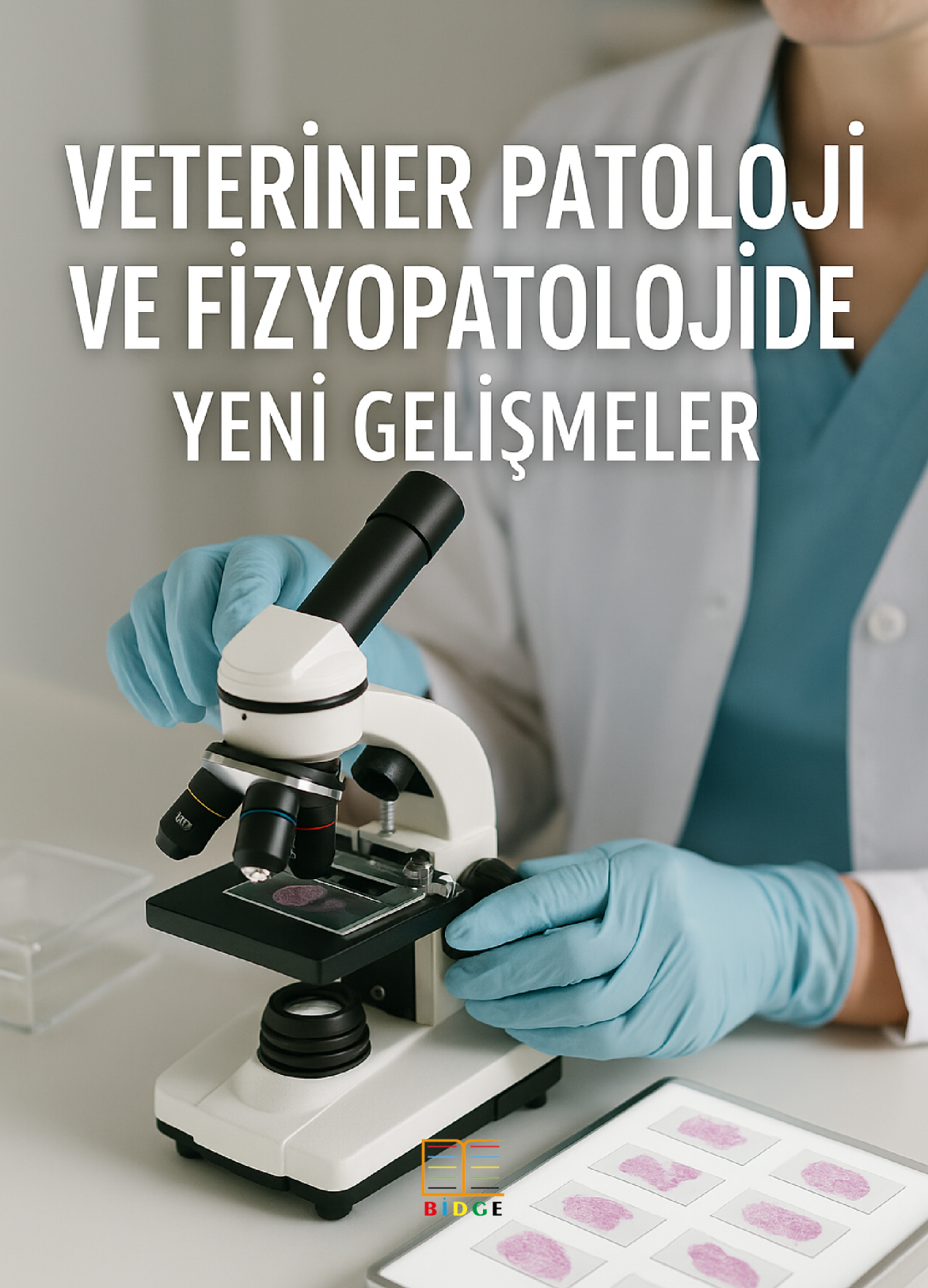

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