



ATATÜRK
UNIVERSITY
PUBLICATIONS

Veterinary Sciences *and Practices*

Formerly: Atatürk University Journal of Veterinary Sciences
Official journal of Atatürk University Veterinary Sciences

Volume 18 • Issue 1 • April 2023

Veterinary Sciences and Practices

Editor-in-Chief

Mustafa Sinan AKTAŞ

Department of Internal Medicine, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Associate Editors

Hakan AYDIN

Department of Virology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Murat GENÇ

Department of Zootechnics, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Uğur ÖZENTÜRK

Department of Zootechnics, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Technical Editors

Ekrem LAÇİN

Department of Zootechnics, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Foreign Language Services

M. Gökhan ŞENOCAK

Department of Surgery, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Advisory Board

Ardita JAHJA-HOXHA

Kosovo

Aleksandra GORECKA-BRUZDA

Department of Animal Behavior, Polish Academy of Sciences, Institute of Genetics and Animal Biotechnology, Warsaw, Poland

Daniel ZAHNER

Faculty of Veterinary Medicine, Justus Liebig University Giessen, Giessen, Germany

Eva VOŠLAROVA

Department of Animal Protection and Welfare and Veterinary Public Health, Veterinary and Pharmaceutical Sciences University, Czech Republic

Mustafa ALİŞARLI

Department of Food Hygiene and Technology, Bolu Abant İzzet Baysal University, Faculty of Veterinary Medicine, Bolu, Turkey

Mustafa ATASEVER

Department of Food Hygiene and Technology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Tanvir RAHMAN

Department of Microbiology and Hygiene, Bangladesh Agricultural University, Bangladesh

Zekai HALICI

Department of Medical Pharmacology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey



Founder

İbrahim Kara

General Manager

Ali Şahin

Finance Coordinator

Elif Yıldız Çelik

Journal Managers

İrem Soysal

Bahar Albayrak

Deniz Kaya

İrmak Berberoğlu

Publications Coordinators

Gökhan Çimen

Arzu Arı

Alara Ergin

Hira Gizem Fidan

İrem Özmen

Derya Azer

Burcu Demirer

Project Coordinators

Doğan Oruç

Sinem Fehime Koz

Contact

Publisher: Atatürk University

Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES

Address: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Turkey

Phone: +90 212 217 17 00

E-mail: info@avesyayincilik.com

Webpage: www.avesyayincilik.com

Veterinary Sciences and Practices

AIMS AND SCOPE

Veterinary Sciences and Practices (Vet Sci Pract) is a scientific, open access, online-only periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is published triannually in April, August, and December. The publication languages of the journal are Turkish and English. The articles submitted in English will be given priority in evaluation.

Veterinary Sciences and Practices aims to contribute to the literature publishing manuscripts at the highest scientific level on all fields of veterinary medicine. The journal publishes original articles, invited reviews, case reports that are prepared in accordance with ethical guidelines.

The scope of the journal includes but not limited to; basic and clinical veterinary sciences, raising livestock, veterinary genetics, animal nutrition and nutritional diseases, zoonoses, veterinary medicinal products and public health, and food hygiene, technology, exotic animal science and laboratory animal science.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

Veterinary Sciences and Practices currently indexed in Scopus, DOAJ, EBSCO, CABI, China National Knowledge Infrastructure (CNKI) and TUBITAK ULAKBIM TR Index.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Open Access Statement

Veterinary Sciences and Practices is an open access publication, and the journal's publication model is based on Budapest Access Initiative (BOAI) declaration. All published content is available online, free of charge at <https://veterinarysciences-ataunipress.org/>. Veterinary Sciences and Practices is content is licensed under a Creative Commons Attribution-NonCommercial (CC BY-NC) 4.0 International License which permits third parties to share and adapt the content for non-commercial purposes by giving the appropriate credit to the original work.

You can find the current version of the Instructions to Authors at <https://veterinarysciences-ataunipress.org/>.

Editor-in-Chief: Mustafa Sinan AKTAŞ

Address: Atatürk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Erzurum, Turkey

E-mail: vetdergisi@atauni.edu.tr

Publisher: Atatürk University

Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES

Address: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Turkey

Phone: +90 212 217 17 00

E-mail: info@avesyayincilik.com

Webpage: www.avesyayincilik.com

Veterinary Sciences and Practices

AMAÇ VE KAPSAM

Veterinary Sciences and Practices; (Vet Sci Pract), bağımsız, tarafsız ve çift-kör hakemlik ilkelerine uygun olarak yayınlanan, bilimsel, açık erişimli, yalnızca çevrimiçi bir süreli yayındır. Dergi yılda üç kez Nisan, Ağustos ve Aralık aylarında yayımlanır. Derginin yayın dili Türkçe ve İngilizce'dir. İngilizce olarak gönderilen makalelere değerlendirmede öncelik verilecektir.

Veterinary Sciences and Practices; veteriner hekimliğin tüm alanlarında bilimsel olarak en üst düzeyde makaleler yayınlayarak literatüre katkı sağlamayı amaçlamaktadır. Dergi, etik kurallara uygun olarak hazırlanmış özgün makaleler, davetli derlemeler, olgu sunumları yayınlar.

Derginin kapsamı bunlarla sınırlı olmamak üzere; temel ve klinik veterinerlik bilimleri, hayvancılık, veteriner genetiği, hayvan besleme ve beslenme hastalıkları, zoonozlar, veteriner tıbbi ürünler ve halk sağlığı ile gıda hijyeni, teknoloji, egzotik hayvan bilimi ve laboratuvar hayvanı bilimidir.

Derginin hedef kitlesi, veteriner hekimliğin tüm disiplinlerinde çalışan ve ilgilenen uzmanlar ve profesyonellerdir.

Veterinary Sciences and Practices; Scopus, DOAJ, EBSCO, CABI, China National Knowledge Infrastructure (CNKI) ve TÜBİTAK ULAKBİM TR Dizin tarafından indekslenmektedir.

Derginin editöryel ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) kılavuzlarına uygun olarak biçimlendirilmiştir. Veterinary Sciences and Practices editöryel ve yayın süreçleri, Akademik Yayıncılıkta Şeffaflık ve En İyi Uygulama (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.

Veterinary Sciences and Practices yayınlanma modeli Budapeşte Açık Erişim Girişimi (BOAI) bildirgesine dayanan açık erişimli bilimsel bir dergidir. Derginin arşivine <https://veterinarysciences-ataunipress.org/> adresinden ücretsiz olarak erişilebilir. Veterinary Sciences and Practices'in içeriği, Creative Commons Atıf-GayriTicari (CC-BY-NC) 4.0 Uluslararası Lisansı ile yayınlanmaktadır.

Yazarlara Bilgi'nin güncel versiyonuna <https://veterinarysciences-ataunipress.org/> adresinden ulaşabilirsiniz.

Baş Editör: Mustafa Sinan AKTAŞ

Adres: Atatürk Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, Erzurum, Türkiye

E-posta: vetdergisi@atauni.edu.tr

Yayıncı: Atatürk Üniversitesi

Adres: Atatürk Üniversitesi, Yakutiye, Erzurum, Türkiye

Yayınevi: AVES

Adres: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Türkiye

Tel: +90 212 217 17 00

E-posta: info@avesyayincilik.com

Web: www.avesyayincilik.com

CONTENTS

REVIEW / DERLEME

- 1 Epidemiology of Animal and Human *Campylobacter* Species Infections in Nigeria: A Retrospective Insight and the Need for One Health Approach in the Prevention and Control**
Nijerya'da Hayvan ve İnsan *Campylobacter* Türleri Enfeksiyonlarının Epidemiyolojisi: Retrospektif Bir Bakış ve Önleme ve Kontrolde Tek Sağlık Yaklaşımına Olan İhtiyaç
Innocent O. NWANKWO, Mohammed D. SALIHU, John A. NWANTA

RESEARCH ARTICLES / ARAŞTIRMA MAKALELERİ

- 12 Köftelerin Bazı Kalite Özelliklerine Bamya Müsilaj Bazlı Yenilebilir Kaplamanın Etkisi**
The Effect of Okra mucilage Based Edible Coating on Some Quality Properties of Meatballs
Enes TERCANLI, Mustafa ATASEVER
- 19 Investigation of Neurotoxicity Oxidative Stress and Oxidative DNA Damage in Cadmium-Induced Brain Injury in Rats**
Sıçanlarda Kadmiyuma Bağlı Beyin Hasarında Nörotoksisite Oksidatif Stres ve Oksidatif DNA Hasarının Araştırılması
İsmail BOLAT, Serkan YILDİRIM, Nesrin CEYLAN, Yavuz Selim SAĞLAM, Ferit Kansu ÖRNEK, Merve BOLAT, Metin KILIÇOĞLU
- 25 Detection of Multidrug-Resistant *Staphylococci* in Beef Processing Line**
Sığır Eti İşleme Hattında Çoklu İlaç Dirençli *Stafilokokların* Belirlenmesi
Akwoba JOSEPH OGUGUA, Catherine UJU OKAFOR, Simeon CHIBUKO OKAFOR, Emmanuel OKECHUKWU NJOGA, Umunna MADUBUIKE, John ANAELOM NWANTA, Gabriel O. EZEİFEKA
- 31 *Sarcoptes canis*'le Doğal Enfeste Köpeklerde Serum Neopterin ve Prokalsitonin Seviyeleri**
Serum Neopterin and Procalcitonin Levels in Dogs Naturally Infested with *Sarcoptes canis*
Yusuf UMUT BATI, Oğuz MERHAN, Nilgün AYDIN, Enes AKYÜZ, Mert SEZER, Ekin EMRE ERKİLİÇ, Zati VATANSEVER, Ali HAYDAR KIRMIZIGÜL
- 35 Multilokus Variable Number of Tandem Repeat Analiz (MLVA) Veri Tabanlarında Türkiye'den İzole Edilen *Bacillus anthracis* Genotiplerinin Moleküler Epidemiyolojisi**
Molecular Epidemiology of *Bacillus anthracis* Genotypes Isolated From Turkey in Multilocus Variable Number of Tandem Repeat Analysis Databases
Ediz Kağan ÖZGEN

CASE REPORT / OLGU SUNUMU

- 44 Surgical Management of Crop Impaction by Inguvotomy in an Aseel Domestic Fowl**
Evcil bir Aseel Tavuğunda Kursak Tıkanıklığının Inguvotomi ile Cerrahi Yönetimi
Kavin RAVIKUMAR, Gowtham RAJAMURUGAN



Epidemiology of Animal and Human *Campylobacter* Species Infections in Nigeria: A Retrospective Insight and the Need for One Health Approach in the Prevention and Control

Nijerya'da Hayvan ve İnsan *Campylobacter* Türleri Enfeksiyonlarının Epidemiyolojisi: Retrospektif Bir Bakış ve Önleme ve Kontrolde Tek Sağlık Yaklaşımına Olan İhtiyaç

Innocent O. NWANKWO¹
Mohammed D. SALIHU²
John A. NWANTA¹

¹Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria

²Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto State, Nigeria

Received/Geliş Tarihi: 21.10.2022

Accepted/Kabul Tarihi: 19.02.2023

Publication Date/Yayın Tarihi: 26.04.2023

Corresponding author/Sorumlu Yazar:

Innocent O. NWANKWO

E-mail: innocent.nwankwo@unn.edu.ng

Cite this article as: Nwankwo IO, Salihu MD, Nwanta JA. Epidemiology of animal and human campylobacter species infections in Nigeria: A retrospective insight and the need for one health approach in the prevention and control. *Vet Sci Pract.* 2023; 18(1), 1-11.

Atıf: Nwankwo IO, Salihu MD, Nwanta JA. Nijerya'da hayvan ve insan campylobacter türleri enfeksiyonlarının epidemiyolojisi: Retrospektif bir bakış ve önleme ve kontrolde tek sağlık yaklaşımına olan ihtiyaç. *Vet Sci Pract.* 2023; 18(1), 1-11.



Copyright@Author(s) - Available online at veterinarysciences-ataunipress.org
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License

ABSTRACT

Campylobacter infection is zoonotic and has remained a global economic and public health concern. The bacteria cause gastroenteritis and other debilitating health and economic problems in both animals and humans. Epidemiological factors and risk practices aiding the acquisition and dissemination of the infection still subsist in developing countries, particularly Nigeria, but epidemiological information on the distribution and control of the infection is sparse. Taking cognizance of the significance of epidemiological data, this study systematically reviewed 53 eligible articles on the prevalence and antimicrobial resistance profile of *Campylobacter* isolates from animals, food matrices, and humans in Nigeria from 1981 to 2022. The 53 articles have a pooled sample population of 9329, 3529, 571, and 5523 from animals, food matrices, water, and humans, respectively. *C. jejuni* and *C. coli* were the most reported species with prevalence range: 5.3%-60.7%, 3.5%-92.7%, 14.3%-27.7%, 8.8%-68.8%, 4.8%-96%, 0.5%-43.8%, and 7%-70% in poultry, other food-producing animals, companion animal, water, food of animal origin, children <5 years old, and other individuals at risk, respectively. A pooled prevalence of 26.8%, 23.1%, 20.7%, 34.8%, 53.7%, 12.7%, and 25.4% in the same order was calculated. Of the 53 articles reviewed, molecular diagnostics tools were employed in 11 (21%) while multidrug resistance was reported in 15 (28%). Intensification of food animal production, use of protective wares by animal and meat handlers, adequate cooking of foods of animal origin, prudent use of antibiotics in animal agriculture, and a coordinated One Health approach to the control of campylobacteriosis has become pertinent in Nigeria.

Keywords: Animal, *Campylobacter*, epidemiology, humans, Nigeria

ÖZ

Campylobacter enfeksiyonu zoonotiktir ve küresel bir ekonomik ve halk sağlığı sorunudur. Bakteriler hem hayvanlarda hem de insanlarda gastroenterit ve diğer sağlığı zayıflatıcı ve ekonomik sorunlara neden olurlar. Enfeksiyonun edinilmesi ve yayılmasına yardımcı olan epidemiyolojik faktörler ve risk uygulamaları gelişmekte olan ülkelerde özellikle Nijerya'da hala varlığını sürdürmektedir, ancak enfeksiyonun dağılımı ve kontrolü hakkında epidemiyolojik bilgi yetersizdir. Epidemiyolojik verilerin önemini göz önünde bulundurarak, bu çalışma 1981-2022 yılları arasında Nijerya'da hayvanlardan, gıda matrislerinden ve insanlardan *Campylobacter* izolatlarının yaygınlığı ve antimikrobiyal direnç profilleri üzerine 53 uygun makaleyi sistematik olarak incelemiştir. 53 makale, sırasıyla hayvanlardan, gıda matrislerinden, sudan ve insanlardan 9329, 3529, 571 ve 5523 örnekleme popülasyonuna sahiptir. *C. jejuni* ve *C. coli* en yaygın rapor edilen türler olup, prevalans aralığı sırasıyla; tavuklarda %5.3-60.7, diğer gıda üretiminde kullanılan hayvanlarda %3.5-92.7, evcil hayvanlarda %14.3-27.7, su kaynaklarında %8.8-68.8, hayvansal kaynaklı gıdalarda %4.8-96, 5 yaşın

altındaki çocuklarda %0.5-43.8 ve diğer risk altındaki bireylerde %7-70'tir. Aynı sırayla hesaplanan birleşik prevalans sırasıyla %26.8, %23.1, %20.7, %34.8, %53.7, %12.7 ve %25.4'tür. İncelenen 53 makalenin 11'inde (%21) moleküler tanı araçları kullanılırken, çoklu ilaç direnci 15'inde (%28) rapor edilmiştir. Nijerya'da, gıda hayvanı üretiminin yoğunlaştırılması, hayvan ve et işleyicileri tarafından koruyucu ekipmanların kullanımı, hayvansal kökenli gıdaların yeterli pişirilmesi, hayvan tarımında antibiyotiklerin özenli kullanımı ve kamflobakteriyozun kontrolünde koordineli bir Tek Sağlık yaklaşımını önemli hale getirmektedir.

Anahtar Kelimeler: Hayvan, *Campylobacter*, epidemiyoloji, insanlar, Nijerya

INTRODUCTION

Campylobacter species are the etiologic agent of campylobacteriosis in both animals and humans. Most *Campylobacter* species, particularly the thermophilic species, are zoonotic and require a microaerophilic culture environment for optimal growth. Generally, *Campylobacter* species are Gram-negative bacteria. The organisms are usually spiral, comma, or rod-shaped and colonize the gastrointestinal tract of most warm-blooded animals, food-producing animals (especially poultry), pets, and humans.¹ *Campylobacter* infections may be asymptomatic, depending on the immune status of the host and the organisms can be discharged into the environments through the feces of infected hosts.² Different animal species especially poultry have been reported as reservoirs of *Campylobacter* species for human infection.³ Foods of animal origin, including processed chicken, other meat products, milk (especially unpasteurized milk), cheese, and untreated water could be major sources of *Campylobacter* infections. Approximately, 70%-80% of cases of campylobacteriosis have been attributed to poultry sources either at the farm level and processing units or due to contact with contaminated carcasses or ingestion of contaminated meat products.⁴ The ability of the organisms to survive over time, even in freezing conditions, and remain viable but not culturable may have contributed to the spread of the infection via contaminated food products.²

Campylobacter species are of immense public health importance due to their ability to cause gastroenteritis and other health problems in humans; especially among risk groups (immunologically naive/compromised and or occupationally exposed individuals). These include children (<5 years old), the elderly, pregnant women, livestock farmers, butchers/animal handlers, and HIV/TB patients.^{1,5} The infection is worldwide in distribution but high prevalence has been reported in animals and humans in many developing countries including Nigeria.¹ The socio-economic burden and intervention cost associated with campylobacteriosis and other zoonotic diseases in Nigeria have been estimated at 8.8% and 10.9%, respectively.⁶ The actual impact in the developed countries has been estimated at 27 million euros per year unlike in the developing countries where the actual impact of the infection has remain indefinable either as a result of deficiency in awareness or complexity in the diagnosis within sensate and paradoxically applied isolation methods.⁷

In Nigeria, the risk practices aiding *Campylobacter* infection are common. Almost every household is involved in subsistent poultry or livestock production for income generation or provision of animal protein.⁸ Furthermore, poor biosecurity, environmental hygienic practices, and contaminated water sources, both at the farm and at the abattoir levels where food-producing animals are reared or processed without good hygiene practices have also contributed to the spread of *Campylobacter* organism

in developing countries.^{4,9} Lack of awareness of the public health implication of *Campylobacter* infection, inaccurate diagnosis, and wrong use of antibiotics in animals including self-medication in the management of gastroenteritis cases in humans may have led to the increase in the development of antimicrobial resistant strains and transfer of resistant genes.¹⁰ In view of the public health and economic impacts of *Campylobacter* infection, there is a need for a retrospective insight on the prevalence and antimicrobial resistance status of the bacteria from both animal and human sources in Nigeria in order to proffer an evidence-based One Health prevention and control strategy.

MATERIALS AND METHODS

Literature Search

A systematic search and review for original articles and research work carried out in Nigeria and published between 1981 and 2022 were accessed using "Pub Med," "Google Scholar," "Research gate," and "African Journal Online." The databases were originally searched for the availability of identical reviews to avoid replication of any earlier research. The systematic search of these databases was performed using the search terms including the prevalence of *Campylobacter* species infections in food-producing animals, companion animals, food of animal origin, water and humans in Nigeria, and resistance to antibiotics. Articles were eligible for review when they: (i) contained data from any geopolitical region in Nigeria and (ii) were published between 1981 and 2022.

Data Extraction

Data such as the first author, year of publication, state, and region of the country in Nigeria where the study was conducted, type of animals (pet or food-producing animals) sampled, description of the sample population, type of samples collected and sample size, *Campylobacter* species prevalence, laboratory diagnostic methods employed and antimicrobials susceptibility testing were collated in the selected articles. Articles for which the sample size or prevalence was not shown or which used archived *Campylobacter* cultures were excluded (Figure 1).

RESULTS

Out of the 6 geopolitical zones in the country, reports on *Campylobacter* infections were found in 4 namely; southeast, southwest, northwest, and north central while no report was found in northeast and south-south parts of the country (Figure 2).

It was revealed that 50.9% (27/53) of studies on *Campylobacter* infection in Nigeria were in animals, followed by 26.4% (14/53) in humans while the food of animal origin and water were at 17.0% (9/53) and 5.7% (3/53), respectively. Out of a pooled population of 9329, 3529, 571, and 5523 samples from animals, food of animal origin, water, and humans, respectively, in the selected articles, *Campylobacter* species infection prevalence range of 5.3-60.7%, 3.5-92.7%, 14.3-27.7%, 8.8-68.8%, 4.8-96%, 0.5-43.8%, and 7-70%

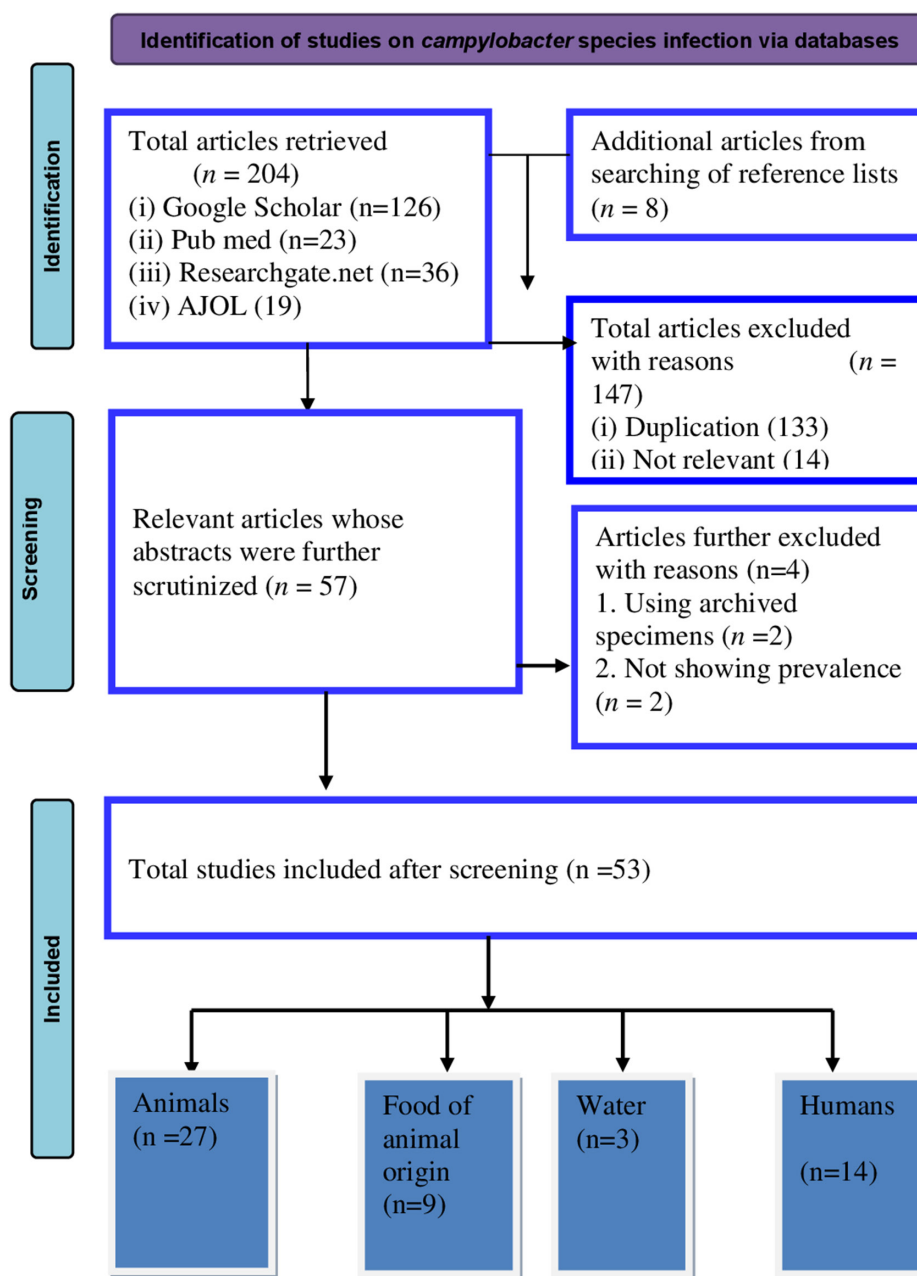


Figure 1. Flow diagram summarizing the process of literature search and article selection.

were in poultry, other food-producing animals, companion animal, water, food of animal origin, children, and other risk groups of individuals, respectively, with a pooled prevalence of 26.8%, 23.1%, 20.7%, 34.8%, 53.7%, 12.7%, and 25.4% in the same order.

In poultry, a pooled prevalence of 19.2%, 24.5%, 25.0%, and 38.5% were in the southeast, southwest, northwest, and north central states of the country, respectively, while 12.6%, 33.6%, and 23.3% were in southwest, northwest, and north central zones, respectively, for other food-producing animals. In pets, 20%, 23%, and 19.5% were the pooled prevalence in the southwest, northwest, and north central, respectively. Furthermore, a pooled prevalence of 8.8% and 60.7% were for water in the southeast and northwest of the country, respectively. In foods of animal origin, a pooled prevalence of 23.3%, 96%, 27.5%, and 68% was revealed in

southeast, southwest, northwest, and north central zones of the country, respectively (Figure 3).

In humans, 8.3%, 14.4%, and 15.3% were the pooled prevalence in children in southeast, southwest, and north-west regions of the country, respectively, while 18.5%, 27.7%, 45.0%, and 10.2% were the rates for other individuals at risk in the southeast, southwest, northwest, and north-central region of the country, respectively (Figure 3). *C. jejuni* and *C. coli* were the most commonly reported species in food-producing animals, foods of animal origin, and humans while *C. upsaliensis* and *C. hyointestinalis* were the most frequently reported species in pets and water, respectively. Multidrug resistant (MDR) strains of *Campylobacter* species and 0%-100% resistance to commonly used antimicrobials in both human and animal sources were reported.

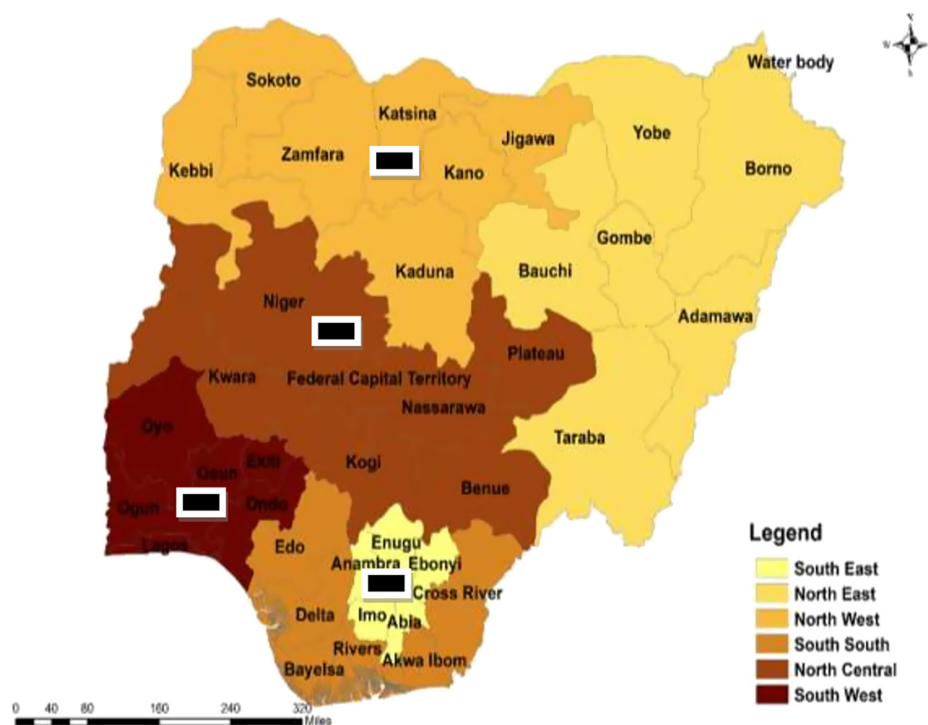


Figure 2. Map of Nigeria showing the constituent 6 geopolitical zones and black rectangles indicating zones where there are published data on *Campylobacter* infections.

Reports in Animals and Food of Animal Origin from Southeast, Nigeria

A prevalence of 37% had been reported in birds in Nsukka with *C. jejuni*, *C. coli*, and *C. lari* accounting for 64%, 23%, and 13% of the isolates, respectively.¹¹ The isolates were resistant at 84%, 61%, 58%, 43%, 43%, 9%, 5%, 5%, and 0% for cephalothin, cephalixin, ampicillin, streptomycin, cotrimoxazole, perfloracin, ofloxacin, ciprofloxacin, and gentamicin, respectively, and 75% MDR.¹¹ The reports of 18.9% and 19.4% prevalence in poultry further prove a significant reduction in the infection rate in Nsukka, Enugu State, Nigeria over a decade.^{3,8} Moreover, a lower prevalence of 14.2% has equally been reported in processed chicken with *C. jejuni* and *C. coli* accounting for 27.6% and 72.4% of the isolates, respectively, in the same study area.⁴ In Imo State, Nigeria, studies have reported

different prevalence rates ranging from 43-56% and 16-22% in beef and chicken, respectively, as well as 13.25%, 12.75%, 12.75%, and 12.25% in ready-to-eat meat, turkey meat, chicken meat, and beef, respectively.^{12,13} A 3-year study in Orlu, Imo State, Nigeria has also revealed the prevalence of *Campylobacter* species at 22.6%, 12.7%, and 8.8% in beef, chicken, and water, respectively.¹⁴ It was further reported that ceftriaxone inhibited the highest number of the isolates while tetracycline inhibited the least number due to their over-usage in the study area¹⁴ (Table 1).

Reports in Humans from Southeast, Nigeria

A report of 8.3% prevalence has been reported in humans in Enugu State, Nigeria of which 93% of the isolates were *C. jejuni*.¹⁵ A 3-year study in Orlu, Imo State, Nigeria has also reported

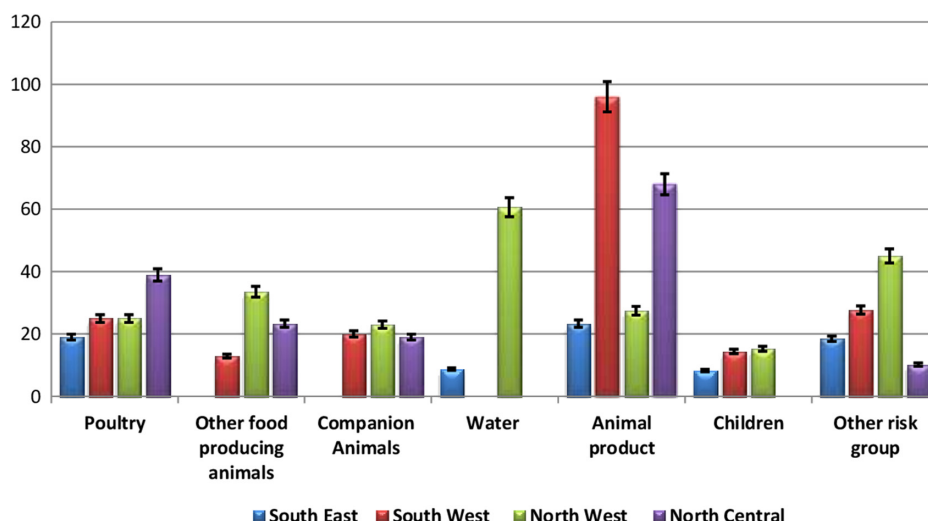


Figure 3. Prevalence and distribution of *Campylobacter* species infections in animals, food matrix, and humans in Nigeria.

Table 1. Prevalence and Distribution of *Campylobacter* Species Infection in Southeast, Nigeria

S. No.	Sources	Prevalence in %	Species	Sample Size	Method of Diagnosis	State	Reference
1	Poultry	19.4	Not indicated	640 fecal	Culture	Enugu	[8]
2	Poultry	18.9	Not indicated	316 fecal	Culture and biochemical test	Enugu	[3]
3	Layers	37	<i>C. jejuni</i> , <i>C. coli</i> , and <i>C. lari</i> at 64%, 23%, and 13%, respectively	275 fecal	Culture and biochemical tests	Enugu	[11]
4	Beef and chicken	22.6 and 12.7	Not indicated	620 beef and 553 chicken	Culture and antibiotics sensitivity	Imo	[14]
5	Beef and chicken	43-56	Not indicated	600 beef and chicken	Culture and biochemical test	Imo	[12]
6	Chicken meat, Turkey, beef, and ready-to-eat meat	12.75, 12.75, 12.25, and 13.25	<i>C. jejuni</i>	400 raw meat	Culture and API campy kits	Imo	[13]
7	Poultry carcass surfaces	14.2	<i>C. jejuni</i> 27.6% and <i>C. coli</i> 72.4%	204 swabs	Phenotypic and PCR	Enugu	[4]
8	Water	8.8		465 water	Culture and antibiotics sensitivity	Imo state	[14]
9	Diarrheic children	8.3	<i>C. jejuni</i> 93%	514 stool	Microscopy and culture	Enugu	[15]
10	Humans	18.5		988 stool	Culture and antibiotics sensitivity	Imo state	[14]

campylobacteriosis as a re-emerging infectious disease of public health importance with 18.5% prevalence in humans¹⁴ (Table 1).

Reports in Animals and Food of Animal Origin from Southwest, Nigeria

A report of 5.3% prevalence had been recorded in Lagos State, Nigeria in a pilot study involving 150 poultry of which 100% of the isolates were resistant to nalidixic acid, chloramphenicol, cloxacillin, and streptomycin while 87.5% and 62.5% were susceptible to amoxicillin/clavulanic acid and erythromycin, respectively.¹⁶ Furthermore, 30% strains of *Campylobacter* isolates from poultry fecal droppings had been characterized as 66.7%, 20.0%, and 6.7% *C. coli* biotype1, *C. jejuni* biotype 1, and biotype II of the 2 species, respectively.¹⁷ In Oyo State, 20.4% prevalence of MDR *C. coli* had been reported in cattle and chicken with 34%, 12%, and 10% in rectal, gall balder, and cloaca samples, respectively.¹⁸ A study has revealed a 96% prevalence for *Campylobacter* species in chicken at Ibadan, Oyo State, and the isolates were resistant to nalidixic acid, gentamicin, and erythromycin at 100% and to enrofloxacin, chloramphenicol, streptomycin, and tetracycline at 38.0%, 46.0%, 50.0%, and 58.0%, respectively.¹⁹ The use of modified charcoal cefoperazone deoxycholate agar m in comparison with Preston broth pre-enrichment and subsequent subculturing on Mueller Hinton agar with growth supplements, has revealed 16.8% and 26% prevalence, respectively, for *Campylobacter* species in layer birds in Ogun State, South Western Nigeria.²⁰ The isolates from the 2 media were confirmed as *Campylobacter* species at zero and 22% in that same order with the use of PCR.²⁰ The PCR-positive isolates were 90%, 10%, and 0% *C. coli*, other *Campylobacter* spp., and *C. jejuni*, respectively. The isolates were resistant to tetracycline, ciprofloxacin, erythromycin, spectinomycin, and tylosin at 100%, 90%, 60%, 90%, and 80%, respectively.²⁰ Furthermore, a study in the western part of Nigeria reported the prevalence of *Campylobacter* spp. at 15.4%, 15%, 20%, 6.25%, 12.5%, and 27.5% in poultry, cattle, dogs, sheep, goats, and pigs, respectively, with the isolates as *C. jejuni*, *C. coli*, and *C. fecalis* at 79.9%, 12.7%, and 7.2% prevalence, respectively.²¹ A study in Lagos, has reported a prevalence of 14% with 7.1%, 25%, 7.1%, and 60.7% in pig, chicken, sheep, and guinea fowl, respectively.²² The isolates were positive for *C. coli* biotype II, *C. coli* biotype I, and *C. jejuni* at 17%, 100% and 1%, respectively²² (Table 2).

Reports in Humans from Southwest, Nigeria

A prevalence of 0.5% has been reported in children with diarrhea, aged between 0 and 36 months in Osogbo, Osun State, Nigeria.²³ A case-control study has also reported a prevalence of 19.1% among diarrheic children in Ile-Ife, Nigeria with *C. coli* accounting

for 53.3% of the isolates which were all susceptible to erythromycin but without evidence of beta-lactamase production.²⁴ Furthermore, in another study in Osun State, 25 out of 57 *Campylobacter* strains from 815 diarrheic stool samples were identified as 72% *C. jejuni* and 28% *C. coli*.²⁵ The *C. jejuni* was classified into biotypes I (44.4%) and II (55.6%) while all the *C. coli* were of biotype I.²⁵ A study has reported the prevalence of 68% for *Campylobacter* spp among HIV patients in Ibadan, Oyo State, Nigeria with higher occurrence in male than the female HIV patients.²⁶ From the same report, *C. upsaliensis* (26.7%) was the most prevalent species followed by *C. jejuni* (25.0%), *C. lari* (19.2%), *C. coli* (16.7%), and *C. fetus* (12.5%). The isolates were sensitive to amikacin, amoxicillin/clavulanic acid, ciprofloxacin, ertapenem, nalidixic acid, and aztreonam at 92.5%, 70.0%, 92.5%, 90.0%, 69.2%, and 55.0%, respectively, while resistance to gentamycin, chloramphenicol, cefixime, and cephalothin was at 19.2%, 26.7%, 31.7%, and 39.2% in the same order.²⁶ A study has also reported the prevalence of *Campylobacter* species at 16.5% among diarrheic children at health centers in Lagos, Nigeria with *C. jejuni* accounting for 87.5% of the isolates which were resistant to erythromycin and produces betalactamase at 79.2% and 12.5%, respectively.²⁷ Furthermore, a study in the western part of Nigeria had reported the prevalence of *Campylobacter* spp. at 7% in humans²¹ (Table 2).

Reports in Animals, Food of Animal Origin, and Water from Northwest, Nigeria

The prevalence of 30% for *Campylobacter* species in poultry has been reported in Sokoto State, Nigeria where the possibility of improper identification using conventional method alone was revealed.¹⁷ In another study, a prevalence of 81.9% for *Campylobacter* species in raw poultry meat has been reported with *C. jejuni*, *C. coli*, and *C. lari* accounting for 60.9%, 28%, and 7% of the isolates, respectively, while "other thermophilic species" were at 4.1%.²⁹ Furthermore, a prevalence of 20.1% in goats has been reported in Sokoto State with *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. sputorum* accounting for the 62.1%, 21.3%, 8.8%, 4.8%, and 3.0% of the isolates, respectively.²⁸ Meanwhile, *Campylobacter* species have been considered a potential agent of ovine enteritis and abortion with the report of 18.0% prevalence in sheep in Sokoto State.³⁰ The isolates were 79.6%, 44.6%, and 72.7% *C. jejuni*, *C. jejuni* biotype, and *C. coli* biotype, respectively.³⁰ There has been a report of 92.67% *Campylobacter* species prevalence in pigs with *C. coli*, *C. jejuni*, *C. upsaliensis*, and *C. hyointestinalis* accounting for the 78.71%, 14.03%, 5.40%, and 1.50% of the isolates, respectively.³¹ *Campylobacter* infection has been reported in dogs and cats at 27.7% and 18.3%, respectively,

Table 2. Prevalence and Distribution of *Campylobacter* Species Infection in Southwest, Nigeria

S.No.	Sources	Prevalence in %	Species	Sample Size	Method of Diagnosis	State	Reference
1	Poultry	30	<i>C. coli</i> biotype I at 66.66%, <i>C. jejuni</i> biotype I and II at 20.00% and 6.67%, respectively	50 fecal	Culture and biochemical test	Lagos	[17]
2	Poultry	5.3	<i>C. coli</i>	150 fecal	Culture & API campy kits	Lagos	[16]
3	Layers	16.8-22%	<i>C. coli</i> , other <i>Campylobacter</i> spp. and <i>C. jejuni</i> were 90%, 10%, and 0% respectively	550 cloacal swab	Modified culture and PCR, genotypic characterization, and antibiotics resistance profiling	Ogun State	[20]
4	Animals, pig, chicken sheep, and guinea fowl	14 7.1 25 7.1 60.7	<i>C. coli</i> biotype I and II at 100% and 17%, respectively, and <i>C. jejuni</i> biotype 1%.	200 animals	Culture and biochemical test	Lagos	[22]
5	Cattle and chicken	20.4	<i>C. coli</i> 100%	250	Culture and biochemical test	Oyo	[18]
6	Animals, poultry, cattle, sheep, goat, pig, dog, and humans	15.4, 15, 6.25, 12.5, 27.5, 20, and 7	<i>C. jejuni</i> biotypes I and II at 70.9% and 9.0%, respectively. <i>C. coli</i> and <i>C. fecalis</i> at 12.7% and 7.2%, respectively	248 fecal samples, 156 poultry, 20 cattle, 16 sheep, 6 goats, 40 pigs, 10 dogs, and 200 humans	Culture and biochemical test		[21]
7	Chicken	96.0	<i>C. jejuni</i> 96%	Meat	Culture and biochemical test	Oyo	[19]
8	Children with diarrhea	16	<i>C. jejuni</i> 87.5% and resistance to erythromycin and betalactamase production at 79.2% and 12.5%, respectively	145 stools		Lagos	[27]
9	Children below 5 years	0.5	<i>C. coli</i>	602	Culture	Osun	[23]
10	Diarrheic children	0.6 by culture and 43.8 molecular	<i>C. jejuni</i> 72% and <i>C. coli</i> 28%. <i>C. jejuni</i> biotypes I (44.4%) and II (55.6%) and all <i>C. coli</i> biotype I	57 cultured samples and 815 stools	Culture and PCR	Osun	[25]
11	Diarrheic patients	8.2	<i>C. jejuni</i> 56%. <i>C. coli</i> 44%	306 stools	Culture and biochemical test	Kwara	[47]
12	Diarrheic children and without diarrhea	19.1 and 6	<i>C. coli</i> 53.3%	300 and 100 stools	Culture	Ile-Ife, Nigeria	[24]
13	HIV patients	68	<i>C. upsaliensis</i> , <i>C. jejuni</i> , <i>C. lari</i> , <i>C. coli</i> , <i>C. fetus</i> at 26.7%, 25.0%, 19.2%, 16.7%, and 12.5%, respectively	100 stool	Culture and Kirby-Bauer disk Diffusion	Oyo	[26]

with *C. upsaliensis* and *C. jejuni* accounting for 89.5% and 23.1% of the isolates in dogs, respectively, while 74.4% and 21.1% were in cat in the same order.³² A prevalence of *Campylobacter* species at 4.8% in raw milk samples from lactating cows in Sokoto has been reported with *C. jejuni* and *C. jejuni* biotype I, as the contaminating species with the highest contamination rates at 5.4% in White Fulani breed and 6.1% in the dry season.³² In Sokoto, State, Nigeria, a study has also revealed a 52.70% prevalence of *Campylobacter* in groundwater samples with *C. jejuni*, *C. coli*, and *C. hyointestinalis* accounting for the 58.97%, 28.21%, and 12.82% of the isolates, respectively.⁹ Meanwhile, there had been an earlier reported 68.75% prevalence for *Campylobacter* species in surface water with *C. jejuni*, *C. coli*, *C. fetus*, and *C. hyointestinalis* at 54.55%, 36.36%, 4.55%, and 4.55% prevalence, respectively;³³ a pointer to the significant of the use of treated water in the prevention of campylobacteriosis nationwide. The prevalence of *Campylobacter fetus* subsp. *jejuni* at 12.3% and 7.1% in live birds and eviscerated carcasses, respectively, have been reported in Zaria, Nigeria, with high rates in free-ranged birds and in slaughtered poultry immersed in boiling water before dressing.³⁴ A study of thermophilic campylobacter species in broilers had reported 51.1% prevalence with *C. jejuni*, *C. coli*, and *C. lari* constituting 62.6%, 21.6% and, and 15.8% of the isolates, respectively, and were resistant to the tested antibiotics except chloramphenicol.³⁵

In Zaria, Kaduna State, a prevalence of 16.1% of *Campylobacter* species in milk product “nunu” had been reported.³⁶ *C. coli* and *C. jejuni* were at 10.5% and 5.6% prevalence, respectively, and the isolates were resistant to imipenem, gentamicin, and erythromycin at 0%, 5%, and 100%, respectively, while MDR was at 39%.³⁶ (Table 3).

Reports in Humans from Northwest, Nigeria

A study in Sokoto has reported *Campylobacter* infection in humans at 55% prevalence with 39%, 37%, and 24% prevalence for *C. coli*, *C. lari*, and *C. jejuni*, respectively.¹ Furthermore, 62.67% prevalence had also been reported in Kebbi, State with *C. coli*, *C. jejuni*, *C. upsaliensis*, *C. hyointestinalis*, and *C. lari* accounting for the 60.63%, 24.50%, 7.45%, 5.32%, and 2.13% of the isolates, respectively.³⁰ The prevalence of 70% and 43% have been reported in pregnant and nonpregnant women of reproductive age, respectively, in Sokoto State, Nigeria with *C. lari*, *C. coli*, and *C. jejuni* accounting for 56%, 32%, and 13% of the isolates, respectively, among the pregnant women and 16%, 60%, and 24% in non-pregnant women in the same order.⁵ A study has reported a 19.6 % prevalence in diarrhoeic HIV patients in Kaduna State with the use of culture and API campy kits.³⁷ Furthermore, a prevalence of 15.3% had been reported in diarrheic children in a study on the role of *Campylobacter* as an agent of diarrhea and their characteristics in Zaria, Nigeria.³⁸ The consumption of chicken within the household, contact with animals, and drinking of untreated well water and canned milk were independently found to be possible sources and risk factors for the infection. Meanwhile, the isolates were reported very sensitive to gentamicin, nalidixic acid, tetracycline, ciprofloxacin, chloramphenicol, erythromycin, co-trimoxazole, and amoxicillin at 100%, 100%, 95%, 92.5%, 90%, 82.5%, 55%, and 50%, respectively³⁸ (Table 3).

Reports in Animals and Food of Animal Origin from North Central, Nigeria

A study in Plateau State had reported a prevalence of 28% for *Campylobacter* infection in cattle of which 7% and 21% were *C. jejuni* and *C. coli*, respectively.³⁹ A lower prevalence of 18.5% has also been revealed for *Campylobacter* species in cattle in Plateau

Table 3. Prevalence and Distribution of *Campylobacter* Species Infection in Northwest, Nigeria

S.No.	Sources	Prevalence in %	Species	Sample Size	Method of Diagnosis	State	Reference
1	Poultry	30	<i>C. coli</i> , <i>C. lari</i> and <i>C. jejuni</i> at 53, 28, and 18%, respectively	506 cloacal swab	Culture and biochemical test	Sokoto	[1]
2	Poultry	12.3	<i>C. fetus</i> subsp. <i>jejuni</i>	487 cloacal swabs	Culture and biochemical test	Kadunna	[34]
3	Broilers	51.1	<i>C. jejuni</i> , <i>C. coli</i> , and <i>C. lari</i> at 62.6%, 21.6%, and 15.8%, respectively	270 cloacal swab	Culture and biochemical test	Sokoto	[35]
4	Pig	92.67	<i>C. coli</i> , <i>C. jejuni</i> , <i>C. upsaliensis</i> , and <i>C. hyointestinalis</i> at 78.71%, 14.03%, 5.40%, and 1.50%, respectively	300 fecal samples	Culture and biochemical test	Kebbi	[31]
5	Goat	20.1	<i>C. jejuni</i> 62.1%, <i>C. coli</i> 21.3%, <i>C. lari</i> 8.8%, <i>C. upsaliensis</i> 4.8%, and <i>C. sputorum</i> 3.0%.	1312 rectal swab	Culture and biochemical test	Sokoto	[28]
6	Sheep	18.0	<i>C. jejuni</i> at 79.6% and <i>C. jejuni</i> biotype I (44.6%), and <i>C. coli</i> biotype I (72.7%)	518 rectal swabs	Culture and biochemical test	Sokoto	[30]
7	Slaughtered Sheep	3.54	<i>C. fetus</i> subsp. <i>jejuni</i> , <i>C. coli</i> , and <i>C. laridis</i> were at 79%, 13%, and 8.0%, respectively	1100 samples,	Culture, Gram staining, biochemical test, and liorbiototyping	Kaduna	[41]
8	Dog Cat	27.7 18.3	<i>C. upsaliensis</i> 89.5% and 74.4% in dog and cat, respectively, <i>C. jejuni</i> at 23.1% and 21.1% in dog and cat, respectively.	141 and 104 fecal from Dog and cats, respectively	Culture and biochemical test	Sokoto	[32]
9	Raw chicken	81.9	<i>C. jejuni</i> , <i>C. coli</i> <i>lari</i> , and others at 60.9%, 28%, 7%, and 4.1%, respectively	681 raw poultry	Culture and biochemical test	Sokoto	[29]
10	Milk	16.1	<i>C. coli</i> 10.5% and <i>C. jejuni</i> 5.6%.	180 nunu	Membrane filtration biochemical test and API campy kits	Zaria, Kaduna	[36]
11	Milk	4.8	<i>C. jejuni</i> and <i>C. jejuni</i> biotype I	146 raw milk	Culture and biotyping	Sokoto	[32]
12	Eviscerated carcasses	7.1	<i>C. fetus</i> subsp. <i>jejuni</i>	70 swabs	Culture and biochemical test	Kadunna	[34]
13	Water	52.7	<i>C. jejuni</i> , <i>C. coli</i> , and <i>C. hyointestinalis</i> at 58.97%, 28.21%, and 12.82%, respectively	74 ground water sample	Culture and biochemical test	Sokoto	[9]
14	Sokoto River.	68.75	<i>C. jejuni</i> , <i>C. coli</i> , <i>C. fetus</i> , and <i>C. hyointestinalis</i> at 54.55%, 36.36%, 4.55%, and 4.55%, respectively	32 water	Culture and biochemical test	Sokoto	[33]
15	Diarrheic children& without diarrhea	15.3		261 and 100 stools	Culture and antibiotics sensitivity	Zaria	[38]
16	Children and Adult	55	<i>C. coli</i> , <i>C. lari</i> , and <i>C. jejuni</i> at 39%, 37%, and 24%, respectively	292 fecal swab	Culture and biochemical test	Sokoto	[1]
17	Poultry& Humans	39.1	<i>C. jejuni</i> , <i>C. coli</i> , and <i>C. lari</i> at 23.8%, 39.4%, and 36.9%, respectively, in poultry at 19.1%, 52.0%, and 28.9% in humans in same order	798 fecal	Culture, biochemical test, and PCR	Sokoto	[7]
18	Pregnant women Nonpregnant women	70 43	<i>C. lari</i> , <i>C. coli</i> , and <i>C. jejuni</i> at 56%, 32%, and 13%, respectively, <i>C. coli</i> , <i>C. jejuni</i> , <i>C. lari</i> at 60% 24%, and 16% respectively	292 fecal swab	Culture and biochemical test	Sokoto	[2]
19	Humans	62.67%.	<i>C. coli</i> , <i>C. jejuni</i> , <i>C. upsaliensis</i> , <i>C. hyointestinalis</i> , and <i>C. lari</i> at 60.63%, 24.50%, 7.45%, 5.32%, and 2.13%, respectively	150 fecal samples	Culture and biochemical test	Kebbi	[31]
20	HIV patients	19.6	<i>C. jejuni</i> , <i>C. coli</i> , <i>C. fetus</i> , and <i>C. hyointestinalis</i>	230 fecal samples	Culture, biochemical test, and API campy kits	Kaduna	[37]

State, Nigeria with 80% and 20% as *C. jejuni* and *C. coli*, respectively, even as 25% and 12.2% prevalence rates were reported in calves and cows, respectively.⁴⁸ An overall prevalence rate of 63.5%, 61.7%, and 66.7% in poultry, ducks around wells, and ponds, respectively, have been reported in Makurdi, Benue State, Nigeria.⁴⁰ A prevalence of 3.54% has been reported in slaughtered sheep in Kaduna State with isolation rates of 6.8%, 4%, 2.8%, and 0% from intestinal contents, gallbladders, vaginal, and fetuses, respectively.⁴¹ The isolates were characterized as *C. fetus* subsp *jejuni*, *C. coli*, and *C. laridis* at 79%, 13%, and 8%, respectively.⁴¹ A study on cattle offals slaughtered within Gwagwalada abattoir in Abuja, Nigeria, has reported a prevalence of 68% with the isolates sensitive to gentamycin and amoxil but resistant to norfloxacin, rifampicin, chloramphenicol, streptomycin, and ampiclox.⁴² A study in Plateau State has also revealed dogs as a carrier of the zoonotic *Campylobacter* infection with a reported prevalence of 14.3%. A significant association was found between the infection and the age of dogs using a standard bacteriological assay.⁴³ The prevalence of 23.8% has been reported in dogs sampled at Veterinary Clinics Jos, Nigeria, and the isolates were identified with

PCR as *C. jejuni*, *C. coli*, and mixed infections at 50.6%, 38.3%, and 11.1%, respectively.⁴⁴ Furthermore, a prevalence of 22.2% has also been reported with *C. jejuni* and *C. coli* at 76.8% and 23.2% in poultry, respectively, and 35.8% and 18.5% in cattle in the same order and study area.⁴⁵ Apparently healthy Japanese quails at slaughter have been considered a potential risk for *Campylobacter* infection in humans with a reported 31.1% prevalence in Jos, Plateau State, of which the isolates were identified as 81% *C. jejuni* and 19% *C. coli*⁴⁶ (Table 4).

Reports in Humans from North Central, Nigeria

A very recent study has revealed the prevalence of *Campylobacter* infection in humans at 9% with *C. jejuni*, *C. coli*, and *C. hyointestinalis* accounting for 66%, 28%, and 6% of the isolates, respectively,, and some of the dominant strains among the isolates were of genetically diverse origin.³⁹ The isolates were resistant to beta-lactams, fluoroquinolones, tetracyclines, macrolides, and MDR at 42%, 41%, 15%, 2%, and 13%, respectively.³⁹ A prevalence of 8.2% has been reported in children with diarrheic in Kwara State.⁴⁷ In Plateau State, a prevalence of 11.3% has been reported with 52.9%

Table 4. Prevalence and Distribution of *Campylobacter* Species Infection in North Central, Nigeria

S. No.	Sources	Prevalence in %	Species	Sample Size	Method of Diagnosis	State	Reference
1	Duck around drinking water and water	63.5	<i>C. jejuni</i> 76.7%, well water and pond water 66.75% and 83.3%, respectively	192 fecal	Culture and biochemical test	Makurdi	[40]
2	Japanese quails	31.1	<i>C. jejuni</i> 81% and <i>C. coli</i> 19%	135 intact caeca	Culture and PCR	Plateau	[46]
3	Cattle	18.5	<i>C. jejuni</i> 80% as <i>C. coli</i> 20%	352 rectal swab	Culture, biochemical test, and PCR	Plateau	[48]
4	Poultry and cattle	22.2	<i>C. jejuni</i> 76.8% <i>C. coli</i> 23.2%	1012 fecal	Culture, PCR, multilocus sequence typing, and antibiotics sensitivity	Plateau	[45]
5	Livestock	28	<i>C. jejuni</i> and <i>C. coli</i> were 7% and 21%, respectively	472 fecal	Culture and whole-genome sequencing	Plateau	[39]
6	Dog	23.8	<i>C. jejuni</i> 50.6%, <i>C. coli</i> in 38.3% and 11.1%	341 faecal	Culture and multiplex PCR	Plateau	[44]
7	Dog	14.3	Not indicated	105 fecal	Culture and Gram staining	Plateau	[43]
8	Beef	68	Not indicated	75 swabs	Culture and biochemical test	Abuja	[42]
9	Humans	9	<i>C. jejuni</i> , <i>C. coli</i> , and <i>C. hyointestinalis</i> at 66%, 28%, and 6%, respectively	586 human	Culture and whole-genome sequencing	Plateau	[39]
10	Humans	11.3	<i>C. jejuni</i> and <i>C. coli</i> at 52.9% and 47.1%, respectively	300 human stool	Culture, PCR, multilocus sequence typing, and antibiotics sensitivity	Plateau	[45]

and 47.1% of the isolates as *C. jejuni* and *C. coli*, respectively.⁴⁵ The isolates were susceptible to clindamycin, however, the resistance to nalidixic acid, tetracycline, ciprofloxacin, sulfamethoxazole/trimethoprim, erythromycin, azithromycin, streptomycin, gentamicin, and chloramphenicol was reported at 60.7%, 57.2%, 53.1%, 46.2%, 41.4%, 27.6%, 13.1%, 6.9%, and 4.8%, respectively⁴⁵ (Table 4).

Reports on Regional Subgrouping of the Studies and Challenges

From the studies, it was revealed that the highest prevalence was in food of animal origin in both southwest and north central regions of the country with 96% and 68% prevalence, respectively, while the highest prevalence was in the water (60.7%) and other risk individuals (45%) in Northwest, Nigeria. On the detection methods, PCR or molecular analysis was done in 11 (21%) of the 53 studies that used culture and phenotypic identification methods. Resistance to antibiotics including MDR was reported in 15 (28%) of the 53 reviewed studies. High frequency of resistance

to erythromycin, ciprofloxacin, and tetracycline was reported in food-producing animals and animal products while resistance to cefixime, cephalothine, and erythromycin was more in humans. Meanwhile, high susceptibility was reported for gentamycin, imipenem, and chloramphenicol in animals and clindamycin, amoxi/clavulanic acid, and amikacin in humans (Table 5).

There is still the issue of noninclusion of *Campylobacter* species testing in the routine laboratory check on gastroenteritis in Nigeria. The public health and economic impact including the antibiotics resistance development and resistance gene transfer is believed to be under-reported as a result of discrepancies in isolation methods. There was a paucity of data on the level of awareness of campylobacteriosis by farmers and animal handlers in Nigeria. Moreover, there is a lack of interest or discouragement among researchers due to the ubiquitous nature of the pathogen and difficulties in species isolation. These were associated with the much time and labor, nonavailability of research reagents,

Table 5. Antimicrobial Profile of *Campylobacter* Species from Different Sources in Nigeria

S.No.	Isolates Sources	Antimicrobial Susceptible	Antimicrobial Resistant	Region	Authors
1	Water, chicken, and beef	Ceftriaxone	Tetracycline	Southeast	[14]
2	Chicken and cattle		Erythromycin and ciprofloxacin	Southwest	[18]
3	Chicken meat		Nalidixic acid, gentamicin, and erythromycin	Southwest	[19]
4	Layers		Tetracycline, ciprofloxacin, erythromycin, spectinomycin, and tylosin	Southwest	[20]
5	Children	Erythromycin		Southwest	[24]
6	HIV patients	Amikacin (92.5%), amoxicillin/clavulanic acid (70.0%), ciprofloxacin (92.5%), ertapenem (90.0%), nalidixic acid (69.2%), and aztreonam (55.0%)	Gentamycin (19.2%), chloramphenicol (26.7%), cefixime (31.7%), and cephalothin (39.2%)	Southwest	[26]
7	Children		Erythromycin and betalactamase production at 79.2% and 12.5%, respectively	Southwest	[27]
8	Broilers	Chloramphenicol	All isolates were resistant to the tested antibiotics	Northwest	[35]
9	Nunu	Imipenem	Gentamicin 5% and erythromycin 100%	Northwest	[36]
10	Cattle	Gentamycin and amoxicillin	Norfloracin, rifampicin, chloramphenicol, streptomycin, and ampiclox but showed some effect to ciprofloxacin, levofloxacin and erythromycin	Northwest	[42]
11	Children	Gentamicin (100%), nalidixic acid (100%), tetracycline (95%), ciprofloxacin (92.5%), chloramphenicol (90%), and erythromycin (82.5%).		Southwest	[38]
12	Birds	Gentamycin	Cephalothin 84%, cephalixin 61%, ampicillin 58%, streptomycin 43%, cotrimoxazole 43%, perfloxacin 9%, ofloxacin 5%, ciprofloxacin 5%	North central	[11]
13	Cattle	Gentamycin and amoxicillin	Norfloracin, rifampicin, chloramphenicol, streptomycin, and ampiclox but showed some effect to ciprofloxacin, levofloxacin, and erythromycin	North central	[42]
14	Humans		Betalactams (42%) fluoroquinolones (41%), tetracyclines (15%), and lmacrolides (2%)	North central	[39]
15	Humans	Clindamycin	Nalidixic acid (60.7%), tetracycline (57.2%), ciprofloxacin (53.1%), sulfamethoxazole/trimethoprim (46.2%), and erythromycin (41.4%)	North central	[45]

high cost of molecular diagnostic equipment, poor technical know-how, and inadequate research facilities and enabling environment.

DISCUSSION

This is the first attempt in providing a retrospective insight on the range and mean prevalence of *Campylobacter* species infection in animals, food sources, and humans in Nigeria, with emphasis on the need for a One Health approach to the prevention and its control. The report has revealed the need to narrow the gap between *Campylobacter* infection research in humans and animals, especially poultry which currently stood at 29% and 49%, respectively, and to further expand the studies in food of animal origin as well as water. It has also revealed the likely under-reported burden of campylobacteriosis in Nigeria as data were only found in 4 out of the 6 geopolitical zones of the country. Meanwhile, the risk practices including open defecation especially in rural communities could have contributed to the spread of *Campylobacter* organisms through human feces and general contamination of the environment.¹ Moreover, high numbers of human populations in many of the states in the regions were not provided with basic healthcare facilities including good water supply which has been reported as potential carriers of *Campylobacter* species.⁹ The prevalence range of 5.3–60.7% in poultry was in agreement with the reports of 22.5% in Ghana.⁴⁹ Similar prevalence reports including that of 6.87% and 18.3% in children and dogs, respectively, in Brazil, were within the revealed range of 0.5%–43.8% and 14.3%–27.7% in children and companion animals in the same order.⁵⁰ The similarities or differences have been associated with many factors including the systems of livestock production, the level of personal, and environmental hygiene.

In Nigeria, the differences in the prevalence; the least 12.6%, in southwest, followed by 23.3% and 33.6% in the north central and northwest regions, respectively, could be attributed to many factors including the sample population, geographical location, species of animal, and management system. The extensive system of broiler production which discourages environmental contamination and disease transmission is practiced more in the southwest and southeast regions than in the northwest and north central regions where local birds are mostly raised on free range and some are transported into the country from the border states of Niger republic.^{1,34}

The highest prevalence of 23% for companion animals in the northwest, followed by 20% in the southwest and the least 19.5% in north central can also be a reflection of keeping the practice of pet animals and the consequential environmental contamination in these regions, especially in the north where cats are kept for companionship and rodent control.⁵⁰ Furthermore, the high prevalence of 60.7% in water from northwest of the country compared to 8.8% in southeast could be a reflection of higher environmental contamination in the north where 85% of the surface water and rivers have been reported contaminated with *Campylobacter* spp. This may be associated with interactions of waterfowl and wild birds in the water bodies, disposal of untreated sewage, and contamination via rain-related runoff.^{33,40} The differences in levels of *Campylobacter* prevalence in the carcass and other food of animal origin could be a product of colonization in slaughter animals, meat, and milk processing hygiene as well as the sampling techniques.⁴ This was further revealed with the highest prevalence of 96% in southwest region where poultry production

and processing are much in practice. Furthermore, age, diarrheic conditions, and exposure to risk practices may have influenced the results in humans. This can be explained by the highest prevalence of 15.3% and 45.0% in children and other risk groups, respectively, in the northwest region followed by 14.4% and 27.7% in the same order in the southwest region. These 2 regions are the areas with much interaction between animals and humans, especially in the production of large and small animals, respectively. Other factors like small sample size may have affected the prevalence as seen in north central and southeast with 10.2% and 18.5% prevalence rates, respectively.

The detection of *C. jejuni* and *C. coli*, the well-known pathogenic species as the most prevalent species in humans and animals in this study, was in agreement with a similar report in Ethiopia and this could pose a huge burden of campylobacteriosis in Nigeria including the development and transfer of antibiotics resistant gene. Since most of the isolates were reported with a high frequency of resistance to the commonly used antibiotics in the management of gastroenteritis, such as erythromycin, ciprofloxacin, and tetracycline, regular antibiotics sensitivity testing should be encouraged in the management of campylobacteriosis.

This study has revealed the range and pooled prevalence with a retrospective insight on the distribution of *Campylobacter* species infection in an animal, food matrix, and humans in Nigeria. It has given credence that the infection in animals could have significantly resulted in cases of acute gastroenteritis and antimicrobial resistance in humans, especially in children and other at-risk individuals in the country. Reduction and control strategies should be targeted toward reduction in the risk practices, provision of adequate social amenities, and improved personal and environmental hygiene and sanitation in the country. There is a need for increased collaboration between human medics and veterinarians on *Campylobacter* research to the level of molecular and comparative analysis of isolates from animals and humans in Nigeria, especially in the south-south and northeast regions of the country where no data is in existence. Accurate diagnosis and rational use of antimicrobials must be prioritized as well as awareness creation on infection prevention and control using the “One Health” approach.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – I.O.N.; Design – I.O.N.; Supervision – M.D.S., J.A.N.; Resources – I.O.N.; Materials – I.O.N.; Data Collection and/or Processing – I.O.N.; Analysis and/or Interpretation – I.O.N.; Literature Search – I.O.N.; Writing Manuscript – I.O.N.; Critical Review – M.D.S., J.A.N.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– I.O.N.; Tasarım – I.O.N.; Denetleme – M.D.S., J.A.N.; Kaynaklar – I.O.N.; Malzemeler – I.O.N.; Veri Toplanması ve/veya İşlemesi – I.O.N.; Analiz ve/veya Yorum – I.O.N.; Literatür Taraması – I.O.N.; Yazıyı Yazan – I.O.N.; Eleştirel İnceleme – M.D.S., J.A.N.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

- Nwankwo IO, Faleke OO, Salihu MD, Magaji AA, Musa U, Garba J. Epidemiology of *Campylobacter* species in poultry and humans in the four agricultural zones of Sokoto State, Nigeria. *Public Health Epidemiol.* 2016;8(9):184-190.
- Nwankwo IO, Faleke OO, Salihu MD et al. Detection and viability of *Campylobacter* species isolates from different species of poultry and humans from Sokoto State, Nigeria. *Int J One Health.* 2016;2:19-23.
- Njoga EO, Ezenduka EV, Nwanta JA. Surveillance of *Campylobacter* infection in indigenous poultry reared at Nsukka, Nigeria. *Not Sci Biol.* 2020;2(2):242-250.
- Nwankwo IO, Ezenduka EV, Nwanta JA, Ogugua AJ, Audu BJ. Prevalence of *Campylobacter* spp. and antibiotics resistant *E. coli* on poultry carcasses and handlers' hands at Ikpa slaughter, Nsukka, Nigeria. *Not Sci Biol.* 2021;13(2):2, 10866. [\[CrossRef\]](#)
- Nwankwo IO, Faleke OO, Salihu MD, Magaji AA. Prevalence of *Campylobacter* species in out patients and pregnant women attending Government clinics in Sokoto, Nigeria. *JPMC.* 2016;1(2):8-15.
- Ihekweazu C, Michael CA, Nguku PM, et al. Prioritization of zoonotic diseases of public health significance in Nigeria using the one-health approach. *One Health.* 2021;13:100257. [\[CrossRef\]](#)
- Nwankwo IO, Faleke OO, Salihu MD, et al. Prevalence and molecular identification of *Campylobacter* species isolates from poultry and humans in Sokoto, Nigeria. *Sok. J Vet Sci.* 2017;15:1-8.
- Njoga EO, Nwankwo IO, Ugwuwarua JC. Epidemiology of thermotolerant *Campylobacter* infection in poultry in Nsukka agricultural zone, Nigeria. *Int J One Health.* 2019;5:92-98. [\[CrossRef\]](#)
- Ugboma AN, Salihu MD, Magaji AA, Abubakar MB. Prevalence of *Campylobacter* species in ground water in Sokoto, Sokoto state, Nigeria. *Vet World.* 2013;6(6):285-287. [\[CrossRef\]](#)
- Nwankwo IO, Salihu MD, Faleke OO, Magaji AA, John G. Seasonal variation in prevalence and antimicrobial resistance of *Campylobacter* species isolates from the feces of free range chickens and humans in Sokoto, north western Nigeria. *Animal Science Reporter (e-edition).* 2018;11(4):11-21.
- Akwuobu C, Oboegbulem SI, Ofukwu RA. Characterization and antibiogram of local isolates of *Campylobacter* species from Chicken in Nsukka area, Southeast Nigeria. *Am. -Eurasian J. Sustain Agric.* 2010; 4(2):117-121.
- Adeleye SA, Braide W, Chinakwe EC, Esonu CE, Uzoh CV. Chicken meat, beef and vegetables: potential sources of *Campylobacter* jejuni contamination in Imo State, Nigeria. *Sustain Food Prod.* 2017; 3:63-71. [\[CrossRef\]](#)
- Braide W, Adeleye SA, Ohanusi I, Ibegbulem CR, Mike-Anaosike EE, Madu LC. Incidence of *Campylobacter* species in poultry products and beef sold in Owerri, Imo State, Nigeria. *EC Microbiol.* 2017;10(4): 147-155.
- Obiajuru IOC, Anolue FC, Adogu POU. Emergence of campylobacteriosis in Orlu, Imo State South Eastern Nigeria and Its antibiotic Susceptibility. *Carib. J Sci Tech.* 2015;3:798-804.
- Ohanu ME, Offune J. The prevalence of *Campylobacter* in childhood diarrhea in Enugu State of Nigeria. *J Commun Dis.* 2009;41(2): 117-120.
- Ogbor O, Ajayi A, Zautner AE, Smith SI. Antibiotic susceptibility profiles of *Campylobacter coli* Isolated from Poultry Farms in Lagos Nigeria – A Pilot Study. *Eur J Microbiol Immunol (Bp).* 2019;9(2):32-34. [\[CrossRef\]](#)
- Aboaba OO, Smith SI. Occurrence of *Campylobacter* species in poultry farms in Lagos area of Nigeria. *J Environ Biol.* 2005;26(2)(suppl): 403-408.
- Ogunlade AO, Ogunleye AO, Jeminlehin FO, Ajuwape ATP. Occurrence of *Campylobacter* species in beef cattle and local chickens and their antibiotic profiling in Ibadan, Oyo State, Nigeria. *Afr J Microbiol Res.* 2015;9(22):1473-1479. [\[CrossRef\]](#)
- Olufemi O, Oluseye O. Prevalence and antibiotics resistance of *Campylobacter jejuni* in retail chickens in Oyo State, Nigeria. *Food Sci Qual Manag.* 2016;48:7-11.
- Kehinde OO, Dipeolu MA, Awoyomi OJ, et al. Isolation Methods for Molecular detection and antibiotic resistance pattern of *Campylobacter* spp in Layer Chickens. *J Natl Sci Engrg Tech.* 2020;19(1):149-159. [\[CrossRef\]](#)
- Olubunmi PA, Adeniran MOA. Isolation of *Campylobacter* sp. from man and domestic animals in western Nigeria. *Bull Anim Hlth Prod Afr.* 1986;32:224-248.
- Uaboi-Egbenni PO, Okolie PN, Adesanya OD, Omonigbehin E, Sobande AO. Epidemiological studies of the incidence of pathogenic *Campylobacter* spp. amongst animals in Lagos metropolis. *Afr J Biotechnol.* 2008;7(16):2852-2956.
- Adekunle OC, Coker AO, Kolawole DO. Incidence, isolation and characterization of *Campylobacter* species in Osogbo. *Biol Med.* 2009; 1(1):24-27.
- Aboderin AO, Smith SI, Oyelese AO, Onipede AO, Zailani SB, Coker AO. Role of *Campylobacter jejuni/coli* in diarrhoea in Ile-Ife, Nigeria. *East Afr Med J.* 2002;79(8):423-426.
- Adekunle OC, Onilude AA, Sanusi TO. Molecular identification of *Campylobacter* species from positive cultural stool samples of diarrhoeic children in Osun State. *Open J Med Microbiol.* 2019;9:8-15. [\[CrossRef\]](#)
- Adedapo AE. *Prevalence and Drug Resistance Patterns of Campylobacter and Listeria species from the Stool Samples of HIV Patients in Ibadan, Nigeria* [Dissertation]. Ibadan: University of Ibadan; 2018.
- Coker AO, Adefeso AO. The changing patterns of *Campylobacter jejuni/coli* in Lagos, Nigeria after ten years. *East Afr Med J.* 1994; 71(7):437-440.
- Salihu MD, Junaidu AU, Oboegbulem SI, Egwu GO, Tambuwai FM, Yakubu Y. Prevalence of *Campylobacter* species in apparently healthy goats in Sokoto state (Northwestern) Nigeria. *Afr J Microbiol Res.* 2009;3(9):572-574.
- Salihu MD, Junaidu AU, Magaji AA, Abubakar MB, Adamu AY, Yakubu AS. Prevalence of *Campylobacter* in poultry meat in Sokoto, Northwestern Nigeria. *J Public Health Epidemiol.* 2009;1(2):041-045.
- Salihu MD, Junaidu AU, Oboegbulem SI, Egwu GO. Prevalence and Biotypes of *Campylobacter* species isolated from Sheep in Sokoto State, Nigeria. *Int J Animvet Adv.* 2009;1(1):6-9.
- Gwimi PB, Faleke O, Salihu MD et al. Prevalence of *Campylobacter* species in faecal samples of pigs and humans from Zuru Kebbi State, Nigeria. *Int J One Health.* 2015;1:1-5. [\[CrossRef\]](#)
- Salihu MD, Junaidu AU, Magaji AA, Rabi ZM. Study of *Campylobacter* in raw cow milk in Sokoto State, Nigeria. *Br J Dairy Sci.* 2010;1(1):1-5.
- Ugboma AN, Salihu MD, Magaji AA, Abubakar MB, Saulawa MA. Prevalence of *Campylobacter* species in Sokoto River, Sokoto State, Nigeria. *J Vet Adv.* 2012;2:55-59.
- Adekeye JO, Abdu PA, Bawa EK. *Campylobacter fetus* Subsp. *jejuni* in Poultry reared under Different Management Systems in Nigeria. *Avian Dis.* 1989;33(4):801-803. [\[CrossRef\]](#)
- Salihu MD, Junaidu AU, Magaji AA, Yakubu Y. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolates from commercial broiler flocks in Sokoto, Nigeria. *Res J Vet Sci.* 2012;5(2): 51-58. [\[CrossRef\]](#)
- Ogbomon EO, Akpomie OO, Enenya RP, Obonor O, Morka E. Prevalence and antibiotic susceptibility patterns of *Campylobacter* species in locally pasteurized milk product (Nunu) sold in Zaria Metropolis, Kaduna State, Nigeria. *Int J Microbiol Biotechnol.* 2018; 3(3):89-94.
- Ogbomon EO, Whong CMZ, Doko MHI, Magaji SN, Addai TI, Orukotan YF. Prevalence of *Campylobacter* spp. Among diarrhoeic HIV-patients in Kaduna, Nigeria. *Int J Appl Microbiol Biotechnol Res.* 2019; 7:70-78.
- Ibrahim A. *Campylobacter Enteritis in Zaria, North Western Nigeria* [Dissertation]. National Post Graduate Medical College of Nigeria; 2014.

39. Audu BJ, Norval S, Bruno L, Meenakshi R, Marion M, Forbes KJ. Genomic diversity and antimicrobial resistance of *Campylobacter* spp. from humans and livestock in Nigeria. *J Biomed Sci.* 2022;29(1):7. [\[CrossRef\]](#)
40. Ofukwu RA, Okoh AEJ, Akwuobu CA. Prevalence of *Campylobacter jejuni* in duck faeces around drinking water sources in Makurdi, north-central Nigeria. *Sok. J Vet Sci.* 2007;7(2):27-30.
41. Raji MA, Adekeye JO, Kwaga JKP, Bale JOO. Bioserogroups of *Campylobacter* species isolated from sheep in Kaduna State, Nigeria. *Small Rumi Res.* 2000;37(3):215-221. [\[CrossRef\]](#)
42. Olabode HOK, Mailafia SM, Ogbale ME, I.B. Ugbaja HOO. Isolation and antibiotic susceptibility of *Campylobacter* species from cattle offals in Gwagwalada abattoir, Abuja-FCT Nigeria. *Int J Curr Microbiol App Sci.* 2017;6(4):324-333. [\[CrossRef\]](#)
43. Olabode MP, Ogbu KI, Sabo JA, et al. Prevalence of *Campylobacter* species in dogs in Bassa, Plateau State, Nigeria. *Ann. Microbiol. Infect Dis.* 2019;2(3):21-25.
44. Karshima SN, Bobbo AA. Isolation and PCR characterisation of thermophilic *Campylobacter* species in Dogs Presented to Selected Veterinary Clinics in Jos, Nigeria. *Alex J Vet Sci.* 2016;50(1):70-77. [\[CrossRef\]](#)
45. Ngulukun SS. *Molecular Epidemiology of Thermophilic Campylobacter Infection in Poultry, Cattle and Humans in Plateau State, Nigeria.* Nsukka: University of Nigeria; 2010.
46. Ngulukun SS, Oboegbulem SI, Fagbamila IO, et al. Isolation of thermophilic *Campylobacter* species from Japanese quails (*Coturnix-coturnix*) in Vom, Nigeria. *Vet Rec.* 2010;166(5):147-148. [\[CrossRef\]](#)
47. Samuel SO, Oboderin AO, Akanbi AA, Adegboro B, Smith SI, Coker AO. *Campylobacter* enteritis in Ilorin, Nigeria. *East Afr Med J.* 2009; 9:478-484.
48. Ngulukun SS, Oboegbulem SI, Fagbamila IO, Bertu W, Odugbo MO. Prevalence and molecular characterization of thermophilic *Campylobacter* species isolated from cattle in Plateau State, Nigeria. *Nig. Vet J.* 2011;32(4):349-356.
49. Akosua BK, Kwasi O, Enoch HF, Karen AK. Multidrug resistant *Campylobacter* in faecal and carcasses of commercially produced poultry. *Afr J Microbiol Res.* 2017;11(7):271-277. [\[CrossRef\]](#)
50. Rodrigues CG, Melo RT, Fonseca BB, et al. Occurrence and characterization of *Campylobacter* spp. isolates in dogs, cats and children. *Pesq Vet Bras.* 2015;35(4):365-370. [\[CrossRef\]](#)



Köftelerin Bazı Kalite Özelliklerine Bamya Müsilaj Bazlı Yenilebilir Kaplamanın Etkisi

The Effect of Okra mucilage Based Edible Coating on Some Quality Properties of Meatballs

Enes TERCANLI^{ID}
Mustafa ATASEVER^{ID}

Atatürk Üniversitesi, Veteriner
Fakültesi, Besin-Gıda Hijyeni ve
Teknolojisi Anabilim Dalı, Erzurum,
Türkiye



*Bu çalışma Enes Tercanlı'nın Yüksek
Lisans tez çalışmasından üretilmiştir.

Geliş Tarihi/Received: 02.10.2022

Kabul Tarihi/Accepted: 14.12.2022

Yayın Tarihi/Publication Date: 26.04.2023

Sorumlu yazar/Corresponding author:
Enes TERCANLI
E-mail: enestercanli@outlook.com

Atıf: Tercanlı E, Atasever M. Köftelerin
bazı kalite özelliklerine bamya müsilaj
bazlı yenilebilir kaplamanın etkisi.
Vet Sci Pract. 2023; 18(1), 12-18.

Cite this article as: Tercanlı E,
Atasever M. The effect of okra mucilage
based edible coating on some quality
properties of meatballs. *Vet Sci Pract.*
2023; 18(1), 12-18.



Copyright©Author(s) - Available online at
veterinarysciences-ataunipress.org
Content of this journal is licensed under a
Creative Commons Attribution-
NonCommercial 4.0 International License

Öz

Köfte tipi et ürünleri kolay hazırlanabilmesi ve beğenilerek tüketilmesinden dolayı fazla tercih edilen besinlerdendir. Ancak bu tip besinler buzdolabı şartlarında (4°C) kısa sürede bozulabilmektedir. Bu çalışmada hazırlanan kaplama malzemeleri köftelerin depolama stabilitelerini geliştirmek ve raf ömürlerini uzatmak amacıyla oluşturuldu. Bamya meyvesinden elde edilen müsilaj; nar çekirdeği yağı (*Punica granatum*) ve kekik yağı (*Origanum heracleoticum* L.) ile kombine edilerek farklı yenilebilir kaplama malzemeleri [Kontrol (K0) ve bamya müsilaj—% 1 gliserol (K1), bamya müsilaj—% 1 gliserol—% 1 nar çekirdeği yağı (K2), bamya müsilaj—% 1 gliserol—% 1 kekik yağı (K3)] oluşturuldu ve köftelere uygulandı. Depolamanın (4°C) 1., 3., 5., 7. ve 9. günlerinde köftelerin bazı mikrobiyolojik, kimyasal, fiziksel ve duyu analizleri yapıldı. K1 kaplama grubunun K0 grubuna göre tbars değerleri genel olarak daha düşük bulundu. Bu durum kaplama malzemesinin gaz bariyeri sağlayabileceğini göstermektedir. K3 grubunda; tbars değerinin daha düşük olduğu ve *Lactobacillus* spp. sayısındaki değişim günler arasında anlamlı bir farklılığa neden olmadığı belirlendi ($P > .05$). Kaplama malzemelerinin duyu yönünden olumsuz bir etkisi gözlemlenmedi. Sonuçta kaplama malzemelerinin köftelerin raf ömrünü uzatmada çok fazla etkili olmadığı; ancak bamya müsilajının yenilebilir film ve kaplama potansiyelinin olduğu saptandı. Bununla birlikte kaplama malzemelerinin farklı gıdalar üzerine etkisinin incelenmesine ihtiyaç olduğu düşünülmektedir.

Anahtar Kelimeler: Bamya müsilajı, kekik yağı, köfte, nar çekirdeği yağı, yenilebilir kaplama

ABSTRACT

Meatballs are among the most preferred foods because they can be easily prepared and consumed with pleasure. Meatball-type meat products can deteriorate in a short time, even under refrigerator conditions. The coating materials applied in this study were created to increase the meatballs' storage resistance and extend their shelf life. The mucilage obtained from okra was combined with pomegranate seed oil (*Punica granatum*) and thyme oil (*Origanum heracleoticum* L.) to make different edible coating materials. These different edible coating materials [Control (K0); okra mucilage—1% glycerol (K1); okra mucilage—1% glycerol—1% pomegranate seed oil (K2); okra mucilage—1% glycerol—1% thyme oil (K3)] were applied to the meatballs. Some microbiological, chemical, physical, and sensory analyses of the meatballs were performed on the 3rd, 5th, 7th, and 9th days of storage. The tbars values of the K1 coating group were generally lower than the K0 group. This indicates that the coating material can provide a gas barrier. It was determined that the tbars value was lower in the K3 group, and the change in the number of *Lactobacillus* spp. did not cause a significant difference between days ($P > .05$). There was no negative sensory effect of the coating materials. As a result, it was determined that okra mucilage has edible film and coating potential. However, the coating materials applied in this study did not have much effect on extending the shelf life of meatballs. It is thought that there is a need to examine the effect of coating materials on different foods.

Keywords: Edible coating, meatballs, okra mucilage, pomegranate seed oil, oregano oil

GİRİŞ

Et biyolojik değeri yüksek proteinler ve bazı besin öğeleri (örn., demir, B12 vitamini, çinko, selenyum) açısından zengindir.¹ Ancak et; su aktivitesi, pH ve içerdiği besin öğeleri bakımından mikrobiyal, kimyasal

ve duysal bozulmaya duyarlıdır. Mikrobiyal faaliyetler ve oksidatif reaksiyonlar etin işlenmesi ve depolanması esnasında bazı kalite kayıplarına (örn., duysal, besinsel) neden olabilmektedir. Bu durum hem halk sağlığını tehdit edebilmekte hem de ekonomik kayıplar oluşturabilmektedir.²

Köfte tipi et ürünleri hazır ve kolay tüketilebilen bir ürün olduğundan tüketiciler tarafından fazla tercih edilmektedir. Özellikle son yıllarda tüketicilerin hazır ve kolay tüketilebilen besinlere yönelik artan taleplerinin yanısıra sağlıklı beslenme ilgileri de artmıştır. Tüketici tercihleri; et ve ürünlerinin mikrobiyolojik olarak güvenli, raf ömrü uzun, minumun düzeyde işlenmiş ve daha az gıda katkı maddeleri kullanılmış olması yönündedir.^{3,4} Güvenli ve sağlıklı et ürünlerinin tüketimi; et ürününün kalitesine, besin içeriğine ve mikrobiyal güvenliğini sağlamak için uygulanan işlemlere ve koruma yöntemlerine bağlıdır. Et endüstrisi artan rekabet ve daha sıkı maliyet marjları için, et ürünlerinin kalitesini ve duysal özelliklerini olumsuz etkilemeden bazı muhafaza yöntemleri bulmak ve geliştirmek için çalışmaktadır.⁵ Özellikle yenilebilir film ve kaplamalar bu yöntemler içerisinde giderek ilgi görmektedir. Hem ambalaj hem de bir gıda bileşeni olan yenilebilir film ve kaplamaların; gıdaları mikrobiyolojik ve fiziksel etkilere koruyan, tüketildiğinde iyi bir duysal izlenim bırakan, toksik olmayan, ucuz ve kolay uygulanabilir özellikte olması istenir.⁶ Ayrıca bazı yenilebilir film ve kaplamaların antioksidan ve antimikrobiyal maddeleri taşıma özelliklerinden de yararlanılmaktadır. Bu amaçla da kaplama malzemelerine bazı antioksidan ve antimikrobiyal katkıları eklenip koruyucu özellikleri geliştirilmektedir.⁷ Bamya müsilağından elde edilen kaplama malzemesinin tüm bu özellikleri sağlayabileceği ile ilgili literatür bilgileri olsa da farklı gıdalar üzerine uygulanması ile ilgili çalışmalar kısıtlıdır.⁸⁻¹⁰

Son yıllarda yapılan çalışmalarda, köfte tipi ürünlerin soğuk depolama boyunca raf ömrünün artırılması, mikrobiyal gelişimin önlenmesi ve oksidasyonun engellenmesi gibi bazı kriterlerin iyileştirilmesi için stratejiler geliştirilmektedir.^{3,11} Bu stratejiler doğrultusunda bu çalışmada, bamya müsilağından elde edilen yenilebilir kaplama malzemeleri nar çekirdeği ve kekik yağı ile kombine edilerek köftelere uygulanmıştır. Depolama süresince kaplama malzemelerinin köftelerin mikrobiyolojik, kimyasal, fiziksel ve duysal özelliklerine etkisi incelenmiştir.

MATERYAL VE METOT

Materyal

Araştırmada kullanılacak köfteler Erzurum Et ve Süt Kurumu'ndan temin edildi. Köfte hamuru; dana eti ve yağı (%89), kuzu eti ve yağı (%10) (yağ miktarı en çok %25, kolajenin et proteinine oranı en çok %15) ve köfte harcından (%) (soğan, galeta unu, baharat karışımı, tuz ve sodyum bikarbonat) oluşmaktadır. Köfteler, soğuk zincirde Atatürk Üniversitesi Veteriner Fakültesi Besin-Gıda Hijyeni ve Teknolojisi Bölümü Laboratuvarına getirildi ve kaplama yapıldı kadar 4°C'de bekletildi.

Bamya Müsilağı Bazlı Kaplama Malzemesinin Hazırlanışı

Bamya müsilağı, Ghorı ve ark¹² 'nın yöntemi modifiye edilerek elde edildi. Bu çalışmada uygulanan modifiye yöntemde; Erzurum'da yerel bir pazardan alınan taze bamya meyvelerinin uç kısımları kesilip temizlendi. %2 oranında sirkeli suda yarım saat bekletilip yıkandıktan sonra dilimlenen bamya meyveleri 1 : 3 oranında saf su içerisinde 4°C'de 24 saat bekletildikten sonra süzldü. Elde edilen müsilağı steril kaplara alınarak 4°C'de bekletildi.

Bamya müsilağından biri kaplamasız (KO, kontrol grubu) olmak üzere üç farklı kaplama çözeltisi hazırlanarak dört grup

oluşturuldu. Birinci kaplama (K1) çözeltisinin hazırlanmasında bamya müsilağına %1 oranında gliserol ilave edildi. Gliserol, bamya müsilağına elastikiyet özellik (plastikleştirici) kazandırması amacıyla eklendi.⁹ İkinci kaplama (K2) çözeltisinin hazırlanmasında bamya müsilağına %1 gliserol ve %1 nar çekirdeği yağı eklendi. Üçüncü kaplama (K3) çözeltisinin hazırlanmasında bamya müsilağına %1 gliserol ve %1 kekik yağı ilave edildi. Hem nar çekirdeği yağı hem de kekik yağının kaplama çözeltisi içinde homojen şekilde dağılmasını sağlamak için; yağlar %0,5'lik Tween 80 ile karıştırıldıktan sonra çözeltiye ilave edildi.¹³ Kaplama çözeltisine ilave edilen maddelerin bamya müsilağına iyice karışması için çözelti; magnetik karıştırıcıda 60°C'de 30 d (dakika) karıştırıldı ve kullanılabacağı zamana kadar 4°C'de bekletildi.

Kaplama Malzemelerinin Uygulanması

Numuneler önceden hazırlanmış kaplama çözeltisinde 10 d bekletildi sonra steril ızgara üzerinde 2 d süzldü daha sonra tekrar kaplama çözeltilerine alınıp 10 dk bekletildi. Köfteler farklı günlerde analizler için hazırlanmış steril ağzı kapaklı köpük tabaklara alındı.

Depolamanın (4°C) 1., 3., 5., 7. ve 9. günlerinde mikrobiyolojik [toplam mezofilik aerobik bakteri (TMAB), toplam psikrofilik aerobik bakteri (TPAB), *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, maya/küf sayıları], kimyasal ve fiziksel [tiyobarbitürik asit reaktif substans değeri (tbars), pH, a_w (su aktivitesi), renk analizleri], duysal analizleri üç tekerrürlü olarak yapıldı.

Metot

Mikrobiyolojik Analizler

Çalışmada uygun dilüsyonlardan 1 ml alınarak dökme plak yöntemi ile besiyerlerine ekim yapıldı. TMAB sayısı için Plate Count Agar (PCA) besiyerinde 37°C 'de 48 saat, TPAB sayısı için PCA besiyerinde 5 ± 1°C'de 10 gün, *Lactobacillus* spp. sayısı için De Man Rogosa Sharpe Agar (MRS) besiyeri anaerobik ortamda (Anaerocult A) 37°C'de 72 saat inkübe edildikten sonra sayıldı. *Pseudomonas* spp. sayısı için CFC (Cephalovidine Fucidin Cetrimide) supplementli *Pseudomonas* Agar besiyerine uygun dilüsyonlardan ekim yapıldıktan sonra 20 °C'de 48 saat inkübasyona bırakıldı ve 1 mm çapından büyük ve katalaz pozitif olan koloniler sayıldı.¹⁴

Kimyasal ve Fiziksel Analizler

Tiyobarbitürik Asit Reaktif Substans (TBARS) Değerinin Belirlenmesi

Homojen hale getirilen köfte numunelerinden 2 g alınarak üzerine 12 ml Trikloro Asitik Asit (TCA) çözeltisi (%7,5 TCA, %0,1 EDTA, %0,1 Propil galat 3 ml etanolde çözülür) ilave edildi. Ultra-turraxda homojenize edilen karışım daha sonra Whatman 1 filtre kağıdından süzldü. Süzüntüden 3 ml alınarak deney tüpüne aktarıldı, üzerine 3 ml Tiyobarbitürik Asit (TBA) (0,02M) çözeltisi konuldu ve karıştırıldı. Karışım su banyosunda 100°C'de 40 d bekletildikten sonra hemen soğutuldu ve 2000 rpm de 5 d santrifüj edildi. Daha sonra spektrofotometrede (Aquamate Thermo electron corporation, England) 530 nm dalga boyunda okundu. Elde edilen absorbans değerleri formülde yerine konularak hesaplama yapıldı.¹⁵

TBARS = ((absorbans/ k (0,06) × 2/1000) × 6,8) × 1000/örnek ağırlığı

pH Değerinin Belirlenmesi

Bamya müsilağı, kıyma ve köfte numunelerinden 10 g tartıldı. Üzerine 100 ml saf su ilave edilerek homojenize edildi. Daha sonra pH metre (inolab WTW) ile pH değeri ölçldü.¹⁶

Renk Analizi

Kıyma ve köfte numunelerinin renk analizi Chroma Meter (Konica Minolta Japonya) cihazı kullanılarak yapıldı. Sonuçlar L* (parlaklıktan koyuluğa), a* (+a kırmızılığa, -a yeşilliğe) ve b* (+b sarılığa, -b maviliğe) parametrelerine göre açıklandı.¹⁶

Su Aktivitesi Tayini

Kıyma ve köfte numunelerinin su aktivitesi (aw) değerinin belirlenmesinde Aqua LAB 4TE cihazı kullanıldı.¹⁶

Duyusal Analizler

Duyusal analiz Atatürk Üniversitesi Veteriner Fakültesi Besin Hijyeni ve Teknolojisi Anabilim Dalı'nda eğitimli panelistler tarafından yapıldı. Panelistler köfte numunelerini sıra gözetmeden rastgele değerlendirdi. Köfte gruplarının duysal analizinde; lezzet, koku, aroma tekstür, görünüş ve genel edilebilirlik özellikleri incelendi. Hedonik skala kullanılarak yapılan analizlerde 1-5 arası puanlama uygulandı. Buna göre; 5 "çok iyi", 4 "iyi", 3 "ne iyi ne kötü", 2 "kötü" ve 1 "çok kötü" olarak değerlendirildi.¹⁷

İstatistiksel Analizler

Araştırma sonunda elde edilen verilerin IBM SPSS Statistics 20 programı kullanılarak varyans analizi (ANOVA) yapıldı ve analiz sonuçları istatistiki olarak değerlendirildi. Sonuçlar Duncan Çoklu Karşılaştırma Testi ile değerlendirildi, uygulama grupları ve depolama günleri arasında farklılık belirlendi.¹⁸

BULGULAR

Mikrobiyolojik Analizler

Köfte numunelerinin depolama (4°C) süresince TMAB, TPAB, *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacteriaceae*, maya/küf sayılarında meydana gelen değişimler Tablo 1'de gösterilmiştir.

Depolama süresince TMAB, TPAB ve *Lactobacillus* spp. sayısındaki değişim, gruplar arasında anlamlı bir farklılığa neden olmamıştır ($P > ,05$). Tüm gruplarda; TMAB sayısı depolamanın 7. gününde, TPAB sayısı ise depolamanın 5. gününde anlamlı bir artış göstermiştir ($P < ,05$).

Lactobacillus spp. sayısında, KO grubunda 9. günde; K1 ve K2 grubunda ise 7. günde anlamlı bir artış gözlemlenmiştir ($P < ,05$). K3 grubunda ise *Lactobacillus* spp. sayısındaki değişim günler arasında anlamlı bir farklılığa neden olmadığı belirlenmiştir ($P > ,05$). *Pseudomonas* spp., *Enterobacteriaceae*, maya/küf sayılarında, depolama süresince gruplar ve günler arasında istatistiki olarak anlamlı bir farklılık bulunmamıştır ($P > ,05$).

Bamya müsilağında TMAB, TPAB, *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacteriaceae* ve maya/küf üremesi gerçekleşmemiştir.

Kimyasal ve Fiziksel Analizler

Farklı kombinasyonlarda hazırlanmış kaplama malzemelerinin uygulandığı köfte numunelerinin depolama (4°C) süresince tbars, pH a_w ve renk değerlerinde meydana gelen değişim Tablo 2'de gösterilmektedir.

KO grubunda 5. ve 9. günlerde, K1 grubunda 7. ve 9. günlerde, K2 ve K3 grubunda ise 5. ve 9. günlerde tbars değerlerinde anlamlı bir artış saptanmıştır ($P < ,05$). Tbars değeri KO grubunda 5. günde anlamlı bir artış gösterirken K1 grubunda ise bu artışın 7. günde olduğu bulunmuştur. Ayrıca KO grubunun 1. ve 3. günlerde tbars değerlerinin diğer gruplara göre daha yüksek olduğu saptanmıştır ($P < ,05$). Diğer günlerde de ortalama TBARS değerleri KO grubunda daha fazladır.

Tablo 1. Köfte numunelerinin depolama süresince (4°C) bazı mikrobiyal değerlerindeki değişimler (log kob) ($\bar{x} \pm S.S$)

Mikrobiyolojik Analizler	Gruplar	Depolama Süresi (4°C)				
		1.Gün	3.Gün	5.Gün	7.Gün	9.Gün
TMAB	K0	5,14 $\pm$ 0,20b	5,91 $\pm$ 0,58ab	6,12 $\pm$ 0,64ab	6,74 $\pm$ 0,37a	6,64 $\pm$ 0,77a
	K1	5,28 $\pm$ 0,65b	5,45 $\pm$ 0,60b	6,08 $\pm$ 0,74ab	6,78 $\pm$ 0,12a	6,61 $\pm$ 0,73a
	K2	5,35 $\pm$ 0,59b	5,66 $\pm$ 0,12b	6,16 $\pm$ 0,89ab	6,93 $\pm$ 0,6a	6,60 $\pm$ 0,95ab
	K3	5,14 $\pm$ 0,49b	5,62 $\pm$ 0,11ab	6,04 $\pm$ 0,77ab	6,78 $\pm$ 0,10a	6,24 $\pm$ 1,05ab
TPAB	K0	6,52 $\pm$ 0,16c	6,84 $\pm$ 0,14bc	7,49 $\pm$ 0,32a	7,31 $\pm$ 0,48ab	7,81 $\pm$ 0,11a
	K1	6,59 $\pm$ 0,06c	6,80 $\pm$ 0,26bc	7,47 $\pm$ 0,35a	7,27 $\pm$ 0,58ab	7,77 $\pm$ 0,09a
	K2	6,26 $\pm$ 0,64c	6,82 $\pm$ 0,27bc	7,50 $\pm$ 0,31ab	7,25 $\pm$ 0,71ab	7,75 $\pm$ 0,09a
	K3	6,56 $\pm$ 0,05c	6,87 $\pm$ 0,24bc	7,41 $\pm$ 0,42ab	7,22 $\pm$ 0,79abc	7,73 $\pm$ 0,08a
<i>Lactobacillus</i> spp.	K0	5,00 $\pm$ 0,33b	5,93 $\pm$ 1,29ab	6,38 $\pm$ 0,57ab	6,20 $\pm$ 0,65ab	6,69 $\pm$ 0,29a
	K1	5,06 $\pm$ 0,43b	5,57 $\pm$ 1,08ab	6,37 $\pm$ 0,35a	6,42 $\pm$ 0,59a	6,58 $\pm$ 0,65a
	K2	5,13 $\pm$ 0,72b	5,56 $\pm$ 1,07ab	6,44 $\pm$ 0,45ab	6,59 $\pm$ 0,42a	6,59 $\pm$ 0,57a
	K3	4,78 $\pm$ 0,80	5,63 $\pm$ 0,99	6,00 $\pm$ 0,63	6,32 $\pm$ 0,33	6,37 $\pm$ 1,09
<i>Pseudomonas</i> spp.	K0	4,38 $\pm$ 1,20	4,88 $\pm$ 0,66	4,97 $\pm$ 0,76	4,95 $\pm$ 0,46	5,65 $\pm$ 0,85
	K1	4,26 $\pm$ 0,86	4,74 $\pm$ 0,61	4,85 $\pm$ 0,68	4,98 $\pm$ 0,55	5,55 $\pm$ 0,72
	K2	4,31 $\pm$ 0,85	4,62 $\pm$ 0,62	4,83 $\pm$ 0,73	5,02 $\pm$ 0,53	5,26 $\pm$ 0,30
	K3	4,42 $\pm$ 0,79	4,77 $\pm$ 0,56	4,79 $\pm$ 0,61	4,95 $\pm$ 0,56	5,40 $\pm$ 0,47
<i>Enterobacteriaceae</i>	K0	3,93 $\pm$ 0,49	3,28 $\pm$ 1,13	3,85 $\pm$ 0,47	3,79 $\pm$ 0,57	3,39 $\pm$ 0,15
	K1	3,77 $\pm$ 0,47	3,46 $\pm$ 0,96	3,65 $\pm$ 0,39	3,68 $\pm$ 0,53	3,42 $\pm$ 0,07
	K2	3,86 $\pm$ 0,65	3,37 $\pm$ 0,91	3,63 $\pm$ 0,37	3,51 $\pm$ 0,77	3,48 $\pm$ 0,16
	K3	3,85 $\pm$ 0,74	3,03 $\pm$ 0,28	3,54 $\pm$ 0,42	3,46 $\pm$ 0,68	3,27 $\pm$ 0,09
Maya/Küf	K0	5,06 $\pm$ 0,62	5,16 $\pm$ 0,33	5,51 $\pm$ 0,54	5,29 $\pm$ 0,52	5,47 $\pm$ 0,30
	K1	4,99 $\pm$ 0,71	5,19 $\pm$ 0,21	5,54 $\pm$ 0,61	5,52 $\pm$ 0,75	5,77 $\pm$ 0,62
	K2	4,98 $\pm$ 0,73	5,28 $\pm$ 0,26	5,69 $\pm$ 0,79	5,64 $\pm$ 0,59	5,93 $\pm$ 0,02
	K3	4,99 $\pm$ 0,75	5,19 $\pm$ 0,34	5,44 $\pm$ 0,63	5,48 $\pm$ 0,72	5,53 $\pm$ 0,16

K0=Kaplama suz

K1=Bamya Müsilağı + %1 Gliserol

K2=Bamya Müsilağı + %1 Gliserol + %1 Nar Çekirdeği Yağı

K3=Bamya Müsilağı + %1 Gliserol + %1 Kekik Yağı

Her satırda a,b,c (-) ve her sütunda A,B (I) aynı olmayan günler ve gruplar arasında p<0.05 düzeyinde anlamlı farklılığı göstermektedir.

Log kob=logatırmık koloni oluşturan birim

$\bar{x} \pm S.S$ =ortalama değer $\pm$ standart sapma

Tablo 2. Köfte numunelerinin depolama süresince (4°C) tbars, pH, a_w ve renk değerlerinde meydana gelen değişimler ($\bar{x} \pm S.S$)

Bazı kimyasal değerler	Gruplar	Depolama Süresi (4°C)				
		1.Gün	3.Gün	5.Gün	7.Gün	9.Gün
TBARS	K0	12,25 ± 1,20Ac	11,75 ± 0,97Ac	17,48 ± 7,18Ab	20,92 ± 6,54Ab	28,52 ± 3,62Ba
	K1	11,19 ± 1,36ABcd	10,03 ± 0,70Bd	14,68 ± 7,72Ac	19,82 ± 6,68Ab	26,50 ± 4,86Ba
	K2	10,29 ± 1,84Bc	10,79 ± 1,77ABc	17,20 ± 7,66Ab	20,70 ± 6,92Ab	28,07 ± 2,06Ba
	K3	10,49 ± 1,15Bc	10,34 ± 1,28Bc	13,28 ± 1,37Ab	14,18 ± 1,95Bab	15,57 ± 2,15Aa
pH	K0	6,27 ± 0,10a	5,92 ± 0,27ab	5,45 ± 0,24bc	5,52 ± 0,60bc	5,22 ± 0,51c
	K1	6,40 ± 0,03a	5,96 ± 0,24b	5,58 ± 0,26c	5,38 ± 0,44c	5,32 ± 0,38c
	K2	6,20 ± 0,27a	5,80 ± 0,39ab	5,52 ± 0,25bc	5,37 ± 0,46bc	5,26 ± 0,40c
	K3	6,31 ± 0,10a	5,77 ± 0,40b	5,55 ± 0,29bc	5,34 ± 0,50bc	5,24 ± 0,38c
a _w	K0	0,9801 ± 0,002A	0,9768 ± 0,003A	0,9772 ± 0,005	0,9753 ± 0,006	0,9765 ± 0,002A
	K1	0,9834 ± 0,003BA	0,9809 ± 0,002BA	0,9814 ± 0,003	0,9801 ± 0,002	0,9842 ± 0,002B
	K2	0,9861 ± 0,003B	0,9852 ± 0,002B	0,9812 ± 0,004	0,9789 ± 0,003	0,9820 ± 0,001B
	K3	0,9831 ± 0,001BA	0,9811 ± 0,001BA	0,9787 ± 0,005	0,9801 ± 0,004	0,9826 ± 0,001B
Renk (L *)	K0	53,10 ± 3,09B	55,12 ± 1,76AB	52,08 ± 3,96	52,24 ± 4,13	52,21 ± 5,59
	K1	56,34 ± 1,76A	55,93 ± 1,64A	54,48 ± 2,92	53,56 ± 3,08	56,52 ± 6,69
	K2	56,86 ± 1,83A	54,32 ± 1,72AB	54,43 ± 2,12	54,00 ± 4,82	56,08 ± 5,94
	K3	57,32 ± 3,05Aa	53,13 ± 2,04Bb	52,81 ± 1,28b	54,52 ± 4,31ab	55,94 ± 2,10ab
Renk (a *)	K0	11,93 ± 1,99	8,20 ± 1,70	7,26 ± 3,17	7,21 ± 5,95	7,07 ± 6,78
	K1	11,40 ± 0,55	9,43 ± 0,87	7,85 ± 4,00	6,89 ± 5,25	6,69 ± 5,85
	K2	11,64 ± 1,12	7,48 ± 2,28	7,26 ± 4,02	6,99 ± 6,14	6,63 ± 6,08
	K3	11,27 ± 1,25a	7,99 ± 1,89ab	7,09 ± 3,15ab	6,82 ± 5,20ab	6,21 ± 4,76b
Renk (b *)	K0	20,33 ± 1,19a	20,07 ± 1,28ab	18,59 ± 1,77b	16,98 ± 1,32c	16,18 ± 0,80c
	K1	20,70 ± 2,28a	20,78 ± 1,73a	18,88 ± 2,30ab	17,75 ± 1,19b	16,74 ± 0,83b
	K2	20,94 ± 1,65a	19,37 ± 1,42ab	18,10 ± 1,33bc	17,08 ± 1,96c	17,01 ± 0,99c
	K3	20,60 ± 1,47a	18,86 ± 1,48b	18,60 ± 1,68bc	17,56 ± 1,23bc	17,03 ± 0,88c

K0=Kaplama

K1=Bamya Müsiliği + % 1 Gliserol

K2=Bamya Müsiliği + % 1 Gliserol + % 1 Nar Çekirdeği Yağı

K3=Bamya Müsiliği + % 1 Gliserol + % 1 Kekik Yağı

Her satırda a,b,c (→) ve her sütunda A,B (↓) aynı olmayan günler ve gruplar arasında p<0.05 düzeyinde anlamlı farklılığı göstermektedir.

 $\bar{x} \pm S.S$ =ortalama değer ± standart sapma

Depolamanın ilk gününde tüm gruplarda pH değerleri birbirine yakın olup 6,27-6,40 arasındadır. Bu durum mikrobiyal üreme için uygun bir değer aralığıdır. Bamya müsiliğinin pH değeri 4,2 ± 0,12 olmasına karşın, kaplamalı gruplar ile kontrol grubu arasında pH değerinin; depolama süresince gruplar arasında anlamlı bir fark oluşturmadığı saptanmıştır ($P > ,05$). Depolamanın 5. gününde pH değeri tüm gruplarda anlamlı bir şekilde azalmıştır. ($P < ,05$).

Depolamanın 1. ve 3. günlerinde K2 grubunun K0 grubuna göre a_w değerinin anlamlı olarak daha yüksek olduğu saptanmıştır ($P < ,05$). Depolamanın 9. gününde K0 grubunun a_w değerinin anlamlı olarak diğer gruplara nazaran daha düşük olduğu saptanmıştır ($P < ,05$).

Depolamanın 1. gününde L* (parlaklık) değerinin kaplamalı gruplarda kontrol grubuna göre daha yüksek olduğu saptanmıştır ($P < ,05$).

Duyusal Analizler

Farklı kombinasyonlarda hazırlanmış kaplama malzemelerinin uygulandığı köfte numunelerinin depolama (4°C) süresince gerçekleştirilen duyusal analiz değerleri Tablo 3'te gösterilmektedir.

Depolama süresi boyunca gruplar arasındaki; lezzet, koku, aroma, tekstür, görünüş ve genel kabul edilebilirlik değerleri arasında istatistiki olarak anlamlı bir fark bulunmamıştır ($P > ,05$). Tüm gruplarda depolamanın 7. ve 9. gününde tüm duyusal değerlerin anlamlı bir şekilde azaldığı saptanmıştır ($P < ,05$).

TARTIŞMA

Et ve ürünleri içerdiği besin öğeleri, su aktivitesi ve pH değerleri bakımından mikrobiyal bozulmaya çok duyarlıdır. Soğukta

muhafaza süresince oksijen, sıcaklık, ışık ve metal iyonları protein ve lipidlerin oksidasyonuna neden olarak oksidatif katalistlerin oluşmasına neden olmaktadır. Bu durum besin bileşimi, lezzet, tekstür ve renk gibi bazı kalite kriterlerini etkilemektedir.^{19,20} Bunun için araştırmalarda soğukta muhafaza boyunca bakteriyel faaliyeti kontrol altında tutmak ve oksidasyonu geciktirmek ana hedef olmalıdır. Çünkü et ve ürünlerinde oksidasyon, mikrobiyal yük ve duysal özellikler önemli kalite kriterleridir. Yenilebilir kaplamalar; et ve ürünlerinde meydana gelen oksidasyonu, mikrobiyal gelişmeyi, istenmeyen renk ve duysal özellikleri engellemek ve geciktirmek amacı ile kullanılabilir.^{21,22} Bu çalışmada kaplama malzemeleri köftelere uygulanarak soğuk depolama (4°C) boyunca numunelerde; mikrobiyal yük ve oksidasyon kontrol edilmeye çalışılmıştır.

TMAB sayısı gıdaların kalitesindeki bozulmayı gösteren önemli bir parametredir.²³ Bu çalışmada, depolama süresince TMAB sayısı gruplar arasında anlamlı bir farklılığa neden olmamıştır ($P > ,05$). K0, K1, K2 ve K3 grubunda 7. günde TMAB sayısı anlamlı bir şekilde artış göstermiştir ($P < ,05$). Tüm gruplarda ortalama TMAB sayısı 9. günde azalmıştır ($P > ,05$). Bu durum farklı mikroorganizmaların ortamda daha baskın hale gelmesi ile açıklanabilir. Gıdaların mikrobiyal florası oldukça dinamikdir. TPAB sayısında da gruplar arasında depolama süresince anlamlı bir fark bulunmamıştır ($P > ,05$). Tüm gruplarda TPAB sayısı depolamanın 5. gününde anlamlı bir şekilde artış göstermiştir ($P < ,05$).

Laktik asit bakterileri et ve ürünlerinin mikroflorasında doğal olarak bulunabilmektedir.²⁴ Depolama süresince *Lactobacillus* spp. sayısındaki değişim, gruplar arasında anlamlı bir farklılığa neden olmamıştır ($P > ,05$). Bu durum Emiroğlu ve ark⁷ 'nın yaptıkları

Tablo 3. K fte numunelerinin depolama s resince (4 C) duysal deęerlerindeki deęişimler ($\bar{x} \pm S.S$)

Duysal Analizler	Gruplar	Depolama S�resi (4�C)				
		1.G�n	3.G�n	5.G�n	7.G�n	9.G�n
Lezzet	K0	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	3,66 $\pm$ 0,48b	2,83 $\pm$ 0,38c
	K1	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	3,69 $\pm$ 0,47b	2,87 $\pm$ 0,33c
	K2	4,91 $\pm$ 0,28a	4,91 $\pm$ 0,28a	4,79 $\pm$ 0,41a	3,62 $\pm$ 0,49b	2,83 $\pm$ 0,38c
	K3	4,91 $\pm$ 0,28a	4,87 $\pm$ 0,33a	4,75 $\pm$ 0,44a	3,54 $\pm$ 0,50b	2,75 $\pm$ 0,44c
Koku	K0	4,91 $\pm$ 0,28a	4,91 $\pm$ 0,28a	4,79 $\pm$ 0,50a	3,66 $\pm$ 0,48b	2,79 $\pm$ 0,41c
	K1	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	3,73 $\pm$ 0,44b	2,87 $\pm$ 0,33c
	K2	4,83 $\pm$ 0,38a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	3,66 $\pm$ 0,56b	2,75 $\pm$ 0,44c
	K3	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	4,79 $\pm$ 0,41a	3,50 $\pm$ 0,58b	2,75 $\pm$ 0,44c
Aroma	K0	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,87 $\pm$ 0,33a	3,66 $\pm$ 0,48b	2,83 $\pm$ 0,48c
	K1	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,87 $\pm$ 0,33a	3,78 $\pm$ 0,42b	3,00 $\pm$ 0,51c
	K2	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	3,75 $\pm$ 0,44b	2,87 $\pm$ 0,44c
	K3	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	4,79 $\pm$ 0,41a	3,62 $\pm$ 0,64b	2,79 $\pm$ 0,41c
Tekst�r	K0	4,83 $\pm$ 0,38a	4,95 $\pm$ 0,20a	4,95 $\pm$ 0,20a	4,25 $\pm$ 0,60b	3,20 $\pm$ 0,41c
	K1	4,87 $\pm$ 0,33a	4,95 $\pm$ 0,20a	4,87 $\pm$ 0,33a	4,30 $\pm$ 0,55b	3,33 $\pm$ 0,48c
	K2	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	4,41 $\pm$ 0,50b	3,37 $\pm$ 0,49c
	K3	4,83 $\pm$ 0,38a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	4,29 $\pm$ 0,62b	3,20 $\pm$ 0,50c
G�r�n�ş	K0	4,95 $\pm$ 0,20a	4,91 $\pm$ 0,28a	5,00 $\pm$ 0,00a	4,33 $\pm$ 0,56b	3,25 $\pm$ 0,44c
	K1	4,87 $\pm$ 0,33a	4,87 $\pm$ 0,33a	4,83 $\pm$ 0,38a	4,43 $\pm$ 0,58b	3,25 $\pm$ 0,53c
	K2	4,87 $\pm$ 0,33a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	4,50 $\pm$ 0,51b	3,16 $\pm$ 0,48c
	K3	4,91 $\pm$ 0,28a	4,91 $\pm$ 0,28a	4,83 $\pm$ 0,38a	4,25 $\pm$ 0,60b	3,04 $\pm$ 0,46c
Genel kabul edilebilirlik	K0	5,00 $\pm$ 0,00a	5,00 $\pm$ 0,00a	4,87 $\pm$ 0,33a	3,66 $\pm$ 0,48b	2,91 $\pm$ 0,50c
	K1	5,00 $\pm$ 0,00a	5,00 $\pm$ 0,00a	4,91 $\pm$ 0,28a	3,78 $\pm$ 0,51b	3,04 $\pm$ 0,55c
	K2	5,00 $\pm$ 0,00a	5,00 $\pm$ 0,00a	4,87 $\pm$ 0,33a	3,75 $\pm$ 0,44b	2,87 $\pm$ 0,44c
	K3	4,95 $\pm$ 0,20a	4,95 $\pm$ 0,20a	4,83 $\pm$ 0,38a	3,58 $\pm$ 0,50b	2,75 $\pm$ 0,49c

Duysal analiz testi 5 ( ok iyi), 4 (iyi), 3 (ne iyi ne k t ), 2 (k t ) ve 1 ( ok k t ) olarak puanlandırılmıřtır.

K0=Kaplama

K1=Bamya M silaęı + % 1 Gliserol

K2=Bamya M silaęı + % 1 Gliserol + % 1 Nar  ekirdeęi Yaęı

K3=Bamya M silaęı + % 1 Gliserol + % 1 Kekik Yaęı

Her satırda a,b,c ( ) ve her s t nde A,B ( ) aynı olmayan g nler ve gruplar arasında $P < ,05$ d zeyinde anlamlı farklılıęı g stermektedir.

$\bar{x} \pm S.S$ =ortalama deęer $\pm$ standart sapma

 alıřma ile benzerlik g stermektedir. *Lactobacillus* spp. sayısı KO grubunda 9. g nde; K1 ve K2 grubunda ise 7. g nde anlamlı bir řekilde artış g stermiřtir ($P < ,05$). K3 grubunda ise *Lactobacillus* spp. sayısındaki deęişim g nler arasında anlamlı bir farklılıęa neden olmamıřtır ($P > ,05$). Bu durum kekik yaęı katkılı kaplama malzemesinin, laktik asit bakterilerinin geliřimi  zerine baskılayıcı bir etkiye neden olması ile a ıklanabilir. Bir  alıřmada²⁴ farklı bitkisel yaę katkılı kaplama malzemelerinin laktik asit bakterilerinin  reme hızını azalttıęı bildirilmiřtir ($P < ,05$). Sonu larda meydana gelen bu farklılık; kullanılan esansiyel yaęın konsantrasyonu, t r  ve kaplama malzemesinin antimikrobiyal ajanları taşıma kapasitesi ile ilgili olabilir.

Pseudomonas spp. suřları, etin d ř k sıcaklıklarda depolanma esnasında bozulmasına sebep olan mikroorganizmalar arasında g sterilmektedir.²⁵ Bu  alıřmada, *Pseudomonas* spp. sayısında depolama s resince gruplar ve g nler arasında istatistiki olarak anlamlı bir farklılık saptanmamıřtır ($P > ,05$) Emiroęlu ve ark⁷ 'ı %5 oranında kekik yaęı eklenen soya protein kaplama malzemelerinin; depolama s resi boyunca *Pseudomonas* spp. sayısını azalttıęını bildirmiřtir ($P < ,05$). Bu farklılık kaplama malzemesine eklenen kekik yaęının oranı ile ilgili olabilir.

Enterobacteriaceae familyası gıdalarda hijyen g stergesi olarak kabul edilmektedir.²⁶ *Enterobacteriaceae* familyasından  zellikle soęuęa dayanıklı t rlerin etlerde bozulmaya neden olduęu ifade edilmektedir.²⁷ Maya/k fler gıdalarda bozulmaya neden olan mikroorganizmalar arasında g sterilmektedir. Bu  alıřmada, *Enterobacteriaceae* ve maya/k f sayısının depolama s resince gruplar ve g nler arasında istatistiki olarak anlamlı bir farklılıęa neden olmadıęı saptanmıřtır ($P > ,05$).

Et ve  r nlerinde lipit oksidasyonunun bir g stergesi olan TBARS analizi; etlerdeki malonaldehid miktarının belirlenmesinde kullanılan bir y ntemdir. Lipit oksidasyonunun nedeni etlerdeki  oklu doymamıř yaę asidi i erięinin y ksek olmasıdır.²⁸

KO grubunda 5. ve 9. g nlerde, K1 grubunda 7. ve 9. g nlerde, K2 ve K3 grubunda ise 5. ve 9. g nlerde TBARS deęerlerinde anlamlı bir artış saptanmıřtır ($P < ,05$). TBARS deęeri KO grubunda 5. g nde anlamlı bir artış g sterirken K1 grubunda ise 7. g nde anlamlı bir artış bulunmuřtur ($P < ,05$). Kaplama malzemesinin oksijen ve nem bariyeri saęlayarak k ftelerde oksidasyonu geciktirdięi d ř n lmektedir.

K3 grubunun 7. ve 9. g nlerde TBARS deęerlerinin dięer gruplara g re daha d ř k olduęu saptanmıřtır ($P < ,05$). Bu  alıřmada kekik yaęı i eren gruplardaki TBARS deęerleri, Granheimer²⁹ ve Vital ve ark³⁰ 'nın yaptıkları  alıřma bulgularıyla benzerlik g stermektedir. Kekik yaęı eklenen grupta TBARS deęerlerinin daha az olması, kekik esansiyel yaęının antioksidan  zellięinden kaynaklanmaktadır.^{29,30}

Nar  ekirdeęi yaęı eklenen K2 grubunda TBARS deęerlerinin y ksek bulunması; nar  ekirdeęi yaęının yaę asidi kompozisyonu ile alakalı olabilir.   nk  nar  ekirdeęi yaęı; oksidasyona duyarlı  oklu doymamıř yaę asitleri (%69,42-91,96) bakımından zengindir.^{28,31}

pH et ve  r nleri i in  nemli bir kalite kriteridir. Taze etin pH deęeri mikroorganizma geliřimi i in uygundur.³² Depolamanın ilk g n nde t m gruplarda pH deęeri birbirine yakın olup 6,27-6,40 arasındadır. Bu durum mikrobiyal  reme i in uygun bir deęer aralıęıdır.  alıřmada elde edilen veriler neticesinde bamya m silaęının pH deęeri 4,2 $\pm$ 0,12 olmasına karřın, kaplamalı gruplar

ile kontrol grubu arasında pH değerinin anlamlı bir fark oluşturmadığı saptanmıştır. Depolamanın 5. gününde pH değeri tüm gruplarda anlamlı bir şekilde azalmıştır ($P < ,05$). Bu durum Xiong ve ark³³'nın yaptıkları çalışma bulguları ile benzerlik göstermektedir. Depolama süresi boyunca pH değerinin düşmesi, köftelerdeki laktik asit bakterilerinin mikrobiyal faaliyetlerine bağlanabilir.³⁴

Artan su aktivitesine bağlı olarak lipid oksidasyonu, enzimatik aktivite ve mikroorganizmaların gelişmesi de artmaktadır.³⁵ Depolamanın 1. ve 3. günlerinde K2 grubunun K0 grubuna göre a_w değerinin anlamlı olarak daha yüksek olduğu saptanmıştır ($P < ,05$). Depolamanın 9. gününde K0 grubunun a_w değerinin anlamlı olarak diğer gruplara göre daha düşük olduğu bulunmuştur ($P < ,05$). Bamya müsillajı ile kaplanan köftelerde artan su aktivitesinin; köftelerin depolama stabilitesine olumsuz etki yapabileceği düşünülse de, mikrobiyolojik ve kimyasal analizlerde bu durumun olumsuz bir etkiye neden olmadığı düşünülmektedir. Ayrıca su aktivitelerindeki artış köftelerin sululuk düzeyini artırmaktadır. Et ve ürünlerindeki sululuk, duyuşal beğeni yönünden istenilen bir durumdur.

Tüketici tercihinde renk çok önemli bir yere sahiptir. Et ve ürünlerinin kendisine özgü rengi depolama süresince önem arz etmektedir. Çünkü etin rengini oluşturan myogloblin ve hemoglobin pigmentleri oksidasyona oldukça duyarlıdır. Lipid peroksidasyon ürünleri ve serbest radikaller, oksimiyoglobinin metmyoglobine dönüşmesine neden olmaktadır. Bu durum renk değişiminin ana nedenidir.^{36,37} Bu çalışmada, depolamanın 1. gününde L* (parlaklık) değerinin kaplamalı gruplarda kontrol grubuna göre daha yüksek olduğu saptanmıştır ($P < ,05$). Kaplama malzemelerinin L* değeri için kısmi bir yararlılık sağladığı ifade edilebilir. Ayrıca et ve ürünlerinde rengin soluklaşma nedeni pigment-indirgeme sistemlerine hasar veren lipid oksidasyonu sonucu oluşan radikallerdir. Bu radikallerin okside pigment oranını artırdığı ifade edilmektedir.³⁸ Bu veri kontrol grubuna göre K1, K2 ve K3 gruplarında daha az serbest radikal oluştuğu fikri uyandırır da, durumun tespitine yönelik biyokimyasal çalışmalara ihtiyaç vardır.

Lipit oksidasyonunun artması sonucu oluşan ürünler, miyogloblinin oksidasyonunu da artırmaktadır. Bu durum a^* (kırmızılık, relative redness) değerinin düşmesi ile sonuçlanmaktadır.^{36,39} Depolama süresi boyunca a^* değeri değişiminin, gruplar arasında anlamlı bir farklılığa neden olmadığı görülmüştür ($P > ,05$). Artan depolama süresi boyunca tüm gruplarda a^* değerinin azaldığı görülmüştür ($P < ,05$).

Gıdalarda duyuşal özellikler tüketici tercihinde önem arz etmektedir. Bu çalışmada gruplar arasındaki lezzet, koku, aroma, tekstür, görünüş ve genel kabul edilebilirlik değerleri arasında depolama süresi boyunca istatistiki olarak anlamlı bir fark bulunmamıştır ($P > ,05$).

Depolama süresinin artışına bağlı olarak tüm grupların lezzet, koku, aroma, tekstür, görünüş ve genel kabul edilebilirlik değerlerinde depolamanın 7. ve 9. günlerinde azalma olduğu saptanmıştır ($P < ,05$). Radha krishnan ve ark²⁴'nın farklı kaplama malzemeleri ile kapladıkları etlerin duyuşal analiz sonuçları bu çalışma ile benzerdir.

Panelistler depolamanın 7. ve 9. günlerinde K0, K1 ve K2 gruplarında oksidatif lezzet algıladıklarını ifade etmişlerdir. Oksidatif lezzet algısının depolamanın 7. ve 9. günlerinde olması; TBARS değerlerinin K0, K1 ve K2 gruplarında daha fazla olmasıyla

açıklanabilir. Çünkü lipit oksidasyonunda oluşan hidroperoksitler kokusuz olmalarına rağmen parçalandıkları zaman; furan, sübsitü, alkol ve hidrokarbon ikincil yapılarını oluştururlar. Bu yapılar etin lezzetinde kayıplara neden olmaktadır.^{38,39}

Ayrıca panelistler depolamanın 7. ve 9. günlerinde kekik kokusunun daha belirgin olduğunu ifade etmişlerdir. Depolama süresinin uzamasına bağlı olarak kekik yağının uçucu bileşenlerinin köfte içerisinde çözünmesi ve dağılması bu durum üzerinde etkili olabilir.

Sonuç olarak kaplama malzemeleri köftelerin raf ömrünü uzatmada çok etkili bulunmamıştır. Ancak kekik yağı katkılı kaplama malzemesinin oksidasyonu azalttığı saptanmıştır. K1 kaplama grubunun K0 kontrol grubuna göre TBARS değerlerinin ortalama olarak daha düşük olması; kaplama malzemesinin oksijen bariyeri sağlayabileceğini göstermektedir. Ayrıca kaplama malzemeleri duyuşal yönden olumsuz bir etkiye neden olmamıştır. Kaplama malzemeleri tüketici beğenisi için önemli olan parlaklık değerine kısmen olumlu bir katkı sağlamıştır. Bamya müsillajının yenilebilir film ve kaplama potansiyeli bulunmaktadır. Ayrıca kaplama malzemesinde kullanılan bamyanın besin değerinden fayda sağlanabileceği göz ardı edilmemelidir. Fakat farklı gıdalar üzerine etkisinin incelenmesine ihtiyaç vardır. Bamya müsillajı farklı hidrokolloid (örn., kitosan, soya protein) ve farklı antimikrobiyal, antioksidan özellik gösteren maddelerle kombine edilerek koruyucu özelliği geliştirilebilir.

Etik Komite Onayı: Bu çalışma Atatürk Üniversitesi Veteriner Fakültesi Birim Etik Kurulu'ndan onay alınmıştır. (Tarih: 27.11.2019, Karar No: 2019/17).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Konsept – E.T.; Tasarım – E.T.; Denetim – M.A.; Kaynaklar – E.T.; Malzemeler – E.T.; Veri Toplama ve/veya İşleme – E.T.; Analiz ve/veya Yorum – M.A.; Literatür Taraması – E.T.; Yazma – E.T.; Eleştirel İnceleme – M.A.

Çıkar Çatışması: Yazarlar, çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ataturk University Faculty of Veterinary. (Date: 27.11.2019, Decision No: 2019/17).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.T.; Design – E.T.; Supervision – M.A.; esources – E.T.; Materials – E.T.; Data Collection and/or Processing – E.T.; Analysis and/or Interpretation – M.A.; Literature Search – E.T.; Writing Manuscript – E.T.; Critical Review – M.A.

Declaration of Interests: The authors declare that they have no conflicts of interest.

Funding: The authors declared that this study has received no financial support.

KAYNAKLAR

1. Tercanlı E, Besin Alerjileri AM. Academic platform journal of halal life-style. 2021;3(1):31-53.
2. Bekhit AE-DA, Holman BWB, Giteru SG, Hopkins DL. Total volatile basic nitrogen (TVB-N) and its role in meat spoilage: a review. *Trends Food Sci Technol*. 2021;109:280-302. [CrossRef]

3. Meng X, Wu D, Zhang Z, et al. An overview of factors affecting the quality of beef meatballs: processing and preservation. *Food Sci Nutr*. 2022;00:1-14.
4. Bhagath Y, Manjula K. Influence of composite edible coating systems on preservation of fresh meat cuts and products: a brief review on their trends and applications. *Int Food Res J*. 2019;26(2):377-392.
5. Echegaray N, Hassoun A, Jagtap S, et al. Meat 4.0: principles and applications of Industry 4.0 technologies in the meat industry. *Appl Sci*. 2022;12(14):6986. [\[CrossRef\]](#)
6. Sun X, Wang J, Dong M, Zhang H, Li L, Wang L. Food spoilage, bioactive food fresh-keeping films and functional edible coatings: research status, existing problems and development trend. *Trends Food Sci Technol*. 2022;119:122-132. [\[CrossRef\]](#)
7. Emiroğlu ZK, Yemiş GP, Coşkun BK, Candoğan K. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. *Meat Sci*. 2010;86(2):283-288. [\[CrossRef\]](#)
8. Araújo A, Galvão A, Filho CS, et al. Okra mucilage and corn starch bio-based film to be applied in food. *Polym Test*. 2018;71:352-361. [\[CrossRef\]](#)
9. de Alvarenga Pinto Cotrim M, Mottin AC, Ayres E. Preparation and characterization of okra mucilage (*Abelmoschus esculentus*) edible films. *Macromol Symp*. 2016;367(1):90-100. [\[CrossRef\]](#)
10. Olawuyi IF, Lee WY. Structural characterization, functional properties and antioxidant activities of polysaccharide extract obtained from okra leaves (*Abelmoschus esculentus*). *Food Chem*. 2021;354:129437. [\[CrossRef\]](#)
11. Wulandari D, Yuliatmo R, Sugiyanto S. The effect of coating of edible film from bovine split hide gelatin on beef meatballs properties. *J Indones Trop Anim Agric*. 2018;43(2):177-183. [\[CrossRef\]](#)
12. Ghorri MU, Alba K, Smith AM, Conway BR, Kontogiorgos V. Okra extracts in pharmaceutical and food applications. *Food Hydrocoll*. 2014;42:342-347. [\[CrossRef\]](#)
13. Yemiş GP, Emiroğlu ZK, Mercanköşk CK. (*Oreganum heracleoticum* L.) ve *Bahçe Kekiği* (*Thymus vulgaris* L.) uçucu yağı içeren soya bazlı yenilebilir filmlerin patojen bakterilere karşı antimikrobiyel etkileri. *Gıda*. 2017;42(3):268-276.
14. Afshar Mehrabi F, Sharifi A, Ahvazi M. Effect of chitosan coating containing *Nepeta pogonosperma* extract on shelf life of chicken fillets during chilled storage. *Food Sci Nutr*. 2021;9(8):4517-4528. [\[CrossRef\]](#)
15. Lemon D. *An improved TBA test for rancidity new series circular*. Halifax, NS, Canada: Halifax-Laboratory. 1975;51.
16. İmlik H, Gümüş R, Özlü H, Gelen SU, Atasever MA. The effect of the supplementation of lamb feed with *Yucca schidigera* on the lipid oxidation, color, physico-chemical and microbiological properties in meat. *Asian J Med Biol Res*. 2018;4(1):86-94. [\[CrossRef\]](#)
17. Milli TC. Eğitim bakanlığı, duyu analizi test teknikleri. Available at: http://megep.meb.gov.tr/mte_program_modul/moduller_pdf/Duyusal%20Test%20Teknikleri.pdf. Accessed May 2, 2022.
18. Arbuckle JL. *IBM SPSS Amos 20 User's Guide*. Chicago: Amos Development Corporation, SPSS Inc.; 2011.
19. Zhang Y, Holman BWB, Ponnampalam EN, et al. Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Sci*. 2019;149:114-119. [\[CrossRef\]](#)
20. Al-Dalali S, Li C, Xu B. Effect of frozen storage on the lipid oxidation, protein oxidation, and flavor profile of marinated raw beef meat. *Food Chem*. 2021;376:131881. [\[CrossRef\]](#)
21. Noshad M, Alizadeh Behbahani B, Jooyandeh H, Rahmati-Joneidabad M, Hemmati Kaykha ME, Ghodsi Sheikhjan M. Utilization of *Plantago major* seed mucilage containing Citrus limon essential oil as an edible coating to improve shelf-life of buffalo meat under refrigeration conditions. *Food Sci Nutr*. 2021;9(3):1625-1639. [\[CrossRef\]](#)
22. Alves Mauricio R, Alvares Duarte Bonini Campos J, Tieko Nassu R. Meat with edible coating: acceptance, purchase intention and neophobia. *Food Res Int*. 2022;154:111002. [\[CrossRef\]](#)
23. Desvita H, Faisal M, Mahidin, Suhendrayatna. Preservation of meatballs with edible coating of chitosan dissolved in rice hull-based liquid smoke. *Heliyon*. 2020;6(10):e05228. [\[CrossRef\]](#)
24. Radha Krishnan K, Babuskin S, Rakhavan KR, et al. Potential application of corn starch edible films with spice essential oils for the shelf life extension of red meat. *J Appl Microbiol*. 2015;119(6):1613-1623. [\[CrossRef\]](#)
25. Wickramasinghe NN, Ravensdale J, Coorey R, Chandry SP, Dykes GA. The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. *Compr Rev Food Sci Food Saf*. 2019;18(5):1622-1635. [\[CrossRef\]](#)
26. Cegar S, Kuruca L, Vidovic B, et al. Risk categorisation of poultry abattoirs on the basis of the current process hygiene criteria and indicator microorganisms. *Food Control*. 2022;132:108530. [\[CrossRef\]](#)
27. Odeyemi OA, Alegbeleye OO, Strateva M, Stratev D. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Compr Rev Food Sci Food Saf*. 2020;19(2):311-331. [\[CrossRef\]](#)
28. Mun HS, Rathnayake D, Dilawar MA, Jeong M, Yang CJ. Effect of ambient temperature on growth performances, carcass traits and meat quality of pigs. *J Appl Anim Res*. 2022;50(1):103-108. [\[CrossRef\]](#)
29. Granheimer K. *Different Parameters Affecting Lipid Oxidation in Meatballs* [Dissertation]. Lund University; 2017. https://scholar.googleusercontent.com/scholar?q=cache:FiyGoWPYsFOJ:scholar.google.com/+Granheimer+K.+Different+Parameters+Affecting+Lipid+Oxidation+in+Meatballs+%5BDissertation%5D.+Lund+University%3B+2017&hl=tr&as_sdt=0,5 and <https://lup.lub.lu.se/student-papers/record/8912102/file/8913213.pdf>
30. Vital ACP, Guerrero A, Monteschio Jde O, et al. Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PLoS One*. 2016;11(8):e0160535. [\[CrossRef\]](#)
31. Paul A, Radhakrishnan M. Pomegranate seed oil in food industry: extraction, characterization, and applications. *Trends Food Sci Technol*. 2020;105:273-283. [\[CrossRef\]](#)
32. Luong NM, Coroller L, Zagorec M, et al. A bayesian approach to describe and simulate the pH evolution of fresh meat products depending on the preservation conditions. *Foods*. 2022;11(8):1114. [\[CrossRef\]](#)
33. Xiong Y, Li S, Warner RD, Fang Z. Effect of oregano essential oil and resveratrol nanoemulsion loaded pectin edible coating on the preservation of pork loin in modified atmosphere packaging. *Food Control*. 2020;114:107226. [\[CrossRef\]](#)
34. Barcenilla C, Ducic M, López M, Prieto M, Álvarez-Ordóñez A. Application of lactic acid bacteria for the biopreservation of meat products: a systematic review. *Meat Sci*. 2022;183:108661. [\[CrossRef\]](#)
35. Yu HH, Chin Y-W, Paik H-D. Application of natural preservatives for meat and meat products against food-borne pathogens and spoilage bacteria: a review. *Foods*. 2021;10(10):2418. [\[CrossRef\]](#)
36. Wang X, Wang Z, Zhuang H, et al. Changes in color, myoglobin, and lipid oxidation in beef patties treated by dielectric barrier discharge cold plasma during storage. *Meat Sci*. 2021;176:108456. [\[CrossRef\]](#)
37. Kondjoyan A, Sicard J, Badaroux M, Gatellier P. Kinetics analysis of the reactions responsible for myoglobin chemical state in meat using an advanced reaction-diffusion model. *Meat Sci*. 2022;191:108866. [\[CrossRef\]](#)
38. Mohan A, Roy A, Duggirala K, Klein L. Oxidative reactions of 4-oxo-2-Nonenal in meat and meat products. *LWT*. 2022;165:113747. [\[CrossRef\]](#)
39. Chan WKM, Faustman C, Decker EA. Oxymyoglobin oxidation as affected by oxidation products of phosphatidylcholine liposomes. *J Food Sci*. 1997;62(4):709-712. [\[CrossRef\]](#)

Investigation of Neurotoxicity Oxidative Stress and Oxidative DNA Damage in Cadmium-Induced Brain Injury in Rats

Sıçanlarda Kadmiyuma Bağlı Beyin Hasarında Nörotoksisite Oksidatif Stres ve Oksidatif DNA Hasarının Araştırılması

İsmail BOLAT¹ 
Serkan YILDIRIM¹ 
Nesrin CEYLAN² 
Yavuz Selim SAĞLAM¹ 
Ferit Kansu ÖRNEK¹ 
Merve BOLAT³ 
Metin KILIÇOĞLU¹ 

¹Department of Pathology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

²Department of Pediatrics, Yıldırım Beyazıt University, Ankara, Turkey

³Department of Physiology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

ABSTRACT

Cadmium, which is one of the toxic metals widely used in many areas of the world, is taken into the body in different ways. In this study, the damage caused by cadmium in the brain and how this damage affects the levels of glial fibrillar acidic protein, 8-Oxo-2'-deoxyguanosine (8-OHdG), neuronal nitric oxide synthase, Süperoksit dismutaz (SOD), Glutasyon (GSH), glutasyon peroksidaz (GPX), inducible nitric oxide synthase, and malondialdehid (MDA) expression in the brain tissue were investigated. A total of 16 male Wistar albino rats 200-220 g were used in the experimental study. Rats were divided into 2 groups. A single cadmium i.p. dose of 0.025 mmol/kg was given to rats in the cadmium group. Rat brain tissue samples were analyzed using biochemical analyses as well as histopathological and immunohistochemical methods. In the histopathological examination of the brain tissues, normal histological structure was observed in the brain samples belonging to the control group, while necrosis and degeneration of neurons in the brain, as well as hyperemia in the parenchyma and meningeal vessels were observed in the cadmium group. In immunohistochemical examinations, while glial fibrillar acidic protein, 8-OHdG, and neuronal nitric oxide synthase expression was not observed in the brain samples of control group, severe expression of glial fibrillar acidic protein, 8-OHdG, and neuronal nitric oxide synthase was observed in the brain tissue of the group receiving cadmium. In the biochemical analyses performed, it was observed that SOD, GSH, and GPx enzyme levels increased in cadmium groups, inducible nitric oxide synthase and MDA enzyme levels were decreased. As a result of this study, it is thought that markers' expression levels are important in understanding the oxidative stress in pathogenesis of cadmium toxicity and will provide a guide and important contributions to future studies.

Keywords: Biochemical, cadmium, histopathology, immunohistochemistry

ÖZ

Dünyada birçok alanda yaygın olarak kullanılan toksik metallerden biri olan kadmiyum, vücuda farklı şekillerde alınmaktadır. Bu çalışmada kadmiyumun beyinde oluşturduğu hasar ve bu hasarın beyin dokusunda GFAP, 8-OHdG, nNOS, SOD, GSH, GPx, iNOS ve MDA ekspresyon düzeylerini nasıl etkilediği araştırıldı. Bu deneysel çalışmada 200-220 gr ağırlığında 16 adet erkek Wistar albino sıçan kullanıldı. Sıçanlar iki gruba ayrıldı. Kadmiyum grubuna 0.025 mmol/kg'lık tek bir intraperitoneal Cd uygulandı. Rat beyin dokuları biyokimyasal, histopatolojik ve immünohistokimyasal boyama yöntemi ile incelendi. Beyin dokularının histopatolojik incelemesinde kontrol grubuna ait beyin örneklerinde normal histolojik yapı gözlenirken, kadmiyum grubunda beyinde nöronlarda nekroz ve dejenerasyon ile parankim ve meningeal damarlarda hiperemi gözlemlendi. İmmünohistokimyasal incelemelerde kontrol grubunun beyin örneklerinde GFAP, 8-OHdG ve nNOS ekspresyonu izlenmezken, Cd uygulanan grubun beyin dokusunda şiddetli GFAP, 8-OHdG ve nNOS ekspresyonu gözlemlendi. Yapılan biyokimyasal analizlerde Cd gruplarında SOD, GSH ve GPx enzim seviyeleri artarken, iNOS ve MDA enzim seviyelerinin düştüğü gözlemlendi. Bu çalışma sonucunda markerlerin ekspresyon düzeylerinin Cd toksisitesinin patogenezinde oksidatif stresin anlaşılmasında önemli olduğu ve gelecekte yol gösterici ve önemli katkılar sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Biyokimya, histopatoloji, immünohistokimya, kadmiyum

Received/Geliş Tarihi: 21.07.2022

Accepted/Kabul Tarihi: 14.12.2022

Publication Date/Yayın Tarihi: 26.04.2023

Corresponding author/Sorumlu Yazar:
Serkan YILDIRIM
E-mail: syildirim@atauni.edu.tr

Cite this article as: Bolat İ, Yıldırım S, Ceylan N, et al. Investigation of neurotoxicity oxidative stress and oxidative DNA damage in cadmium-induced brain injury in rats. *Vet Sci Pract.* 2023; 18(1), 19-24.

Atif: Bolat İ, Yıldırım S, Ceylan N, et al. Sıçanlarda kadmiyuma bağlı beyin hasarında nörotoksisite oksidatif stres ve oksidatif dna hasarının araştırılması. *Vet Sci Pract.* 2023; 18(1), 19-24.



Copyright©Author(s) - Available online at
veterinarysciences-ataunipress.org
Content of this journal is licensed under a
Creative Commons Attribution-
NonCommercial 4.0 International License

INTRODUCTION

In the developing world, many conveniences have been provided in our lives with the innovations in various industries. However, this development has also caused many health problems. Among the causes of these health problems, heavy toxic metals such as iron, lead, arsenic, copper, and cadmium, which are industrial wastes, have an important place.¹⁻³ Cadmium (Cd) is a heavy metal with mutagenic, carcinogenic, and toxic effects on living organisms. Cadmium is taken into body through digestion, respiration, and skin. Although it varies according to the animal species, 0.5%-12% of Cd was taken through digestion. Almost all of the Cd vapor is absorbed from the lungs. This makes this element in the cigarette important to us. Moreover, Cd salts can be absorbed up to 4% through the skin.^{1,4,5} Cd taken into the body is transported by binding to proteins and blood cells in the blood, and most of it accumulates in the liver and kidney, and a small part in the spleen.⁶ Apart from these organs, Cd has also been reported to accumulate in the brain due to its ability to cross the blood brain barrier (BBB) and cause serious neurological damage.⁷ Recent studies on cadmium have reported that it is also a risk factor for occurrence of Parkinson's⁸ and Alzheimer's disease.⁹⁻¹¹

Glial cells, one of the central nervous system cells, are of great importance in supporting neurons. Yet another cell structure, astrocytes act as a bridge between the BBB and neurons, providing great support to neurons and preventing foreign substances from entering the brain.¹²⁻¹⁵ In addition, it has been reported that some toxic metals inhibit the cytotoxic effects they can create in brain tissue.¹⁶ Glial fibrillar acidic protein (GFAP) is a protein that is an intermediate filament in mature astrocytes. It is also an important protein that plays a role in structure of astrocytes and many functions during the development of astrocytes.¹⁷⁻²⁰ In some toxication studies conducted in brain tissue, it has been reported that determining the level of GFAP expression is important in determining the severity of toxicity.²¹ It has been reported that GFAP expression increases in brain tissue in diseases such as Scrapie, Creutzfeldt-Jakob and Alzheimer's.²¹ In some studies, in animals exposed to heavy metals such as Cd, Zn, Pb, and Tl, it was found that heavy metals accumulate in glial cells and cause morphological changes in these cells as a result of their toxic effects.^{22,23}

Oxidative stress of cells in many tissues and organs causes permanent damage to the membranes, protein structures, and lipids of DNA.²⁴ Toxic substances are known to cause serious oxidative damage in the body. In particular, cadmium has been reported to cause serious damage to many tissues especially brain tissue, by increasing oxidative stress in the body.²⁵ It has been reported that cadmium causes significant decreases in SOD, GPx, and GSH enzyme levels, which are oxidative stress parameters in brain tissue.^{26,27} 8-OHdG and 8-oxodG in nuclear and mitochondrial DNA determine the damage caused by oxidative stress on cell DNA in the body and are known to be the most common of the free radical-derived forms.²⁴ 8-OHdG marker was used to determine the oxidative damage caused by cadmium in the brain.^{25,28}

Nitric oxide (NO) is synthesized by a reaction catalyzed by the nitric oxide synthase (NOS) enzyme in many cells, and L-arginine and O₂ molecules are converted into NO and citrulline molecules. The NOS enzyme has 3 isoforms, the first 2 being structural (cNOS) and the third being inducible (iNOS). While iNOS can be stimulated by cytokines and other compounds, cNOS is divided into 2 groups as vasodilation endothelial NOS (eNOS) in

endothelial cells and neuronal NOS (nNOS) in the nervous system.²⁹ Neuronal NOS is reported as a highly sensitive marker in detecting the damage caused by oxidative stress in brain tissue.³⁰⁻³² Although the cellular and biochemical effects of cadmium in the brain have been revealed in many studies in the literature reviews, the pathogenesis of the disorders it causes has not been fully elucidated.³³ In this study, it was aimed to investigate the effects of GFAP, 8-OHdG, nNOS, SOD, GSH, GPx, iNOS, and MDA expression levels in brain damage caused by Cd toxicity by biochemical and immunohistochemical analyses in order to determine the pathogenesis of Cd intoxication in brain tissue. This study aimed to reveal the pathogenesis of oxidative stress caused by cadmium in the body and subsequent oxidative DNA damage caused in brain tissue.

MATERIALS AND METHODS

As the research material, 16 male Wistar-albino rats with a weight of 200-220 g were used. Rats were provided by Atatürk University, Medical Experimental Application and Research Center (ATADEM) unit. The rats were kept for a week in order to adapt to their environment, and ad libitum nutrition was applied with pellet feed and water. In the study using CdCl₂·5H₂O (cadmium chloride pentahydrate, CAS NO: 7790-78-5, Sigma, US) as a cadmium source, rats were divided into 2 groups, with n=8 in each group.

Group I: Control group: The animals in this group were given standard pellet feed, drinking water, and saline (SF) intraperitoneally (i.p.).

Group II: Cadmium applied group: A single dose of i.p. 0.025 mmol/kg Cd was given to each animal.³⁴

It was prepared by dissolving Cd in saline (SF). Group II was administered SF for 5 days, and on the 5th day, Cd i.p. was administered 1 hour after SF application. Animals were euthanized 24 hours after the last administration to collect brain tissue samples. In 6 days, rats were necropsied and brain tissue samples were taken (Ethics Number: 2014/12-b).

Biochemical Analysis

Homogenization was performed for brain tissues using Magna Lyser (Roche, Switzerland) homogenizer device. The obtained homogenates were centrifuged at 15 000 rpm. Then the supernatant was used for biochemical analysis. MDA level, GSH, CAT, iNOS, and GPx activity in the brain tissues were measured using the corresponding anti-rat ELISA kits (YL Biotech, Shanghai, China) according to the manufacturer's instructions.³⁴

Histopathological Examination

Brain tissue samples were detected in 10% buffered formalin solution. Paraffin blocks were prepared after routine tissue follow-up procedures. Sections of 4 µm thickness were taken from the blocks. The preparations for histopathological examination were stained with hematoxylin-eosin and examined with a microscope (OLYMPUS BX51, Tokyo, Japan). Examined brain cortex areas were evaluated as none (–), mild (+), moderate (++), and severe (+++) according to their pathological findings.

Immunohistochemical Examination

Tissue sections were deparaffinized and dehydrated. The sections were kept in 3% H₂O₂ for 10 minutes and then incubated for 5 minutes with protein block. Primary antibody (GFAP Cat No: ab68428, dilution ratio (DR): 1/100, UK; 8-OHdG Cat No: sc-66036, DR: 1/100, US; nNOS Cat No: ab5586, DR: 1/100, UK) was dropped onto the sections and incubated for 1 hour. 3-3'

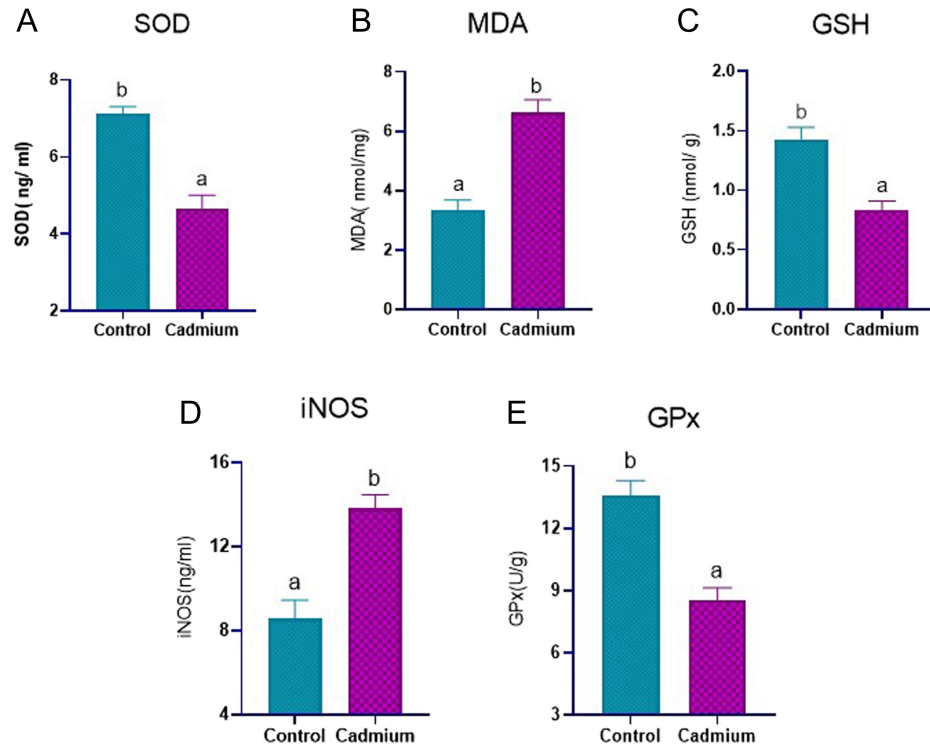


Figure 1. SOD, GSH, MDA, GPx, and nNOS levels in brain tissues. (A) SOD enzyme level, (B) MDA level, (C) GSH level, (D) iNOS level, and (E) GPx level; statistically, the differences between the groups were shown with letters ($P < .05$). The obtained values were expressed as mean $\pm$ SEM. nNOS, neuronal nitric oxide synthase; SEM, standard error of mean.

Diaminobenzidine (DAB) was used as a chromogen. The slides were kept in Mayer's hematoxylin and then washed. Then the prepared sections were examined with microscope (ZEISS AXIO, Jena, Germany).³⁴

Statistical Analysis

For histopathological examination, Mann Whitney U test was used to compare binary groups using the Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA). The statistical comparison of the values obtained as a result of the biochemical analyses performed in the study was made with the GraphPad Prism 8.0.1 data program. One-way ANOVA and Tukey test were used to compare the mean values of more than 2 independent groups.

To determine the intensity of positive staining obtained as a result of immunohistochemical staining, 5 areas were selected from images and evaluated in the ZEISS Zen Imaging software. One-way ANOVA and Tukey test were used. As a result of the test, P value of $<.05$ was considered significant.

RESULTS

Biochemical Analyses Findings

As a result of the biochemical analyses performed on the brain tissues, it was observed that SOD, GSH, and GPx enzyme levels in the cadmium group decreased significantly when compared to the control group. The levels of nNOS and MDA showed a significant increase in the cadmium groups compared to the control group ($P < .05$) (Figure 1).

Histopathological Findings

In the histopathological examination, normal histological structure was observed (Figure 2) in group I (control group). In group II

(cadmium applied group), in the brain tissues, severe degeneration and necrosis in the neurons and hyperemia in parenchyma and meningeal vessels were detected (Figure 2) When compared with the control group, a statistically significant difference ($P < .05$) was found. Histopathological results were shown in Table 1.

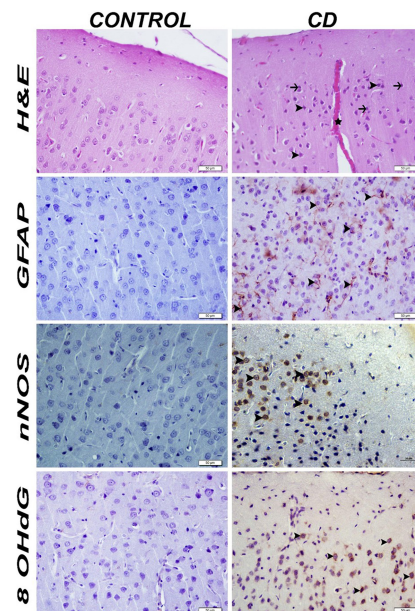


Figure 2. Brain tissue, degeneration in neurons (arrowheads), necrosis (arrows), hyperemia in vessels (stars), hematoxylin-eosin, severe level of GFAP, nNOS and 8 OHdG expression (arrowheads), IHC-P, Bar: 50 μ m. CD, cadmium applied group.

Table 1. Scoring of Histopathological Findings in Brain Tissue

Parameters	Control group	Cadmium Applied Group
Degeneration in neurons	–	+++
Necrosis in neurons	–	++
Hyperemia of meningeal and parenchymal vessels	–	+++

Table 2. Scoring of Immunohistochemical Findings in Brain Tissue

Parameters	Control Group	Cadmium Applied Group
Glial fibrillar acidic protein	21.52 ± 4.11 ^a	62.85 ± 5.46 ^b
Neuronal nitric oxide synthase	19.42 ± 4.96 ^a	58.55 ± 6.62 ^b
8-OHdG	20.39 ± 6.62 ^a	60.74 ± 7.45 ^b

^{a,b}Differences between means with different letters in the same column are significant ($P < .05$).

Immunohistochemical Findings

In the control group, there was no GFAP, nNOS, and 8 OHdG expression in the brain tissues (Figure 2). In the Cadmium applied group, there was severe GFAP, nNOS, and 8 OHdG expression in the brain tissues (Figure 2). When compared with the control group, a statistically significant difference ($P < .05$) was found. Immunohistochemical results are shown in Table 2.

DISCUSSION

Cadmium, which is widely used in industry, accumulates in the environment and creates serious problems for human health. Volcanic mountains are reported to be the most important pollutants as a source of Cd. Cadmium, either intentionally or unintentionally, is taken into the body with contaminated food, water, soil, dust, or air,³⁵ digestion, respiration, and skin.¹ After entering the body, it is transported by binding to proteins and blood cells in the blood and most of it accumulates in the liver and kidney.⁶ Apart from these organs, Cd has also been reported to accumulate in the brain due to its ability to cross the BBB and cause serious neurological damage.⁷

Cadmium causes necrotic hepatitis, hyperemia in the vessels, and mononuclear cell infiltration in the liver.³⁴ It has been reported to cause fatty degeneration, hydropic degeneration, and fibrosis³⁶ in animals treated with a daily dose of 1 mg/kg CdCl₂. It has been revealed that it causes severe intertubular hemorrhages in the medulla and cortex in the kidneys and degeneration and necrosis in the tubular epithelium.³⁴ It has been reported that neuronal atrophy, degeneration, and necrosis were observed in rats administered Cd at 5 mg/kg to brain tissues.³⁶ Podarcis sicutus was shown to cause widespread edema in the brain tissue administered 2 mg/kg Cd daily for 7 days.³³ Histopathologically, in rat brain tissues where Cd toxicity was induced, it has been reported that neuronophagia, perineuronal vacuolization, vascular hyperemia, and satellitosis were observed especially in the cerebral cortex.² In this study, in accordance with the literature, degeneration and necrosis in neurons and hyperemia in parenchymal and meningeal vessels were detected in Cd toxicity in brain tissues.

Glial fibrillar acidic protein (GFAP) is a protein that is an intermediate filament in mature astrocytes. It is also an important protein that plays a role in the formation of the skeleton of astrocytes and many functions during the development of astrocytes.^{17,18} Astrocytes become reactive and react as a result of traumas that cause astrogliosis, various diseases, genetic disorders, or toxic effects of chemical substances. It has been reported that GFAP expression started to increase rapidly with the formation of astrogliosis.³⁷ In

some studies, they have defined that the increase in GFAP levels in trauma or disease situations that cause astrogliosis is the most serious finding of this process.³⁸ In rats with severe traumatic brain injury, an increase in GFAP expression level was found in astrocytes in the cortex where brain tissue damage occurred 3 days after the injury.³⁹ It was also reported that GFAP expression increased in gliosis models created experimentally in rat brain tissues.³⁷ In this study, it was determined that GFAP expressions in brain tissues increased significantly in Cd toxicity.

It was reported in studies that oxidative stress occurring in the body causes neurotoxicity in brain tissue as well as in many tissues and organs. Studies have shown that many substances, including Cd, increase oxidative stress in the body and cause oxidative damage to the brain tissue. In some studies performed by creating neurotoxicity with Cd, while it has been reported that Cd causes a decrease in SOD, GSH, and GPx enzyme levels in brain tissues, it has also been reported to cause serious increases in iNOS and MDA enzyme levels.^{26,27} The results of the present study and the literature were found to be compatible. With this result, the negative effects of Cd on some oxidative stress parameters in brain tissues were revealed.

8-OHdG is widely used in toxication studies to show DNA damage caused by oxidative stress.^{24,40,41} There are many studies in which 8-OHdG shows DNA damage in cadmium toxicity.^{25,28,42} In a study, it was shown that the expression level of 8-OHdG increased significantly as a result of oxidative stress in the brain tissues of rats administered 5 mg/kg cadmium chloride.²⁵ In another study, it was determined that cadmium administration at 5 mg/kg increased the expression level of 8-OHdG in rat brain tissues.²⁸ In this study, it was determined that 8-OHdG expressions increased in brain tissues with Cd application.

Neuronal NOS is a very sensitive marker in detecting neurological damage in the brain due to oxidative stress.⁴³ In many toxicity studies, it has been reported that the level of nNOS expression in neurons in brain tissue increases depending on the severity of Cd toxicity.^{44–46} In a study conducted in rats treated with Cd at concentrations 0, 5, 10, and 20 µmol/L, it was detected that the expression level of nNOS increased significantly in rats treated with 5 and 10 µmol/L Cd.⁴⁷ In this study, in accordance with the literature, it was determined that the level of nNOS expression in neurons increased due to the damage caused by Cd in rat brain tissues in which acute Cd intoxication was created.

As a result, it was observed in the study that a single dose of 0.025 mmol/kg Cd caused severe damage to the brain tissues and this damage increased GFAP, 8-OHdG, nNOS, iNOS, and MDA expression and decreased SOD, GSH, and GPx expression. The GFAP, 8-OHdG, nNOS, iNOS, MDA, SOD, GSH, and GPx expression levels has been an important study in understanding the pathogenesis of Cd toxicity, and it is considered and evaluated to provide a guiding and important contribution to future studies.

Ethics Committee Approval: Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Van Yüzüncü Yıl University (Date: 01.02.2014, Decision No: 12-B).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – I.B., S.Y.; Design – I.B., S.Y.; Supervision – I.B., S.Y., Y.S.; Resources – I.B., S.Y., Y.S., M.B.; Materials – I.B., S.Y., Y.S.,

N.C.; Data Collection and/or Processing – I.B., S.Y., F.O., M.K.; Analysis and/or Interpretation – I.B., S.Y.; Literature Search – I.B., S.Y.; Writing Manuscript – I.B., S.Y.; Critical Review – I.B., S.Y., Y.S.

Declaration of Interests: The authors declare that they have no competing interest.

Etik Komite Onayı: Bu çalışma için etik komite onayı Van Yüzüncü Yıl Üniversitesi'nden (Tarih: 01.02.2014, Sayı: 12-B) alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – I.B., S.Y.; Tasarım – I.B., S.Y.; Denetleme – I.B., S.Y., Y.S.; Kaynaklar – I.B., S.Y., Y.S., M.B.; Malzemeler – I.B., S.Y., Y.S., N.C.; Veri Toplanması ve/veya İşlemesi – I.B., S.Y., F.O., M.K.; Analiz ve/veya Yorum – I.B., S.Y.; Literatür Taraması – I.B., S.Y.; Yazıyı Yazan – I.B., S.Y.; Eleştirel İnceleme – I.B., S.Y.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

REFERENCES

- Baldwin DR, Marshall WJ. Heavy metal poisoning and its laboratory investigation. *Ann Clin Biochem.* 1999;36(3):267-300. [CrossRef]
- Akkoyun HT, Bengu AS, Ulucan A, et al. Effect of astaxanthin on rat brains against oxidative stress induced by cadmium. *Biochemical, histopathological evaluation. J Sci Techno.* 2018;8:33-39.
- Hocaoğlu ÖA, Genç BN. Cadmium in plants, humans and the environment. *Front Life Sci.* 2020;1:12-21.
- Taysı MR, Kırıcı M, Kırıcı M, Söğüt B, Bozdayı MA, Taysı S. Gökkuşluğu Alabalıklarında (*Oncorhynchus mykiss*) kadmiyum Toksisitesi: kalp ve kas üzerine bir araştırma. *Türk J Agri Nat Sci.* 2020;7:983-987. [CrossRef]
- Evcimen M, Aslan R, Gulay MS. Protective effects of polydatin and grape seed extract in rats exposed to cadmium. *Drug Chem Toxicol.* 2020;43(3):225-233. [CrossRef]
- Thévenod F. Nephrotoxicity and the proximal tubule. Insights from cadmium. *Nephron Physiol.* 2003;93(4):p87-p93. [CrossRef]
- Li M, Pi H, Yang Z, et al. Melatonin antagonizes cadmium-induced neurotoxicity by activating the transcription factor EB-dependent autophagy-lysosome machinery in mouse neuroblastoma cells. *J Pineal Res.* 2016;61(3):353-369. [CrossRef]
- Okuda B, Iwamoto Y, Tachibana H, Sugita M. Parkinsonism after acute cadmium poisoning. *Clin Neurol Neurosurg.* 1997;99(4):263-265. [CrossRef]
- Panayi AE, Spyrou NM, Iversen BS, White MA, Part P. Determination of cadmium and zinc in Alzheimer's brain tissue using inductively coupled plasma mass spectrometry. *J Neurol Sci.* 2002;195(1):1-10. [CrossRef]
- Notarachille G, Arnesano F, Calò V, Meleleo D. Heavy metals toxicity: effect of cadmium ions on amyloid beta protein 1-42. Possible implications for Alzheimer's disease. *Biometals.* 2014;27(2):371-388. [CrossRef]
- Adali A, Yirun A, Kocer Gümüşel B, Erkekoğlu P. Alzheimer Hastalığının Gelişiminde Biyolojik Ajanların Olası Etkileri. *J Fac Pharm Ankara.* 2019;44(1):167-187.
- Dimou L, Götz M. Glial cells as progenitors and stem cells: new roles in the healthy and diseased brain. *Physiol Rev.* 2014;94(3):709-737. [CrossRef]
- Terni B, López-Murcia FJ, Llobet A. Role of neuron-glia interactions in developmental synapse elimination. *Brain Res Bull.* 2017;129:74-81. [CrossRef]
- Kim Y, Park J, Choi YK. The role of astrocytes in the central nervous system focused on BK channel and heme oxygenase metabolites: a review. *Antioxidants (Basel).* 2019;8(5):121. [CrossRef]
- Huang L, Nakamura Y, Lo EH, Hayakawa K. Astrocyte signaling in the neurovascular unit after central nervous system injury. *Int J Mol Sci.* 2019;20(2):282. [CrossRef]
- Tiffany-Castiglioni E, Sierra EM, Wu JN, Rowles TK. Lead toxicity in neuroglia. *Neurotoxicology.* 1989;10(3):417-443.
- Middeldorp J, Hol EM. GFAP in health and disease. *Prog Neurobiol.* 2011;93(3):421-443. [CrossRef]
- Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res.* 2000;25(9-10):1439-1451. [CrossRef]
- Hagemann TL, Powers B, Mazur C, et al. Antisense suppression of glial fibrillary acidic protein as a treatment for Alexander disease. *Ann Neurol.* 2018;83(1):27-39. [CrossRef]
- van Bodegraven EJ, van Asperen JV, Robe PAJ, Hol EM. Importance of GFAP isoform-specific analyses in astrocytoma. *Glia.* 2019;67(8):1417-1433. [CrossRef]
- McLendon RE, Bigner DD. Immunohistochemistry of the glial fibrillary acidic protein: basic and applied considerations. *Brain Pathol.* 1994;4(3):221-228. [CrossRef]
- Hossain S, Liu HN, Nguyen M, Shore G, Almazan G. Cadmium exposure induces mitochondria-dependent apoptosis in oligodendrocytes. *Neurotoxicology.* 2009;30(4):544-554. [CrossRef]
- Rai A, Maurya SK, Khare P, Srivastava A, Bandyopadhyay S. Characterization of developmental neurotoxicity of As, Cd, and Pb mixture: synergistic action of metal mixture in glial and neuronal functions. *Toxicol Sci.* 2010;118(2):586-601. [CrossRef]
- Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Stud.* 2020;27:120-139.
- Alnahdi HS, Sharaf IA. Possible prophylactic effect of omega-3 fatty acids on cadmium-induced neurotoxicity in rats' brains. *Environ Sci Pollut Res Int.* 2019;26(30):31254-31262. [CrossRef]
- Al Olayan EM, Aloufi AS, AlAmri OD, El-Habit OH, Abdel Moneim AE. Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: role of oxidative stress, inflammation and apoptosis. *Sci Total Environ.* 2020;723:137969. [CrossRef]
- Chen J, Xu Y, Han Q, Yao Y, Xing H, Teng X. Immunosuppression, oxidative stress, and glycometabolism disorder caused by cadmium in common carp (*Cyprinus carpio* L.): application of transcriptome analysis in risk assessment of environmental contaminant cadmium. *J Hazard Mater.* 2019;366:386-394. [CrossRef]
- Al Omairi NE, Radwan OK, Alzahrani YA, Kassab RB. Neuroprotective efficiency of *Mangifera indica* leaves extract on cadmium-induced cortical damage in rats. *Metab Brain Dis.* 2018;33(4):1121-1130. [CrossRef]
- Fidancı ŞB, Gümüş LT, Nitrik Oksit Ölçüm Yöntemleri. *Mersin Üni Sağ Bil Derg.* 2011;4(3):1-8.
- Liang HY, Chen ZJ, Xiao H, et al. nNOS-expressing neurons in the vmPFC transform pPVT-derived chronic pain signals into anxiety behaviors. *Nat Commun.* 2020;11(1):2501. [CrossRef]
- Qu W, Liu NK, Wu X, et al. Disrupting nNOS-PSD95 interaction improves neurological and cognitive recoveries after traumatic brain injury. *Cereb Cortex.* 2020;30(7):3859-3871. [CrossRef]
- Lechado i Terradas A, Vitadello M, Traini L, Namuduri AV, Gastaldello S, Gorza L. Sarcolemmal loss of active nNOS (Nos1) is an oxidative stress-dependent, early event driving disuse atrophy. *J Pathol.* 2018;246(4):433-446. [CrossRef]
- Favorito R, Monaco A, Grimaldi MC, Ferrandino I. Effects of cadmium on the glial architecture in lizard brain. *Eur J Histochem.* 2017;61(1):2734. [CrossRef]
- Yildirim S, Celikezen FC, Oto G, et al. An investigation of protective effects of lithium borate on blood and histopathological parameters in acute cadmium-induced rats. *Biol Trace Elem Res.* 2018;182(2):287-294. [CrossRef]
- Meeker JD, Rossano MG, Protas B, Rossano MG, Protas B et al. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. *Environ Health Perspect.* 2008;116(11):1473-1479. [CrossRef]
- Karaca Ö, Sunay FB, Kuş MA, Gülçen B, Özcan E, Ögetürk M, Kuş İ. Kadmiyum ile Oluşturulan Deneyel Karaciğer Hasarına Karşı Melatoninin Etkilerinin Biyokimyasal ve Histopatolojik Düzeylerde İncelenmesi. *Firat Med J.* 2014;19(3):110-115.

37. Eddleston M, Mucke L. Molecular profile of reactive astrocytes—implications for their role in neurologic disease. *Neuroscience*. 1993;54(1):15-36. [\[CrossRef\]](#)
38. Messing A, Brenner M. GFAP at 50. *ASN Neuro*. 2020;12:1759091420949680. [\[CrossRef\]](#)
39. Trautz F, Franke H, Bohnert S, et al. Survival-time dependent increase in neuronal IL-6 and astroglial GFAP expression in fatally injured human brain tissue. *Sci Rep*. 2019;9(1):11771. [\[CrossRef\]](#)
40. Baran A, Sulukan E, Türkoğlu M, et al. Is sodium carboxymethyl cellulose (CMC) really completely innocent? It may be triggering obesity. *Int J Biol Macromol*. 2020;163:2465-2473. [\[CrossRef\]](#)
41. Sulukan E, Ghosigharehagaji A, Baran A, Yildirim S, Bolat İ, Ceyhun SB. A versatile toxicity evaluation of ethyl carbamate (urethane) on zebrafish embryos: morphological, physiological, histopathological, immunohistochemical, transcriptional and behavioral approaches. *Tox Let*. 2021;353:71-78. [\[CrossRef\]](#)
42. Varmazyari A, Taghizadehghalehjoughi A, Sevim C, et al. Cadmium sulfide-induced toxicity in the cortex and cerebellum: in vitro and in vivo studies. *Tox Rep*. 2020;7:637-648. [\[CrossRef\]](#)
43. Korneev SA, Park JH, O'Shea M. Neuronal expression of neural nitric oxide synthase (nNOS) protein is suppressed by an antisense RNA transcribed from an NOS pseudogene. *J Neurosci*. 1999;19(18):7711-7720. [\[CrossRef\]](#)
44. Liu W, Li J, Sun X, et al. Repetitive hyperbaric oxygen exposures enhance sensitivity to convulsion by upregulation of eNOS and nNOS. *Brain Res*. 2008;1201:128-134. [\[CrossRef\]](#)
45. Bonthius DJ, Bonthius NE, Li S, Karacay B. The protective effect of neuronal nitric oxide synthase (nNOS) against alcohol toxicity depends upon the NO-cGMP-PKG pathway and NF-κB. *Neurotoxicology*. 2008;29(6):1080-1091. [\[CrossRef\]](#)
46. Bonthius DJ, Mckim RA, Koele L, et al. Pantazis NJ. Severe alcohol-induced neuronal deficits in the hippocampus and neocortex of neonatal mice genetically deficient for neuronal nitric oxide synthase (nNOS). *J Comp Neurol*. 2006;499(2):290-305. [\[CrossRef\]](#)
47. Yuan Y, Jiang C, Sun Y, et al. Effect of cadmium on the apoptosis and mRNA transcription of nitric oxide synthase of cerebral cortical neurons in embryonic rat. *Chin J Vet Sci*. 2013;33:102-106.



Detection of Multidrug-Resistant *Staphylococci* in Beef Processing Line

Siğir Eti İşleme Hattında Çoklu İlaç Dirençli *Stafilokokların* Belirlenmesi

Akwoba JOSEPH OGUGUA¹
Catherine UJU OKAFOR¹
Simeon CHIBUKO OKAFOR¹
Emmanuel OKECHUKWU
NJOGA¹
Umunna MADUBUIKE¹
John ANAELOM NWANTA¹
Gabriel O. EZEIFEKA²

¹Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka, Nigeria

²Department of Veterinary Microbiology, Michael Okpara University of Agriculture, Umudike, Nigeria

ABSTRACT

Some *Staphylococcus* species are zoonotic and the non-zoonotic species may harbor antibiotic-resistance genes for transmission to humans via the food chain. The study aimed at determining the staphylococci contamination of beef processed for human consumption and the antibiogram of the organisms. Isolation and identification of *Staphylococcus* from beef and the meat contact surfaces were done following standard microbiological protocols, including the Application Programmed Interface. Disc diffusion method was used to test the susceptibility of the staphylococci to 14 commonly used antimicrobial agents. The mean staphylococci load of the beef before processing was $5.0 \times 10^9 \pm 1.0 \times 10^5$ and $7.1 \times 10^9 \pm 1.0 \times 10^6$ cfu/cm² after. Of the 200 samples tested, *Staphylococcus* spp. were isolated in 25 (12.5%). The isolates were *Staphylococcus aureus* (12%), *Staphylococcus xylosus* (56%), *Staphylococcus cohnii* (16%), *Staphylococcus saprophyticus* (12%), and *Staphylococcus hominis* (4%). Twenty-two (88%) of the isolates were resistant to antimicrobials, including those listed in World Health Organization's list of "high" and "highest" priority antibiotics. Eighteen isolates (81.8%) were multidrug resistant while 4 (21%) were resistant to at least 1 antimicrobial agent. Isolation of multidrug-resistant *Staphylococcus* from beef and the meat contact surfaces portends significant food safety and public health risks as the organisms or their resistance determinants are transmissible to humans via the food chain. This emphasizes the need for the adoption of the "farm to fork" concept of food safety in beef production and processing lines to forestall staphylococci meat contamination and hence the untoward public health and economic consequences thereof.

Keywords: Beef, contact surfaces, multidrug-resistant *Staphylococcus*, Kwata slaughterhouse, Nigeria

ÖZ

Bezelye İşleme Hattında Çoklu İlaç Dirençli Stafilokokların Belirlenmesi Bazı *Staphylococcus* türleri zoonotiktir ve zoonotik olmayan türler de antibiyotik direnci genlerini barındırabilir ve gıda zinciri yoluyla insanlara iletebilir. Bu çalışmanın amacı, insan tüketimi için işlenen siğir etindeki stafilokok kirliliğini ve organizmaların antibiyogramını belirlemektir. Siğir eti ve et temas yüzeylerinden *Staphylococcus* izolasyonu ve tanımlaması yapılması için Aplikasyon Programlı Arayüz gibi standart mikrobiyolojik protokoller kullanıldı. Stafilokokların 14 yaygın olarak kullanılan antimikrobiyal ajanlara duyarlılığı disk difüzyon yöntemiyle test edildi. İşlemden önceki ortalama stafilokok yükü $5.0 \times 10^9 \pm 1.0 \times 10^5$ idi ve işlemden sonra $7.1 \times 10^9 \pm 1.0 \times 10^6$ cfu/cm² oldu. Test edilen 200 örnekten 25'inde (%12,5) *Staphylococcus* spp. izole edildi. İzolatlar *Staphylococcus aureus* (%12), *Staphylococcus xylosus* (%56), *Staphylococcus cohnii* (%16), *Staphylococcus saprophyticus* (%12) ve *Staphylococcus hominis* (%4) idi. İzolatların 22'si (%88) Dünya Sağlık Örgütü'nün "yüksek" ve "en yüksek" öncelikli antibiyotiklerinin listesinde yer alanlar da dahil olmak üzere antimikrobiyallere dirençliydi. On sekiz izolat (%81,8) multidrug-resistance iken 4'ü (%21) en az bir antimikrobiyal ajana dirençliydi. Siğir eti ve et temas yüzeylerinden çoklu ilaç dirençli *Staphylococcus* izole edilmesi, organizmaların veya direnç belirleyicilerinin gıda zinciri yoluyla insanlara taşınması bakımından önemli gıda güvenliği ve halk sağlığı riskleri taşımaktadır. Bu sonuç da, siğir üretimi ve işleme hatlarında gıda güvenliği "çiftlikten çatala" kavramının benimsenmesinin stafilokok et kirliliğini önlemek ve buna bağlı olarak olumsuz halk sağlığı ve ekonomik sonuçları engellemenin gerekliliğine vurgu yapmaktadır.

Anahtar Kelimeler: Siğir eti, temas yüzeyleri, çoklu ilaca dirençli *Staphylococcus*, Kwata mezbahası, Nijerya

Received/Geliş Tarihi: 04.09.2022

Accepted/Kabul Tarihi: 15.01.2023

Publication Date/Yayın Tarihi: 26.04.2023

Corresponding author/Sorumlu Yazar:

Akwoba Joseph Ogugua

E-mail: ogugua.akwoba@unn.edu.ng

Atıf: Joseph Ogugua A, Uju Okafor C, Chibuko Okafor S, et al. Siğir eti ileme hattında çoklu ilaç dirençli *Stafilokokların* belirlenmesi. *Vet Sci Pract.* 2023; 18(1), 25-30.

Cite this article as: Joseph Ogugua A, Uju Okafor C, Chibuko Okafor S, et al. Detection of multidrug-resistant *Staphylococci* in beef processing line. *Vet Sci Pract.* 2023; 18(1), 25-30.



Copyright©Author(s) - Available online at veterinarysciences-ataunipress.org
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License

INTRODUCTION

Foodborne pathogens are the leading causes of illnesses and death globally, costing billions of dollars in medical care and social costs.¹ Although foodborne diseases affect persons of all ages, the burden is more in children below the age of 5, immunologically compromised individuals and people residing in developing and low-income countries.² Globally, many foodborne disease outbreaks have been associated with the consumption of contaminated foods of animal origin. Of these outbreaks, staphylococcus food diseases (staphyloenterotoxigenesis or staphyloenterotoxemia) are prominent,³ although campylobacteriosis and salmonellosis are also in the front burners.⁴

In developing countries, majority of meats consumed are slaughtered at homes or in clandestine slaughterhouses.⁵ This makes the bacteriological qualities of these meats uncertain due to poor sanitary conditions of the staff, equipment, meat contact surfaces, and the environment in most slaughterhouses.⁶ Further meat contamination occurs in the slaughterhouses as a result of poor planning during the construction of slaughterhouse, lack of required amenities, and non-adherence to rules guiding food animal slaughter or processing for human consumption.⁷

Consequently, quite a good number of bacterial meat contaminants have been reported in Nigerian slaughterhouses.⁸⁻¹⁰ Most of these contaminants may be zoonotic, pathogenic, or harbor antimicrobial-resistance determinants transmissible to humans via the food chain. Of all bacterial meat contaminants, emphasis has been on the *Staphylococcus* species, especially coagulase-positive staphylococci (*Staphylococcus aureus* and *Staphylococcus pseudintermedius*) due to their zoonotic and pathogenic potentials. However, the coagulase-negative *Staphylococcus* (CoNS) species are equally important. Their importance lies in the fact that CoNS cause opportunistic infections which may degenerate into severe diseases in immune-compromised hosts.³ Additionally, the CoNS are reservoirs of antimicrobial resistance genes, due to frequent exposure to low doses of antimicrobials occasioned by the imprudent use of the drugs in medical and veterinary practices.¹¹

Meats contaminated with *Staphylococcus* species are major causes of foodborne disease outbreaks as the moisture and nutritional contents of meat facilitate bacterial growth and proliferation.³ In addition, the organisms are capable of surviving as commensals on the skin and nares of meat processors and food handlers, as well as on inanimate surfaces like clothing, meat contact surfaces, and meat processing equipment.¹²

Staphylococcus ranks high as a major cause of foodborne intoxication globally because most toxigenic species are capable of elaborating toxins. Some of these toxins are heat stable, resistant to the activities of proteolytic enzymes and can cause intoxication even at low doses.¹³ Unfortunately, the ambient temperature in most tropical climates, including Nigeria, favors the proliferation of *Staphylococcus* and the production of toxins in the toxigenic species.³ In both *Staphylococcus* food poisoning and intoxication, the onset of clinical manifestations depends on the immune status of the host and includes nausea, vomiting, stomach aches, chills, fever, and diarrhea as well as cause abscess, fatal sepsis, endocarditis, meningitis, and toxic shock.^{14,15}

Considering the state of some slaughterhouse facilities and the role of slaughterhouse workers in the spread of zoonotic

pathogens in the meat supply chain in Nigeria;^{6,16} meat processed for human consumption in Anambra State, Nigeria, may be contaminated with *Staphylococcus* species in view of the ubiquitous nature of the organisms. Detection of drug-resistant *Staphylococcus* in the meat and abattoir environment could provide a scientific basis for improvement in sanitary conditions of the facilities which will bring about great economic and public health gains to humanity. In addition, the antimicrobial resistance profile of the staphylococci contaminants could guide empirical antibiotic treatment in cases of suspected *Staphylococcus* foodborne diseases or intoxication.

Published data on staphylococcal contamination of beef and/or the meat contact surfaces, as well as the antibiogram of the bacteria in Anambra State are sparse and far between. Therefore, this study determined *Staphylococcus* contamination of beef and meat contact surfaces in Anambra State, Nigeria, and also the antimicrobial-susceptibility profile of the isolates in order to recommend appropriate and implementable public health action.

MATERIALS AND METHODS

Study Area

The study was conducted in Kwata slaughterhouse (KSH), Anambra State, Nigeria. The KSH is an open system with neither roof nor doors. Therefore, visitors as well as scavenging animals and birds have unrestricted access to the facility. The slaughterhouse is the major source of meat supply to Awka, the capital city of Anambra State. The demographics of the state have been published,¹⁷ and the geographical location of Awka is shown in Figure 1.

Study Design and Sampling

The study adopted a cross-sectional study design. Using the prevalence of 9.4% earlier recorded,³ a minimum sample size of 72 was calculated as earlier described.¹⁸ However, 200 samples were used in this study for buoyancy and accuracy of data. Swab samples were collected from 2 cm² of the groin skin before processing and from the same site of the meat carcass after processing and different meat contact surfaces (before and after contact with dressed beef) in the slaughterhouse using sterile swab sticks moistened with 0.1% peptone water. A systematic random sampling (1 in 5) was used in the selection of beef and the contact surfaces for swab sample collection. The surfaces sampled were slaughter floor, meat dressing table, washing bucket, bleeding knife, butchers' footwear, knife sharpener, wheelbarrow, washing water, and meat display table. In all, 200 samples were collected over a period of 10 weeks.

Determination of *Staphylococcus* Contamination

The *Staphylococcus* load of the beef and the contact surfaces were determined by aerobic bacterial count (enumeration) as described.¹⁹ Briefly, swab samples were transferred aseptically into a sterile stomacher bag containing 225 mL of 0.85% sterile saline solution and homogenized in a stomacher (Stomacher® 400 Circulator, Seward, Ltd., UK) for 2 minutes at room temperature to achieve a 10⁻¹ dilution. Microbial extracts were serially diluted in sterile distilled water. Each diluted 1 mL sample was plated individually and spread thoroughly on mannitol salt agar and incubated at 37°C for 48 hours for staphylococcal counting. Glistening whitish/creamy and yellow colonies were recorded and counted as *Staphylococcus*. All analyses were performed in triplicate, and results were expressed as the logarithm of colony-forming units per cm² (Log CFU/cm²). Plate counts were determined and converted to log₁₀ CFU values using standardized plate count

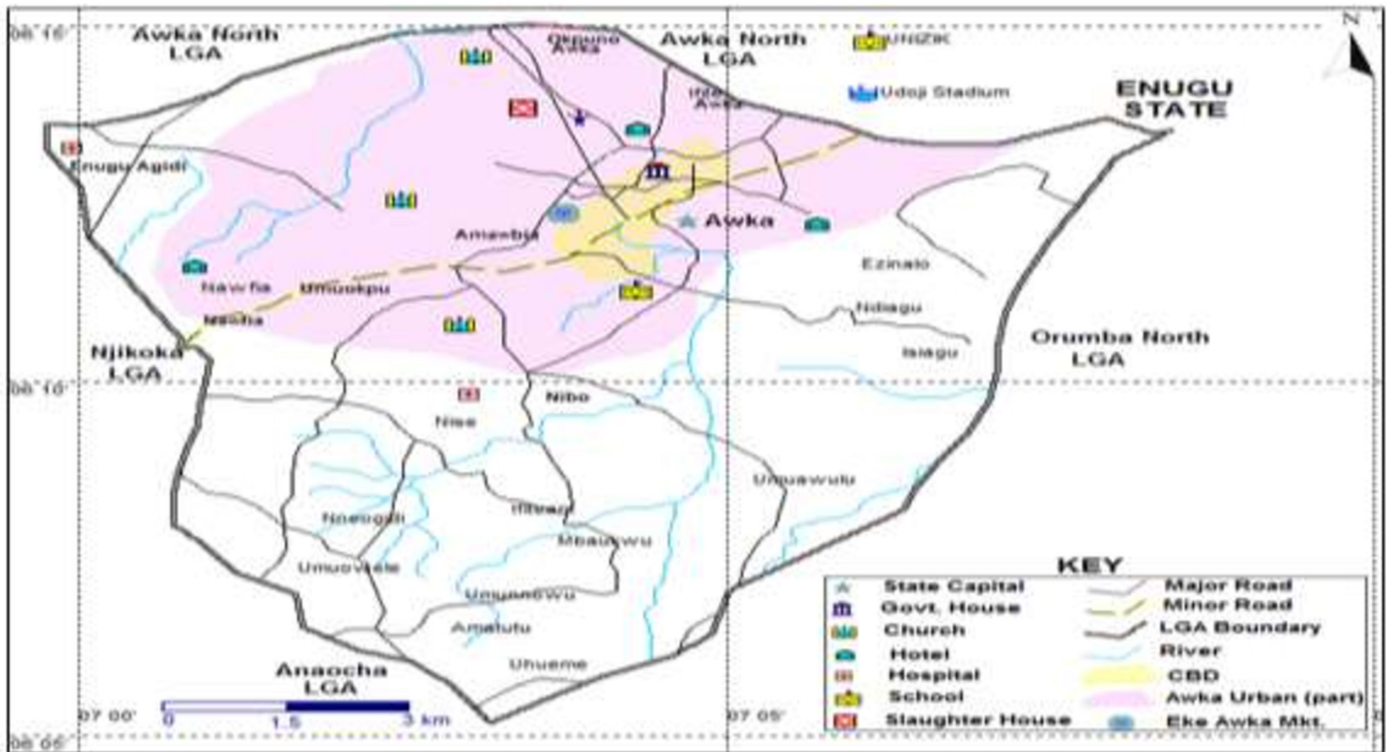


Figure 1. Map of Anambra State showing the study area.

rules.¹⁹ Isolation of *Staphylococcus* species was done using mannitol salt agar (Oxoid, Basingstoke, UK) according to the method of Cheesbrough.²⁰ At first, the swab samples were pre-enriched in nutrient broth supplemented with 7.5% NaCl for 24 hours at 37°C. Thereafter, a loop full of the pre-enriched samples was streaked on mannitol salt agar and incubated at 37°C for another 24 hours. In plates that yielded growth, 1 or 2 whitish/creamy and yellow colonies on the agar were purified on nutrient agar (Oxoid, Basingstoke, UK). Putative *Staphylococcus* colonies, appearing cream on nutrient agar, were further subjected to Gram-staining and catalase tests. Gram-positive cocci in bunches that produced vigorous bubbles on emulsification with 3% H₂O₂ were subjected to further characterization and speciation using the Application Programmed Interface Staph kit (Biomérieux®, France) according to the manufacturer's instruction.

Antimicrobial Resistance Profiling

Antimicrobial susceptibility testing of the staphylococcal isolates was performed by disc diffusion method according to the guidelines of the Clinical Laboratory Standards Institute²¹ with discs (Oxoid Hampshire England) impregnated with the following 13 antimicrobial agents belonging to 6 classes: β -lactam—ceftriaxone (30 μ g), cefixime (5 μ g), ampicillin (10 μ g), and amoxicillin (10 μ g), macrolides—erythromycin (15 μ g), fluoroquinolones—ciprofloxacin (5 μ g), levofloxacin (5 μ g), norfloxacin (10 μ g), and ofloxacin (5 μ g), aminoglycosides—gentamicin (10 μ g) and streptomycin (10 μ g), ansamycin—rifampicin (5 μ g), and phenicols—chloramphenicol (30 μ g). *Staphylococcus aureus* ATCC 25923 was used as a quality-control strain for susceptibility. Inhibition zone diameters were interpreted in accordance with the breakpoints for *Staphylococcus*.²¹ An isolate resistant to at least 1 antimicrobial agent in 3 or more classes/categories of antimicrobial agents was considered multidrug-resistant.

Statistical Analyses

Data bothering on the prevalence and antimicrobial resistance profile of the isolates were analyzed descriptively. Results obtained for the bacteria count were summarized as mean $\pm$ standard error of mean. Mean values of the bacterial load for beef and the various contact surfaces were compared using a 1-way analysis of variance. Duncan's multiple range test was used to separate variant means. Values were considered significant at $P < .05$. All the statistical analyses were performed using IBM® Statistical Package for Social Sciences statistics version 23 (SPSS Inc., Chicago, Ill, USA).

Ethical Approval

The research was conducted in accordance with the guidelines laid down by local laws and regulations, and the swab samples used for the study were collected from surfaces of carcasses, floors, and materials used for their processing in the slaughterhouses. The University of Ibadan Animal Care and Use Research Ethics Committee gave ethical permission for the work with the number UI-ACUREC/APP/2015/047 issued on 15/11/2015.

RESULTS

The mean staphylococci load of the beef before and after contact with processing equipment and surfaces were $0.50 \times 10^{10} \pm 0.10 \times 10^6$ and $0.71 \times 10^{10} \pm 1.0 \times 10^6$ cfu/cm², respectively. Results on the bacterial loads of the different beef contact surfaces are presented in Table 1. There was a significant ($P < .05$) increase in the *Staphylococcus* load in the washing water after beef immersion. Also, there was a significant ($P < .05$) increase in *Staphylococcus* contamination of the beef after processing.

Out of 200 samples processed, *Staphylococcus* species were isolated in 25 (12.5%) samples. The isolates were *S. aureus*, 3

Table 1. Mean *Staphylococcus* Counts in Different Beef Contact Surfaces (n=20 Each) Collected from Kwata Slaughterhouse, Anambra State, Nigeria

Contact Surfaces	Number of Samples that Yielded Growth (%)	Mean <i>Staphylococcus</i> Count (cfu/cm ²)	
		Before Beef Contact	After Beef Contact
SF	3 (15)	1.1 × 10 ⁹ ± 1.1 × 10 ^{6a}	1.0 × 10 ⁹ ± 1.1 × 10 ^{5b}
MDT	4 (20)	1.1 × 10 ⁹ ± 1.0 × 10 ^{6a}	1.1 × 10 ⁹ ± 1.0 × 10 ^{6a}
WBu	2 (10)	1.1 × 10 ⁹ ± 1.1 × 10 ^{5a}	1.0 × 10 ⁹ ± 1.0 × 10 ^{5b}
BK	2 (10)	1.1 × 10 ⁹ ± 1.1 × 10 ^{5a}	1.0 × 10 ⁹ ± 1.0 × 10 ^{6b}
BFW	2 (10)	1.0 × 10 ⁹ ± 1.1 × 10 ^{5a}	1.1 × 10 ⁹ ± 1.0 × 10 ^{6b}
KS	2 (10)	1.0 × 10 ⁹ ± 1.1 × 10 ^{5a}	1.0 × 10 ⁹ ± 1.1 × 10 ^{5b}
WB	3 (15)	1.1 × 10 ⁹ ± 1.1 × 10 ^{5a}	1.0 × 10 ⁹ ± 1.0 × 10 ^{5b}
BDT	3 (15)	6.0 × 10 ⁹ ± 1.1 × 10 ^{5a}	7.1 × 10 ⁹ ± 1.1 × 10 ^{6b}
WW	1 (5)	7.1 × 10 ⁹ ± 1.0 × 10 ^{5a}	1.1 × 10 ¹⁰ ± 1.0 × 10 ^{5b}

BDT, beef display table; BFW, butchers footwear; BK, bleeding knife; CFU, colony-forming unit; KS, knife sharpener; MDT, meat dressing table; SF, slaughter floor; WB, wheelbarrow; WBu, washing bucket; WW, washing water.
Different superscripts across the rows (within contact surfaces) indicate statistical significance.

(12%); *S. xylosus*, 14 (56%); *S. cohnii sub-species cohnii*, 4 (16%); *S. saprophyticus*, 3 (12%); and *S. hominis*, 1 (4%). The only *S. hominis* isolated was resistant to ceftriaxone, cefixime, ofloxacin, and clindamycin. Three isolates (2 *S. xylosus* and 1 *S. saprophyticus*) were susceptible to all the 14 antimicrobial agents used in the susceptibility testing. Details on the resistance profile of the other 22 isolates are presented in Table 2.

Results on the susceptibility of the *Staphylococcus* species to each of the antimicrobials are shown in Table 3. Of the 25 isolates, 18 (81.8%) were multidrug-resistant, following their non-susceptibility to at least 1 antimicrobial agent in 3 or more classes or groups of antimicrobials. Four isolates (21%) were resistant to at least 1 antimicrobial agent used in the susceptibility test. The resistance of the isolates to the antimicrobials in decreasing order were ofloxacin (14/25, 56%) > clindamycin (13/25, 52%) > norfloxacin (12/25, 48%) > ceftriaxone (10/25, 40%) > amoxicillin and rifampicin (9/25, 36%) > ampicillin and cefixime (8/25, 32%) > streptomycin (7/25, 28%) > chloramphenicol (6/25, 12%) > erythromycin (3/25, 12%) > ciprofloxacin (2/25, 8%). All the isolates were susceptible to levofloxacin and gentamicin (Table 3).

Twenty-two resistance patterns/phenotypes were observed for the antimicrobial-resistant isolates (Table 4). The patterns ranged from 1 to 10 antimicrobials and none had the same resistant

Table 2. Antimicrobial Resistance Profile of *Staphylococcus* species (n=25) Isolated from Beef and Meat Contact Surfaces and Processing Equipment at Kwata Slaughterhouse, Nigeria

Antibiotics	Number of Resistant <i>Staphylococci</i> Species (%)			
	<i>S. aureus</i> (n=3)	<i>S. xylosus</i> (n=14)	<i>S. cohnii</i> (n=4)	<i>S. saprophyticus</i> (n=3)
Erythromycin	0 (0.0)	2 (14.3)	1 (25.0)	0 (0.0)
Ceftriaxone	2 (66.7)	3 (21.43)	3 (75.0)	2 (66.7)
Ampicillin	0 (0)	6 (42.9)	1 (25.0)	1 (33.3)
Cefixime	2 (66.7)	2 (14.3)	3 (75.0)	1 (33.3)
Levofloxacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Norfloxacin	2 (66.7)	5 (35.7)	3 (75.0)	2 (66.7)
Ciprofloxacin	0 (0.0)	1 (7.1)	1 (25.0)	0 (0.0)
Gentamicin	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)
Ofloxacin	3 (100.0)	6 (42.9)	3 (75.0)	2 (66.7)
Clindamycin	3(100.0)	5 (35.7)	4 (100.0)	1 (33.3)
Amoxicillin	0 (0.0)	5 (35.7)	3 (75.0)	1 (33.3)
Streptomycin	0 (0.0)	4 (28.6)	1 (25.0)	2 (66.7)
Rifampicin	0 (0.0)	5 (35.7)	2 (50.0)	2 (66.7)
Chloramphenicol	1(33.3)	3 (21.43)	1 (25.0)	1 (33.3)

Table 3. Susceptibility of *Staphylococcus* Species (n=25) Isolated from Beef and Meat Contact Surfaces at Kwata Slaughterhouse to Different Antimicrobial Agents

Antimicrobials (µg)	Number of resistant isolates (%)
Ofloxacin (5)	14 (56)
Clindamycin (2)	13 (52)
Norfloxacin (10)	12 (48)
Ceftriaxone (30)	10 (40)
Amoxicillin (10)	9 (36)
Rifampicin (5)	9 (36)
Ampicillin (10)	8 (32)
Cefixime (5)	8 (32)
Streptomycin (10)	7 (28)
Chloramphenicol (30)	6 (24)
Erythromycin (15)	3 (12)
Ciprofloxacin (5)	2 (9)
Levofloxacin (5)	0
Gentamicin (10)	0

Table 4. Antimicrobial Susceptibility Profile of *Staphylococcus* Species (n=25) Isolated from Beef and Meat Contact Surfaces and Processing Equipment at Kwata Slaughterhouse, Nigeria

Antimicrobial Resistance Phenotypes	Number (%) of Isolates Exhibiting the Pattern
E	1 (4)
OF	1 (4)
CE	1 (4)
AP	1 (4)
O-CD	1 (4)
N-S-RD	1 (4)
AP-S-RD	1 (4)
N-OF-CD	1 (4)
E-AP-N-AMX	1 (4)
CT-CE-OF-CD	1 (4)
CT-CE-OF-CD-S	1 (4)
CE-OF-CD-AMX-RD	1 (4)
CT-N-OF-CD-S-RD	1 (4)
CT-CE-N-OF-CD-CH	1 (4)
CT-AP- N-OF-CD-AMX	1 (4)
CT-AP-CIP-OF-CD-AMX-CH	1 (4)
AP-N-OF-CD-AMX-S-RD-CH	1 (4)
CT-CE-N-CIP-OF-CD-AMX-RD	1 (4)
E-CE-N-CIP-O-CD-AM-RD-CH	1 (4)
E-CE-N-CIP-OF-CD-AMX-RD-CH	1 (4)
CT-AP-CE-LV-N-OF-AMX-S-R-CH	1 (4)
CT-AP- CE-N-OF-CD-AMX-S-RD-CH	1 (4)

AMP, ampicillin; AMX, amoxicillin; C, chloramphenicol; CD; clindamycin, CE, cefixime; CIP, ciprofloxacin; CN, gentamicin; CRO, ceftriaxone; E, erythromycin; LEV, levofloxacin; N, norfloxacin; OF, ofloxacin; RD, rifampicin; S, streptomycin.

pattern as the other. The resistance phenotypes indicated that 7, 10, and 5 isolates exhibited resistance to 8-10, 3-6, and 1-2 antimicrobials, respectively (Table 4).

DISCUSSION

The high *Staphylococcus* contamination of beef and the processing facilities found may be attributed to poor hygienic practices at farm and slaughterhouse levels.²² Non-adherence to hygiene practices in all stages of meat production causes adverse health effects on animal health and decreases the microbial quality of the meat,²³ thereby raising serious food safety and public health concerns. In developing countries, poor hygiene practices in livestock farms or during transportation, marketing, or processing of animal is very common.^{22,24} Moreover, the unsanitary conditions in which cattle in transit or those awaiting slaughter in lairage

could also have contributed to the meat contamination with *Staphylococcus*.

Additionally, the practices of flaying and evisceration on cattle carcasses on the bare floor, immersion in beef in water of unproven bacteriological quality, and lack of routine decontamination of slaughterhouses and slaughter equipment may have contributed to the high bacterial loads found in the beef, contact surfaces, and processing equipment. In the KSH disposal of wastes into streams and the use of water from the same streams to wash carcasses are common practices. This may have also accounted for the high staphylococcal counts found, which were higher than the recommended maximum permissible limits as cited by Edward et al.²⁵

In addition, the colony counts of *Staphylococcus* in this study are higher than that recorded in Abuja and Abattoirs.^{25,26} The high counts found are of public health significance as consumption of meat heavily contaminated with *Staphylococcus* species, may overwhelm the host immune defense system, and hence the onset of staphylococcal diseases, especially in immune-deficient individuals.²⁷

Furthermore, the contaminated meat or meat processing equipment can contaminate ready-to-eat foods in the kitchens of food vendors and meat buyers. This may enhance further transmission of the *Staphylococcus* species through ingestion of the contaminated foods. The infection can also spread via contact with abraded skin. This is of great public health importance knowing that most abattoir workers in Nigeria, including KSH workers, do not use protective equipment during routine duties.²⁸

Although the toxigenic potentials of the isolates were not determined, the isolation of known toxigenic staphylococcal species from the meats and the contact surfaces casts aspersion on the toxicological safety of the beef. While most Africans have the culture of proper meat cooking (heating at 80-100°C for over 30 minutes) which may be sufficient to kill off most pathogens in meats,²⁹ it does not deactivate heat-labile toxin if present, no matter the heating temperature or duration of cooking.³

The multidrug resistance noted in the isolates as well as the non-susceptibility to vital antibiotics such as quinolones and ceftriaxone portends great public health problems. The resistance may be due to large-scale indiscriminate use of antibiotics in medical practice and animal agriculture as antimicrobial use is not well regulated in Nigeria.³ Although some of the isolates were not pathogenic species, their ingestion could result in the compromise of antimicrobial therapy in individuals colonized by them.

As a result of the observations of the study, the clinical importance and pathogenic potentials of different *Staphylococcus* isolates found are noteworthy. The recovery of *S. saprophyticus*, *S. xylosus*, *S. aureus*, and *S. homini* from beef and slaughterhouse contact surfaces in this study calls for serious concern since these staphylococcal species have been implicated in a wide range of diseases, especially in immunocompromised persons.³³ Infections with *S. aureus* usually result in a large range of clinical manifestations including infertility, food poisoning and intoxication, skin and soft tissue diseases, surgical site abnormalities, pleuro-pneumonia, and several other life-threatening conditions that resulted in a large number of deaths annually.³⁰ Cognizant that human immunodeficiency virus burden and other immunomodulating infections are high in Nigeria,³¹ the synergy of these infections and Staphylococcosis could overwhelm the health of

immune-deficient individuals and further pressurize the already precarious health facilities in Nigeria.

Beef processed for human consumption in Anambra State was contaminated with multidrug-resistant *Staphylococcus*, and this may pose deleterious health effects on the meat consumers. The contamination may be due to unhygienic practices in the meat production and processing lines hence the need for the adoption of the farm-to-fork concept of food safety. In the meantime, proper cooking of the meat is recommended to limit the odds of bacterial infection and the untoward health and economic effects thereof.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of University of Ibadan (Date: November 15, 2015, Decision No: UI-CUREC/APP/2015/047).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.U.O., U.M., J.A.N., G.O.E.; Design – U.M., J.A.N., G.O.E.; Supervision – J.A.N., G.O.E.; Resources – C.U.O., S.C.O.; Materials – C.U.O., S.C.O.; Data Collection and/or processing – C.U.O., S.C.O., U.M.; Analysis and/or Interpretation – A.J.O., U.M., J.A.N.; Literature Search – C.U.O., E.O.N., A.J.O., U.M.; Writing Manuscript – A.J.O., E.O.N.; Critical Review – A.J.O., E.O.N., U.M.

Declaration of Interests: The authors declare no conflict of interest.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı XXXX Üniversitesi'nden (Tarih: 15 Kasım 2015, Karar No: UI-ACUREC/APP/2015/047) alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– C.U.O., U.M., J.A.N., G.O.E.; Tasarım – U.M., J.A.N., G.O.E.; Denetleme – J.A.N., G.O.E.; Kaynaklar – C.U.O., S.C.O.; Malzemeler – C.U.O., S.C.O.; Veri Toplanması ve/veya İşlemesi – C.U.O., S.C.O., U.M.; Analiz ve/veya Yorum – A.J.O., U.M., J.A.N.; Literatür Taraması – C.U.O., E.O.N., A.J.O., U.M.; Yazıyı Yazan – A.J.O., E.O.N.; Eleştirel İnceleme – A.J.O., E.O.N., U.M.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Lee H, Yoon Y. Etiological agents implicated in foodborne illness worldwide. *Food Sci Anim Resour.* 2021;41(1):1-7. [\[CrossRef\]](#)
2. Pires SM, Desta BN, Mughini-Gras L, et al. Burden of foodborne diseases: think global, act local. *Curr Opin Food Sci.* 2021. [\[CrossRef\]](#); 39:152-159. [\[CrossRef\]](#)
3. Okoli CE, Njoga EO, Enem SI, Godwin EE, Nwanta JA, Chah KF. Prevalence, toxigenic potential and antimicrobial susceptibility profile of *Staphylococcus* isolated from ready-to-eat meats. *Vet World.* 2018;11(9):1214-1221. [\[CrossRef\]](#)
4. Njoga EO, Nwankwo IO, Ugwuwarua JC. Epidemiology of thermotolerant *Campylobacter* infection in poultry in Nsukka agricultural zone, Nigeria. *Int J One Health.* 2019;5:92-98. [\[CrossRef\]](#)
5. Herenda D, Chambers PG, Ettriqui A, Seneviratna P. *Manual on meat inspection for developing countries.* FAO Anim Prod Health Pap 119. Rome, Italy: FAO, Viale delle Terme di Caracalla. 2011.
6. Ekere SO, Njoga EO, Onunkwo JI, Njoga U. Serosurveillance of Brucella antibody in food animals and role of slaughterhouse workers

- in spread of *Brucella* infection in Southeast. *Vet World*. 2018;11:1171-1178.
7. Stuart SA. Code of Hygienic Practice for Meat; (2005). [\[CrossRef\]](#)
 8. Ogugua AJ, Onunkwo JI, Nwankwo IO, Gugu LE, Basil-Ejidiye RC, Nwanta JA. Tubercles in cattle carcasses and risk behaviours for zoonotic tuberculosis transmission among workers in a municipal slaughterhouse. *Not Sci Biol*. 2021;13(2):10811. [\[CrossRef\]](#)
 9. Bikom PM, Nwankwo IO, Ogugua AJ, et al. Prevalence and distribution of bovine tuberculosis among slaughtered cattle in Cross River State, Nigeria. *Anim Res Int*. 2021;18(1):3977-3989.
 10. Nwankwo IO, Ezenduka EV, Nwanta JA, Ogugua AJ, Audu BJ. Prevalence of *Campylobacter* spp. and antibiotics resistant *E. coli* on poultry carcasses and handlers' hands at Ikpa slaughter, Nsukka, Nigeria. *Not Sci Biol*. 2021;13(2):Article No. 10866. [\[CrossRef\]](#)
 11. Njoga EO, Onunkwo JI, Okoli CE, Ugwuoke WI, Nwanta JA, Chah KF. Assessment of antimicrobial drug administration and antimicrobial residues in food animals in Enugu State, Nigeria. *Trop Anim Health Prod*. 2018;50(4):897-902. [\[CrossRef\]](#)
 12. Kadariya J, Smith TC, Thapaliya D. Staphylococcus aureus and Staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed Res Int*. 2014;2014:Article ID 827965, 9 pages. [\[CrossRef\]](#)
 13. Fletcher S, Boonwaat L, Moore T, Chavada R, Conaty S. Investigating an outbreak of staphylococcal food poisoning among travellers across two Australian states. *Western Pac Surveill Response J*. 2015; 6(2):17-21. [\[CrossRef\]](#)
 14. Abebe E, Gugsu G, Ahmed M. Review on major foodborne zoonotic bacteria pathogens. *J Trop Med*. 2020;2020:Article ID 4674235, 19 pages. [\[CrossRef\]](#)
 15. MMWR. *Outbreak of Staphylococcal Food Poisoning from a Military Unit Lunch Party — United States*. 2013; 2012.
 16. Njoga EO, Onunkwo JI, Ekere SO, Njoga U, Okoro WN. Seroepidemiology of equine brucellosis and role of horse carcass processors in spread of *Brucella* infection in Enugu State, Nigeria. *Int J Curr Res Rev*. 2018;10:39-35.
 17. Onunkwo JI, Njoga EO, Njoga UJ, Ezeakafor E, Ekere SO. *Brucella* seropositivity in chicken and risk factors for *Brucella* infection at the animal-human interface in Anambra State, Nigeria. *Int J One Health*. 2018;4:28-34. [\[CrossRef\]](#)
 18. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Bench*. 2013;6(1):14-17.
 19. Cappucciono M, Sherma S. *Manual of Laboratory Microbiology*. Oxford, Philadelphia: Blackwell Publishing Company; 2011:414.
 20. Cheesbrough M. *Medical Laboratory Manual for Tropical Countries*. Oxford, JH: Linacre House; 2000.
 21. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing* CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019:58-68.
 22. Ademola IA, McCrindle CM, Adejuwon TA. Losses associated with mortality of cattle and camels during transportation to Oke-Oba abattoir, Lagos State, Nigeria. *Eur J Transl Myol - Basic Appl Myol*. 2010;1:13-16.
 23. Njisane YZ, Muchenje V. Farm to abattoir conditions, animal factors and their subsequent effects on cattle behavioural responses and beef quality — a review. *Asian-Australas J Anim Sci*. 2017;30(6):755-764. [\[CrossRef\]](#)
 24. Oloruntoba EO, Adebayo AM, Omokhodion FO. Sanitary conditions of abattoirs in Ibadan, Southwest Nigeria. *Afr J Med Med Sci*. 2014;43(3):231-237.
 25. Edward KC, Nnochiri OA, Owuamalam PO, Onyekachi OV, Akumah CN. Microbial quality assessment of an abattoir effluent discharged into Waterside River in Aba, Abia State, Nigeria. *J Pharm Biol Sci*. 2017;12(5):38-41.
 26. Nafarnda WD, Ajayi IE, Shawulu JC, et al. Bacteriological quality of abattoir effluents discharged into water bodies in Abuja, Nigeria. *Int Sch Res Netw ISRN Vet Sci*. 2012;2012:Article ID 515689, 5 pages. [\[CrossRef\]](#)
 27. Pollitt EJG, Szkuta PT, Burns N, Foster SJ. *Staphylococcus aureus* infection dynamics. *PLoS Pathog*. 2018;14(6):e1007112.
 28. Ajibo FE, Njoga EO, Azor N, Idika IK, Nwanta JA. Epidemiology of infections with zoonotic pig parasites in Enugu State, Nigeria. *Vet Parasitol Reg Stud Reports*. 2020;20:100397. [\[CrossRef\]](#)
 29. Szewczyk EM, Nowak T, Cieřlikowski T, Szewczyk EM, Nowak T, Cies T. Potential role of *Staphylococcus cohnii* in a hospital environment. *Microb Ecol Health Dis*. 2003;15(1):51-56. [\[CrossRef\]](#)
 30. Lo J, Nwafor SU, Schwitters AM, et al. Key population hotspots in Nigeria for targeted HIV program planning: mapping, validation, and reconciliation. *JMIR Public Health Surveill*. 2021;7(2):e25623. [\[CrossRef\]](#)
 31. Ukaegbu E, Alibekova R, Ali S, Crape B, Issanov A. Trends of HIV/AIDS knowledge and attitudes among Nigerian women between 2007 and 2017 using Multiple Indicator Cluster Survey data. *BMC Public Health*. 2022;22(1):440. [\[CrossRef\]](#)

Sarcoptes canis'le Doğal Enfeste Köpeklerde Serum Neopterin ve Prokalsitonin Seviyeleri

Serum Neopterin and Procalcitonin Levels in Dogs Naturally Infested with *Sarcoptes canis*

Yusuf UMUT BATI¹ 
Oğuz MERHAN² 
Nilgün AYDIN³ 
Enes AKYÜZ¹ 
Mert SEZER¹ 
Ekin EMRE ERKİLİÇ¹ 
Zati VATANSEVER³ 
Ali HAYDAR KIRMIZIGÜL¹ 

¹Department of Internal Medicine,
Faculty of Veterinary Medicine,
Kafkas University, Kars, Turkey

²Department of Biochemistry,
Faculty of Veterinary Medicine,
Kafkas University, Kars, Turkey

³Department of Parasitology,
Faculty of Veterinary Medicine,
Kafkas University, Kars, Turkey

Geliş Tarihi/Received: 21.10.2022

Kabul Tarihi/Accepted: 19.02.2023

Publication Date/Yayın Tarihi: 26.04.2023

Sorumlu Yazar/Corresponding Author:
Yusuf UMUT BATI
E-mail: umutbatibati@gmail.com

Atıf: Bati YU, Merhan O, Aydın N, Akyüz E, Sezer M, Erkiliç EE, Vatansever Z, Kirmizigül AH. *Sarcoptes canis*'le Doğal Enfeste Köpeklerde Serum Neopterin ve Prokalsitonin Seviyeleri. *Vet Sci Pract.* 2023; 18(1), 31-34.

Cite this article as: Umut Bati Y, Merhan O, Aydın N, et al. Serum neopterin and procalcitonin levels in dogs naturally infested with *Sarcoptes canis*. *Vet Sci Pract.* 2023; 18(1), 31-34.



Copyright©Author(s) - Available online at
veterinarysciences-ataunipress.org
Content of this journal is licensed under a
Creative Commons Attribution-
NonCommercial 4.0 International License

ÖZ

Bu çalışmada *Sarcoptes canis*'le doğal enfeste köpeklerde serum neopterin ve prokalsitonin seviyelerinin belirlenmesi amaçlanmıştır. Çalışmanın hayvan materyalini farklı yaş ve cinsiyette 15 *Sarcoptes canis*'li ve sağlıklı 10 köpek oluşturdu. Hem *Sarcoptes canis*'li hemde sağlıklı köpeklerden bir kez kan alınarak 3000 devirde 10 dakika santrifüj edilerek serumları ayrıştırıldı. Elde edilen serumlar ölçümler yapıldı kadar -20 °C'de muhafaza edildi. Yapılan ölçümler sonucunda hasta köpeklerin neopterin (5,48 nmol/L $\pm$ 0,37 nmol/L) ve prokalsitonin (59,71 ng/L $\pm$ 2,45 ng/L) değerleri sağlıklı köpeklerin neopterin (4,27 nmol/L $\pm$ 0,30 nmol/L) ve prokalsitonin (47,63 ng/L $\pm$ 3,16 ng/L) değerlerine göre anlamlı derecede $P < ,05$ yüksek bulundu. Sonuç olarak *Sarcoptes canis*'li köpeklerde deride şiddetli bir yangı gelişmektedir. Deride şekillenen bu yangı sonucu ortaya çıkan hücresel immun yanıt ve akut faz yanıtın belirleyicisi olarak neopterin ve prokalsitonin biyobelirteçlerinin yükseldiği tespit edilmiştir.

Anahtar Kelimeler: *Sarcoptes Canis*, neopterin, prokalsitonin

ABSTRACT

This study aimed to determine serum neopterin and procalcitonin levels in dogs naturally infested with *Sarcoptes canis*. The animal material of the study consisted of 15 dogs with *S. canis* and 10 healthy dogs of different ages and genders. Blood was taken once from both *S. canis* infested dogs and healthy dogs, and their serums were separated by centrifugation at 3000 rpm for 10 minutes. The obtained sera were stored at -20 °C until the analyses. As a result of the analyses, neopterin (5,48 nmol/L $\pm$ 0,37 nmol/L) and procalcitonin (59,71 ng/L $\pm$ 2,45 ng/L) levels of the sick dogs were found to be significantly higher ($P < ,05$) than the neopterin (4,27 nmol/L $\pm$ 0,30 nmol/L) and procalcitonin (47,63 ng/L $\pm$ 3,16 ng/L) levels of the healthy dogs. As a result, dogs with *S. canis* develop a severe skin inflammation. It has been determined that neopterin and procalcitonin biomarkers increased as the cells that emerged as a result of this fire formed in the skin, as the speech of the immune response and acute phase response.

Keywords: *Sarcoptes Canis*, neopterin, procalcitonin

GİRİŞ

Uyuz hastalığı evcil hayvanlarda oldukça ciddi problemlere neden olmaktadır ve insanlara da bulaşma riski bulunmaktadır.¹ Uyuz ya da akariazis olarak adlandırılan zoonoz karakterli enfestasyon hemen hemen tüm hayvan türlerinde görülmekte² yaş, ırk, cinsiyet predispozisyonu bulunmamaktadır.³ Direkt temas aracılığı ile bulaşmakta ve dermatit ile sonuçlanarak önemli sağlık sorunlarına ve ekonomik kayıplara yol açmaktadır.²

Köpek uyuz vakalarının çoğuna *Sarcoptes scabiei* (var. *canis*) neden olur. Sarkoptik uyuz, insanlar dahil birçok memeli türünde gözlenen, yoğun kaşıntı ile karakterize, mevsimsel olmayan zoonotik bir hastalıktır. Etkin genellikle karın bölgesi ve bacakların iç yüzeyi gibi daha az tüylü bölgelere lokalize olur. Hastalık göz çevresi derisinde, kulak kepçesinde, dirseklerde ve bunların iç yüzeyinde milier dermatit şeklinde gözlenmekte ve şiddetli doku hasarı ve kaşıntı ile seyretmektedir.⁴ Sarkoptik uyuz vakalarında akar deri içerisinde tünel kazarak ilerler. Kaşıntı büyük oranda akara ve akarın salgılarına yanıt olarak

oluşan bir hipersensitivite reaksiyonu sonucu gelişir. Hastalarda alopesi mevcut olup karakteristik erken deri lezyonları eritemli ve papüüllü erupsiyonlardır. Tipik olarak puritrus, sarı renkli kabuklanmalar ve ekskoriasyon oluşabilir. Devamında etkilenen deri bölgelerinde hiperpigmentasyon meydana gelebilir. Çoğu hastada generalize lenfadenopati bulunmaktadır.⁵

Biyobelirteçler genel anlamda kimyasal, biyolojik ve fiziksel ajanlar ile biyolojik sistemler arasındaki etkileşimi yansıtan hemen bütün ölçümleri içine almaktadır.⁶ Neopterin (NP) hücre aracı bağıışıklığın biyokimyasal bir belirteci olarak kabul edilir. Kimyasal yapısı nedeniyle canlı hücrelerde yaygın olarak bulunan pteridinler, pirazino-(2,3-d)-pirimidin bileşikler grubuna aittir. Pteridinler ilk olarak 1889 yılında biyolojik bir materyalden izole edilmiştir.⁷ Neopterin, aktif T lenfositlerden salınan interferon-gamayı (IFN- γ) uyarması sonucu monositler ve makrofajlar tarafından sentezlenir.⁸⁻¹¹ Neopterin salınımı, T hücre proliferasyonu maksimum seviyeye ulaşmadan 3 gün önce başlar ve spesifik antikorların ortaya çıkmasından 1 hafta önce de artar. Bu nedenle neopterin erken inflamasyonun bir göstergesi olarak gösterilmektedir.^{7,9} Ayrıca Neopterin, hücre aracı bağıışıklığın da hassas bir belirleyicisidir.^{8,9} Neopterin böbrekler yoluyla herhangi bir reaksiyona uğramadan organizmadan atılır.⁷

Bakteriyel ve viral enfeksiyonlarda¹¹ otoimmün hastalıklar ve malign progresyonun çeşitli aşamaları olan hastalarda da çeşitli vücut sıvılarında artan bir neopterin konsantrasyonu gözlenmiştir. Ayrıca malign tümörleri olan hastalarda yüksek bir neopterin konsantrasyonu da bulunmuştur.^{12,13} Neopterin beyin omurilik sıvısı, sinovial sıvı, pankreas sıvısı, idrar, tükürük, gibi çeşitli vücut sıvılarında da saptanabilir.⁷

Prokalsitonin, tiroid bezindeki parafoliküler hücreler tarafından üretilen, 13 kDa ağırlığında ve 116 amino asitten oluşan küçük bir peptittir. Üretimi 11. kromozomdaki kalsitonin-1 (CALC-1) geni tarafından düzenlenir.^{10,11,14,15} Tiroid bezindeki parafoliküler hücrelerde kalsitonin prohormonu olarak üretilir. Kalsitonine dönüştürülerek dolaşıma salındığı için dolaşımdaki düzeyi çok düşüktür. Ayrıca enfeksiyonlar sırasında veya bazı patolojik durumlarda parankimal hücreler tarafından üretilmeye başlar. Parankimal dokular oldukça yaygın olduğu için inflamasyon sırasında bol miktarda salgılanır.¹⁶ Pankreas, karaciğer, dalak, adrenal bez, akciğerler, böbrekler, beyin, medulla spinalis, testisler, mide, ince bağırsaklar, kolon, abdominal yağlar ve beyaz kan hücreleri, ekstratiroid üretiminin ana kaynaklarıdır.¹⁵

Prokalsitonin, mikrobiyal toksinlere ve spesifik proinflamatuvar mediatörlere yanıt olarak her yerde salınır ve konsantrasyonları, enfeksiyöz bir uyarana maruz kaldıktan hemen sonra yükselir.¹⁷ Sepsisli köpeklerde plazma prokalsitonin konsantrasyonları önemli ölçüde artar.¹⁸ Babesia canis ile enfekte köpeklerde,¹⁹ sepsisli köpeklerde ve şiddetli bakteriyel enfeksiyonu olan köpeklerde¹⁸ yükseldiği bildirilmiştir. Fakat viral enfeksiyonlarda ve *Anaplasma phagocytophilum* veya *Ehrlichia canis* ile enfekte köpeklerdeki yükselişin istatistiksel olarak anlamlı bulunmadığı bildirilmiştir.¹⁵

Bu çalışmada *Sarcoptes canis*'le doğal enfeste köpeklerde serum neopterin ve prokalsitonin seviyelerinin belirlenmesi amaçlanmıştır.

MATERYAL ve METOT

Hayvan Materyali

Bu çalışmanın materyalini Kafkas Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı kliniklerine getirilen 3-15 aylık

yaşlarda farklı ırk ve cinsiyette deri kazıntısının incelenmesi sonrasında *Sarcoptes canis* tanısı konulan 15 hasta köpek (Grup I) ve 10 da sağlıklı köpek (Grup II) oluşturdu. KAÜ-HADYEK/2022-172 onay alındıktan sonra çalışmaya başlanmıştır.

YÖNTEM

Uyuz şüpheli köpeklerin derilerindeki lezyonlu bölgelerden bistüri ile kazıntı alındı. Alınan kazıntı örnekleri %10'luk KOH ile bir lam üzerinde ezilerek direkt mikroskopta *Sarcoptes canis* etkenlerinin görülmesi ile tanı konuldu.¹ Kesin tanı konulan hayvanlardan kan alındıktan sonra 200 μ g/kg dozda ivermectin (Baymec®, Bayer) 15 gün ara ile iki doz ivermektin uygulandı.

Kan Örneklerinin Alınması

Kan örnekleri bir kez *V. cephalica antebraçii*'den serum tüplerine (8,5 mL, BD Vakutainer®, BD, UK) holder başlığı ve uyumlu bir steril holder iğne ucu (Vacuette®, Greiner Bio-One GmbH, Avusturya) kullanılarak alındı. Serum örnekleri elde etmek için 3000 devirde 10 dakika (Hettich Rotina 380R®, Hettich, Almanya) santrifüj edildi. ELISA ölçümleri için kullanılacak serum örnekleri analize kadar -20 °C'de saklandı.

Serum prokalsitonin ve neopterin konsantrasyonları, köpeklerle özel ticari ELISA kitleri (Canine Neopterin ELISA Kit®, Canine Procalcitonin ELISA Kit®, BT Lab, Shanghai Korain Biotech Co, Çin) kullanılarak belirlendi. ELISA testleri üreticinin önerdiği şekilde yapıldı ve ELISA mikrolapla okuyucusunda 450 nm dalga boyunda (Epoch®, Biotek, ABD) optik yoğunluklar belirlendi. Regresyon analizi yapılarak neopterin ve prokalsitonin değerleri okundu.

İstatistiksel Analiz

Elde edilen sonuçlar Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) paket programında T testi yapılarak istatistiksel olarak değerlendirildi. $P < ,05$ istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışmaya alınan köpeklerin yapılan klinik muayenelerinde alopesi, kabuklanma, kepeklenme ve kaşıntı görülen klinik bulgular arasındadır. Hasta gruptaki köpeklerin tedavi öncesinde klinik muayeneleri yapıldı. Vücut sıcaklığı, solunum sayısı/ dakika, nabız sayısı/ dakika istatistiksel olarak değerlendirildi (Tablo 1).

Uyuz tanısı konulan köpeklerin vital bulguları kontrol grubu ile karşılaştırıldı. Elde edilen sonuçlara göre istatistiksel açıdan önemli olmadığı belirlendi ($P > ,05$).

Uyuz tanısı konulan ve kontrol grubu köpeklerde serum neopterin ve prokalsitonin seviyelerine bakıldı. Hasta gruptaki köpeklerin neopterin ve prokalsitonin seviyelerinin kontrol grubuna göre belirgin bir şekilde yüksek olduğu tespit edildi ($P < ,05$). Sonuçlar Tablo 2' de gösterilmiştir.

TARTIŞMA

Uyuzlu köpeklerde şiddetli kaşıntı, alopesi, deride kalınlaşma, kızarıklık ve kepeklenme gözlenmekte akarın salgılarına karşı

Tablo 1. Uyuz tanısı konulan ve kontrol grubu köpeklerin vital bulgularının ortalama ve standart hata değerleri

Vital Parametreler	Hasta Grup (n=15)	Kontrol Grubu (n=10)	P
Nabız Sayısı (Dk)	113,60 $\pm$ 5,28	115,70 $\pm$ 5,07	,78
Solunum sayısı (Dk)	29,20 $\pm$ 1,21	29,30 $\pm$ 1,61	,96
Vücut Sıcaklığı (°C)	38,46 $\pm$ 0,18	38,32 $\pm$ 0,16	,58

$P < ,05$ ifadesi hasta ve kontrol grupları arasındaki istatistiksel farkı ifade eder.

Tablo 2. Uyuz tanısı konulan ve kontrol grubu köpeklerin Neopterin ve Prokalsitonin ortalama ve standart hata değerleri

Biyobelirteçler	Hasta Grup (n = 15)	Kontrol Grubu (n = 10)	P
Neopterin (nmol/L)	5,48 ± 0,37	4,27 ± 0,30	<,05
Prokalsitonin (ng/L)	59,71 ± 2,45	47,63 ± 3,16	<,05

P <,05 ifadesi hasta ve kontrol grupları arasındaki istatistiksel farkı ifade eder.

görülen hipersensivite reaksiyonları gibi dermatolojik bulgular ortaya çıkmaktadır.^{4,5} Çalışmaya alınan köpeklerin yapılan klinik muayenesinde alopesi, kabuklanma, kepeklenme ve kaşıntı görülmesi literatür bilgileriyle uyumluluk göstermektedir.

Uyuzlu köpeklerde yapılan çalışmada solunum ve nabız sayılarının değişiklik göstermediği, vücut sıcaklığının ise ara sıra hafif değişiklikler gösterdiği belirtilmiştir.²⁰ Yapılan bu çalışmada hasta gruptaki köpeklerde nabız ve solunum sayısının değişiklik göstermediği, vücut sıcaklığının ise istatistiksel açıdan anlamlı olmayacak kadar hafif değişiklikler gösterdiği tespit edilmiştir.

Uyuz etkenlerinin bir çoğunda kesin tanı deri kazıntısı ile konmaktadır.⁵ Kazıntıda mikroskop altında akarın veya akar yumurtasının görülmesi tanıyı kesinleştirmektedir.³ Yapmış olduğumuz çalışmamızda teşhis klinik bulguların yanı sıra deri kazıntısı ile birlikte kesinleştirilmiştir.

Sunulan bu çalışmada uyuzlu köpeklerde serum neopterin, prokalsitonin seviyelerinin araştırılması ve bu biyobelirteçlerin hastalığın tanısında önemi ortaya konmaya çalışılmıştır. Neopterin hücre aracılı bağışıklığın biyokimyasal bir belirteci olarak kabul edilir. Neopterin, aktif T lenfositlerden salınan interferon-gamayı uyarması sonucu monositler ve makrofajlar tarafından üretilir. Neopterin üretiminin, spesifik antikorların ortaya çıkmasından önce artış gösterdiği bildirilmektedir. Bu nedenle neopterin erken inflamasyonun göstergesi olduğu düşünülmektedir.^{7,9} Yapığımız çalışmada hasta gruptaki yüksek neopterin konsantrasyonunun nedeni hastalığın yaptığı yangı neticesinde olduğunu düşünmekteyiz.

Akyüz ve ark¹¹ bakteriyel ve viral hastalıklarda serum neopterin seviyesinde artış olduğunu bildirmiştir. Literatürdeki bilgilerle uyumlu olarak hasta grubumuzdaki köpeklerde neopterin seviyesinin sağlıklılara göre artış gösterdiğini tespit etmiş bulunmaktayız. Bu artışın muhtemel nedeninin hasta köpeklerde uyuz enfestasyonu öncesi var olan immünsupresyon ve sonrasında deride meydana gelen sekonder bakteriyel enfeksiyonlar kaynaklı olduğunu düşünmekteyiz.

Prokalsitonin mikrobiyal toksinlere ve spesifik proinflamatuvar mediatörlere yanıt olarak salınır ve konsantrasyonları, enfeksiyöz bir uyarana maruz kaldıktan hemen sonra yükselmektedir.^{17,18} Sepsisli köpeklerde plazma prokalsitonin konsantrasyonlarının önemli ölçüde yükseldiğini bildirmiştir. Verilen literatür bilgi ile uyumlu olarak uyuz etkeni tespit edilen hayvanlarda, uyuz enfestasyonundan önce immün sistemin suprese olduğu uyuz ile birlikte kaçınılmaz görülen dermal bakteriyel kontaminasyona binaen artmış olabileceği kanısı burada da geçerlidir.

Sonuç olarak *Sarcoptes canis*'li köpeklerde deride şekillenen yangıya bağlı olarak serum neopterin ve prokalsitonin değerlerinin yükseldiği kanısına varılmış olup hastalığın şiddetinin değerlendirilmesinde önemli parametreler olabilecekleri düşünülmektedir.

Etik Komite Onayı: Bu çalışma için etik komite onayı Kafkas Üniversitesi'nden (Tarih: 25.10.2022, Karar No: KAÜ-HADYEK/2022-172) alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – Y.U.B.; A.H.K.; Tasarım – M.S.; Denetleme – E.A.; M.S.; E.E.E.; Kaynaklar – O.M.; Malzemeler – O.M.; Veri Toplanması ve/veya İşlemesi – N.A.; Z.V.; Analiz ve/veya Yorum – O.M.; N.A.; Literatür Taraması – M.S.; E.A.; Yazıyı Yazan – Y.U.B.; Eleştirel İnceleme – A.H.K.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Kafkas University (Date; 25.10.2022, Approval no; KAÜ-HADYEK/2022-172).

Informed Consent: Informed consent was received from patent ships.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.U.B.; A.H.K.; Design – M.S.; Supervision – E.A.; M.S.; E.E.E.; Resources – O.M.; Data Collection and/or Processing – N.A.; Z.V.; Analysis and/or Interpretation – O.M.; N.A.; Literature Review – M.S.; E.A.; Writer – Y.U.B.; Critical Review – A.H.K.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

KAYNAKLAR

- Meletis G, Oustas E, Kemanetzi C, Botziori C. Is the simple saline mount technique more effective than potassium hydroxide for the microscopic detection of *Sarcoptes scabiei*? *J Parasitol.* 2018;104(1): 109-109. [CrossRef]
- Yipel Altınok F. *Kedilerde Kulak Uyuzu (Otodectes Cynotis) Sağaltımında Ozonlanmış Zeytinyağı ve Bazı Esansiyel Yağların (allium sativum l, Origanum majorana l.) Etkileri.* Doktora Tezi]. Afyon; 2014.
- Curtis CF. Current trends in the treatment of *Sarcoptes*, cheylitiella and otodectes mite infestations in dogs and cats. *Vet Dermatol.* 2004;15(2):108-114. [CrossRef]
- Arslan A, Kırmızıgül A. Serum amyloid-a, haptoglobin and ceruloplasmin levels in dogs infected with *Sarcoptes canis*. *Atatürk Üniv Vet Bilim Derg.* 2020;15(1):1-6.
- Değer TB. *Köpeklerde Sarkoptik Uyuzun Sağaltımında Topikal Eprinomektin ile Permetrinin Etkinliklerinin Karşılaştırılması* [Yüksek Lisans Tezi]. Aydın; 2012.
- Baydar T, Palabıyık S, Neopterin ŞG. Günümüzün popüler biyogöstergesi mi? *Türk Klin J Med Sci.* 2009;29(5):1280-1291.
- Berdowska A, Zwirski-Korczala K. Neopterin measurement in clinical diagnosis. *J Clin Pharm Ther.* 2001;26(5):319-329. [CrossRef]
- Cesur S, Aslan T, Hoca NT, et al. Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. *Int J Mycobacteriol.* 2014;3(1):5-8. [CrossRef]
- Basbug O, Aydogdu U, Agaoglu ZT. Neopterin and soluble urokinase type plasminogen activator receptor as biomarkers in dogs with systemic inflammatory response syndrome. *J Hellenic Vet Med Soc.* 2020;71(1). [CrossRef]
- Akyüz E, Gökçe GG. Neopterin, procalcitonin, clinical biochemistry, and hematology in calves with neonatal sepsis. *Trop Anim Health Prod.* 2021;53(3):354. [CrossRef]

11. Akyüz E, Merhan O, Aydın U, et al. Neopterin, procalcitonin, total sialic acid, paraoxonase-1 and selected haematological indices in calves with aspiration pneumonia. *Acta Vet Brno*. 2022;91(2):115-124. [\[CrossRef\]](#)
12. Yıldırım Y, Günel N, Coskun U, Pasaoglu H, Aslan S, Cetin A. Serum neopterin levels in patients with breast cancer. *Med Oncol*. 2008;25(4):403-407. [\[CrossRef\]](#)
13. Tascene N, Kismalı G, Vural S, Karagul H, Sel T. Serum neopterin, sialic acid and nitric oxide levels in dogs with malignant mammary tumours. *Vet Comp Oncol*. 2012;10(2):155-159. [\[CrossRef\]](#)
14. Becker KL, Snider R, Nylen ES. Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. *Crit Care Med*. 2008;36(3):941-952. [\[CrossRef\]](#)
15. Matur E, Dokuzeylül B, Mukaddes Ö, et al. Can procalcitonin be used as a clinical biomarker during bacterial, viral and parasitic infections in dogs? *Jpn J Vet Res*. 2021;69(1):5-17.
16. Christ-Crain M, Müller B. Biomarkers in respiratory tract infections: diagnostic guides to antibiotic prescription, prognostic markers and mediators. *Eur Respir J*. 2007;30(3):556-573. [\[CrossRef\]](#)
17. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2013;13(5):426-435. [\[CrossRef\]](#)
18. Troia R, Giunti M, Goggs R. Plasma procalcitonin concentrations predict organ dysfunction and outcome in dogs with sepsis. *BMC Vet Res*. 2018;14(1):111. [\[CrossRef\]](#)
19. Brkljacic M, Brkljacic I, Torti M, et al. The concentrations of the inflammatory markers the amino-terminal portion of C-type pro-natriuretic peptide and procalcitonin in canine babesiosis caused by *Babesia canis*. *Vet Arh*. 2014;84:575-589.
20. Sharma S, Pokharel. Diagnosis and Therapeutic Management of Mixed Demodex and Sarcoptes Mite Infestation in Dog. *ASAG* 2019;3:6.



Multilokus Variable Number of Tandem Repeat Analiz (MLVA) Veri Tabanlarında Türkiye'den İzole Edilen *Bacillus anthracis* Genotiplerinin Moleküler Epidemiyolojisi

Molecular Epidemiology of *Bacillus anthracis* Genotypes Isolated From Turkey in Multilocus Variable Number of Tandem Repeat Analysis Databases

Ediz Kağan ÖZGEN 

Tarım ve Orman Bakanlığı,
Erzurum Veteriner Kontrol
Enstitüsü, Erzurum, Türkiye



Geliş Tarihi/Received: 15.02.2023

Kabul Tarihi/Accepted: 20.03.2023

Publication Date/Yayın Tarihi: 26.04.2023

Sorumlu Yazar/Corresponding Author:
Ediz Kağan ÖZGEN
E-mail: edizkagan.ozgen@tarimorman.gov.tr

Atıf: Özgen EK. Multilokus variable number of tandem repeat analiz (MLVA) veri tabanlarında Türkiye'den izole edilen *Bacillus anthracis* genotiplerinin moleküler epidemiyolojisi. *Vet Sci Pract.* 2023; 18(1), 35-43.

Cite this article as: Özgen EK. Molecular epidemiology of *Bacillus anthracis* genotypes isolated from Turkey in multilocus variable number of Tandem repeat analysis databases. *Vet Sci Pract.* 2023; 18(1), 35-43.



Copyright@Author(s) - Available online at
veterinarysciences-ataunipress.org
Content of this journal is licensed under a
Creative Commons Attribution-
NonCommercial 4.0 International License

ÖZ

Antraks, dünya genelinde insan ve hayvan sağlığını tehdit eden *Bacillus anthracis*'in neden olduğu önemli bir hastalıktır. *B. anthracis* doğada uzun süreler boyunca spor halinde kalması nedeniyle genetik olarak homojen bir yapı göstermektedir. Ancak epidemiyolojik olarak multiple-locus variable-number tandem repeat analysis (MLVA), single nucleotide repeat (SNR), genome-wide single nucleotide polymorphism (SNP) gibi moleküler metotlar ile suşlar arasında ayırım yapılabilmektedir. Bu çalışmada, MLVA Bank for Genotyping Microbes veri tabanına yüklenmiş olan *B. anthracis* MLVA-8 genotiplerinin analiz edilmesi amaçlandı. Bu çalışmada veri tabanında bulunan 49 ülkeye ait 2313 genotip profili değerlendirildi. Toplamda 402 genotip, 24 klonal grup içerisinde dağılım gösterdi. Türkiye'ye ait genotipler ise, 8 grup içerisinde dağılım gösterdi. Türkiye genotiplerinden en yüksek sayıdaki genotip (%31) XXIV klonal grubunda yerleşim gösterdi. Analiz sonucunda tespit edilen genotiplerin %63,2'si IX, XXIII ve XXIV klonal grupları içerisinde yer aldı. Sonuç olarak, MLVA Bank for Genotyping Microbes veri tabanında bulunan genotipler içerisinde Türkiye genotipleri birçok ülkeden bildirilen genotiplerle yakın genotipik yapıya sahip olduğu belirlendi.

Anahtar Kelimeler: Antraks, *Bacillus anthracis*, MLVA-8, moleküler epidemiyoloji

ABSTRACT

Anthrax is an important disease caused by *Bacillus anthracis* that threatens human and animal health worldwide. *B. anthracis* shows a genetically homogeneous structure because it can remain in the form of spores for a long time in nature. However, epidemiologically, it is possible to distinguish between strains with molecular methods such as multiple-locus variable-number tandem repeat analysis, single nucleotide repeat, and genome-wide single nucleotide polymorphism. In this study, it was aimed to analyze the *B. anthracis* MLVA-8 genotypes uploaded to the Bank for Genotyping Microbes database by multiple-locus variable-number tandem repeat analysis method. In this study, 2313 genotype profiles of 49 countries in the database were evaluated. In total, 402 genotypes were distributed within 24 clonal groups. Genotypes belonging to Turkey, on the other hand, show distribution in 8 groups. The highest number of genotypes (31%) among Turkey genotypes were localized in the XXIV clonal group. A total of 63.2% of the genotypes detected as a result of the analysis were included in the clonal groups IX, XXIII, and XXIV. As a result, among the genotypes in the MLVA Bank for Genotyping Microbes database, it was determined that the genotypes of Turkey were close to the genotypes reported from many countries.

Keywords: Anthrax, *Bacillus anthracis*, MLVA-8, molecular epidemiology

GİRİŞ

Bacillus anthracis (*B. anthracis*), Gram pozitif, sporlu, hareketsiz, basil şeklinde ve antraksın etiyolojik etkenidir.¹⁻³ Evcil ve yabani tüm memeli hayvanlarda enfeksiyon oluşturabilmektedir.² Ant-raks, dünya genelinde hayvan ve insan sağlığını tehdit eden genel olarak akut formda görülen bir hastalıktır.^{3,4} Hastalık şarbon, woollsorters' disease, ragpickers' disease, malignant carbuncle, malignant pustule ve Siberian ulcer isimleriyle de bilinmektedir.⁴ Bakteri sporlarının konak vücuduna girmesi sonrasında vejetatif hale dönüşmektedir. Vejetatif forma döndükten sonra bakteri tarafından salgılanan toksinler nedeniyle şiddetli bir hastalık tablosu oluşturmaktadır.² Enfekte hayvanlar genellikle 24 saat içerisinde ölmüş olarak bulunur.⁴ İnsanlara enfeksiyonun bulaşması enfekte hayvanlarla direkt temas veya etken ile kontamine et, kıl ya da deri ile temas sonucunda meydana gelmektedir.^{3,5}

Bakterilerin genomik karakterleri dinamiktir ve fenotipik karakterlerini belirlemektedir. Geçmişte bakteriler, fenotipik özelliklerindeki farklılıklarına göre ayırım sınıflandırılmaktaydı ancak moleküler metotların geliştirilmesiyle bakterilerin veya suşların ayırımında moleküler metotlar daha fazla kullanılmaktadır.³ Dünya genelinde *B. anthracis*'in toplanan izolatlarından yürütülen moleküler araştırmalarda etkenin suşları arasında homoloji olduğu belirlenmiştir.³ *B. anthracis* suşları arasında homolojinin olması nedeniyle yüksek ayırım gücü olan multiple-locus variable-number tandem repeat analysis (MLVA), single nucleotide repeat (SNR), genome-wide single nucleotide polymorphism (SNP) analizleri ve tüm genom sekans analizi, *B. anthracis*'in ayırımında etkin kullanılan metotlardır.² Moleküler epidemiyolojik incelemelerde öncelikle canonical SNP (canSNP) analizi kullanılmaktadır. Bu analize göre, incelenen tüm *B. anthracis* izolatlarında yalnızca A, B ve C olmak üzere üç filogenetik hat oluşmaktadır. Bu üç hat içerisinde 14 filogenetik grup oluşmaktadır. A grubunda: A.Br.Ames, A.Br.Australia 94, A.Br.003/004, A.Br.Vollum, A.Br.005/006, A.Br.001/002, A.Br.Western, A.Br.WNA, A.Br.008/009, A.Br.011/009, B grubunda: B.Br.001/002, B.Br.KrugerB, B.Br.CNEVA, C grubunda ise yalnızca C.Br.A1055 genotipi bulunmaktadır. Tespit edilen canSNP tiplerinin alt türlerinin ayırımında MLVA metodu kullanılmaktadır.^{6,7} MLVA metotları arasından MLVA-8 ilk olarak geliştirilen ve *vrnA*, *vrnB1*, *vrnB2*, *vrnC1*, *vrnC2*, *CG3*, *pXO1*-aat ve *pXO2*-at bölgelerindeki tekrarları analiz eden metot olmuştur.³ Bu metotla 30 ülkedeki 426 izolatın 89 genotip olduğu belirlenmiştir.² Daha sonraki dönemlerde ise MLVA-15, MLVA-25 ve MLVA-31 metotları geliştirilmiştir.^{2,3}

B. anthracis'in moleküler tiplendirmesinde MLVA son 10 yılda yaygın bir metot haline gelmiş olup bir ülke içerisinde *B. anthracis*'in yayılımının anlaşılmasını sağlayan bir metottur.^{2,8} Kromozomal DNA üzerinde variable number tandem repeat (VNTR) lokusları, lokuslardaki tekrar sayılarına göre suşlar arasında büyük ölçüde farklılıkların gözlemlenebildiği bölgelerdir.² Bu çeşitliliğin nedeni, bakteriyel DNA'nın replikasyonu sırasında slip-strand mismatch repair mutasyonu veya DNA polimeraz enzimi DNA'nın replikasyonunda geriye doğru kayarak belirli bir bölgeyi iki kez tekrar edebilmesi sonucunda meydana gelmektedir.^{2,9} Bu durum sonucunda oluşan tekrarlı bölgeler nesil olarak aktarılır.⁹

Bakterinin yayılımları canSNP metoduna göre isimlendirmesine göre incelenmektedir. Türkiye'de baskın genotip olan A.Br.Aust94 genotipi Avrupa ülkelerinde tespit edilen genotipler arasında çok düşük orandadır. Avrupa'nın kuzey ülkelerinde daha baskın olan B.Br.CNEVA, güney ülkelerinde ise yaygın olarak A3 grubu

genotipler görülmektedir. Avrupa ülkelerinde yaygın olarak bildirilen A3 grubu genotipler Türkiye'de düşük orandadır. Türkiye'nin doğudaki komşusu olan Gürcistan'da da A3 grubu genotipler bildirilmektedir.² Türkiye'ye ait MLVA verilerinin tümünün MLVA-8 metoduna göre genotiplendirilmesi sebebi ile bu araştırmada dünya geneli *B. anthracis* MLVA-8 profilleri içerisinde Türkiye'den izole edilen *B. anthracis* genotiplerinin moleküler epidemiyolojinin belirlenmesi ve Türkiye genotiplerinin diğer ülkelere göre benzerliklerin belirlenmesi amaçlanmıştır.

MATERYAL VE METOT

B. anthracis MLVA-8 verilerinin toplanması

Araştırmanın veri tabanının oluşturulması amacıyla Keim ve ark¹⁰ in MLVA-8 metoduna göre analiz edilmiş toplam 2313 adet insan ve hayvanlardan izole edilen *B. anthracis* seçildi ve *vrnA*, *vrnB1*, *vrnB2*, *vrnC1*, *vrnC2*, *CG3*, *pXO1* ve *pXO2* lokuslarındaki tekrar sayıları toplandı (<https://microbesgenotyping.i2bc.paris-saclay.fr/da/tabases/view/9> Erişim Tarihi:17.08.2022). MLVA-8 verileri MLVA Bank for Microbes Genotyping veri tabanından alındı.¹¹ Toplam 49 ülkedeki *B. anthracis* verileri değerlendirmeye alındı. Alınan MLVA-8 verileri içerisinde aynı ülke içerisinde dublike olan veriler tek örnek haline getirilerek toplam 402 adet veri kümesi oluşturuldu. Veri kümesindeki her *B. anthracis* izolatı için izole edildiği ülke adıyla tablo oluşturuldu.

Çalışmada analiz edilen 2313 genotip içerisinde Türkiye'den toplam 336 adet *B. anthracis* genotipin MLVA Bank for Microbes Genotyping sistemine yüklenen ancak şehir bilgisi bildirilmeyen 32 genotip dışındaki 304 adet genotip şehir bilgileri ve izole edilen konak veya örnek ile değerlendirildi.

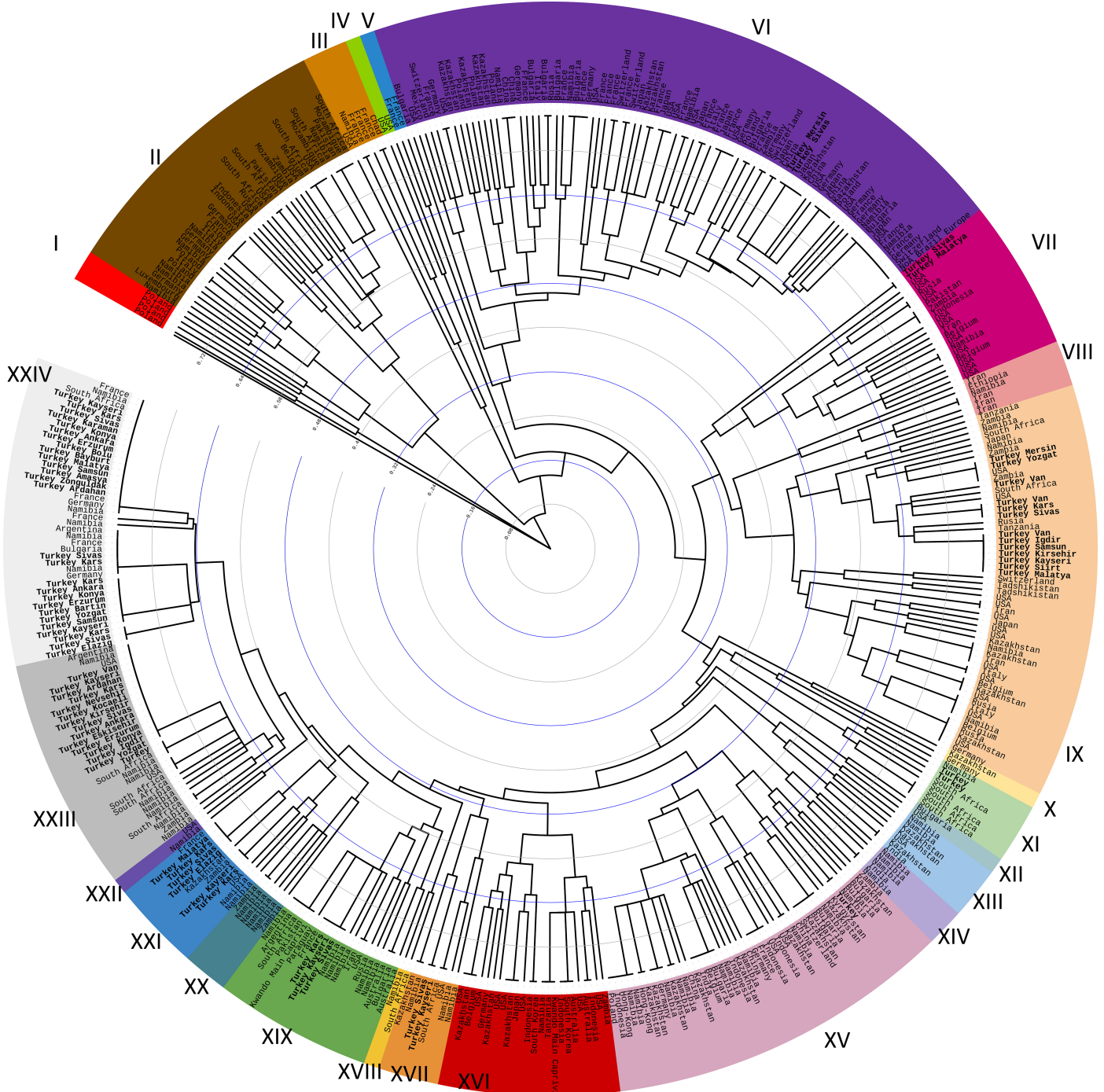
Veri Tabanının Kalıplarının Analizi

Verileri toplanan ve birleştirilen 402 adet *B. anthracis* MLVA-8 kalıplarının kümüleme analizleri için Past Software 4.06 kullanıldı. Veriler programa aktarıldıktan sonra unweighted pair group method with mathematical averaging (UPGMA) metoduna göre analiz edildi. Benzerlik katsayısı olarak "Dice" kullanıldı.¹² UPGMA analizi sonucunda elde edilen clustering verilerine ait nexus dosyası elde edilerek Interactive Tree of Life (iTOL) v6 sistemine yüklenerek sirküler dendrogram oluşturuldu.¹³ Aynı zamanda Türkiye'den bildirilen *B. anthracis* genotiplerinin izole edildikleri şehir, izole edildiği konak veya ortam ve genotip dağılımlarının gösterilmesinde chord dendrogram <http://www.datasmith.org/> web sitesinde oluşturuldu.

BULGULAR

Toplam 2313 *B. anthracis* MLVA-8 verilerinin analizleri sonucunda toplam 24 klonal grup oluşturuldu (Şekil 1). MLVA verileri değerlendirildiğinde Türkiye'nin de aralarında yer aldığı 16 farklı ülkeden 88 genotipin bulunduğu görüldü. VI. grup, en geniş klonal grup olarak belirlendi. Analiz sonucunda, 49 farklı ülkeden elde edilen izolatların ülkelere göre dağılımı Tablo 1'de gösterilmiştir.

Türkiye'den analiz edilen *B. anthracis* izolatlarının 32'sinin şehir bilgisi bulunmamakta olup, Durmaz ve ark¹⁴ tarafından verileri sağlanan 251 ile Ortatlatlı ve ark¹⁵ tarafından verileri sağlanan 53 olmak üzere toplam 304 *B. anthracis*'in 25 farklı şehirden alındığı belirlendi. Veri tabanına yüklenmiş olan Türkiye genotipleri en fazla Kars ilinden tespit edilen genotipler olup Türkiye genotiplerinin %32,2 (98/304)'sini oluşturmaktadır. Şehirler içerisinde en fazla farklı MLVA profiline sahip şehir ise, 39 *B. anthracis* izolatında 10 MLVA profiline sahip Sivas ilidir (Şekil 2). Türkiye'den yüklenen *B. anthracis* MLVA verilerinin 11 farklı klonal gruba dağılım gösterdiği



Şekil 1. *B. anthracis* genotiplerinin sirküler dendrogramı.

belirlenmiştir. Toplam 402 genotip değerlendirmesi sonucunda 24 klonal grup oluşmuştur (Şekil 1). Oluşturulan dendrogramdaki klonal gruplar ve gruplar içerisinde bulunan genotiplerin izole edildiği ülkelere ait veriler Tablo 2'de verildi.

İncelenen Türkiye MLVA profillerinin (Tablo 3.) en büyük kısmı Amasya, Ankara, Ardahan, Bartın, Bayburt, Bolu, Elazığ, Erzurum, Karaman, Kars, Kayseri, Konya, Malatya, Samsun, Sivas, Yozgat ve Zonguldak illerinden 27 MLVA genotipi XXIV klonal grubunda yerleşim gösterdiği belirlendi (Şekil 3). Diğer MLVA genotiplerinin klonal gruplara dağılımları sırasıyla 14 şehirden 14 genotip XXIII klonal grubu, 11 şehirden 13 genotip IX klonal grubu, beş farklı

şehir altı genotip XXI klonal grubu, üç farklı şehirden üç genotip XIX klonal grubunda dağılım gösterdi. VI, VII ve XVII klonal gruplarının her birinde iki farklı şehirden genotipler olduğu tespit edildi.

Verilerin alındığı sistemde bildirilen *B. anthracis* genotiplerinin izole edildikleri konaklar hakkında bilgi mevcut değildi. Ancak, Durmaz ve ark¹⁴ ile Ortatatlı ve ark¹⁵ verilerinden Türkiye genotiplerinin %32,2 (98/304)'i insan ve %67,8 (206/304)'i hayvan genotipi (163 siğir, 34 koyun, 6 keçi, 3 çevresel) olduğu belirlendi. İzole edilen konak veya ortam ile genotipler arasında değerlendirme yapıldığında; insan genotiplerinin en yoğun olarak IX ve XXIII klonal gruplarına yerleşim gösterdi bunun yanında siğir

Tablo 1. MLVA bank for Microbes Genotyping Veri Tabanından Araştırmaya Alınan Genotiplerin Ülkelere Göre İzolat Sayıları ve Farklı Genotip Sayıları

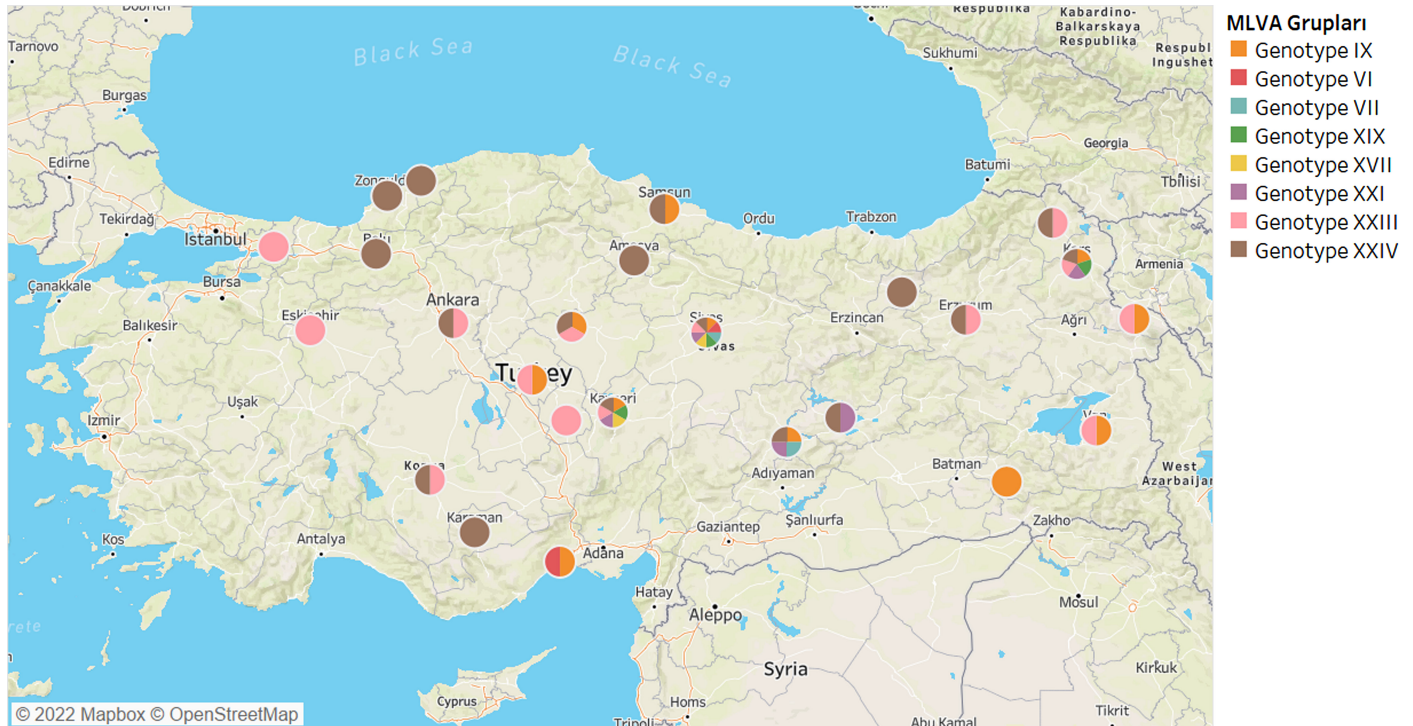
Ülke	İzolat Sayısı	Farklı Genotip Sayısı	Ülke	İzolat Sayısı	Farklı Genotip Sayısı
ABD	85	55	İsviçre	10	4
Afganistan	28	4	İtalya	164	20
Afrika	1	1	Japonya	12	8
Almanya	84	33	Kamerun	23	2
Arjantin	12	8	Kanada	27	7
Arnavutluk	4	3	Kazakistan	20	11
Avustralya	6	3	Kırgızistan	1	1
Avusturya	2	2	Lüksemburg	1	1
Belçika	4	3	Macaristan	4	2
Birleşik Krallık	22	13	Meksika	2	1
Botsvana	1	1	Mozambik	7	3
Bulgaristan	40	7	Namibya	919	35
Çad	16	1	Norveç	11	5
Çin	213	31	Pakistan	12	6
Endonezya	8	4	Paraguay	1	1
Etiyopya	1	1	Polonya	32	12
Fransa	97	17	Rusya	13	2
Güney Afrika	11	19	Slovakya	4	3
Güney Kore	23	12	Tacikistan	3	2
Gürcistan	13	3	Tanzanya	4	2
Haiti	3	1	Tayland	4	3
Hindistan	6	4	Türkiye	336	33
Hong-Kong	2	2	Zambiya	6	3
İran	6	2	Zimbabve	7	4
İrlanda	2	1	TOPLAM	2313	402

genotiplerinin ise en fazla sayıda XXIII ve XXIV klonal gruplarında yerleşim gösterdi.

TARTIŞMA

B. anthracis'in doğal klonal yapısı, doğada uzun süre spor halinde kalması ve sporların uzun süre çoğalmaması etkenin genetik

homojenlik göstermesi nedeniyle geleneksel metotlarla suşların ayrımı zordur. Dünya genelinde *B. anthracis*'in genotipik ayrımında MLVA ve canSNP analizleri kullanılmaktadır.⁸ Bu araştırmada MLVA Bank for Genotyping veri tabanından seçilen 2313 adet *B. anthracis*'in 402 genotipi içerisinde Türkiye genotiplerinin dağılımları incelendi. İncelenen genotipler toplam 24 klonal grupta dağılım



Şekil 2. Türkiye'den bildirilen MLVA profillerin dağılımı genotiplerden 8 klonal grup oluşmaktadır. Şehirlerde bulunan genotipik gruplar farklı renklerle gösterilmiştir (Tableau Software 2022.2).

Tablo 2. Dendrogramda Tespit Edilen Klonak Gruplar ve Klonal Grup İçerisindeki Genotiplerin İzole Edildiği Ülkeler

Klonal Grup	Grup İçinde Bulunan Ülkeler	Klonal Grup	Grup İçinde Bulunan Ülkeler	Klonal Grup	Grup İçinde Bulunan Ülkeler	Klonal Grup	Grup İçinde Bulunan Ülkeler
I	Polonya	IX	ABD	XV	ABD	XX	Namibya
II	ABD		Almanya		Almanya		Zambiya
	Almanya		Belçika		Belçika	XXI	ABD
	Belçika		Güney Afrika		Bulgaristan		Fransa
	Çin		İran		Çin		Kazakistan
	Endonezya		İsviçre		Endonezya		Namibya
	Fransa		İtalya		Fransa		Türkiye Elazığ
	Güney Afrika		Japonya		Hindistan		Türkiye Kars
	İtalya		Kazakistan		Hong-Kong		Türkiye Kayseri
	Lüksemburg		Namibya		İsviçre		Türkiye Malatya
	Mozambik		Rusya		Kazakistan		Türkiye Sivas
	Namibya		Tacikistan		Kırgızistan		Zambiya
	Pakistan		Tanzanya		Namibya	XXII	ABD
	Polonya		Türkiye Iğdır		Polonya		Namibya
	Rusya		Türkiye Kars		Türkiye	XXIII	ABD
	Zambiya		Türkiye Kayseri		Zambiya		Arjantin
III	ABD		Türkiye Kırşehir	XVI	ABD		Güney Afrika
	Çad		Türkiye Malatya		Almanya		Namibya
	Fransa		Türkiye Mersin		Avustralya		Türkiye Ankara
	Namibya		Türkiye Samsun		Belçika		Türkiye Ardahan
IV	ABD		Türkiye Siirt		Endonezya		Türkiye Erzurum
V	Fransa		Türkiye Sivas		Güney Kore		Türkiye Eskişehir
VI	ABD		Türkiye Van		Japonya		Türkiye Iğdır
	Almanya		Türkiye Yozgat		Kazakistan		Türkiye Kars
	Bulgaristan		Zambiya		Namibya		Türkiye Kayseri
	Çin	X	Almanya		Tanzanya		Türkiye Kırşehir
	Fransa		Kazakistan		Zambiya		Türkiye Kocaeli
	İsviçre	XI	Güney Afrika	XVII	ABD		Türkiye Konya
	İtalya		Namibya		Güney Afrika		Türkiye Malatya
	Japonya		Türkiye		Kazakistan		Türkiye Nevşehir
	Kazakistan	XII	ABD		Namibya		Türkiye Sivas
	Meksika		Bulgaristan		Türkiye Kayseri		Türkiye Van
	Namibya	XIII	ABD		Türkiye Sivas		Zambiya
	Polonya		Hindistan	XVIII	Güney Afrika	XXIV	Almanya
	Rusya		Kazakistan		Namibya		Arjantin
	Türkiye Mersin		Namibya	XIX	ABD		Bulgaristan
	Türkiye Sivas	XIV	Hindistan		Arjantin		Fransa
VII	ABD		Namibya		Avustralya		Güney Afrika
	Belçika				Bulgaristan		Namibya
	Endonezya				Fransa		Türkiye Ankara
	İran				Güney Afrika		Türkiye Bartın
	Namibya				İran		Türkiye Elazığ
	Pakistan				Namibya		Türkiye Erzurum
	Rusya				Pakistan		Türkiye Erzurum
	Türkiye Malatya				Paraguay		Türkiye Kars
	Türkiye Sivas				Rusya		Türkiye Kayseri
	Zambiya				Türkiye Kars		Türkiye Konya
VIII	Etiyopya				Türkiye Kayseri		Türkiye Samsun
	İran				Türkiye Sivas		Türkiye Sivas
	Namibya						Türkiye Yozgat

gösterdi. Klonal gruplar içerisinde en büyüğü olan VI. grup içerisinde Bulgaristan,¹⁶ Amerika Birleşik Devletleri,^{10,17,18} Meksika,¹⁸ İsviçre,¹⁹ Fransa,^{18,20} Almanya (MLVA Bank), Kazakistan,²¹ Polonya,^{18,22} Namibya,²³ Çin,¹⁸ İtalya,^{24,25} Rusya,²⁶ Japonya,²⁷ Zambiya,¹⁸ Brezilya'dan¹⁰ genotiplerle Türkiye'nin Mersin ve Sivas illerinden iki genotipinde aynı grupta yer aldığı tespit edildi. Bu klonal grup çok geniş coğrafi dağılım göstermesine rağmen analize dahil edilen Durmaz ve ark¹⁴ ve Ortatatlı ve ark¹⁵ tarafından bildirilen 69 genotip içerisinden, Mersin ve Sivas illerinden olmak üzere yalnızca iki (%2,9) genotip profili bu grupta yerleşim göstermiştir. *B. anthracis*'in

uzun yıllar doğada spor halinde bulunması genetik olarak homojen kalmasının en önemli sebebidir.¹⁸ *B. anthracis*'in ülkeler arasında yayılımında tarihsel yönden hayvansal ürün ticaretinin etkili olduğu bildirilmektedir. Van Ert ve ark¹⁸ tarafından Hindistan'dan alınan 10 adet *B. anthracis* izolatlarının aynı zamanda Avustralya'da da baskın genotip A.Br.Aust94 genotipi olduğu ve Avustralya'ya Asya üzerinden hayvan ürünlerin ticaretiyle yayıldığı bildirilmektedir.¹⁸ Aynı zamanda A.Br.Aust94 genotipi Türkiye'de de baskın genotiptir.² Türkiye'nin farklı şehirlerden Durmaz ve ark¹⁴ ile Ortatatlı ve ark¹⁵ bildirdiği *B. anthracis* genotiplerinin dağılımları

Tablo 3. MLVABank for Microbes Genotyping sistemindeki Türkiye'ye ait *Bacillus anthracis* MLVA-8 Genotip Profilleri

Coğrafi Kaynak	Referans	vrrA	vrrB1	vrrB2	vrrC1	vrrC2	CG3	pXO1	pXO2
Türkiye	10	4	20	15	57	21	1	10	8
Türkiye	10	4	20	13	57	21	1	9	8
Türkiye	10	4	20	14	57	21	1	10	8
Türkiye	10	4	20	14	57	21	2	13	8
Türkiye	10	4	20	13	57	21	2	5	8
Türkiye	10	4	20	14	57	17	2	7	10
Türkiye	10	4	20	14	57	17	2	10	10
Türkiye	10	4	20	14	57	17	2	10	12
Türkiye	10	4	20	14	57	17	2	10	11
Türkiye	10	3	20	14	57	17	2	10	10
Türkiye	10	4	20	14	44	17	2	10	10
Türkiye	10	4	20	14	57	17	2	11	10
Türkiye	10	4	20	14	57	17	2	8	10
Türkiye	10	4	20	14	57	17	2	9	10
Türkiye	17	4	20	14	53	17	2	8	0
Türkiye	17	2	20	14	57	17	2	9	10
Türkiye	17	4	20	14	57	17	2	5	10
Türkiye	18	4	20	13	56	21	2	5	8
Türkiye: Amasya	14, 27	4	20	14	57	17	2	9	10
Türkiye: Ankara	14, 27	4	20	14	57	17	2	9	10
Türkiye: Ankara	14, 27	4	20	14	57	17	2	7	10
Türkiye: Ankara	14, 27	4	20	14	57	17	2	10	10
Türkiye: Ardahan	14	4	20	14	57	17	2	10	10
Türkiye: Ardahan	14	4	20	14	57	17	2	9	10
Türkiye: Bartın	14, 27	4	20	14	57	17	2	7	10
Türkiye: Bayburt	14, 27	4	20	14	57	17	2	9	10
Türkiye: Bolu	14, 27	4	20	14	57	17	2	9	10
Türkiye: Elazığ	14	4	20	14	57	17	2	9	12
Türkiye: Elazığ	14	4	20	14	57	17	2	6	10
Türkiye: Erzurum	14, 27	4	20	14	57	17	2	9	10
Türkiye: Erzurum	14, 27	4	20	14	57	17	2	7	10
Türkiye: Erzurum	14, 27	4	20	14	57	17	2	10	10
Türkiye: Eskişehir	14, 27	4	20	14	57	17	2	10	10
Türkiye: Iğdır	14, 27	4	20	14	57	21	2	9	9
Türkiye: Iğdır	14	4	20	14	57	17	2	10	10
Türkiye: Karaman	14, 27	4	20	14	57	17	2	9	10
Türkiye: Kars	14	4	20	14	57	21	2	9	11
Türkiye: Kars	14	4	20	14	57	17	2	9	10
Türkiye: Kars	14	4	20	14	57	17	2	9	9
Türkiye: Kars	14	4	20	14	57	17	2	7	10
Türkiye: Kars	14	4	20	14	57	17	2	10	10
Türkiye: Kars	14	4	20	14	57	17	2	6	10
Türkiye: Kars	14	4	20	14	57	17	2	9	12
Türkiye: Kars	14	4	20	14	57	17	2	8	10
Türkiye: Kars	14	4	20	14	57	17	2	9	11
Türkiye: Kayseri	14, 27	4	20	14	57	21	2	9	9
Türkiye: Kayseri	14	4	20	14	57	17	2	9	10
Türkiye: Kayseri	14	4	20	14	57	17	2	9	11
Türkiye: Kayseri	14, 27	4	20	14	57	17	2	7	10
Türkiye: Kayseri	14, 27	4	20	14	57	17	2	10	10
Türkiye: Kayseri	14	3	20	14	57	17	2	9	10
Türkiye: Kayseri	14	4	20	14	57	17	2	9	9
Türkiye: Kırşehir	14	4	20	14	57	21	2	9	9
Türkiye: Kırşehir	14	4	20	14	57	17	2	10	10
Türkiye: Kocaeli	14	4	20	14	57	17	2	10	10
Türkiye: Konya	14, 27	4	20	14	57	17	2	9	10
Türkiye: Konya	14, 27	4	20	14	57	17	2	7	10
Türkiye: Konya	14, 27	4	20	14	57	17	2	10	10
Türkiye: Malatya	14	4	20	14	57	21	2	9	9
Türkiye: Malatya	14	4	20	13	57	21	2	4	8
Türkiye: Malatya	14	4	20	14	57	17	2	9	12
Türkiye: Malatya	14	4	20	14	57	17	2	9	10

(Continued)

Tablo 3. MLVABank for Microbes Genotyping sistemindeki Türkiye'ye ait Bacillus anthracis MLVA-8 Genotip Profilleri (Continued)

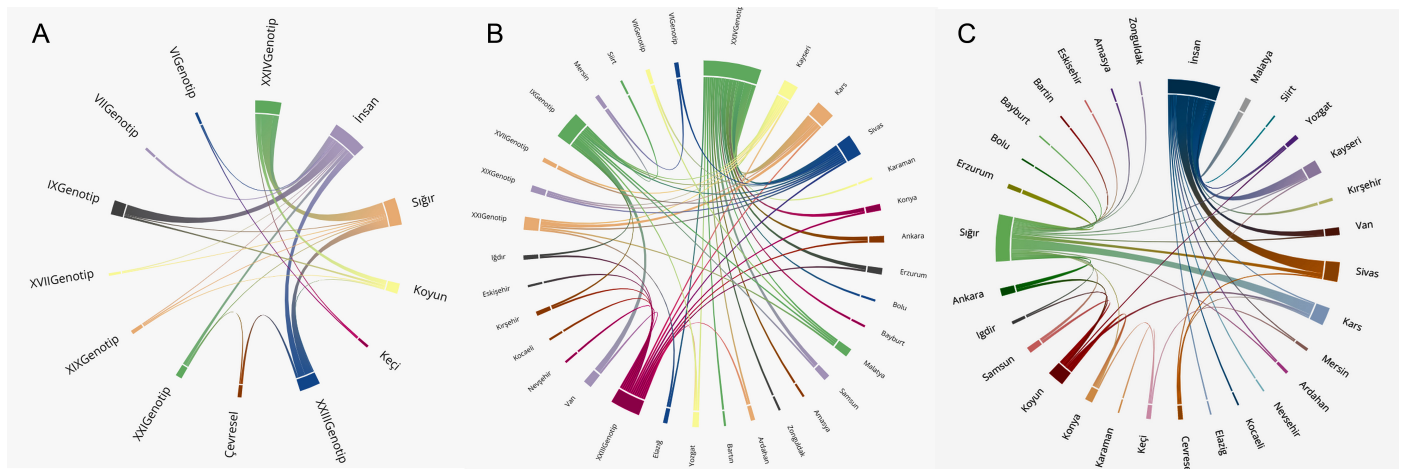
Coğrafi Kaynak	Referans	vrrA	vrrB1	vrrB2	vrrC1	vrrC2	CG3	pXO1	pXO2
Türkiye: Mersin	14, 27	4	20	14	57	21	1	9	8
Türkiye: Mersin	14	4	20	14	57	21	2	9	8
Türkiye: Nevşehir	14	4	20	14	57	17	2	10	10
Türkiye: Samsun	14, 27	4	20	14	57	21	2	9	9
Türkiye: Samsun	14, 27	4	20	14	57	17	2	7	10
Türkiye: Samsun	14, 27	4	20	14	57	17	2	9	10
Türkiye: Siirt	14	4	20	14	57	21	2	9	9
Türkiye: Sivas	14	4	20	14	57	21	2	9	11
Türkiye: Sivas	14	4	20	14	57	21	1	9	8
Türkiye: Sivas	14	4	20	13	57	21	2	4	8
Türkiye: Sivas	14, 27	4	20	14	57	17	2	9	10
Türkiye: Sivas	14	4	20	14	57	17	2	10	10
Türkiye: Sivas	14	3	20	14	57	17	2	9	10
Türkiye: Sivas	14	4	20	14	57	17	2	9	12
Türkiye: Sivas	14	4	20	14	57	17	2	9	9
Türkiye: Sivas	14	4	20	14	57	17	2	8	10
Türkiye: Sivas	14	4	20	14	57	17	2	6	10
Türkiye: Van	14	4	20	14	57	21	2	9	9
Türkiye: Van	14	4	20	14	57	21	2	9	11
Türkiye: Van	14	4	20	14	57	17	2	10	10
Türkiye: Van	14	4	20	14	57	21	2	9	10
Türkiye: Yozgat	14, 27	4	20	14	57	21	2	9	8
Türkiye: Yozgat	14	4	20	14	57	17	2	7	10
Türkiye: Yozgat	14	4	20	14	57	17	2	10	10
Türkiye: Zonguldak	14, 27	4	20	14	57	17	2	9	10

incelendiğinde insan, sığır, koyun ve keçilerden izole edilen genotiplerin oluşturduğu XXIV klonal grubun en yüksek sayıda Türkiye genotipi bulundurduğu saptanmıştır. Bu grup içerisinde Amasya, Ankara, Ardahan, Bartın, Bayburt, Bolu, Elazığ, Erzurum, Karaman, Kars, Kayseri, Konya, Malatya, Samsun, Sivas, Yozgat ve Zonguldak illerinden toplam 27 genotip Arjantin,¹⁷ Fransa (MLVA Bank), Namibiya,²³ Güney Afrika,¹⁸ Almanya¹⁸ ve Bulgaristan'dan¹⁶ genotiplerle aynı grupta yerleşim gösterdi.

XXIII klonal grup, Durmaz ve ark¹⁴ ile Ortatatlı ve ark¹⁵ tarafından bildirilen genotiplerin yoğunluk gösterdiği ikinci en büyük genotip grubudur. Bu grup içerisinde Ankara, Ardahan, Erzurum, Eskişehir,

Iğdır, Kars, Kayseri, Kırşehir, Kocaeli, Konya, Nevşehir, Sivas, Van ve Yozgat illerinden genotipler bulunmakta olup, aynı grup içerisinde Arjantin,¹⁸ Namibiya,^{10,18} ABD,^{17,18} Güney Afrika¹⁸ ve Zambiya¹⁸ gibi ülkelerden bildirilen genotipler de bulunmaktadır. Ülkemizden bildirilen *B. anthracis* izolatları ortak atadan gelmekte olup, minor mutasyonlar nedeniyle farklı MLVA profilleri göstermekte olduğu düşünülmektedir.¹⁴ Ülkemizin de içerisinde yer aldığı Akdeniz ve Avrupa'nın doğu ülkelerinde antraks yaygındır.²

Analiz edilen genotipler arasında Türkiye'ye komşu ülkelerden yalnızca Bulgaristan ve İran'a ait genotipler bulunmaktadır. Bulgaristan ile VI, XV ve XXIV klonal gruplarında Türkiye genotipleri de



Şekil 3. Türkiye'den bildirilen *B. anthracis* genotiplerinin konak, şehir ve genotip dağılımlarını gösterir chord dendrogram. (A) Türkiye'de izole edilen *B. anthracis*'lerin 8 genotip gruplarının insan, sığır, koyun, keçi ve çevresel orijinlerine göre dağılımları, (B) *B. anthracis* genotip grupları ile tespit edilen şehirlere göre dağılımları, (C) Türkiye'den bildirilen genotiplerin insan, sığır, koyun, keçi ve çevresel orijinlerine göre tespit edildiği şehirlere göre dağılımları.

bulunmuştur. Bunlar Amasya, Ankara, Ardahan, Bartın, Bayburt, Bolu, Elazığ, Erzurum, Karaman, Kars, Kayseri, Konya, Malatya, Mersin, Samsun, Sivas, Yozgat ve Zonguldak illerinden elde edilen genotiplerdir.^{14,16} Türkiye ile İran genotipleri VII, IX ve XIX klonal gruplarında ortak olarak bulunmakta olup, bu genotipler ülkemizde de içidir, Kars, Kayseri, Kırşehir, Malatya, Mersin, Samsun, Siirt, Sivas, Van ve Yozgat illerinde tespit edilmiştir.^{14,18} Ortatlatlı ve ark¹⁵ Türkiye’de tespit edilen 55 adet *B. anthracis*’in MLVA metodu ile genotiplendirmesini gerçekleştirmiştir. Araştırmacılar tespit edilen genotiplerin bölgeler arasındaki hayvan hareket rotalarına göre dağılım gösterdiğini belirtmektedir.¹⁵ Gerçekleştirilen analiz sonucunda, İran ile aynı klonal grup içerisinde bulunan genotiplerin genel olarak ülkemizin iç Anadolu, Doğu Anadolu ve Güneydoğu Anadolu illerinden olmasına rağmen Bulgaristan ile yakınlık tespit edilen genotiplerin Türkiye’de belirli bir bölgede kümelenmediği belirlendi.

B. anthracis’in genotipik yapılarının araştırılması Amerika Birleşik Devletleri’ndeki biyoterörizm vakasından sonra daha da önem kazanmıştır.²⁸ MLVA yöntemi, *B. anthracis*’in genotiplendirmesinde kullanılan sağlam, hızlı ve kolay aktarılabilir bir metottur.¹⁰ MLVA-8 metodu *B. anthracis*’in genotiplendirilmesinde kullanılan bir metottur.²⁹ *B. anthracis*’in tiplendirmesinde canSNP ve MLVA’nın ikisinin birlikte kullanımı moleküler epidemiyoloji için yararlı olmasının yanında tarihsel sürecin izlenmesini de sağlamaktadır.¹⁸ Ülkeler içerisinde *B. anthracis*’in genomik farklılıkların açıklaması olarak hayvan hareketlerinin yoğunluğu ile ilgili olabileceği bildirilmektedir.⁸ Türkiye’den bildirilen genotiplerin 8 klonal grup içerisinde olması ise hayvan hareketlerinin yoğunluğu nedeniyle olduğu düşünüldü. Bunun yanında ülkeler içerisinde hayvan ürünlerinin ithal edilmesinde de ülkede görülmemiş genotiplerin görülmesine neden olduğu bildirilmektedir.³⁰

Sonuç olarak; MLVA Bank for Genotyping veri tabanında bulunan 2313 adet *B. anthracis* genotipinin verileri, 49 ülke ve 402 MLVA genotipi olacak şekilde daraltıldıktan sonra analiz edilerek Türkiye genotiplerinin dağılımları değerlendirilmiştir. Bu araştırma ile ülkemizden bildirilen genotiplerin dağılımlarının 8 klonal grupta yer aldığı belirlenmiş olup, ülkeler arası coğrafi dağılımda dünya genelinde yayılım göstermektedir. *B. anthracis*’in genotipleri homojen bir yapı göstermesine rağmen MLVA analizleri yapılarak epidemiyolojik takiplerinin sürekli yapılmasının faydalı olacağı düşünülmektedir.

Etik Komite Onayı: Çalışmada herhangi bir hayvan veya insan örneği analiz edilmemiş olup MLVA Bank for Microbes Genotyping veritabanında bulunan veriler analiz edilmiştir. Bu nedenle Etik Kurul onayı alınmamıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Çıkar Çatışması: Yazar çıkar çatışması bildirmemiştir.

Finansal Destek: Yazar bu çalışma için finansal destek almadığını beyan etmiştir.

Ethics Committee Approval: No animal or human samples were analyzed in the study, and data in the MLVA Bank for Microbes Genotyping database were analyzed. Therefore, Ethics Committee approval was not obtained.

Peer-review: Externally peer-reviewed.

Declaration of Interests: The author has no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

KAYNAKLAR

1. Koehler TM. *Bacillus anthracis* physiology and genetics. *Mol Aspects Med.* 2009;30(6):386-396. [CrossRef]
2. Derzelle S, Thierry S. Genetic diversity of *Bacillus anthracis* in Europe: genotyping methods in forensic and epidemiologic investigations. *Biosecur Bioterror.* 2013;11(suppl 1):S166-S176. [CrossRef]
3. Pilo P, Frey J. Pathogenicity, population genetics and dissemination of *Bacillus anthracis*. *Infect Genet Evol.* 2018;64:115-125. [CrossRef]
4. WOA. *Anthrax* (Terrestrial Manual, 8th edn, pp. 1-14). 2018.
5. Zasada AA. Detection and identification of *Bacillus anthracis*: from conventional to molecular microbiology methods. *Microorganisms.* 2020;8(1) [CrossRef]
6. Anisimova EA, Fakhrutdinov NA, Mirgazov DA, et al. *Bacillus anthracis* strain differentiation based on SNP and VNTR loci. *Vavilovskii Zhurnal Genet Selektii.* 2022;26(6):560-567. [CrossRef]
7. Wang D, Wang B, Zhu L, et al. Genotyping and population diversity of *Bacillus anthracis* in China based on MLVA and canSNP analysis. *Microbiol Res.* 2020;233:126414. [CrossRef]
8. Rondinone V, Serrecchia L, Parisi A, et al. Genetic characterization of *Bacillus anthracis* strains circulating in Italy from 1972 to 2018. *PLoS One.* 2020;15(1):e0227875. [CrossRef]
9. Keim P, Gruendike JM, Klevytska AM, Schupp JM, Challacombe J, Okinaka R. The genome and variation of *Bacillus anthracis*. *Mol Aspects Med.* 2009;30(6):397-405. [CrossRef]
10. Keim P, Price LB, Klevytska AM, et al. Multiple-locus variable-number tandem repeat analysis reveals genetic relationships within *Bacillus anthracis*. *J Bacteriol.* 2000;182(10):2928-2936. [CrossRef]
11. Grissa I, Bouchon P, Pourcel C, Vergnaud G. On-line resources for bacterial micro-evolution studies using MLVA or CRISPR typing. *Biochimie.* 2008;90(4):660-668. [CrossRef]
12. Aslan S. Klinik örneklerden izole Edilen *Brucella* izolatlarının epidemiyolojik özelliklerinin multi-locus Variable Number Tandem Repeat Analysis ve Pulsed Field Gel Electrophoresis Yöntemleri ile Tespiti. Dissertation. Çukurova University; 2015.
13. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 2021; 49(W1):W293-W296. [CrossRef]
14. Durmaz R, Doganay M, Sahin M, et al. Molecular epidemiology of the *Bacillus anthracis* isolates collected throughout Turkey from 1983 to 2011. *Eur J Clin Microbiol Infect Dis.* 2012;31(10):2783-2790. [CrossRef]
15. Ortatatlı M, Karagoz A, Percin D, Kenar L, Kilic S, Durmaz R. Antimicrobial susceptibility and molecular subtyping of 55 Turkish *Bacillus anthracis* strains using 25-loci multiple-locus VNTR analysis. *Comp Immunol Microbiol Infect Dis.* 2012;35(4):355-361. [CrossRef]
16. Antwerpen M, Ilin D, Georgieva E, Meyer H, Savov E, Frangoulidis D. MLVA and SNP analysis identified a unique genetic cluster in Bulgarian *Bacillus anthracis* strains. *Eur J Clin Microbiol Infect Dis.* 2011; 30(7):923-930. [CrossRef]
17. Sue D, Marston CK, Hoffmaster AR, Wilkins PP. Genetic diversity in a *Bacillus anthracis* historical collection (1954 to 1988). *J Clin Microbiol.* 2007;45(6):1777-1782. [CrossRef]
18. Van Ert MN, Easterday WR, Huynh LY, et al. Global genetic population structure of *Bacillus anthracis*. *PLoS One.* 2007;2(5):e461. [CrossRef]
19. Pilo P, Perreten V, Frey J. Molecular epidemiology of *Bacillus anthracis*: determining the correct origin. *Appl Environ Microbiol.* 2008; 74(9):2928-2931. [CrossRef]
20. Fouet A, Smith KL, Keys C, et al. Diversity among French *Bacillus anthracis* isolates. *J Clin Microbiol.* 2002;40(12):4732-4734. [CrossRef]

21. Aikembayev AM, Lukhnova L, Temiraliyeva G, et al. Historical distribution and molecular diversity of *Bacillus anthracis*, Kazakhstan. *Emerg Infect Dis*. 2010;16(5):789-796. [\[CrossRef\]](#)
22. Gierczyński R, Kałużewski S, Rakin A, et al. Intriguing diversity of *Bacillus anthracis* in eastern Poland – the molecular echoes of the past outbreaks. *FEMS Microbiol Lett*. 2004;239(2):235-240. [\[CrossRef\]](#)
23. Beyer W, Bellan S, Eberle G, et al. Distribution and molecular evolution of *Bacillus anthracis* genotypes in Namibia. *PLOS Negl Trop Dis*. 2012;6(3):e1534. [\[CrossRef\]](#)
24. Fasanella A, Van Ert M, Altamura SA, et al. Molecular diversity of *Bacillus anthracis* in Italy. *J Clin Microbiol*. 2005;43(7):3398-3401. [\[CrossRef\]](#)
25. Lista F, Faggioni G, Valjevac S, et al. Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-number tandem repeats analysis. *BMC Microbiol*. 2006;6:33. [\[CrossRef\]](#)
26. Wattiau P, Klee SR, Fretin D, et al. Occurrence and genetic diversity of *Bacillus anthracis* strains isolated in an active wool-cleaning factory. *Appl Environ Microbiol*. 2008;74(13):4005-4011. [\[CrossRef\]](#)
27. Okutani A, Sekizuka T, Boldbaatar B, Yamada A, Kuroda M, Inoue S. Phylogenetic typing of *Bacillus anthracis* isolated in Japan by multiple locus variable-number tandem repeats and the comprehensive single nucleotide polymorphism. *J Vet Med Sci*. 2010;72(1):93-97. [\[CrossRef\]](#)
28. Van Belkum A. Tracing isolates of bacterial species by multilocus variable number of tandem repeat analysis (MLVA). *FEMS Immunol Med Microbiol*. 2007;49(1):22-27. [\[CrossRef\]](#)
29. Yudianingtyas DW, Sumiarto B, Susetya H, et al. Identification of the molecular characteristics of *Bacillus anthracis* (1982-2020) isolates in East Indonesia using multilocus variable-number tandem repeat analysis. *Vet World*. 2022;15(4):953-961. [\[CrossRef\]](#)
30. Lienemann T, Beyer W, Pelkola K, et al. Genotyping and phylogenetic placement of *Bacillus anthracis* isolates from Finland, a country with rare anthrax cases. *BMC Microbiol*. 2018;18(1):102. [\[CrossRef\]](#)



Surgical Management of Crop Impaction by Inguvotomy in an Aseel Domestic Fowl

Evcil bir Aseel Tavuğunda Kursak Tıkanıklığının Inguvotomi ile Cerrahi Yönetimi

Kavin RAVIKUMAR¹
Gowtham
RAJAMURUGAN²

¹Department of Veterinary and Animal, Husbandry Veterinary Hospital, India

²Department of Veterinary Clinical Medicine, Madras Veterinary College, India



ABSTRACT

Based on the history and clinical signs, a 1-year-old Aseel breed domestic fowl reared in backyard farming was diagnosed with crop impaction. An ingluviotomy was performed, and the impacted grass and plastic materials were removed. This case was followed up for 2 months and no complications were reported. The surgery resulted in a favorable prognosis and the case is discussed here.

Keywords: Aseel, crop impaction, ingluviotomy, grass materials

ÖZ

Bahçe kümesinde yetiştirilen 1 yaşındaki Aseel cinsi evcil tavukta, hastanın hikayesi ve klinik belirtilere dayanarak kursak tıkanıklığı teşhisi konuldu. İnklüvyotomi yapıldı ve tıkanıklığa neden olan çim ve plastik materyaller çıkarıldı. Olgu 2 ay boyunca takip edildi ve herhangi bir komplikasyon rapor edilmedi. Cerrahi müdahalenin olumlu bir prognozla neticlendiği bu vakaya ait tartışma bu çalışma içerisinde sunulmaktadır.

Anahtar Kelimeler: Aseel tavuk, kursak tıkanıklığı, ingluviotomi, çimen materyali

INTRODUCTION

Poultry is one of the agriculture sector's subsidiaries for economic and social development. Backyard poultry production is a long-standing tradition among landless farmers in rural India. The majority of backyard poultry production consists of rearing indigenous birds with poor production performance.¹ It requires little investment, produces high economic returns, and is simple to manage. This type of farming is marked by an indigenous night shelter system, scavenging the natural hatching of chicks, poor bird productivity, scarce supplementary feed, local marketing, and minimum healthcare procedures.

The esophagus of birds is highly distensible due to longitudinal folds that allow them to consume large-sized food materials. This could be the cause of esophageal foreign bodies in birds.^{2,3} Inguvotomy is a surgical procedure performed on birds to correct conditions like crop impaction, crop rupture, or the removal of foreign materials.⁴ Previous studies described ingluviotomy procedures in different birds, including the removal of imbibed masses of peanuts in a cumulet pigeon⁴ and the placement of ingluviotomy tubes in 6 free-flying California condors for lead-induced crop stasis.⁵ The different techniques of ingluviotomy are single-layer closure (skin and ingluviotomy incision together), double-layer closure (skin and ingluviotomy incision separately), and the use of surgical glue.⁶ This case report describes the diagnosis and ingluviotomy and its prognosis.

CASE PRESENTATIONS

A 1-year-old Aseel breed of domestic fowl weighing 1.0 kg was brought to Veterinary Dispensary, Devakottai, with a history of dull, depressed behavior, swelling at the distal part of neck, and not taking feed for last 2 days. The owner was practicing backyard farming, and the presented bird was properly vaccinated against Ranikhet Disease. No other abnormality was reported.

On physical examination, the vital parameters (heart rate, respiratory rate, body temperature, and color of mucus membrane) were within normal range and no abnormality was noticed in the oral cavity. The

Received/Geliş Tarihi: 06.12.2022

Accepted/Kabul Tarihi: 24.01.2023

Publication Date/Yayın Tarihi: 26.04.2023

Corresponding author/Sorumlu Yazar:
Kavin Ravikumar
E-mail: kavinlk@gmail.com

Cite this article as: Ravikumar K, Rajamurugan G. Surgical management of crop impaction by ingluviotomy in an Aseel Domestic Fowl. *Vet Sci Pract.* 2023; 18(1), 44-46.

Atif: Ravikumar K, Rajamurugan G. Evcil bir Aseel tavuğunda kursak tıkanıklığının ingluviotomi ile cerrahi yönetimi. *Vet Sci Pract.* 2023; 18(1), 44-46.



Copyright@Author(s) - Available online at veterinarysciences-ataunipress.org
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License

crop was moderately increased in size, and palpation revealed unusual doughy consistency materials without any grains. Based on the above findings, the condition was diagnosed as crop impaction or ingluvius. The condition was explained to the owner, and the surgical option was adopted.

Treatment

After intranasal administration of Butorphanol (Butodol®1, 1 mg/mL, Neon Laboratories Limited, Thane, India) at a dose of 2 mg/kg and midazolam (Mezolan®, 1 mg/mL, Neon Laboratories, VHB Medi Sciences Limited, Uttarakhand, India) at a dose of 1 mg/kg, the head of the bird was held up for 3 minutes to facilitate better absorption of the sedatives. The bird was positioned on the left lateral recumbency, and the surgical site (base of the neck, over the crop) was prepared aseptically (Figure 1). Local analgesia was achieved by infiltrating 1.0 mL of 2% lignocaine (1 : 3) (Lox 2%, 20 mg/mL, Norries Medicine Limited, Gujarat, India) at the desired site.

A longitudinal 1.5-inch incision was performed through the skin followed by wall of the crop at the healthiest site (Figure 2) and the contents were removed (Figure 3). Impacted materials such as undigested grasses and small plastic papers were noticed in



Figure 3. Crop after the contents were emptied.



Figure 4. Undigested and impacted materials such as grass materials and small plastic papers were noticed.



Figure 1. Preparation of the surgical site (base of the neck, over the crop).



Figure 2. A longitudinal incision through the skin followed by wall of the crop.



Figure 5. The crop incision was closed in a simple continuous pattern using polyglycolic acid no 3-0.

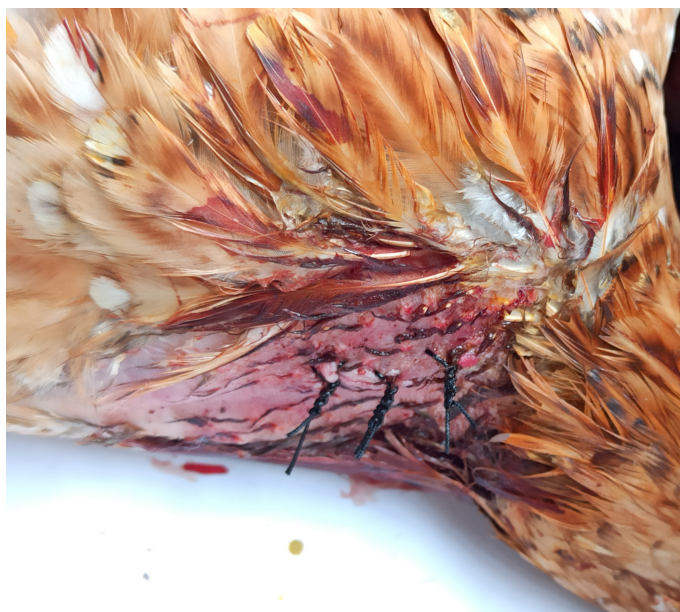


Figure 6. Skin was opposed in an interrupted pattern using silk.

the removed contents (Figure 4). The crop incision was closed in a simple continuous pattern using polyglycolic acid no 3-0 (Figure 5). The sutured site was checked for leakage by giving water orally, and the skin was opposed in an interrupted pattern using silk (Figure 6). Throughout the surgical procedure, the bird was monitored for heart rate, respiratory rate, and rectal temperature, and the values were within the clinical range.

Postoperative Care and Recovery

The bird had free access to water 2 hours after surgery, and a liquid diet was advised for the next 3 days. Watery dropping was noticed 6 hours after surgery. Normal feeding was adopted gradually thereafter. Antibiotic enrofloxacin at a dose of 7.5 mg/kg, I/M for 5 days and analgesic meloxicam at a dose of 0.5 mg/kg, I/M for 4 days were administered. The bird was routinely monitored, and external sutures were removed after 7 days. There were no post-operative complications observed during the 8 weeks follow-up period. This surgery resulted in excellent resolution of clinical signs and the prognosis was favorable.

DISCUSSION

Foreign bodies in the gastrointestinal tract are prevalent in animals. It has been documented in a variety of avian species, including companion birds, zoo birds, poultry, and ostriches, as well as wild and domestic birds.^{7,8} The proventriculus or ventriculus is the most common location for foreign bodies in birds,⁹ and in the present case, impaction was noticed in the crop. After evaluating the fowl's pain sensitivity and restraint characteristics, local analgesia was chosen over general anesthesia in this case. Local infiltration analgesia alone was found to be enough, allowing for the assessment of any leakage following esophageal suturing via oral water administration.^{8,10} The surgery was performed as per standard procedure, and the crop incision was opposed in water-tight simple continuous pattern. Due to the nature of the bird raised and the complications associated with tube placement, such as position slippage and the possibility of developing granulomas or necrosis at the skin and mucosa, feeding tubes were not used in this study.⁵

Double-layer closure resulted in better liquid and feed intake, weight gain, superior healing, reduced leakage-related complications, less narrowing of the lumen, and less fibrosis. Similar findings were observed in this study.⁶ The limitation of this study is that SpO₂ was not monitored during the surgical procedure.

As a result, crop impaction due to various reasons in birds can be efficiently managed by surgical intervention if it is presented at correct time and performed at the earliest.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – K.R., G.R.; Design – K.R.; Supervision – G.R.; Resources – G.R.; Materials, Data Collection and/or Processing – G.R.; Analysis and/or Interpretation – G.R.; Literature Search – K.R.; Writing Manuscript – K.R., G. R.; Critical Review – K.R., G.R.

Declaration of Interests: We certify that we have answered every question and have not altered the wording of any of the questions on this form.

Funding: The authors declared that this study has received no financial support.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – K.R., G.R.; Tasarım – K.R.; Denetleme – G.R.; Kaynaklar – G.R.; Malzemeler – G.R.; Veri Toplanması ve/veya İşlemesi – G.R.; Analiz ve/veya Yorum – G.R.; Literatür Taraması – K.R.; Yazıyı Yazan – K.R., G. R.; Eleştirel İnceleme – K.R., G. R.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Jha BK, Asit C. Backyard poultry farming as a source of livelihood in tribal village: an economic appraisal. *Int J Agric Sci Res.* 2017;267-274.
2. Dyce KM, Sack WO, Wensing CJG. *Textbook of Veterinary Anatomy.* 3rd ed. Philadelphia, PA: Saunders; 2002:799-824.
3. McLelland J. Aves. Digestive system. In: Sisson S, Grossman JD, Getty R, eds. *Sisson and Grossman's the Anatomy of the Domestic Animals.* 5th ed. New Delhi, India: Rekha Printers Pvt. Ltd; 1975: 1857-1882.
4. Laku D, Mutah AA, Mohammed A, Mshelia SH, Mohammed AA. Inguvotomy in a cumulet pigeon following crop impaction: case report. *Int J Vet Sci.* 2021;6(3):01-03.
5. Aguilar RF, Yoshicedo JN, Parish CN. Inguvotomy tube placement for lead-induced crop stasis in the california condor (*Gymnogyps californianus*). *J Avian Med Surg.* 2012;26(3):176-181. [\[CrossRef\]](#)
6. Hussain R, Hussain N, Iqbal N, et al. A study on crop repair by using ingluviotomy techniques in pigeon. *Adv Anim Vet Sci.* 2020;8(7): 753-760.
7. Miller CL, Bischoff KL, Hoff B. Polyacrylamide gel ingestion leading to fatal intestinal obstruction in two birds in a zoological collection. *J Avian Med Surg.* 2009;23(4):286-289. [\[CrossRef\]](#)
8. Ramesh R, Vijayanand V, Gopalakrishnan A, Ayyappan S. Crop impaction and its surgical management in a domestic fowl. *J Entomol Zool Stud.* 2020;8(5):259-260.
9. Lupu C, Robins S. Comparison of treatment protocols for removing metallic foreign objects from the ventriculus of Budgerigars (*Melopsittacus undulates*). *J Avian Med Surg.* 2009;23(3):186-193. [\[CrossRef\]](#)
10. Ninu AR, Uma Rani R, Vishnugurubaran D. Esophagotomy in a domestic fowl: a rare case report. *Iran J Vet Res.* 2019;20(3):218-220.