2025

E-ISSN 2651-3609 | VOLUME 8 | ISSUE 1

IJVAR

INTERNATIONAL JOURNAL OF VETERINARY AND ANIMAL RESEARCH

INTERNATIONAL JOURNAL OF VETERINARY AND ANIMAL RESEARCH

www.ijvar.org

PUBLISHED BY
Anatolia Academy of Sciences

IJVAR

International Journal of Veterinary and Animal Research

E-ISSN: 2651-3609

Owner and Publisher
Anatolia Academy of Sciences

International Journal of Veterinary and Animal Research (IJVAR) is an international non-profit, full open access, double-blind peer-reviewed journal and publishes three issues per year.

IJVAR welcomes article submissions and does not charge any article submission or processing charges.

Authors are completely responsible for the contents of their articles.

Address
Anatolia Academy of Sciences
Selahaddini Eyyubi District, Tekke Street, No: 21, Selcuklu, Konya / TURKEY

e-mail: ijvareditor@gmail.com

http://www.ijvar.org

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Publication date: 31 March 2025

Volume: 8 Issue: 1

Research Articles

1. Determination of Methicillin, Vancomycin, Erythromycin and Clindamycin Susceptibilities in *Staphylococcus aureus* Strains Isolated from Cats' Oral and Nasal Swab Samples
Orkun Babacan 01-05

2. In Vitro Determination of Ovicidal and Larvicidal Activity of Curcumin on *Toxocara canis* Eggs
Selma Kocademir, Kader Yıldız..... 06-11

3. Clinical and Radiologic Evaluation of Dental Diseases in Cats
Nazlıcan Çelik, Birkan Karslı..... 12-17

4. Investigation of Distempervirus and Parvovirus Infections in Dogs
Hatice Pelin Aslım, Irmak Dik.....ş..... 18-23

Case Report

5. Atresia Ani Et Vulva and Rectovaginal Fistula: A Clinical Presentation in a Calf
Sevdet Kılıç, Mustafa Barış Akgül, Kadir Sulu, Ali Gülaydın, Bahar Erden 24-27

Review Article

6. Intersection of Toxicology and Archaeology Sciences
Doga Can Nalbantoglu, Mustafa Yipel 28-31

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Determination of Methicillin, Vancomycin, Erythromycin and Clindamycin Susceptibilities in *Staphylococcus aureus* Strains Isolated from Cats' Oral and Nasal Swab Samples

Orkun Babacan✉

Balıkesir University, Kepsut Vocational School, Department of Veterinary, Balıkesir, Türkiye.

ARTICLE INFO

Received: 08/10/2024
Accepted: 04/02/2025

DOI: 10.5281/zenodo.15109837

✉**Corresponding Author:** orkun_babacan@hotmail.com

Keywords

Antibiotic resistance
Bacterium
Feline

Cite this article as: *Babacan O. 2025. Determination of Methicillin, Vancomycin, Erythromycin and Clindamycin Susceptibilities in Staphylococcus aureus Strains Isolated from Cats' Oral and Nasal Swab Samples. International Journal of Veterinary and Animal Research, 8(1): 01-05. DOI: 10.5281/zenodo.15109837.*

ABSTRACT

The current study aims to investigate the prevalance and antibiotic susceptabilities including methicillin resistance of *S. aureus* in cats' oral and nasal swabs and to assess their potential role in the epidemiology for antibiotic resistance. *Staphylococcus aureus* is a common commensal bacterium found in the skin, nasal microbiota, mucose membrans and respiratory tracts of humans and animals especially dogs and cats, respectively. This trend of increasing pet ownership has raised concerns about the potential for companion animals to spread zoonotic infections, including *S. aureus*. A total of 12 (%25) *S. aureus* isolates were obtained and identified as bright zoned black colonies on RPF-BP agar from 48 nasal and oral swabs collected from cats. According to the evaluation of methicillin susceptibility using the disk diffusion test following the EUCAST method, 2 *S. aureus* isolates were found to be methicillin-resistant. All *S. aureus* strains isolated were determined to be susceptible to vancomycin. In the double-disk diffusion test conducted to determine susceptibility to erythromycin and clindamycin, one (1) of the methicillin-resistant *S. aureus* isolates also exhibited resistance to both erythromycin and clindamycin. In the PCR test, among the 2 methicillin-resistant *S. aureus* isolates, 2 contained the *mecC* gene, while *mecA* gene was not found. Considering that these animals are pets, it suggests that they could transmit MRSA bacteria to their owners or other individuals through various routes such as biting, licking, and close contact. These results are of significant importance from a public health perspective, especially One Health perspective.

INTRODUCTION

Staphylococcus aureus is a prevalent commensal bacterium found on the skin and within the nasal microbiota, mucous membranes, and respiratory tracts of both humans and animals, particularly dogs and cats (Mourabit et al., 2020; Abdullahi et al., 2022; Afhsar et al., 2023; Das et al., 2023). Although these bacteria typically coexist without causing harm, they can become opportunistic pathogens, especially in individuals with compromised immune systems or when they breach other body sites. This capacity to shift from harmless commensals to pathogenic forms underscores the necessity of monitoring *S. aureus* in both human and veterinary health contexts due to its potential as a zoonotic agent (Abrahan et al., 2007; Loeffler and Lloyd, 2010; Algammal et al., 2020; Abdullahi et al., 2022; Das et al., 2023). Its resilience on hands and surfaces further establishes it as a significant opportunistic pathogen, particularly in immunocompromised populations. Infections associated with this bacterium range from food poisoning and skin infections to respiratory tract infections and unique clotting disorders (Abrahan et al., 2007; Abdullahi et al., 2022; Das et al., 2023). Its ability to endure outside the host enhances its risk of causing infections in vulnerable groups (Abdullahi et al., 2022; Das et al., 2023).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) present critical challenges in both clinical and community environments. Community-acquired MRSA (CA-MRSA)

has emerged as a prominent public health concern, leading to infections that require immediate attention (Abdel-Moein and Samir, 2012). MRSA is particularly recognized as a leading antibiotic-resistant pathogen, resulting in severe and often difficult-to-treat infections. The rise of these resistant strains emphasizes the urgent need for effective infection control measures and the development of new therapeutic strategies in healthcare settings. VRSA, in particular, poses a serious threat, as vancomycin is typically considered a last-resort antibiotic for MRSA infections. The emergence of VRSA complicates treatment options and raises alarms regarding potential widespread resistance. This scenario underscores the necessity for ongoing surveillance, judicious antibiotic usage, and the pursuit of alternative treatment options to manage infections caused by these resistant strains (Das et al., 2023).

Companion animals, such as dogs and cats, play a significant role in enhancing the emotional and social well-being of their owners. In Türkiye, pet ownership has grown increasingly common, particularly in larger cities, where having pets-especially cats and dogs-has become more socially accepted and is viewed as a status symbol (Abdullahi et al., 2022; Das et al., 2023). This rising trend in pet ownership raises concerns about the potential transmission of zoonotic infections, including *S. aureus*. MRSA has emerged as a notable issue within veterinary medicine, as pets can carry MRSA strains from humans, leading to a marked increase in reported cases in veterinary hospitals over the past decade. Research indicates that MRSA clones in pets often mirror those found in humans, especially strains associated with hospital settings. Furthermore, MRSA can be transmitted between pets and their owners, and the close interactions in domestic environments facilitate the spread of these antibiotic-resistant bacteria. While MRSA and VRSA have been extensively studied in human populations, research focusing on the prevalence of these pathogens in domestic cats and their human counterparts remains limited, particularly in Bangladesh, where detailed investigations into zoonotic strains of *S. aureus* and their resistance genes have not yet been conducted (Abrahan et al., 2007; Loeffler and Lloyd, 2010; Algammal et al., 2020; Abdullahi et al., 2022; Das et al., 2023).

Clindamycin, part of the lincosamide class of antimicrobial agents, along with macrolides and streptogramin B, is often used to treat various bacterial infections. These antibiotics inhibit bacterial protein synthesis and can exhibit resistance through two main mechanisms: active efflux, mediated by the msrA gene, which pumps out the antibiotic, and modification of the ribosomal target site, encoded by erm genes. Resistance can manifest as either constitutive, permanently expressed against all MLSB antibiotics, or inducible, expressed only in the presence of an inducing agent, such as erythromycin (Rich et al., 2005). Understanding these resistance mechanisms is vital for developing effective treatment approaches and addressing antibiotic resistance in clinical settings.

In veterinary medicine, clindamycin is commonly employed to treat various infections, including those affecting the skin, respiratory tract, and oral cavity, as well as infections caused by anaerobic bacteria. It is particularly valuable for treating staphylococcal infections and is often the preferred treatment option when MRSA is identified. Its efficacy against resistant strains makes it a crucial choice in managing bacterial infections in animals (Rich et al., 2005).

However, some staphylococcal strains may exhibit an inducible form of resistance to clindamycin. These strains may appear susceptible in standard antimicrobial susceptibility tests, yet resistance can develop during treatment, potentially leading to therapeutic failure. This phenomenon highlights the importance of careful monitoring and consideration of resistance patterns when prescribing clindamycin for staphylococcal infections (Faires et al., 2009).

The current study aims to investigate the prevalence and antibiotic susceptibilities, including methicillin resistance of *S. aureus* in cats' oral and nasal swabs, as well as to evaluate their potential role in the epidemiology of antibiotic resistance.

MATERIALS AND METHODS

Sampling

A total of 48 oral and nasal swab samples were examined and evaluated from cats, which were collected and sent by veterinary clinic veterinarians from veterinary clinics in Balıkesir and İzmir for microbiological examination. The swabs were sent to the laboratory by veterinary clinic veterinarians under cold chain conditions in transport medium for microbiological examination. Upon arrival at the laboratory, the swabs were immediately processed for isolation analysis.

Isolation ve identification

Nasal and oral swabs of cats were inoculated onto 5% sheep blood agar (Merck, Germany), MacConkey agar (Merck, Germany), and Rabbit Plasma Fibrinogen-Baired-Parker (RPF-BP) agar (Oxoid, UK). The agars were incubated at 37°C for 24 hours. Bright zoned black colonies on the RPF-BP agar were identified as *S. aureus* (Baired-Parker, 1962; Göçmen et al., 2020). Colonies identified as *S. aureus* were preserved in bead bacterial storage tubes at -20°C for antibiotic susceptibility testing.

Antibiotic susceptability tests

S. aureus isolates preserved in bead bacterial storage tubes at -20°C were inoculated into nutrient broth and incubated at 37°C. The *S. aureus* isolates that grew in the nutrient broth were then subcultured onto RPF-BP agar (Oxoid, Merck) for purity control. After purification, the isolates underwent antibiotic susceptibility testing using the disk diffusion method according to EUCAST standards. (Bauer et. Al., 1966; EUCAST, 2017a; 2017b).

S. aureus isolates were initially diluted according to the McFarland 0.5 standard. Antibiotic susceptibility testing for methicillin and vancomycin was performed on Mueller-Hinton agar (Merck, Germany). Methicillin and vancomycin resistance profiles of *S. aureus* isolates were investigated and evaluated phenotypically according to EUCAST standard and Hallabjaiy et al., 2014, respectively. Methicillin resistance was investigated by disc diffusion method using cefoxitin (30 µg disk, Oxoid, UK). Vancomycin resistance was investigated by disc diffusion test, 30 µg vancomycin discs according to Hallabjaiy et al., 2014 (Oxoid, UK) (Hallabjaiy et al., 2014; EUCAST, 2017a; 2017b).

S. aureus strains with <22 mm zone diameter was recorded as Methicillin-resistant according to EUCAST procedure and Vancomycin-resistance was evaluated

according to Hallabjaiy et al., 2014 (Hallabjaiy et al., 2014; EUCAST, 2017a; 2017b).

For erythromycin and clindamycin, the double-disc diffusion D-test described by Rich et al., (2005) was performed. This test was performed using a 2 mg clindamycin disc (Oxoid, UK) and a 15 mg erythromycin disc (Oxoid, UK) on both blood agar and Mueller-Hinton agar (Merck, Germany) for comparative analysis (Rich et al). Plates were incubated aerobically at 37℃ for 18 h. After incubation, the presence of a flattened zone (D-shape) between the discs, where both antimicrobials have diffused, suggests that the organism exhibits inducible clindamycin resistance (Rich et al., 2005).

Detection of mecA and mecC genes by PCR in isolated phenotypic methicillin resistant S. aureus strains

To assess methicillin resistance, DNA was extracted from *S. aureus* isolates using the GeneJET Genomic DNA Purification Kit (MAN0012663, protocol for isolating genomic DNA from gram-positive bacteria, Thermo, USA) along with a lysis buffer, following the manufacturer's guidelines. The methicillin resistance genes, *mecA* and *mecC*, were analyzed through PCR using previously established primers and amplification protocols (Table 1) (Mehrotra et al., 2000; García-Alvarez et al., 2011; García-Garrote et al., 2014; Doğan et al., 2016).

Table 1. Primer sequences, target genes and references for *mecA and mecC* genes

Primers	Sequences	Target genes	Base pairs	References
GMECAR-1	5'-ACTGCTATCCACCCTCAAAC-3'	*mecA*	163	Mehrotra et al. (2000)
GMECAR-2	5'-CTGGTGAAGTTGTAATCTGG-3'			
Primer-F	5′ -GAA AAA AAG GCT TAG AAC GCC TC-3′	*mecC*	138	Garcı´a-Alvarez et al. (2011)
Primer-R	5′ GAA GAT CTT TTC CGT TTT CAG C-3			Garcı´a-Garrote F. et al. (2014) Doğan et al. (2016)

The PCR mixture for the *mecA* and *mecC* genes was formulated to a total volume of 50 µl for each gene, which included 30 µl of Taq polymerase Master Mix (Ampliqon, Denmark), 0.4 µl of the forward primer, 0.4 µl of the reverse primer, 17.2 µl of PCR-grade water (DNase and RNase free), and 2 µl of DNA (Mehrotra et al., 2000; García-Alvarez et al., 2011; García-Garrote et al., 2014; Doğan et al., 2016).

The amplification protocol for the *mecA* gene commenced with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 2 minutes, annealing at 57°C for 2 minutes, and extension at 72°C for 1 minute. The procedure concluded with a final extension step at 72°C for 7 minutes (Mehrotra et al., 2000).

For the amplification of the *mecC* gene, the conditions included an initial denaturation at 94°C for 15 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, and extension at 72°C for 1 minute. A final elongation step was performed at 72°C for 10 minutes (Doğan et al., 2016).

The PCR products were analyzed by electrophoresis on a 1.5% agarose gel (Prona, USA) containing Novel Juice dye (Thermo Scientific, USA) and a DNA molecular weight marker (Gene Ruler 100 bp DNA Ladder Plus, Thermo Scientific, USA). Gel imaging was conducted using the EBOX CX5 TS EDGE system from Vilber.

Methicillin-resistant *S. aureus* NCTC 12493 (*mecA*) and methicillin-resistant *S. aureus* NCTC 13552 (*mecC*) and *E. coli* ATCC 25923 were utilized as reference strains in PCR as positive and negative controls, obtained from the Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratory.

RESULTS

Isolation ve identification results

A total of 12 (%25) *S. aureus* isolates were obtained and identified as bright zoned black colonies on RPF-BP agar from 48 nasal and oral swabs of cats.

Antibiotic susceptibility tests results

According to the evaluation of methicillin susceptibility using the disc diffusion test following the EUCAST method, 2 *S. aureus* isolates were found to be methicillin-resistant.

All *S. aureus* strains isolated were determined to be susceptible to vancomycin. In the double-disk diffusion test conducted to determine susceptibility to erythromycin and clindamycin, one (1) of the methicillin-resistant *S. aureus* isolates also exhibited resistance to both erythromycin and clindamycin.

Results of mecA and mecC genesby PCR in isolated phenotypic methicillin resistant S. aureus strains

In the PCR test, in 2 methicillin-resistant *S. aureus* isolates the *mecC* gene were detected, while *mecA* gene was not found (Figure 1).

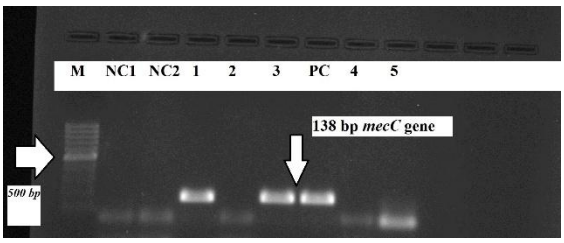

Figure 1. Results of *mecA* and *mecC* genes by PCR in isolated phenotypic methicillin resistant *S. aureus* strains (M: Marker, NC1: *E.coli* ATCC 25923, NC2: *S. aureus* NCTC 12493, PC: Positive control-, *S. aureus* NCTC 13552 (*mecC*) Line 1 and 3: *mecC* positive samples, Line 4-5: Negative samples)

DISCUSSION AND CONCLUSION

The natural oral flora of cats contains a diverse range of bacterial species, including opportunistic pathogens such as *Staphylococcus* spp., which can be transmitted to humans through bites. Among the species of

staphylococci, *S. aureus* and other coagulase-positive staphylococci (CoPS) can act as opportunistic pathogens (Razali et. al., 2022).

Globally, dogs and cats account for the majority of animal bites. Infections can occur in 20-80% of cat bites, primarily due to the oral flora of the biting animal (Razali et. al., 2022).

Despite this, there have not been sufficient studies investigating the oral and nasal carriage of staphylococci in cats.

Among staphylococci species, *S. aureus* is recognized as the most significant pathogen, responsible for a wide range of infections in both humans and animals. Additionally, *S. aureus* is a common resident of the skin and nostrils in humans and can be transmitted to pets through close contact, such as petting, kissing, or licking the skin. Consequently, *S. aureus* is significantly more prevalent in pets than in stray animals. (Razali et. al., 2022). All of the sampled cats were pets (owned cat) in this study. Their regular contact with humans may explain the high isolation rates of *S. aureus* observed in our findings.

According to the results of previous studies, MRSA isolates, which were isolated from cats and dogs, are similar to hospital isolates, indicating that companion animals likely acquire MRSA from humans. It also highlights that both humans and animals are more frequently carriers (colonized) rather than showing symptoms of infection. This means they can serve as reservoirs for MRSA, allowing the bacteria to circulate within the household (Mustapha et. al., 2014).

Ma et al., (2020) reported that they isolated 7 *S. aureus* strains from swab samples taken from the noses, oropharynxes, and perineum of cats, and did not detect MRSA. MRSA were found in two *S. aureus* strains in this study. Considering that these animals were pets (owned cat), it suggests that they could transmit MRSA bacteria to their owners or other individuals through various routes such as biting, licking, and close contact. These results are of significant importance from a public health perspective, especially one health perspective.

The risk factors for the carriage of *mecC* MRSA in humans include contact with animals and the presence of underlying health conditions (EFSA and CDC, 2022). According to EFSA and CDC (2022), the presence of the *mecC* gene in the MRSA strains isolated in this study can be considered an important finding from a public health perspective.

Rimbu et al., (2012) reported isolating 49 *S. aureus* strains from materials collected from 135 cats suffering from gingivitis, periodontitis, abscesses, glossitis, tonsillitis, and dental caries. They declareted to found that 14.3% of these isolates exhibited resistance to clindamycin, while were not detected resistance to erythromycin.

In this study, resistance to clindamycin and erythromycin was detected in only one methicillin-resistant *S. aureus* isolate. Despite being a single isolate, its detection in MRSA is considered to be epidemiologically significant.

In conclusion, the detection of two MRSA strains in *S. aureus* isolates from the oral and nasal swabs of cats in this study was considered to be epidemiologically significant from a public health and One Health perspective. Furthermore, the detection of clindamycin and erythromycin resistance in one MRSA isolate was considered to be potentially epidemiologically significant. Furthermore, in *S. aureus* strains, including those from feline cases, resistance to clindamycin and erythromycin is typically attributed to the presence of specific genes, such as *erm* (which confers MLSB resistance) or *mef*. These genes enable bacteria to modify their ribosomal targets, rendering these antibiotics ineffective. Resistance can pose significant challenges, particularly in the treatment of skin and soft tissue infections, where these antibiotics are commonly used (Rich et. al., 2005). Therefore, monitoring resistance to methicillin, erythromycin, and clindamycin was considered important for effective treatment planning and public health, in alignment with One Health principles.

Acknowledgement

The author thanks to the Ministry of Health, General Directorate of Public Health, Microbiology Reference Laboratory for referance strains.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: Concept: O.B., Design: O.B., Data Collection or Processing: O.B., Analysis and Interpretation: O.B., Literature Search: O.B., Writing: O.B.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Abdel-moein, KA, El-Hariri M, Samir A. 2012. Methicillin-resistant Staphylococcus aureus: an emerging pathogen of pets in Egypt with a public health burden. Transboundary and Emerging Diseases, 59(4): 331-335.

Abdullahi IN, Zarazaga M, Campaña-Burguet A, Eguizábal P, Lozano C, Torres C. 2022. Nasal Staphylococcus aureus and S. pseudintermedius carriage in healthy dogs and cats: a systematic review of their antibiotic resistance, virulence and genetic lineages of zoonotic relevance. Journal of Applied Microbiology, 133(6): 3368-3390.

Abraham JL, Morris DO, Griffeth GC, Shofer FS, Rankin SC. 2007. Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and Staphylococcus schleiferi ssp. schleiferi. Veterinary Dermatology, 18(4): 252-259.

Afshar MF, Zakaria Z, Cheng CH, Ahmad NI. 2023. Prevalence and multidrug-resistant profile of methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius in dogs, cats, and pet owners in Malaysia. Veterinary World, 16(3): 536-545.

Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE, et al. 2020. Methicillin-resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist, 13: 3255-3265.

Bağcıgil AF, İkiz S, Güzel Ö, Parkan Yaramış Ç, Ilgaz A. 2012. Specıes distributıon of methicillin resıstant Staphylococci isolated from anımals, environmental samples and staffs. Journal of Faculty of Veterinary Medicine İstanbul University, 28(2): 151-160.

Baird-Parker AC. 1962. An improved diagnostic and selective medium for isolating coagulase positive Staphylococci. Journal of Applied Bacteriology, 25(1): 12-19.

Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by standardized single disk method. American Journal of Clinical Pathology, 45: 493-496.

Bhat AH. 2021. Bacterial zoonoses transmitted by household pets and as reservoirs of antimicrobial resistant bacteria. Microbial Pathogenesis, 155: 104891.

Cong Y, Yang S, Rao X. 2020. Vancomycin resistant *Staphylococcus aureus* infections: a review of case updating and clinical features. Journal of Advanced Research, 21:169-76.

Das S, Kabir A, Chouhan CS, Shahid MAH, Habib T, Kobir MA, Hossain MZ, Rahman M, Nazir KHMNH. 2023. Antimicrobial resistance patterns of Staphylococcus aureus isolated from apparently healthy pet cats of Bangladesh. Journal of Advanced Veterinary and Animal Research, 10(3): 545-553.

Doğan E, Kılıç A, Türütoğlu H, Öztürk D, Türkyılmaz H. 2016. Screening of Staphylococcus aureus isolates for mecA and mecC genes carriage. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 63: 389-391.

EFSA and ECDC. 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal, 20(3).

European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2017a. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0. Available at http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_ Tables.pdf. (Accessed September 16, 2024).

European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2017b. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0. Available at https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. (Accessed September 16, 2024).

Faires MC, Gard S, Aucoin D, Weese JS. 2009. Inducible clindamycin-resistance in methicillin-resistant Staphylococcus aureus and methicillin-resistant Staphylococcus pseudintermedius isolates from dogs and cats. Veterinary Microbiology, 139(3-4): 419-420.

García-Álvarez L, Holden MTG, Lindsay H, Webb CR, Brown DFJ, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RLR, Larsen AR, Skov RL, Holmes MA. 2011. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: A descriptive study. The Lancet Infectious Diseases, 11(8): 595-603.

García-Garrote F, Cercenado E, Marín M, Bal M, Trincado P, Corredoira J, Ballesteros C, Pita J, Alonso P, Vindel A. 2014. Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. Journal of Antimicrobial Chemotherapy, 69(1): 45-50.

Göçmen H, Tamakan H, Şükür H, Esendal ÖM. 2020. Investigation of multidrug resistance among Staphylococcus species ısolated from cats and dogs. Ataturk Universitesi Veteriner Bilimleri Dergisi, 15(3): 156-166.

Hallabjaiy RMHS, Darogha SNR, Hamad PA. 2014. Vancomycin resistance among methicillin-resistant Staphylococcus aureus isolated from clinical samples in Erbil City-Iraq. Medical Journal of Islamic World Academy of Sciences, 22(4): 168-174.

Loeffler A, Lloyd DH. 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiology and Infection, 138(5): 595–605.

Ma GC, Worthing GA, Ward MP, Norris JM. 2020. Commensal Staphylococci ıncluding methicillin-resistant Staphylococcus aureus from dogs and cats in Remote New South Wales, Australia. Microbial Ecology, 79: 164-174

Mehrotra M, Wang G, Johnson WM. 2000. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of Clinical Microbiology, 38(3): 1032-1035.

Mourabit N, Arakrak A, Bakkali M, Zian Z, Bakkach J, Laglaoui A. 2020. Nasal carriage of Staphylococcus aureus in farm animals and breeders in north of Morocco. BMC Infectious Diseases, 20(1): 1-6.

Mustapha M, Bukar-Kolo Y, Geidam Y, Gulani I. (2014). Review on methicillin-resistant Staphylococcus aureus (MRSA) in dogs and cats. International Journal of Animal and Veterinary Advances. 6: 61-73. 10.19026/ijava.6.5619.

Razali K, Kaidi R, Giarratana F. 2022. Staphylococci and zoonotic potential: oral carriage and antibiotic susceptibility in stray dogs and cats in Algeria Journal of the Hellenic Veterinary Medical Society, 73(1): 3621-3628.

Rich M, Deighton L, Roberts L. 2005. Clindamycin-resistance in methicillin-resistant Staphylococcus aureus isolated from animals. Veterinary Microbiology, 111(3-4): 237-240.

Rimbu C, Guguianu E, Horhogea C, Carp-Carare E. 2012. Staphylococcus aureus – implications of the oral cavity disease at the dog and cat. Availabe at: https://www.researchgate.net/publication/257934696_Staphylococcus_aureus_-_implications_of_the_oral_cavity_disease_at_the_dog_and_cat?enrichId=rgreq-dd54df73df0c735d6d19a1ce31288a34-XXX&enrichSource=Y292ZXJQYWdlOzI1NzkzNDY5NjtBUzoxMDQ0MzYwNjgyNTc4MDRAMTQwMTkxMDkwNzU0MQ%3D%3D&el=1_x_2&_esc=publicationCoverPdf.

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

In Vitro Determination of Ovicidal and Larvicidal Activity of Curcumin on *Toxocara canis* Eggs

Selma Kocademir[1,2✉], Kader Yıldız[3]

[1]*Kırıkkale University, Institution of Health and Science, Kırıkkale, Türkiye.*
[2]*Ministry of Agriculture and Forestry, Veterinary Control Central Research Institute, Laboratory of Parasitology, Ankara, Türkiye*
[3]*Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale, Türkiye.*

ARTICLE INFO

Received: 01/04/2024
Accepted: 26/02/2025

DOI: 10.5281/zenodo.15109865

✉**Corresponding Author:** selmavetkocademir@hotmail.com

Keywords

Curcumin
Eggs
In Vitro
Larvae
Larvicidal
Ovicidal
Toxocara canis

Cite this article as: *Kocademir S. and Yıldız K. 2025. In Vitro Determination of Ovicidal and Larvicidal Activity of Curcumin on Toxocara canis Eggs. International Journal of Veterinary and Animal Research, 8(1): 06-11. DOI: 10.5281/zenodo.15109865.*

ABSTRACT

Curcuma longa rhizome is the source of turmeric. Curcumin exhibits some encouraging antiparasitic properties in helminths. The purpose of this investigation was to determine the ovicidal and larvicidal activities of curcumin in *Toxocara canis* eggs in vitro. Curcumin dilutions (36.8 mg/ml, 18.4 mg/ml and 3.6 mg/ml) were prepared by adding RPMI-1640. The eggs and hatched infective-stage larvae were incubated with curcumin dilutions for 6, 12 or 24 hours. The ovicidal activity was evaluated after 28 days. Larvicidal activity was assessed after completing each incubation time. In the present study, no changes in the eggshell structure were observed in all curcumin groups. The lowest embryogenesis rate (75%) was observed only at the highest curcumin dilution (36.8 mg/ml) at the 12- and 24-hour incubations, but the difference was not found statistically significant. No significant larvicidal effect of curcumin was detected. The percentage of moving larvae was 80% at 12 hours and 76% at 24 hours in the highest curcumin dilution (36.8 mg/ml). *T. canis* larvae survived in RPMI-1640 for four days after being incubated with 36.8 mg/ml curcumin for 24 hours. However, the untreated larvae were still active at this time. Further studies focusing on the migration of *T. canis* infective larvae in animal models may shed light on the effect of curcumin, which is rapidly metabolized in the body and absorbed at low levels from the intestine, on the migrating larva.

INTRODUCTION

Turmeric is obtained from the rhizome of *Curcuma longa*, Zingiberaceae family. It has been used in complementary and alternative medicine for a long time (Bigford and Del Rossi, 2014). Curcumin (60-70%), dethoxycurcumin (20-27%), and bisdemethoxycurcumin (10-15%) are the three main curcuminoid chemicals found in turmeric (Nelson et al., 2017). Curcumin is known antioxidant, anti-inflammatory, antibacterial, immune system regulatory, anticarcinogenic, antidiabetic, neuroprotective, and protective agent for cardiovascular system and liver (Wang et al., 2018; Cao et al., 2018).

Curcumin has promising antiparasitic effects on helminths, including *Ascaridia galli* (Bazh and Bahy, 2013), *Trichinella spiralis* (Hamed et al., 2022), *Fasciola gigantica* (Ullah et al., 2017), *Schistosoma mansoni* and *Schistosoma haematobium* (Abou El Dehab et al., 2019), *Raillietina cesticillus* (El-Bahy and Bazh, 2015), *Taenia crassiceps* cysticercus (Martínez-González et al., 2022). Curcumin acts in different ways such as reducing the detoxification ability, increasing the amount of reactive oxygen species (Rehman et al., 2020), and altering the tegument of helminths (Ullah et al., 2017). Due to the tegumental changes, Na+ –K+ transport is impaired in the parasite, possibly resulting in a significant decrease in motility (Abou El Dehab et al., 2019; Faixová et al., 2021).

Toxocara canis, nematode, lives in the small intestine of dogs and other canids (Oge, 2018). There is no intermediate host in the biology of the parasite; however, some animal species and humans play a role as the

paratenic host (Kocademir and Yildiz, 2022). *Toxocara canis* eggs are spherical-oval, 74-80 µm in diameter, brown in color and covered with thick shell (Oge, 2018). Embryogenesis begins in the eggs at air temperature above 15°C, and infective larvae (L3) develop about 3-4 weeks in nature (Abou-El-Naga, 2018). The eggs, including L3, cause infection in dogs and paratenic hosts. The dog is also infected by eating the L3-containing tissues of the paratenic hosts (Oge, 2018). The infective larvae are released in the small intestine and migrate to different tissues, including the liver, heart and lungs in the dog (Kocademir and Yildiz, 2022). The migration route of the larvae varies according to the age and gender of the dogs (Oge, 2018).

Infective larvae maintain their viability in the tissues of their paratenic hosts, and they are also transmitted between paratenic hosts. Infective larvae do not become adults in the paratenic host, but they cause a pathology called visceral larva migrans (VLM) during migration in the paratenic host tissues (Chen et al., 2018). Drugs have limited efficacy in treating VLM in humans. Albendazole is used as the first option for the treatment of VLM. However, the treatment regime has not yet been standardized (Hombu et al., 2019). In addition to drug treatment, the effectiveness of some plant extracts on VLM is being investigated. Several plant extracts have been shown to have ovicidal and larvicidal effects on *T. canis* in both in vitro and in vivo experiments (Mata-Santos et al., 2015; Orengo et al., 2016; El-Sayed, 2017).

This study aimed to determine the effect of curcumin on ovicidal activity in *T. canis* eggs in vitro. In addition, it was also aimed to determine larvicidal activity on *T. canis* infective stage larvae which are responsible for infection in both dogs and paratenic hosts including humans.

MATERIALS AND METHODS

Toxocara canis eggs

Adult *T. canis* were obtained from veterinary clinics in Ankara, Türkiye. The parasites were brought by owners after being excreted in the faeces of dogs naturally infected with toxocariasis. The samples were brought to the Parasitology Laboratory of the Central Veterinary Control Institute in Ankara. The parasites were carefully washed in distilled water and diagnosed as *T. canis* under a stereo microscope based on their morphological features. Female *T. canis* were washed three times with distilled water in a Petri dish. The uterine part was dissected with a sterile scalpel, and the eggs were collected in distilled water. After sieving the egg suspension through a sieve (200 µm pore), it was centrifuged three times using sterile distilled water (3 mins, 500 x g). Then the number of eggs were counted and adjusted to 1000 eggs per ml with sterile distilled water.

Preparation of the curcumin dilutions

Curcumin powder (Sigma C1386) was stored at -18°C and protected from light until used in the experiments. The curcumin dilutions were prepared by adding RPMI-1640 (Sigma) (36.8 mg/ml, 18.4 mg/ml and 3.6 mg/ml). They were prepared just before the experiments and protected from light until use.

Experimental design

A total 5 groups were consisted (3 different curcumin dilutions, the positive and negative controls) for each incubation time of this study. These groups were created separately for three different incubation times (6, 12 and 24 hours).

Determination of the ovicidal activity

In this assay, the experimental groups were consisted with *T. canis* eggs obtained from female parasites. The egg suspension (100 µl) was added to the microcentrifuge tubes. Three curcumin dilutions (36.8 mg/ml, 18.4 mg/ml and 3.6 mg/ml) were added (100 µl) separately to the tubes and gently dispersed by pipetting. The positive control was prepared from a commercial drug containing pyrantel pamoate at 725 µg/ml in RPMI-1640 and it was added (100 µl) to the eggs and gently dispersed. The untreated eggs were used as the negative control in the experiments. The tubes protected from light were incubated for 6, 12, and 24 hours in the incubator (28°C). After completing each incubation step, the supernatant was removed from the tubes and they were centrifuged with sterile distilled water three times (3 mins, 500 x g). Final centrifugation was performed with formalin solution (0.5%). The eggs were placed into the wells of sterile polystyrene microplates with a lid. The plates were put in the incubator (28°C) and mixed daily. Larvae development was evaluated using a light microscope (Leica ICC50) for 28 days.

Determination of the larvicidal activity

In this assay, the experimental groups were consisted with *T. canis* larvae after hatchled from the eggs after complated 28-day incubation. To obtain hatched larvae, *T. canis* eggs were incubated for 28 days (28°C). The eggs were washed with sterile distilled water at the end of the incubation period. To remove the protein cover on the eggshell, the hypochlorite solution was added to the eggs. Then the eggs were centrifuged twice with sterile distilled water, the final centrifugation was performed with RPMI-1640 (3 minutes at 500 xg). The hatched larvae number was counted and adjusted as 1000 larvae per ml in the larvae suspension.

To determine the larvicidal effect, the larvae suspension (100 ml) was placed in the microcentrifuge tubes. 100 µl of each curcumin dilutions (36.8 mg/ml, 18.4 mg/ml and 3.6 mg/ml) were added to the tubes and mixed gently. The larvae in RPMI-1640 and pyrantel pamoate served as the negative and the positive controls, respectively. The tubes protected from light were incubated at 37°C for 6, 12, and 24 hours. After completing each incubation period, the larvae were washed three times with RPMI-1640. The trypan blue dye test was used to determine the larvae viability (Sena Lopes et al., 2020). Larvae that did not move were considered dead (Reis et al., 2010).

To assess the impact of curcumin on the larvae's life span, the larvae were washed with RPMI-1640 after being incubated with the highest curcumin dilutions (36.8 mg/ml) for 24 hours. The larvae left untreated served as the negative control. They were incubated with daily replacement of RPMI-1640 (28°C). Larval viability was examined daily by a light microscope.

Statistical analysis

Data were analyzed using the Chi-square test. $P<0.05$ were considered as statistically significant.

RESULTS

The ovicidal activity of curcumin

At the 6-hour incubation, the ovicidal activity was not detected in all curcumin dilutions in this study ($P>0.05$).

The lowest embryogenesis rate (75%) was observed only in the highest curcumin dilution (36.8 mg/ml) at the 12 and 24 hours-incubations (Table 1). Only one or two damaged blastomers were observed in the undeveloped eggs of curcumin groups. The embryogenesis rate was lower in the curcumin dilutions than in the positive controls at the 12 and 24 hours-incubations, but the difference was not found statistically significant (P>0.05). Concerning the larval development, there was no difference in other curcumin dilutions at these incubation steps (12 and 24 hours). No changes in shell structure were observed in the eggs in all groups. Depending on the curcumin concentration and the length of incubation, the eggshell's color ranged from gray to yellow (Figure 1). The eggshell's color was not changed in the positive control (Figure 2). It was observed that the larvae were moved inside the eggs in all groups.

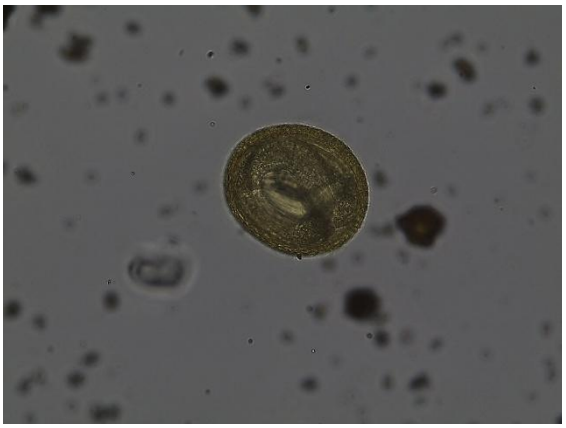

Figure 1. The larvae developed in *Toxocara canis* eggs after being incubated with the curcumin dilution (36.8 mg/ml) for 6 hours (x 40)

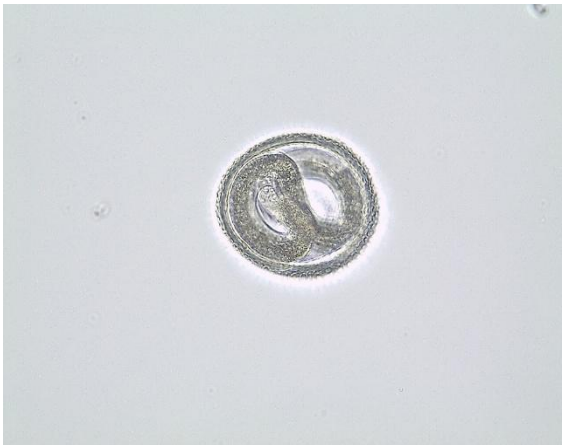

Figure 2. The larvae developed in *Toxocara canis* eggs after being incubated with pyrantel pamoate (725 µg/ml) for 6 hours (x40)

The larvicidal activity of curcumin

At the six-hour incubation, the motile larvae rate was similar (89-92%) in all curcumin dilutions, while motile larvae were not observed in the positive control at this time (Table 1). The motile larvae rates were detected as 80% and 76% in the highest curcumin dilution (36.8 mg/ml) at 12 and 24 hours incubations, respectively (Figure 3). Dead larvae were observed immobile and straight in shape in the curcumin groups (Figure 4). All larvae were observed as motionless and flat in the positive control at 12 and 24 hours incubations, and stained with trypan blue dye solution.

After incubating with 36.8 mg/ml curcumin for 24 hours, the larvae can live only for four days in RPMI-1640. But the untreated larvae were more motile at this incubation step.

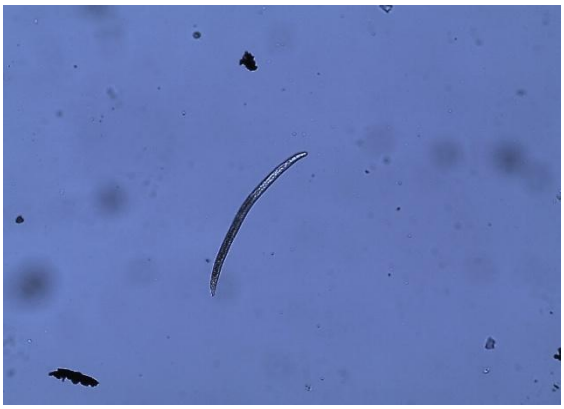

Figure 3. Dead *Toxocara canis* larva after being incubated with the curcumin dilution (36.8 mg/ml) for 24 hours (x10)

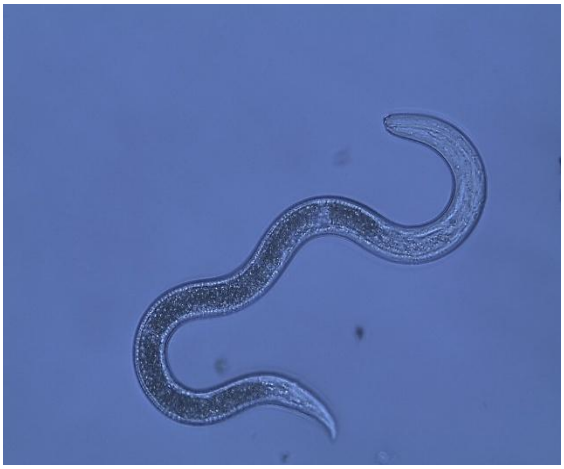

Figure 4. Live *Toxocara canis* larva after being incubated with the curcumin dilution (36.8 mg/ml) for 12 hours (x40)

Table 1. Larval development rate in *Toxocara canis* eggs and the viability rate of the larvae incubated with different doses of curcumin for different times

Incubation time (hours)	Curcumin dilutions (mg/kg)	The larvae development rate in the eggs (%)	The viability rate of the larvae (%)
6	36.8	90[a]	89[x]
	18.4	85[a]	92[x]
	3.6	93[a]	90[x]
	PC	92	0
	NC	95	90
12	36.8	75[b]	80[y]
	18.4	91[b]	93[y]
	3.6	88[b]	90[y]
	PC	93	0
	NC	98	85
24	36.8	75[c]	76[z]
	18.4	91[c]	84[z]
	3.6	89[c]	93[z]
	PC	97	0
	NC	92	84

PC: Positive control
NC: Negative control
[a,b,c,x,y,z] The difference was not found statistically significant (P>0.05)

DISCUSSION AND CONCLUSION

Curcumin reduces egg production by the female parasite (Magalhaes et al., 2009). It alters the activity in many genes in the parasite, including significant signaling pathways that affect embryogenesis and oogenesis (Morais et al., 2013). Curcumin disrupts the shell structure of Schistosoma spp. eggs and adversely affects the larvae inside (Abou El Dehab et al., 2019). In this study, disruption of the eggshell was not detected depending on curcumin incubation. *T. canis* egg possesses a strong eggshell consisting of five layers (Bouched et al., 1986). The shell protects the eggs from the detrimental effects of disinfectants and chemicals besides strong environmental conditions (Aycicek et al., 2001). Curcumin dilutions in the current investigation had some negative effects on the development of *T. canis* eggs. The highest ovicidal activity (75%) was observed in the eggs at 12-hour incubation with 36.8 mg/ml curcumin dilutions. A similar result was observed in the 24-hour incubation. The larva development rate was lower in all curcumin dilutions than in the positive controls at the 12- and 24 hours-incubations.

Flavonoids, including curcumin, are low molecular-weight polyphenols and they have therapeutic potential for some diseases. The potential anthelmintic effects of curcumin are reported on mainly trematodes and cestodes (El-Bahy and Bazh, 2015; Ullah et al., 2017; Abou El Dehab et al., 2019; Martínez-González et al., 2022). Few studies have examined the effectiveness of curcumin on nematodes (Bazh and Bahy, 2013; Hamed et al., 2022). The efficacy of curcumin is dependent on in vitro concentration and incubation time, with the highest effect on adult *A. galli* reported after 48 hours of incubation at a dilution of 100 mg/ml curcumin (Bazh and Bahy, 2013). Caroccia et al. (2013) reported that *T. canis* larvae mobility decreased after incubation with curcumin dilutions (0.01, 0.05 and 0.1 mg/ml) for 48 and 72 hours. In the present study, the larvicidal activity of the curcumin dilutions (36.8 mg/ml, 18.4 mg/ml and 3.6 mg/ml) was limited to the infective stage *T. canis* larvae. No significant difference was found between the viability rates of infective stage *T. canis* larvae at all incubations with all curcumin dilutions, and the viable larvae rate was still higher (75%) even after 24 hours of incubation at the highest curcumin dilution (36.8 mg/ml).

Curcumin can regulate certain parasites' ion channels, receptors, and enzyme structures and functions, thus leading to deterioration in the physiology and death of parasites in vitro. In addition, it penetrates the tegument of platyhelminths, affects glycogen stores, and leading disruption of energy metabolism. It is also reported to interfere with enzymatic systems involved in muscle coordination (Faixova et al., 2021). After being incubated with curcumin at 60 µM concentration in vitro, adult *F. gigantica* were alive: however, a significant decrease in motility, tegumental distortions in the anterior and posterior regions, and erosion of the tegumentary spines of the parasite have been reported (Ullah et al., 2017). At doses of 50 and 100 µM, curcumin kills *S. mansoni*, and some morphological abnormalities have been observed on the parasite's surface (Magalhaes et al., 2019). Curcumin decreased the movement of *R. cestillus* depending on the concentration (25, 50 and 100 mg/ml) (El-Bahy and Bazh, 2015). In the present study, the viability rate was observed as 74% in the infective stage *T. canis* larvae after being treated with 36.8 mg/ml curcumin for 24 hours: however, the shortened life span was detected in the treated larvae compared to the untreated group. Faixová et al. (2021) reported that decreasing parasite motility triggered possibly impaired Na+ –K+ transport due to the tegumental changes caused by curcumin. Mostly *R. cesticillus* (65-80%) disappeared after 48 hours of exposure to curcumin at concentrations of 25 or 100 mg/ml in vitro. The detrimental effect of curcumin is primarily seen in the tegument of *R. cesticillus*, to cause death by affecting the metabolism of glucose absorption/penetration in the parasite (El-Bahy and Bahzy, 2013).

Tegumental damage affects excretory/secretory processes, alters signalling pathways, and affects metabolic pathways in the parasite (Ullah et al., 2017; Abou El Dehab et al., 2019; Rehman et al., 2020; Faixová et al., 2021). *Toxocara canis* infective stage larvae require minimal support to survive in vitro (Bowman, 2020). Cell culture mediums like RPMI-1640 contain some chemicals

which important to surviving *T. canis* larvae in vitro. *Toxocara canis* larvae can survive in the cell culture media for a long time (Bowman, 2020). In the current investigation, infective stage *T. canis* larvae only survived in RPMI-1640 for 4 days after being exposed to 36.8 mg/ml curcumin dilution for 24 hours. The untreated larvae were still motile at this point. It was assumed that curcumin could interfere with the energy metabolism of *T. canis* L3, which would cause the larvae to lose all of their glycogen reserves and eventually die.

Curcumin has potential anthelmintic properties against *A. galli* in experimentally infected chickens (Bazh et al., 2013). The antiparasitic activity of curcumin (1000 mg) is relatively low in chickens infected with *R. cesticillus* contrary to in vitro experiments (El-Bahy and Bazh, 2015). Curcumin reduces the parasite load in mice experimentally infected with *T. spiralis*, moreover, the anti-inflammatory, antioxidant, and anti-angiogenic properties of curcumin help to reduce the trichinellosis-related pathology (Hamed et al., 2022). Triggered apoptotic-like activities and increased oxidative stress caused by curcumin, reduce the parasite's ability to survive in the host (De Paula Agular et al., 2016; Rehman et al., 2020; Faixová et al., 2021). In the present study, curcumin shortened the life span of the *T. canis* infective stage larvae in vitro. Shortening the life span of the larvae could affect the migration of the infected larvae of *T. canis* in the host tissues. Most of the infective stage *T. canis* larvae reach the host liver within 24 hours of post-infection and then they migrate to the lungs (Oge, 2018). The shortening life span of infective stage *T. canis* larva depending on curcumin needs to be tested in animal models experimentally infected.

The bioavailability of curcumin is relatively low because of inadequate intestinal absorption and quick metabolism in the liver (Shehzad et al., 2017). Curcumin is mostly converted to conjugated curcumin form in the digestive system after oral ingestion, but it is reduced to dihydrocurcumin, tetrahydrocurcumin and hexahydrocurcumin when administered intraperitoneally or intravenously (Prasad et al., 2014). Data on curcumin bioavailability is very limited in dogs. Liposomal curcumin is metabolised to tetrahydrocurcumin after being administered intravenously in Beagle dogs (total dose of 10 mg/kg for 2 hours) and the plasma half-life is 0.4-0.7 hours (Helson et al., 2012). After feeding with curcumin-rich food, red blood cells, neutrophils and lymphocytes numbers have been increased in Beagle dogs naturally infected with some pathogens, which is reported as the result of the anti-inflammatory effect of curcumin (Campigotto et al., 2020). According to the Author's opinion, a curcumin-rich diet may have negative effects on both the adult *T. canis* residing in the intestine and the infective stage *T. canis* larvae in dogs.

In the present study, the low ovicidal activity was detected in the highest curcumin dilution at 12 hours-incubations. The effect of curcumin on the larval viability was not found statistically significant at the doses and incubation stages determined in this study. The infective stage *T. canis* larvae can only survive for four days after 24 hours-incubation with 36.8 mg/ml curcumin. The effect of curcumin, which is absorbed at low levels from the intestine and metabolized rapidly in the body, on the migration of *T. canis* infective stage larvae in animal models can be revealed by future studies.

Acknowledgement

This study was summarised from the Master's thesis of Selma Kocademir. We would like to thank Vet. Med. Gozde Nur Akkus of the Parasitology Department at Kirikkale University's Faculty of Veterinary Medicine for her valuable assistance with the lab work. We also would like to thank Prof. Adil Korkmaz of Akdeniz University's Econometry Department for statistical analysis.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: S.K., K.Y., Design: S.K., K.Y., Data Collection or Processing: S.K., K.Y., Analysis or Interpretation: S.K., K.Y., Literature Search: S.K., K.Y., Writing: S.K., K.Y.

Financial Support

This study was financially supported by Kirikkale University Scientific Research Projects Coordination Unit (Grant code: 2022/029).

REFERENCES

Abou El Dehab MM, Shahat SM, Mahmoud SSM, Mahan NA. 2019. In vitro effect of curcumin on *Schistosoma* species viability, tegument ultrastructure and egg hatchability. Experimental Parasitology, 199:1-8.

Abou-El-Naga IF. 2018. Developmental stages and viability of *Toxocara canis* eggs outside the host. Biomedica, 38: 189-197.

Aycicek H, Yarsan E, Sarimehmetoğlu H, Tanyuksel M, Girginkardesler N, Ozyurt M. 2001. Efficacy of some disinfectants on embryonated eggs of *Toxocara canis*. Turkish Journal of Medical Sciences, 31: 35-39.

Bazh E, Bahy N. 2013. In vitro and in vivo screening of anthelmintic activity of ginger and curcumin on *Ascaridia galli*. Parasitology Research, 11: 3679-3686.

Bigford GE, Del Rossi G. 2014. Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders. Advances in Nutrition, 5: 394-403.

Bouchet F, Boulard Y, Baccain D, Leger N. 1986. Ultrastructural studies of alterations induced by microwaves in *Toxocara canis* eggs: prophylactic interest. Zeitschrift für Parasitenkunde, 72: 755-764.

Bowman DD. 2020. The anatomy of the third-stage larva of *Toxocara canis* and *Toxocara cati*, in: Bowman DD. (Ed), Toxocara and Toxocariasis. 1st ed, Academic Press, London, England, 39-61.

Campigotto G, Alba DF, Sulzbach MM, Dos Santos DS, Souza CF, Baldissera MD, Gundel S, Ourique AF, Zimmer F, Petrolli TG, Paiano D, Da Silva AS. 2020. Dog food production using curcumin as antioxidant: effects of intake on animal growth, health and feed conservation. Archives of Animal Nutrition, 74: 397-413.

Cao J, Wang T, Wang M. 2018. Investigation of the anti-cataractogenic mechanisms of curcumin through in vivo and in vitro studies. BMC Ophthalmology, 18: 48.

Caroccia GHG, Rodolpho JA, Oliveira SRP, Camillo L, Magalhães LG, Anibal FF. 2013. Atividade dos compostos curcumina e albendazol contra o nematodeo *Toxocara canis* in vitro. Revista de Saude Publica, 7: 1-2 (with an English abstract).

Chen J, Liu Q, Liu GH, Zheng WB, Hong SJ, Sugiyama H, Zhu XQ, Elsheikha HM. 2018. Toxocariasis: a silent threat with a progressive public health impact. Infectious Diseases of Poverty, 7: 59.

De Paula Agular D, Brunetto Moreira Moscardini M, Rezende Morais E, Graciano De Paula R, Ferreira PM, Afonso A, Belo S, Tomie Ouchida A, Curti C, Cunha WR, Rodrigues V, Magalhães LG. 2016. Curcumin generates oxidative stress and induces apoptosis in adult *Schistosoma mansoni* worms. PLoS One, 11: e0167135.

El-Bahy NM, Bazh EKA. 2015. Anthelmintic activity of ginger, curcumin, and praziquantel against *Raillietina cesticillus* (in vitro and in vivo). Parasitology Research, 114: 2427-2434.

El-Sayed NM. 2017. Efficacy of *Zingiber officinale* ethanol extract on the viability, embryogenesis and infectivity of *Toxocara canis* eggs. Journal of Parasitic Diseases, 41: 1020-1027.

Faixová D, Hrčková G, Mačák Kubašková T, Mudroňová D. 2021. Antiparasitic effects of selected isoflavones on flatworms. Helminthologia, 58: 1-16.

Hamed AMR, Abdel-Shafi IR, Elsayed MDA, Mahfoz AM, Tawfeek SE, Abdeltawab MSA. 2022. Investigation of the effect of curcumin on oxidative stress, local inflammatory response, COX-2 expression, and microvessel density in *Trichinella spiralis* induced enteritis, myositis and myocarditis in mice. Helminthologia, 59: 18-36.

Helson L, Bolger G, Majeed M, Vcelar B, Pucaj K, Matabudul D. 2012. Infusion pharmacokinetics of Lipocurc (liposomal curcumin) and its metabolite tetrahydrocurcumin in Beagle dogs. Anticancer Research, 32: 4365-4370.

Hombu A, Yoshida A, Kikuchi T, Nagayasu E, Kuroki M, Maruyama H. 2019. Treatment of larva migrans syndrome with long-term administration of albendazole. Journal of Microbiology Immunology Infection, 52: 100-105.

Kocademir S, Yıldız K. 2022. *Toxocara canis* and visceral larvae migrans. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni, 13: 47-54.

Magalhaes LG, Machado CB, Morais ER, Moreira EB, Soares CS, da Silva SH, Da Silva Filho AA, Rodrigues V. 2009. In vitro schistosomicidal activity of curcumin against *Schistosoma mansoni* adult worms. Parasitology Research, 104: 1197-1201.

Martínez-González JJ, Ríos-Morales SL, Guevara-Flores A, Ramos-Godinez MDP, López-Saavedra A, Rendón JL, Del Arenal Mena IP. 2022. Evaluating the effect of curcumin on the metacestode of *Taenia crassiceps*. Experimental Parasitology, 239: 108319.

Mata-Santos T, Pinto NF, Mata-Santos HA, De Moura KG, Carneiro PF, Carvalho Tdos S, Del Rio KP, Pinto Mdo C, Martins LR, Fenalti JM, Da Silva PE, Scaini CJ. 2015. Anthelmintic activity of lapachol, β-lapachol and its derivatives against *Toxocara canis* larvae. Revista do Instituto de Medicina Tropical de São Paulo, 57: 197-204.

Morais ER, Oliveira KC, Magalhaes LG, Moreira ÉBC, Verjovski-Almeida S, Rodrigues V. 2013. Effects of curcumin on parasite *Schistosoma mansoni*: A transcriptomic approach. Molecular and Biochemical Parasitology, 187: 91–97.

Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. 2017. The essential medicinal chemistry of curcumin. Journal of Medicinal Chemistry, 60: 1620-1637.

Oge S. 2018. Ascaridoidea. In: Helmintoloji, Doğanay A. (Ed), Ankara Nobel Tıp Kitapevleri, Ankara, 248-275.

Orengo KO, Maitho T, Mbaria JM, Maingi N, Kitaa JM. 2016. In vitro anthelmintic activity of *Allium sativum, Allium cepa* and *Jatropha curcas* against *Toxocara canis* and *Ancylostoma caninum*. African Journal of Pharmacy and Pharmacology, 10: 465-471.

Prasad S, Tyagi AK, Aggarwal BB. 2014. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. Cancer Research and Treatment, 46: 2-18.

Rehman A, Ullah R, Gupta D, Khan MAH, Rehman L, Beg MA, Khan AU, Abidi SMA. 2020. Generation of oxidative stress and induction of apoptotic like events in curcumin and thymoquinone treated adult *Fasciola gigantica* worms. Experimental Parasitology, 209: 107810.

Reis M, Trinca A, Ferreira MJU, Monsalve-Puello AR, Gracio MAA. 2010. *Toxocara canis:* potential activity of natural products against second-stage larvae in vitro and in vivo. Experimantal Parasitology, 126: 191-197.

Sena-Lopes A, Remiao MH, Alves MSD, da Rocha Fonseca B, Seixas FK, Collares T, Borsuk S. 2020. Cell viability analysis of *Toxocara cati* larvae with LIVE/DEAD® Viability/Cytotoxicity kit. Experimental Parasitology, 212: 107871.

Shehzad A, Qureshi M, Anwar MN, Lee YS. 2017. Multifunctional curcumin mediate multitherapeutic effects. Journal of Food Science, 82: 2006-2015.

Ullah R, Rehman A, Zafeer MF, Rehman L, Khan YA, Khan MA, Khan SN, Khan AU, Abidi SM. 2017. Anthelmintic potential of thymoquinone and curcumin on *Fasciola gigantica*. PLoS One, 12: e0171267.

Wang R, Li J, Zhao Y, Li Y, Yin L. 2018. Investigating the therapeutic potential and mechanism of curcumin in breast cancer based on RNA sequencing and bioinformatics analysis. Breast Cancer, 25: 206-212.

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Clinical and Radiologic Evaluation of Dental Diseases in Cats

Nazlıcan Çelik✉, Birkan Karslı

Kırıkkale University, Faculty of Veterinary Medicine, Department of Surgery, Kırıkkale, Türkiye.

ARTICLE INFO

Received: 05/06/2024
Accepted: 20/03/2025

DOI: 10.5281/zenodo.15109879

✉**Corresponding Author:** nazlican.ccelik@gmail.com

Keywords

Dental radiography
Feline dental disease
Tooth resorption

Cite this article as: *Çelik N. and Karslı B. 2025. Clinical and Radiologic Evaluation of Dental Diseases in Cats. International Journal of Veterinary and Animal Research, 8(1): 12-17. DOI: 10.5281/zenodo.15109879.*

ABSTRACT

This study investigated the prevalence of dental diseases in cats, with a focus on the distribution of age and gender among effected individuals. A total of 50 cats were included, comprising 21 females (42%) and 29 males (58%). Age distribution revealed that 24 cats (48%) were kitten (0-11 months), 22 (44%) were adults (1-5 years), and 4 (8%) were elderly (≥6 years). Comprehensive clinical and radiographic evaluations were performed to assess the condition of the pulp, resorption, and the overall dental health, including the status of deciduous and permanent teeth, tooth mobility, dental plaque, halitosis, salivation, and mucous membrane appearance. The condition and number of deciduous and permanent teeth, mobility of teeth, dental plaque status, bad breath, salivation, and the appearance of mucous membranes were assessed. The findings showed that 44 cats (88%) presented with at least one dental disease, affecting a total of 125 teeth. Alarmingly, none of the owners practiced routine oral or dental care for their cats, and only a minority had any awareness of feline dental health. These results underscore the widespread neglect of oral hygiene in cats and highlight the urgent need for educational initiatives to improve awareness and management of feline dental health in the general population.

INTRODUCTION

Teeth (dentes) are the formations that enable the tearing, breaking, and crushing of foodstuffs into the mouth. They are the hardest anatomical formations of the body (Dursun, 1995). Domestic animals' morphology and dental configuration exhibit significant variations depending on the species. The formation of teeth is intricately linked to one's dietary habits. Carnivores possess pointed teeth designed for tearing or pulling, while herbivores have flatter teeth adapted for grinding or crushing. The teeth of carnivores also function as effective defensive weapons (Samsar and Akın, 2002).

Cats' most common dental diseases are feline odontoclastic resorptive lesions, periodontal disease, broken teeth, lost teeth, and persistent deciduous teeth. Tooth resorption, also known as feline odontoclastic resorptive lesion (FORL), neck lesions, and feline cavities, is a common condition for cats. Tooth resorption clinically affects slightly less than half of the feline population, according to the majority of studies. This figure can be elevated to 75% through the use of intraoral radiographs. The most significant consequences of tooth resorption are the resorption of the tooth and the proliferation of the gingiva or pulp to conceal the resulting lesion. The main symptom of tooth resorption is pain sensation (Holmstrom, 2013). The roots of deciduous teeth are typically resorbed as the permanent teeth erupt in dogs and cats from 14 weeks of age. Permanent teeth typically emerge at six months of age in the majority of dogs and cats. The mechanism that induces primary root resorption and the mechanism that prevents root resorption still need to be fully comprehended. One of the causes of deciduous tooth loss is the pressure generated by the eruption of the underlying permanent tooth during the eruption (Bellows, 2019).

Enamel and dentin loss are typically detectable on radiography due to crown fractures. A fracture line is visible between the tooth and the portion of the tooth that has not yet fully separated in crown and crown-root fractures. A root fracture may manifest in any region of the tooth. In incisive teeth, root fractures are typically transverse and oblique. Longitudinal crown-root fractures are less common than transverse and oblique fractures. In dogs and cats, root remnants are often seen on radiographs

(Reiter et al., 2018). While tooth fractures affecting the pulp are called complicated, tooth fractures where the pulp is not influenced are called uncomplicated crown fractures. Enamel is formed by cells called ameloblasts (Holmstrom, 2013). Defects in enamel formation may result from hereditary or inflammatory conditions during tooth development. Local inflammation can affect a single tooth, while systemic inflammation can affect all teeth. These events can induce microscopic changes, resulting in enamel hypoplasia, a condition characterized by a tooth with thin enamel that is susceptible to damage. Enamel hypoplasia is caused by insufficient levels of enamel matrix (Bellows, 2019). Periodontal disease is an inflammation and infection of the tissues surrounding the tooth, collectively called the periodontium (Holmstrom, 2013). The process of tissue destruction is caused by subgingival plaque, acute inflammation, and prostaglandin-induced bone resorption (Holmstrom et al., 2004). Plaque is a biofilm formed by glycolic bacteria and saliva. It is not easy to see unless stained using a long-wave ultraviolet light source and fluorescein. The stain darkens as the plaque thickness on the tooth surface increases. A tartar is a calcified deposit made up of minerals that come from saliva. Tartar is predominantly found on the outer side of molars and premolars, as well as on the inner side of incisors near the openings of salivary ducts (Lane, 1981).

Pulpitis may be either reversible or irreversible. Sometimes, pulpitis can lead to dystrophic mineralization of the pulp, which can result in the pulpal cavity being completely lost or narrowed in a localized area. This dystrophic mineralization is not to be confused with pulp stones, which are intrapulpal mineralized structures that are unrelated to the current disease (Reiter and Gracis, 2018). Anodontia, the lack of teeth, can occur in cats and dogs. Teeth may be missing because they never developed in the first place, erupt slowly, or are present but fall out (Holmstrom, 2013). Dental radiographs should be taken in animals suspected to be missing some teeth (Tutt, 2006). Supernumerary teeth are typically observed in incisive teeth; however, the entire tooth may be supernumerary. Supernumerary teeth can result in the misplacement of other teeth, the failure of teeth to erupt, or the accumulation of severe plaque and periodontal disease (Holmstrom, 2013).

The aim of this study is to identify dental problems by performing radiographic and clinical examinations of the oral cavity and dental diseases in cats brought to clinics with dental problems or other complaints.

MATERIALS AND METHODS

Permission was obtained from the “Kırıkkale University Clinical Practices Ethics Committee”, and the animal owners were informed about the study, and informed consent and anesthesia authorization form was obtained for this study. The study used 50 cats of different breeds, ages, sexes, and reasons for complaints in Kırıkkale University Faculty of Veterinary Medicine. All of the patients went through clinic and radiological examination. A standardized dental examination form (Figure 2) was developed to streamline data collection and analysis. This form included information on patient's age, breed, sex, current home care practices, diet, and specific complaints. The anesthesia protocol and the clinical and radiological procedures required for the detailed examination were explained the owner beforehand.

For the examination, anesthesia was induced with 40 µg/kg medetomidine (Domitor, Finland, Zoetis) and 5 mg/kg ketamine HCL (Ketasol, Austria, Interhas), administered intramuscularly. An endotracheal tube was used to intubate patients who were under general anesthesia. All through the anesthesia, patients were administered intravenously 5-10 ml/kg/h of 0.9% isotonic solution.

The patient underwent a comprehensive clinical examination under anesthesia. Assessment included the condition and number of deciduous and permanent teeth, tooth mobility, dental plaque, mucous membranes appearance, salivation, and halitosis. The examination form was utilized to document the clinical examination findings. Findings from the clinical examination were documented using a standardized examination form. Following administration of appropriate anesthetic doses, the patient was positioned for radiographic imaging. In order to image maxillary premolars and molars, the film was placed flat along the palatine and the x-ray was sent laterally at 45° angle (Niemec, 2014). While using bisecting angle technique, arcus zygomaticus maxillary 4th premolar, 1st molar and even maxillary 3rd premolar distal root from being imaged. That is the reason why extra oral technique was used here. The film was placed on the table. The area of the patient to be imaged was positioned facing downwards, and the x-ray was sent at an approximately 30° angle (Loprise et al., 2019).

For maxillary canines and inciciv tooth, the x-ray tube and film were positioned in a parallel technique, then the x-ray tube was given a rostro-caudal at an angle of either 20 or 70° Niemec, 2015). In order mesial root of the maxillary 4th premolar, the tube head was positioned at the degree 45 angle in the vertical plane. It was then rotated approximately at a 30° distally in the horizontaly plane (Niemec, 2015).

While imaging mandibulary canine and inciciv tooth, the patient was put in the ventrodorsal position. Parallel technique was used for mandibular molar and caudal premolar radiographs (Volker, 2019). A 90° angle was used for mandibular premolars and molars. Niemec, 2014) Radiographs were taken using parallel, simplified, bisecting angle technique ans extra-oral techniques (Figure 1). The examination form was also utilized to evaluate and document pulp status and the presence of resorption observed in the radiographic images (Figure 2).

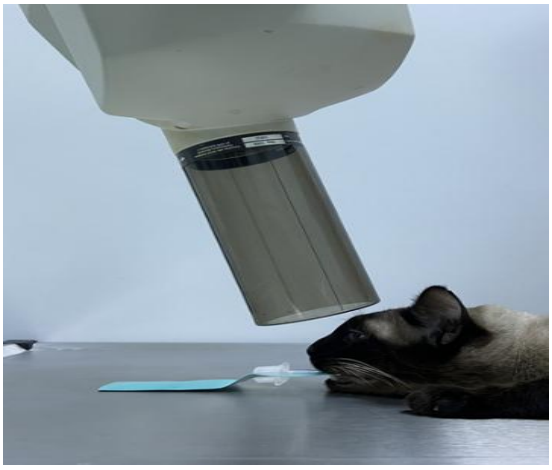

Figure 1. Visualization of maxillary canine and incisive teeth

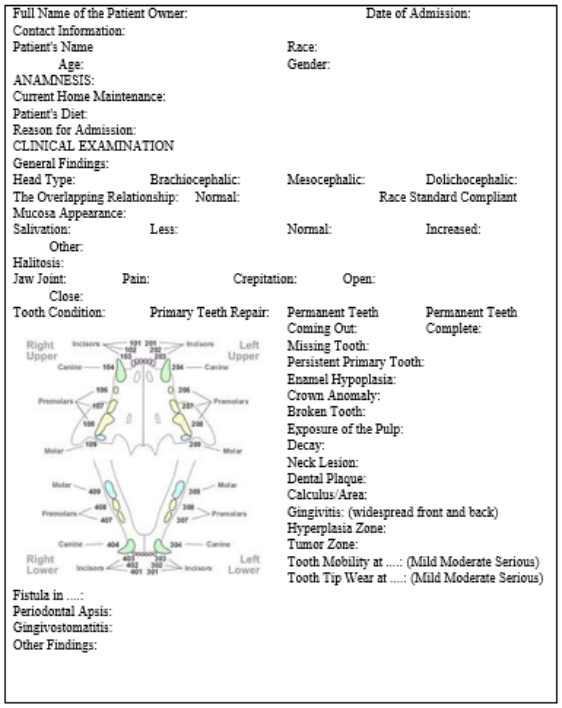
Full Name of the Patient Owner: Date of Admission:
Contact Information:
Patient's Name Race:
Age: Gender:
ANAMNESIS:
Current Home Maintenance:
Patient's Diet:
Reason for Admission:
CLINICAL EXAMINATION
General Findings:
Head Type: Brachiocephalic: Mesocephalic: Dolichocephalic:
The Overlapping Relationship: Normal: Race Standard Compliant
Mucosa Appearance:
Salivation: Less: Normal: Increased:
Other:
Halitosis:
Jaw Joint: Pain: Crepitation: Open:
Close:
Tooth Condition: Primary Teeth Repair: Permanent Teeth Coming Out: Permanent Teeth Complete:
Missing Tooth:
Persistent Primary Tooth:
Enamel Hypoplasia:
Crown Anomaly:
Broken Tooth:
Exposure of the Pulp:
Decay:
Neck Lesion:
Dental Plaque:
Calculus/Area:
Gingivitis: (widespread front and back)
Hyperplasia Zone:
Tumor Zone:
Tooth Mobility at ....: (Mild Moderate Serious)
Tooth Tip Wear at ....: (Mild Moderate Serious)
Fistula in ....:
Periodontal Apsis:
Gingivostomatitis:
Other Findings:

Figure 2. Dental examination form

RESULTS

Data related to the sex and age of the cats used in the research is shown on the table (Table 1).

Table 1. Percentage of disease based on age and sex

Sex and Age Group	Number of Cats Examined	Number of Cats with Disease Detected	Percentage of Disease
Female	21	18	85,7%
Male	29	26	89,6%
Kitten (0-11 mounths old)	24	20	83,3%
Adult (1-5 years old)	22	20	90,9%
Elder (+6 years old)	4	4	100%

Clinical and radiologic examination revealed disease in 44 of 50 cats and 125 teeth in total. There were 31 cases in canines, 22 in incisives, 50 in premolars, and 22 in molars. Most cases of persistent deciduous teeth were seen in canines (Figure 3), while cases of missing teeth were seen in incisives, premolars, and molars (Table 2).

Table 2. Disease distribution based on tooth types

	1st Degree Tooth Resorption	2nd Degree Tooth Resorption	3rd Degree Tooth Resorption	4th Degree Tooth Resorption	5th Degree Tooth Resorption	Missing Tooth	Persistent Deciduous Tooth	Crrown Non-complicated Fractured	Crown Complicated Fractured	Complicated Root Fractured	2nd Degree Plaque	3rd Degree Plaque	4th Degree Plaque	Discolorization
Canin Teeth	-	-	-	-	-	-	16	6	5	-	4	-	-	-
Inciciv Teeth	-	-	1	2	-	19	-	-	-	-	-	-	-	-
Premolary Teeth	1	1	1	2	-	18	-	-	-	1	2	3	13	1
Molary Teeth	1	1	7	1	2	8	-	-	-	-	1	-	1	-

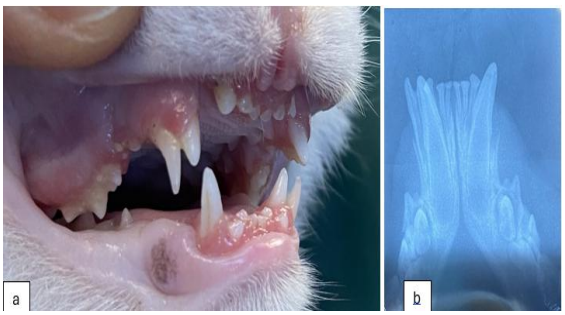

Figure 3. Persistent deciduous tooth case (a) and radiographic image (b)

Periodontal disease was the most prevalent condition, affecting 16 patients. In 14 patients, tooth resorption followed periodontal disease (Figure 4). After radiological examination, loss of density was seen in 14 cats in various tooth (Table 3). The number of cats with broken teeth was 8. 4 of these cats were male, and 3 were female. Of the cats with fractured teeth, 5 were kittens, 2 were adults and 1 was elderly. 11 fractured teeth were canine, and 1 was premolar (Figure 5). After radiographic examination, it was found that 3 cats had uncomplicated crown fractures without pulp exposure, 4 cats had complicated crown fractures, and 1 cat had complicated crown-root fractures. (Table 3) Out of the 11 fractured canine teeth, 6 of them were uncomplicated cron fractures, and 5 of them were

complicated crone fractures. Whereas, complicated cron root fracture was seen in the fractured premolar teeth. Out of the 11 fractured canine teeth, 6 of them were uncomplicated cron fractures, and 5 of them were complicated crone fractures. Whereas, complicated cron root fracture was seen in the fractured premolar teeth. Periodontal disease was seen in 16 of the cats used in the study. Of these cats, 5 were female and 11 were male. 6 of the cases were kittens, 8 were adults, and 2 were elderly. Dental tartar was encountered in 10 of the cats. Of these, 4 were females, 6 were males, 2 were kittens, 7 were adults, and 1 was elderly. 2 cats were level 2, 2 cats were level 3, and 6 cats were level 4. Tartar was seen on 24 teeth in total. Of these teeth, 4 were canines, 18 were premolars, and 2 were molars. A total of 9 cats had cases of missing teeth. Of these cats, 4 were females and 5 were males. Of the cats with missing teeth, 2 were kittens, and 7 were adults. A total of 45 missing teeth were detected in 9 cases. Of these teeth, 19 were incisors, 18 were premolars, and 8 were molars. Spotting was observed in 4 of the cats used in the study. 3 of these cats were male, and 1 was female. 2 of the cats were kittens, and 2 were adults. In these 4 cases, staining was seen in a total of 7 teeth, all of which were premolars. Discoloration of premolar teeth was detected only in 1 kitten and 1 female cat. In addition, an intraoral mass was detected in 1 adult female cat (Figure 6) (Table 3).

Table 3. Disease distribution based on age

	Disease	Number of Cats with Disease Detected	Kitten	Adult	Elder
1	Tooth Resorption	14	3	8	3
	1st Degree	1	-	1	1
	2nd Degree	2	-	2	-
	3rd Degree	9	3	4	2
	4th Degree	4	-	3	1
	5th Degree	2	-	2	-
2	Missing Tooth	9	2	7	-
3	Supernumerary Tooth	-	-	-	-
4	Persistent Deciduous Tooth	6	6	-	-
5	Enamel Hypoplasia	-	-	-	-
6	Fractured Tooth	8	5	2	1
	Enamel Fractured	-	-	-	
	Non-complicated Crown Fractured	3	1	1	1
	Complicated Crown Fractured	4	3	1	-
	Non-complicated Crown – Root Fractured	-	-	-	-
	Complicated Crown – Root Fractured	1	1	-	-
	Root Fractured	-	-	-	-
	Plaque	10	2	7	1
	1st Degree	-	-	-	
	2nd Degree	2	-	2	-
	3rd Degree	2	1	1	-
	4th Degree	6	1	4	1
8	Pulpitis	-	-	-	-
10	Discolorization	1	1	-	-
11	Periodontal Disease	16	6	8	2
	1st Degree	3	2	1	-
	2nd Degree	2	1	2	-
	3rd Degree	6	1	3	2
	4th Degree	5	2	3	-
12	Oral Neoplasia	1	-	1	-

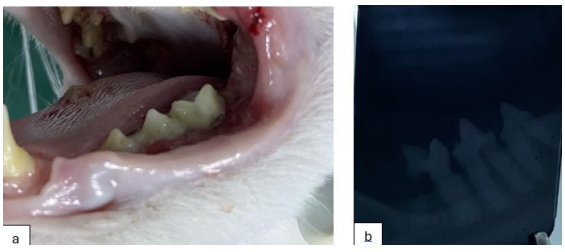

Figure 4. A case of resorption in a mandibular molar (a) and its radiologic appearance (b)

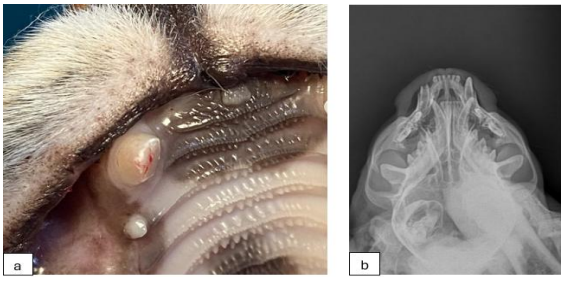

Figure 5. A case of a fractured canine tooth (a) and its radiologic appearance (b)

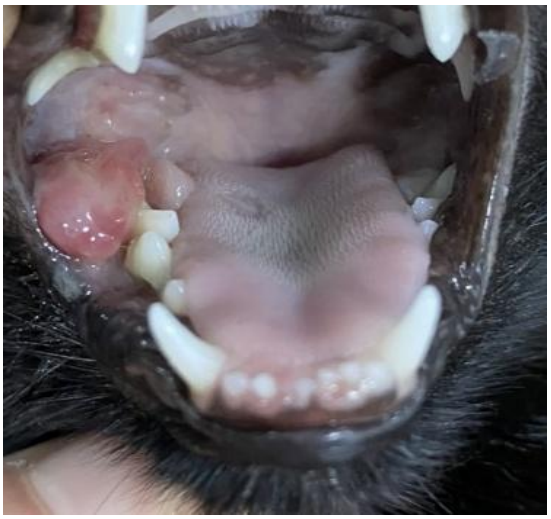

Figure 6. Intraoral mass

It was determined that none of the cases received any oral or dental health care during their daily lives.

DISCUSSION AND CONCLUSION

In a study of 18.249 cats in the United Kingdom, dental diseases were found to be the 3rd most common disease in cats (O'Neill et al., 2023). The literature review found that the incidence of dental diseases in cats increases with age (Larsen, 2010). In the study, 83% of kittens, 90% of adult cats, and 100% of older cats had the disease.

In diagnosis of dental diseases, the importance of dental radiographics is significant, but the radiograph must be interpreted correctly. Factors like bone density, pulpa's condition and monitoring periodontal ligament must be carefully evaluated (Martinez et al., 2022). Radiological imaging was used in all cats in the submitted research. Dental radiographs supported in diagnosing many diseases especially tooth resorption and fractured tooth as well as shaping the proper treatment.

Tooth resorption is commonly seen in cats and radiographic imaging is crucial to diagnose it. Even a very detailed dental examination may not be 100% affective for tooth resorption (Lobprise et al., 2019). Studies have determined that the prevalence of tooth resorption in cats ranges from 28 to 67% (Karslı and Pekcan, 2020). Nevertheless, the disease was not influenced by age or gender; however, it was more prevalent in older cats; tooth resorption was reported in 16% of cats under 6 years of age and 74% of cats over 6 years of age (Coles, 1990, Pistor et al., 2023). Other studies found that premolars and molars had the most common lesions (Pistor et al., 2023), while Ingham et al.'s study on 228 cats found symmetrical resorption cases most frequently in teeth numbered 307 and 407 (Ingham et al., 2001). In the study, we identified Resorption cases in 28% of the cats. Adult patients accounted for 57% of resorption cases, while elderly patients accounted for 21%. The most prevalent type of dental resorption was observed in molars, with most cases being symmetrical and occurring in teeth with numbers 309 and 409. Althouhg there are a couple of theories, the reason for tooth resorption is not known yet. Different theories have been put forward, such as high levels of vitamin D or the abfraction injuries that are caused by violation in dry food diets (Schaer et al., 2019). According to another theory, tooth resorption is caused by the acids occurred while vomiting furball (Holmstrom, 2013). Considering these theories, it can be thought that it is more common in older cats, because the time the cat is exposed to the mentioned situations increases at the same rate as it gets older.

Tutt (2006) has reported that the persistence of mandibular and maxillary canine teeth in cats is uncommon in comparison to dogs. According to another study, persistent deciduous teeth are also more common in dogs, especially in small and miniature breeds (Schaer et al., 2019). A comparison between cats and dogs was not possible due to the absence of dogs in the study, and persistent deciduous teeth were observed in 12% of the cats used in the study. The canine teeth were the only persistent deciduous teeth observed, and all of the cats were between 0 and 11 months old.

The reported prevalence of periodontal disease in cats ranged from 13.9% to 96%. Although many risk factors such as breed, age, sex, and body weight have been reported for periodontal disease in dogs, the literature is very limited in cats (O'Neill et al., 2023). A study conducted in France revealed that Persian and Maine coon cats were more susceptible to periodontal disease. Conversely, other studies could not determine the prevalence of mongrel or purebred cats due to insufficient results (Girard et al., 2009; Lommer et al., 2001). The literature review showed that gender and neutering status were not associated with periodontal disease in cats (Girard et al., 2009). Periodontal disease was observed in 32% of the cats used in the study. No breed discrimination was observed. In the present study, 61% of the 18 cats with periodontal disease were male. But it would not be correct to say that male cats are predisposed just with these results.

In a study conducted in North America, it was reported that the incidence of dental tartar in cats of all ages was 13-24% (Lund et al., 1999). In this study, dental tartar was seen in 20% of the cats. Of the cats with dental tartar, 2 were kittens, 7 were adults and 1 was an elderly cat.

There is a lack of substantial literature regarding feline enamel hypoplasia, pulpitis, supernumerary teeth, lost teeth, and fractured teeth. The majority of the research was conducted on dogs. The frequency of dental diseases in cats, the distribution of age and sex, and the practices of home care were all examined in this study. Although at least one disease was identified in 88% of the cats that participated in the study, it was noted that none of the owners provided home care for oral and dental health. Nearly none of them had any knowledge. It is thought that oral and dental health needs to be sufficiently emphasized in our country, and patient owners need to be informed. We veterinarians have a great job in this regard. The quality of life of a cat is significantly diminished by its poor oral and dental health. Simple home applications can be implemented to safeguard dental and oral health. Particularly when the patient is accustomed to oral care during kittenhood, the patient will permit the care to be administered without causing any issues for the owner in adulthood.

In conclusion, it was observed that dental diseases in cats increase with age. It is also thought that inadequate oral care is effective in this situation. Males were more likely to have dental diseases than females, but since there was no significant difference, gender is not thought to have an effect on dental diseases. Through comprehensive literature reviews, it has been noted that there needs to be more sufficient oral and dental research specifically focused on cats compared to dogs. It was determined that

Turkish patient owners should be educated about oral and dental health. Dental diseases are widely prevalent in cats, and as they advance, they result in intense pain, decreased appetite, and rapid deterioration of overall health.

Acknowledgement

-

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: B.K., N.Ç., **Design:** N.Ç., **Data Collection or Processing:** B.K., N.Ç., **Analysis or Interpretation:** N.Ç., **Literature Search:** N.Ç., **Writing:** B.K., N.Ç.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Bellows J. 2019. Small Animal Dental Equipment, Materials and Techniques, second ed. Wiley Blackwell, USA.

Coles S. 1990. The prevalance of buccal cervical root resorptions in australian cats. Journal of Veterinary Dentistry, 7(4): 14-16.

Dursun N. 1995. Veteriner Anatomi 1, second ed. Medisan, Ankara.

Girard N, Servet E, Biourge V, Hennet P. 2009. Periodontal health status in a colony of 109 cats. Journal of Veterinary Dentistry, 26(3): 147-155.

Holmstrom S, Fitch P, Eisner E. 2004. Veterinary Dental Techniques for the Small Animal Practitioner, third ed. Saunders, USA.

Holmstrom S. 2013. Veterinary Dentistry, A Team Approach, third ed. Elsevier Inc, California.

Ingham KE, Gorrel C. 2001. Prevalence of odontoclastic resorptive lesions in a population of clinically healthy cats. Journal of Small Animal Practice, 42(9): 439-443.

Karslı B, Pekcan Z. 2020. Kedilerde diş rezorpsiyonu, in: Genccelep M, Karasu A. (Eds.), Kedi ve köpeklerde ağız ve diş hastalıkları, 1st ed. Türkiye Klinikleri.

Lane G. 1981. Small animal clinic, Small animal dentistry. British Medical Journal, 3(2): 23-30.

Larsen JA. 2010. Oral products and dental disease. Continuing Education for Veterinarians, 32(9): E1-E3

Lobprise, Heidi B. 2019. Wiggs's Veterinary Dentistry, Second ed. Wiley Blacwell, USA

Lommer MJ, Verstraete FJM. 2001. Radiographic patterns of periodontitis in cats: 147 cases (1998–1999). Journal of the American Veterinary Medical Association, 218(2): 230-234.

Lund EM, Armstrong PJ, Kirk CA, Kolar LM, Klausner JS. 1999. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. Journal of the American Veterinary Medical Association, 214(9): 1336-1341.

Niemiec BA. 2014. Feline Dental Radiography and Radiology. Journal of Feline Medicine and Surgery, 16(14): 887-889.

Niemiec BA. 2015. Techniques for Intraoral Radiology. Practical Dentistry, 5: 59-66.

O'Neill DG, Gunn-Moore D, Sorrell S, McAuslan H, Chruch DB, Pegram C, Brodbelt DC. 2023. Commonly diagnosed disorders in domestic cats in the uk and their associations with sex and age. Journal of Feline Medicine and Surgery, 25(2): 1-9.

Pistor P, Janus I, Janeczek M, Dobrzynski M. 2023. Feline tooth resorption: A description of the severity of the disease in regard to animal's age, sex, breed and clinical presentation. Animals, 13(2500): 2-12.

Reiter MA, Gracis M. 2018. BSAVA Manual of Canine and Feline Dentistry and Oral Surgery, fourth ed. BSAVA, England.

Samsar E, Akın F. 2002. Özel Cerrahi, first ed. Medipress, Ankara.

Schaer M., Gaschen F., Walton S. 2019. Köpek ve Kedilerin Klinik Hekimliği, Altuğ N. (Eds.), 3rd ed. Güneş Tıp Kitapevleri, Turkiye, 83-103.

Tutt C. 2006. Small Animal Dentistry, a Manual of Techniques, first ed. Blackwell, USA.

Volker MK, 2019. Principles and Techniques of Dental Radiology. Fetch Kansas City Meeting, Kansas, USA, September 1 2019, 179-82.

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Investigation of Distempervirus and Parvovirus Infections in Dogs

Hatice Pelin Aslım ✉, Irmak Dik

Selcuk University, Faculty of Veterinary Medicine, Department of Virology, Konya, Türkiye.

ARTICLE INFO

Received: 06/02/2025
Accepted: 29/03/2025

DOI: 10.5281/zenodo.15109891

✉**Corresponding Author:** hpelinucan@gmail.com

Keywords

CDV
CPV
ELISA

Cite this article as: *Aslım H.P. and Dik I. 2025. Clinical and Radiologic Investigation of Distempervirus and Parvovirus Infections in Dogs. International Journal of Veterinary and Animal Research, 8(1): 18-23. DOI: 10.5281/zenodo.15109891.*

ABSTRACT

CDV and CPV are significant viral agents that frequently cause fatal infections in both domestic and wild dogs. In this study, a total of 95 animals, including both healthy individuals and those exhibiting gastrointestinal and neurological symptoms, aged between 6 and 24 months, were serologically examined for CDV and CPV infections using the ELISA test. According to the manufacturer's instructions, the assay range for CDV was specified as 0.7 ng/ml to 200 ng/ml, with a sensitivity value of 0.665 ng/ml. Among the total 95 animals, 48 (50.52%) with good general health had antibody titers ranging between 7 and 20, while 9 (9.47%) had titers between 50 and 85. In animals showing lethargy, diarrhea, vomiting, and neurological symptoms, 22 (23.15%) had titers between 7 and 20, 10 (10.52%) between 20 and 35, 12 (12.63%) between 35 and 50, and 4 (4.2%) between 50 and 85. In terms of CPV antibodies, 88 (92.63%) were found to be positive. In conclusion, the study data indicate the necessity of developing and strictly implementing strategies to combat CDV and CPV infections. Further studies are required to investigate the genetic variability of these viruses, the effectiveness of vaccine-induced antibodies in protecting against local strains, and the pathogenesis of the diseases.

INTRODUCTION

Since its discovery in the 1730s, Distemper infection has been observed in Europe and many other parts of the world (Uhl et al., 2019; Saltık and Kale, 2023). Canine Distemper Virus (CDV), which belongs to the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, and genus *Morbillivirus*, is an enveloped, single-stranded RNA virus that primarily infects carnivores but can also cause infections in a wide range of animal species (ICTV, 2024a). Distemper infection, which leads to a multisystemic disease and often results in mixed infections in dogs, is commonly known as "canine distemper" among the public. In general, CDV has an affinity for epithelial tissue, and the initial clinical manifestation is a severe respiratory problem. Additionally, simultaneous or subsequent symptoms affecting the central nervous system and gastrointestinal tract may develop (Saltık and Kale, 2020; Zhao and Ren, 2022; Saltık and Atlı, 2023). The symptoms in animals vary depending on the duration of viral persistence in the body, viral strain, secondary bacterial infections, the host's age, and its immune response (Skyes and Vandevelde, 2021). CDV is typically transmitted through droplet infection, direct contact, or aerosol routes (Shin et al., 2022).

Canine parvovirus (CPV) has been known since the late 1970s and, despite intensive vaccination efforts, remains one of the leading causes of acute gastroenteritis and mortality in puppies worldwide (Decaro et al., 2020; Saltık and Koç 2024). Shortly after its first detection in 1978, CPV-2 reached pandemic proportions, and new antigenic variants, later named CPV-2a, CPV-2b, and CPV-2c, emerged (Grecco et al., 2024). Canine parvovirus (CPV), a DNA virus, belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Protoparvovirus* (ICTV, 2024b). The transmission of CPV-2 occurs primarily through the oronasal route, direct contact, or exposure to contaminated feces. Clinical signs include lethargy, anorexia, vomiting, fever, and severe diarrhea, which may be bloody or non-bloody. The rapid progression of this disease can lead to fatal outcomes in immunocompromised animals (de Oliveira Santana et al., 2022).

In this study, due to the increasing number of pet animals in the Konya region, the aim was to determine the

epidemiological status of distemper and parvovirus infections, which pose a significant threat to canine health.

MATERIALS AND METHODS

Materials

In this study, a total of 95 unvaccinated animals, both healthy and symptomatic, aged between 6 and 24 months, brought to private clinics in the Konya, İzmit, and Antalya regions, were serologically examined for CDV and CPV infections. Gastrointestinal and neurological problems were commonly observed in the dogs. The clinical findings recorded in the dogs are listed in Table 1.

Among the sampled dogs, 50.52% (48/95) were in good general condition, 44.21% (42/95) exhibited lethargy, 16.84% (16/95) showed neurological symptoms, 38.94% (37/95) had diarrhea as a gastrointestinal issue, 12.63% (12/95) experienced vomiting, and 20% (19/95) suffered from cachexia.

When evaluating clinical findings, some dogs exhibited a single symptom, while others presented with two or even three symptoms simultaneously.

Table 1. Clinical findings in sampled dogs

Clinical findings	Positive/n	%
General Condition Good	48/95	50.52
Weakness	42/95	44.21
Tics	16/95	16.84
Diarrhea	37/95	38.94
Vomiting	12/95	12.63
Cachexia	19/95	20

Preparation of Blood Serum Samples

Blood samples were collected from all dogs via the cephalic vein (vena cephalica antebrachii) into biochemistry tubes (BD Vacutainer®). The collected blood samples were centrifuged at 3,000 rpm for 10 minutes.

Indirect ELISA

The prepared blood serum samples were analyzed for the presence of antibodies against CDV and CPV. For this purpose, commercially available Sunred ELISA (Cat No. SRB0543) and Agrolabo ELISA (Cat No. 27224096) test kits were used, following the manufacturer's instructions.

Statistical Analysis

CDV antibody titers and CPV antibody positivity were examined based on the gender and age distribution of the 95 animals included in the study. The animals were grouped into two categories based on gender (male and female) and three age groups (11-15 months, 16-20 months).

RESULTS

According to the manufacturer's instructions, the test result range was 0.7 ng/ml to 200 ng/ml, with a sensitivity value of 0.665 ng/ml. Among the 95 animals: 48 (50.52%) in good general condition had CDV antibody titers between 7-20, while 9 (9.47%) had titers between 50-85. In animals with symptoms such as lethargy, diarrhea, vomiting, and neurological signs: 22 (23.15%) had antibody titers between 7-20, 10 (10.52%) between 20-35, 12 (12.63%) between 35-50, 4 (4.2%) between 50-85. The range of values observed among the sampled animals is presented in the table below. Regarding CPV antibody positivity, 56 animals in good general condition and 32 animals showing gastrointestinal clinical signs were found to be positive.

Table 2. Relationship between clinical findings of animals and CDV antibody concentration

Clinical Finding	Calculated CDV Ab concentrations (CC)	Number of Animals
General Condition is good	7-30	48
	50-85	9
Weakness, Diarrhea, Nervous symptoms	7-20	22
	20-35	5
	35-50	7
	50-85	4

Statistics

No statistically significant difference was found between CDV and CPV infections and age or gender.

Table 3. The effect of age on CDV antibody titer values in dogs (ANOVA test)

					Concentration					
					7-35		36-50		51-85	
Age	N	%	Mean±SE	P	N	%	N	%	N	%
11-15 months	42	44,2	1,30±0,68		34	81	3	7,1	5	11,9
16-20 months	35	36,8	1,45±0,81		26	74,3	2	5,7	7	20,0
21-24 months	18	18,9	1,27±0,57	0.583	14	77,8	3	16,7	1	5,6
Total	95	100	1,35±0,71		74	77,9	8	8,4	13	13,7

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 4. The effect of gender on CDV antibody titer values in dogs (Independent t-test)

					Concentration					
					7-35		36-50		51-85	
Gender	N	%	Mean±SE	P	N	%	N	%	N	%
Male	41	43,2	1,34±0,72		33	80,5	2	4,9	6	14,6
Female	54	56,8	1,37±0,70	0.846	41	75,9	6	11,1	7	13,0
Total	95	100	1,35±0,71		74	77,9	8	8,4	13	13,7

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 5. The effect of age on CPV antibody titer values in dogs (ANOVA)

					Positive		Negative	
Age	**N**	**%**	**Mean±SE**	**P**	**N**	**%**	**N**	**%**
11-15 months	42	44,2	1,07±0,26		39	92,9	3	7,1
16-20 months	35	36,8	1,14±0,35		31	88,6	4	11,4
21-24 months	18	18,9	1,05±0,23	0.473	18	100	-	-
Total	95	100	1,09±0,29		88	92,6	7	7,4

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 6. The effect of gender on CPV antibody titer values in dogs (Independent t-test)

					Positive		Negative	
Gender	**N**	**%**	**Mean±SE**	**P**	**N**	**%**	**N**	**%**
Male	41	43,2	1,12±0,33		37	90,2	4	9,8
Female	54	56,8	1,07±0,26	0.435	51	94,4	3	5,6
Toplam	95	100	1,09±0,29		88	92,6	7	7,4

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

DISCUSSION AND CONCLUSION

This study reports the results of research on the seroepidemiology of distemper virus and parvovirus infections in dogs in Turkey. Both infections cause contagious and fatal diseases in unprotected domestic and wild carnivores worldwide (Kimpston et al., 2022).

The standard procedure for assessing immunity against canine distemper is to experimentally infect vaccinated animals with a virulent strain. However, an alternative method widely used is the measurement of serum antibody titers (McCaw et al., 1998; Carmichael, 1999; Meazzi et al., 2022; Gonzalez et al., 2023). Nevertheless, the antibody titers considered protective vary between laboratories and methodologies. Meazzi et al., (2022) reported a protective titer of ≥1:32 in a neutralization test. Rima et al., (1991) stated that dogs with neutralizing antibody titers above 50 in an SN test survived distemper. Carmichael (1999) found that a neutralizing antibody titer of ≥1:80 in vaccinated Beagle dogs kept in isolation indicated immunity against CDV. McCaw et al., (1998) considered an SN antibody titer of ≥1:96 in vaccinated dogs to be protective.

ELISA is a preferred method due to its high sensitivity and specificity (Fan et al., 2013). In this study, the ELISA test was used to serologically evaluate distemper infection in animals. Inder et al., (2021), in a study comparing SN tests and ELISA, found a correlation between the two methods. They reported that ELISA titer values provided uncertain levels of protection on day 25 in vaccinated puppies and dropped to a non-protective titer (≤30 AU) by day 45. They emphasized that maternal antibodies play a crucial role in preparing puppies against canine distemper, as maternal immunity is considered the primary reason for vaccine failure in young dogs.

If serum antibody titers reach high levels within 8-9 days after infection, the virus disappears from the lymphatic and other tissues, and the infection remains subclinical or mild. However, if the immune response is weak or delayed, CDV spreads to multiple tissues, leading to an acute or chronic disease with high mortality (Jóźwik, 2004). The results of this study confirm the role of humoral immunity in disease recovery.

Among 12 dogs with lethargy, vomiting, and neurological symptoms, high CDV serum antibody titers 35-50 Calculated CDV Ab concentrations (CC) were detected (Table 1, Table 2). These animals were suspected to be in the acute phase of infection. Additionally, four dogs with clinical symptoms and a strong serological response (50-85 CC) exhibited muscle twitching.

It is known that Feline Leukemia Virus (FeLV) (Cong et al., 2016), Toxoplasma gondii (Simion et al., 2019), and Feline Immunodeficiency Virus (FIV) (Mauler et al., 2014) can cause muscle twitching. If an animal does not receive adequate nutrition, neurological problems may develop depending on the duration and severity of the deficiency. Neurological symptoms can be caused by deficiencies in vitamins B1, B6, B12, and C, Omega-3 fatty acids, hypocalcemia, hyponatremia, hypochloremia, hyperphosphatemia, vitamin D deficiency, and taurine deficiency (Kumar et al., 2024). It is suspected that these four dogs had recovered from distemper but suffered from a secondary infection or vitamin deficiency.

Respiratory, intestinal and dermatological signs are known to appear 10 days after epithelial localisation of distemper virus infection. Symptoms such as purulent nasal discharge, cough, dyspnoea, pneumonia, diarrhoea, vomiting and dermal pustules are often exacerbated by secondary bacterial infections. Hyperkeratosis of the soles of the feet and nose and enamel hypoplasia are typical (Saltık and Kale, 2020; Saltık and Atlı, 2023). Signs of infection can be observed in dogs surviving CDV subclinical or subacute infections (Martella et al., 2008). It was found that 10 animals with slightly higher CDV antibody titre (20-35 CC) and clinical signs of diarrhoea, vomiting and cough were animals with increased antibody production as a result of infection. It was reported that neurological signs such as circling, head tilt, eye tremor, partial or complete paralysis, convulsions and dementia could be seen 20 days after infection. Involuntary sudden twitching or contraction of muscles is considered as a typical example of CDV infection (Green and Appel, 1990). Neurological clinical findings were present in 39 of the sampled animals. Among these, 9 of them (antibody titre 50-85CC) had only neurological symptoms despite their good general condition. Considering the high antibody titres of these animals, in parallel with Jozwik et al., (2004) we determined that these animals were in the peak period of antibody in the 3rd week after infection. Jozwik et al., (2004) in their study, when they tested with Immuno Peroxidase Monolayer Assay (IPMA) in dogs in which CDV was detected by RT PCR, they determined that those with high antibody titres (1280) recovered, those

with relatively medium titres (640, 320) had localised twitching in their muscles, and those with lower titres (40,10,5) died.

In parallel with Vandevelde and Zurbrigge, (2005) who found acute demyelination in the brain at day 20 post CDV infection, we interpreted that these animals with neural symptoms were approximately at day 20 post infection. Litster et al., (2012) determined antibody titres for CDV in ≥4-month-old dogs for 2 weeks following vaccination with modified live vaccine (MLV) and reported that animals still had negative antibody titres at 6-8 days post-vaccination, but turned positive at 13-15 days. Bergmann et al., (2021) reported that they detected the maximum antibody titre increase (≥4-fold titre increase) on the 28th day after vaccination. As a result of this study, it was determined that 12 animals (antibody titre 35-50CC) had acute infection and their antibody titres started to increase slowly. Because clinical findings were also present in these animals. Pardo et al., (1997) reported that clinical signs in CDV started at the earliest on the 9th day after exposure to the virus. It has been reported that CDV-infected dogs with high body temperature (above 39.5°C) become depressed and anorexic 3 or 4 days after infection. In another study, it was reported that all dogs, except those fatally infected, became clinically stable between 14 and 21 days (Appel et al., 1982). According to the antibody titres of the 12 dogs in the study, it was interpreted that it was around the 16th day after exposure to the agent (9+7). The 37 animals with clinical signs and low antibody titres (7-20CC) were thought to be in the peracute period at the onset of infection. The 78 animals with good general condition and low distemper antibody titre (7-20 CC) were thought to have been infected long ago and may not even have clinical signs. The results shown in Table 1 confirm that serological examination of a distemper patient may have a prognostic value.

High antibody titres are known to be associated with protective immunity. Therefore, the critical issue in a study of this nature is to define protective serum antibody titres against CDV. Böhm et al., (2004) stated that CPV HI titres equal to or higher than 1:128 are protective titre values, while another study pointed out that a cut-off titre of 1:80 was used (Waner et al., 2006). Böhm et al., (2004) chose a cut-off SN titre of 1:64 or higher, but SN titres between 1:16 and 1:32 have been shown to be protective in other studies (Olson et al., 1988; Coyne et al., 2001). In the present study, antibody titres of 50CC and above were found to be protective against the disease. Young dogs, especially newborns and recently weaned dogs, are generally more susceptible to CDV infection and there is a relationship between susceptibility and age (Headley and Graça, 2000). In this study, similar to Shabbir et al., (2010), antibody levels were found to be independent of age (Table 3). However, Martella et al., (2008) reported that CDV titre was higher in juveniles. The fact that there was no statistically significant difference between CDV antibody titre and age in the study was interpreted as the animals were not puppies and were at an age that could be considered adult (12-24 months). In addition, in parallel with Luo and Zhang, (2017); Costa et al., (2019); Tavakoli et al., (2021) no statistically significant difference was found between antibody titre and sex (Table 4). Similar results were reported by Brady et al., (2012); Hübner et al., (2010) that there was no relationship between gender and CPV (Table 6). On the contrary, Gamege et al., (2020) reported a negative correlation between age and infection. In the present study, in parallel with Mokhtari et al., (2018), the lack of a significant relationship between age and infection (Table 5) was thought to be due to variables such as sample size or severity of infection, status in the population and habitus of dogs. The severity of the disease, mortality and morbidity rates vary depending on the body's immune system and secondary infections. Especially in animal shelters with collective living conditions, a more favourable environment for the spread of the disease occurs (Şahna et al., 2008). Pets are also likely to come into contact with stray animals when walking on the street and meeting their toilet needs. Therefore, there is always a risk of catching the virus. Since stray animals are unvaccinated and the virus is constantly circulating in them, they are reservoirs and pose a potential risk for pets (Sayın and Erol, 2021). It is known that dogs from the urban population vaccinated with commercial modified live virus distemper vaccines have antibody titres indicating immunity for 2 years and then the antibody level decreases significantly (Jozwik et al., 2004). An unknown history of vaccination should raise suspicion that the dog is not protected against CPV and CDV and there should be a rush for basic vaccination. However, it is currently not logistically or economically feasible to vaccinate all stray dogs. It may therefore be advisable to keep dogs at home, especially in the first phase, and only release them after they have been vaccinated. In addition, control programmes need to be developed to prevent the spread of infectious diseases, such as the adoption and sterilisation of stray dogs and the improvement of hygiene and management of shelters.

In conclusion, the study data show that strategies to combat CDV and CPV infections should be developed and strictly implemented. For this, future studies on the epizootiology (incidence, prevalence, host characteristics) and virological characteristics (genetic variability of the virus, the success of antibodies against vaccinia virus in protecting against existing strains in the region, pathogenesis and the relationship between field isolates, etc.) of these viruses are needed.

Acknowledgement

-

Ethical Declaration

This study, approved by Selcuk University Animal Experiments Local Ethics Committee (SÜVDAMEK) under decision number 2025/11.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: H.P.A., Design: H.P.A., I.D., Data Collection or Processing: H.P.A. Analysis or Interpretation: H.P.A., I.D., Literature Search: H.P.A., Writing: H.P.A., I.D.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Appel MJ, Pearce-Kelling S, Summers BA. 1992. Dog lymphocyte cultures facilitate the isolation and growth of virulent canine distemper virus. Journal of Veterinary Diagnostic Investigation, 4(3): 258-263.

Bergmann M, Freisl M, Zablotski Y, Khan MAA, Speck S, Truyen U, et al. 2021. Prevalence of neutralizing

antibodies to canine distemper virus and response to vaccination in client-owned adult healthy dogs. Viruses, 13(5): 945.

Brady S, Norris JM, Kelman M, Ward MP. 2012. Canine parvovirus in Australia: the role of socio-economic factors in disease clusters. The Veterinary Journal, 193(2): 522-528.

Böhm M, Herrtage ME, Thompson H, Weir A, Hasted AM, Maxwell NS. 2004. Serum antibody titres to canine parvovirus, adenovirus and distemper virus in dogs in the UK which had not been vaccinated for at least three years. Veterinary Record, 154(15): 457-463.

Carmichael LE. 1999. Canine viral vaccines at a turning point – a personal perspective. In: Schultz R.D. (ed.), Veterinary Vaccines and Diagnostics. Advances in Veterinary Medicine, 41: 289-308.

Cong W, Meng QF, Blaga R, Villena I, Zhu XQ, Qian AD. 2016. Toxoplasma gondii, Dirofilaria immitis, feline immunodeficiency virus (FIV), and feline leukemia virus (FeLV) infections in stray and pet cats (Felis catus) in northwest China: co-infections and risk factors. Parasitology Research, 115: 217-223.

Costa VGD, Saivish MV, Rodrigues RL, Lima Silva RFD, Moreli ML, Krüger RH. 2019. Molecular and serological surveys of canine distemper virus: A meta-analysis of cross-sectional studies. PloS One, 14(5): 1-19.

Coyne MJ, Burr JHH, Yule TD, Harding MJ, Tresnan DB, McGavin D. 2001. Duration of immunity in dogs after vaccination or naturally acquired infection. Veterinary Record, 149(17): 509-515.

de Oliveira Santana W, Silveira VP, Wolf JM, Kipper D, Echeverrigaray S, Canal CW, et al., 2022. Molecular phylogenetic assessment of the canine parvovirus 2 worldwide and analysis of the genetic diversity and temporal spreading in Brazil. Infection, Genetics and Evolution, 98: 1-10.

Decaro N, Buonavoglia CBVR, Barrs VR. 2020. Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication?. Veterinary Microbiology, 247: 1-8.

Hübner SDO, Pappen FG, Ruas JL, Vargas GDÁ, Fischer G, Vidor T. 2010. Exposure of pampas fox (Pseudalopex gymnocercus) and crab-eating fox (Cerdocyon thous) from the Southern region of Brazil to Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus (CCoV). Brazilian Archives of Biology and Technology, 53: 593-597.

Gamage BGSS, Dissanayake DRA, Prasada DVP, Silva ID. 2020. Risk, prognosis and causality of parvo viral enteritis in dogs in Sri Lanka. Comparative immunology, microbiology and infectious diseases, 72: 1-6.

Jóźwik A, Frymus T, Mizak B, Rzeżutka A. 2004. Antibody titres against canine distemper virus in vaccinated and unvaccinated dogs. Journal of Veterinary Medicine, Series B, 51(3): 99-103.

Kimpston CN, Hatke AL, Castelli B, Otto N, Tiffin HS, Machtinger ET, et al. 2022. High prevalence of antibodies against canine parvovirus and canine distemper virus among coyotes and foxes from Pennsylvania: Implications for the intersection of companion animals and wildlife. Microbiology Spectrum, 10(1): 1-5.

Kumar GC, Ashwini A, Choudhary S, Sagar RS. 2024. Diseases of the nervous system of dogs and cats. In Introduction to Diseases, Diagnosis, and Management of Dogs and Cats. Academic Press, 239-256.

Litster AL, Pressler B, Volpe A, Dubovi E. 2012. Accuracy of a point-of-care ELISA test kit for predicting the presence of protective canine parvovirus and canine distemper virus antibody concentrations in dogs. The Veterinary Journal, 193(2): 363-366.

Luo H, Li K, Zhang H. 2017. Epidemiology of Canine distemper and Canine parvovirus in pet dogs in Wenzhou, China. Indian Journal of Animal Research, 51(1): 159-161.

ICTV, 2024a. Access address: https://ictv.global/report/chapter/paramyxoviridae/paramyxoviridae/morbillivirus. Access date: 19.09.2024.

ICTV, 2024b. Access address: https://ictv-global.translate.goog/report/chapter/parvoviridae/parvoviridae/protoparvovirus?_x_tr_sl=en&_x_tr_tl=tr&_x_tr_hl=tr&_x_tr_pto=tc. Access date: 14.11.2024.

Inder BM, Kumar MA, Ramesh A, Senthil NR, Srinivas K, Devi T, et al. 2021. Kinetics of Maternal Derived Antibody Status against Canine Distemper in Pups. International Journal of Current Microbiology and Applied Sciences, 10(11): 43-47.

Fan JH, Zuo YZ, Yang Z, Pei LH. 2013. The development of an indirect ELISA for the detection of antibodies to goose parvovirus in blood serum. Letters in Applied Microbiology, 57(1): 26-32.

Gonzalez SE, Gogal RM, Meindl AG, Boyer N, Nelson S, Everett SE, Gonzalez JM. 2023. Influence of age and vaccination interval on canine parvovirus, distemper virus, and adenovirus serum antibody titers. Veterinary Immunology and Immunopathology, 262: 1-6.

Grecco S, Condon E, Bucafusco D, Bratanich AC, Panzera Y, Pérez R. 2024. Comparative genomics of canine parvovirus in South America: Diversification patterns in local populations. Infection, Genetics and Evolution, 123: 1-9.

Green EC, Appel MJ. 1990. Canine distemper virus. In: Green, editor. Infections diseases of the dog and cat. Philadelphia: W.B. Saunders Company, 226-241.

Headley SA, Graça DL. 2000. Canine distemper: epidemiological findings of 250 cases. Brazilian Journal of Veterinary Research and Animal Science, 37: 136-140.

Martella V, Elia G, Buonavoglia C. 2008. Canine distemper virus. Veterinary Clinics of North America: Small Animal Practice, 38(4): 787-797.

Mauler DA, Van Soens I, Bhatti SF, Cornelis I, Martlé VA, Van Ha LM. 2014. Idiopathic generalised tremor syndrome in two cats. Journal of Feline Medicine and Surgery, 16(4): 378-380.

Meazzi S, Filipe J, Fiore A, Di Bella S, Mira F, Dall'Ara P. 2022. Agreement between in-clinics and virus neutralization tests in detecting antibodies against canine distemper virus (CDV). Viruses, 14(3): 517.

McCaw DL, Thompson M, Tate D, Bonderer A, Chen YJ. 1998. Serum distemper virus and parvovirus antibody titers among dogs brought to a veterinary hospital for revaccination. Journal of the American Veterinary Medical Association, 213(1): 72-75.

Mokhtari A, Farmani N, Rajabi M. 2018. Detection of Canine Parvovirus by PCR and its association with some of risk factors. Revista MVZ Córdoba, 23(2): 6607-6616.

Olson P, Klingeborn B, Hedhammar A. 1988. Serum antibody response to canine parvovirus, canine adenovirus-1, and canine distemper virus in dogs with known status of immunization: study of dogs in Sweden. American Journal of Veterinary Research, 49(9): 1460-1466.

Pardo MC, Bauman JE, Mackowiak M. 1997. Protection of dogs against canine distemper by vaccination with a canarypox virus recombinant expressing canine

distemper virus fusion and hemagglutinin glycoproteins. American Journal of Veterinary Research, 58(8): 833-836.

Rima BK, Duffy N, Mitchell WJ, Summers BA, Appel MJG. 1991. Correlation between humoral immune responses and presence of virus in the CNS in dogs experimentally infected with canine distemper virus. Archives of Virology, 121: 1-8.

Saltik HS, Kale M. 2020. Evaluation of infection with N protein-specific Immunoglobulin M and G in naturally occurring distemper in dogs. Veterinární Medicína, 4: 168-173.

Saltık HS, Atlı K. 2023. Approaches to identify canine distemper virus with neurological symptoms on the basis of molecular characterization of hemagglutinin and fusion genes. Virus Genes, 59(4): 591-603.

Saltik HS, Kale M. 2023. Rapid molecular detection and isolation of canine distemper virus in naturally infected dogs. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 70(1):49-56.

Saltık HS, Koç BT. 2024. First identification of canine parvovirus -2a/2b variant in unvaccinated domestic dogs with gastrointestinal signs in Türkiye. Veterinary Medicine and Science, 1-9.

Sayın Y, Nural E. 2021. Investigation of Canine Distemper Virus Infection in Dogs in the Antalya Province. Animal Health Production and Hygiene, 10(2): 45-51.

Shabbir MZ, Rabbani M, Ahmad A, Ahmed A, Muhammad K, Anwar I. 2010. Comparative evaluation of clinical samples from naturally infected dogs for early detection of canine distemper virus. Turkish Journal of Veterinary & Animal Sciences, 34(6): 547-552.

Shin DL, Chludzinski E, Wu NH, Peng JY, Ciurkiewicz M, Sawatsky B, et al. 2022. Overcoming the barrier of the respiratory epithelium during canine distemper virus infection. American Society for Microbiology, 13(1): 1-17.

Simion V, Fernoaga C, Codreanu M. 2019. Comparative aspects regarding the presence of behavioral changes of aggressive type in seropositive Toxoplasma gondii individuals-humans and cats. Scientific Works. Series C, Veterinary Medicine, 65(2): 1-7.

Sykes JE, Vandevelde M. 2021. Canine distemper virus infection. In Greene's Infectious Diseases of the Dog and Cat, WB Saunders. 271-288.

Şahna KC, Gençay A, Atalay O. 2008. Viral etilogy of diarrhoea in puppies from a same shelter in turkey, presence of mixed infections. Revue de Médecine Vétérinaire, 159: 345-347.

Tavakoli ZA, Mokhtari A, Esmailnejad A. 2021. Molecular and immunological investigation of canine distemper virus (CDV) and its co-infection with canine parainfluenza virus type 2. Iranian Journal of Medical Microbiology, 15(2): 212-226.

Uhl EW, Kelderhouse C, Buikstra J, Blick JP, Bolon B, Hogan RJ. 2019. New world origin of canine distemper: Interdisciplinary insights. International Journal of Paleopathology, 24: 266-278.

Vandevelde M, Zurbriggen A. 2005. Demyelination in canine distemper virus infection: a review. Acta Neuropathologica, 109: 56-68.

Waner T, Mazar S, Keren-Kornblatt E. 2006. Application of a dot enzyme-linked immunosorbent assay for evaluation of the immune status to canine parvovirus and distemper virus in adult dogs before revaccination. Journal of Veterinary Diagnostic Investigation, 18(3): 267-270.

Zhao J, Ren Y. 2022. Multiple receptors involved in invasion and neuropathogenicity of canine distemper virus: a review. Viruses, 14(7): 1-17.

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Atresia Ani Et Vulva and Rectovaginal Fistula: A Clinical Presentation in a Calf

Sevdet Kılıç✉, Mustafa Barış Akgül, Kadir Sulu, Ali Gülaydın, Bahar Erden

Siirt University, Faculty of Veterinary Medicine, Department of Surgery, Siirt, Türkiye.

ARTICLE INFO

Received: 01/02/2024
Accepted: 28/01/2025

DOI: 10.5281/zenodo.15109908

✉**Corresponding Author:** sevdet.kilic@siirt.edu.tr

Keywords

Atresia
Calf
Vulva

Cite this article as: *Kılıç S., Akgül M.B., Sulu K., Gülaydın A., Erden B. 2025. Atresia Ani Et Vulva and Rectovaginal Fistula: A Clinical Presentation in a Calf. International Journal of Veterinary and Animal Research, 8(1): 24-27. DOI: 10.5281/zenodo.15109908.*

ABSTRACT

Anomalies are not uncommon in various domestic animal species, particularly among ruminants. However, when examining anomalies collectively, those pertaining to the urogenital system in calves and lambs are relatively infrequent occurrences. Instances of atresia ani, atresia recti, intestinal atresia, and rectovaginal fistula are more prevalent in calves. While these anomalies may manifest individually or in combination, cases involving atresia vulva are notably rare in such occurrences. The subject of this case report was a 5-day-old female Jersey cross calf, weighing 11 kg. The calf was presented to the Surgery clinic at Siirt University Faculty of Veterinary Medicine Animal Hospital, with the primary concerns being the absence of vulvar lips and issues related to anal patency. Following clinical, radiologic, and ultrasonographic evaluations, a diagnosis of atresia ani et vulva, accompanied by a rectovaginal fistula, was established. The decision was made to proceed with operative intervention. During the operative intervention, the anus hole was opened appropriately using the classical atresia sudden operative intervention method and the vulva was opened using the vaginoplasty method. During the operation, feces and urine output were checked, and the wound lips and rectovaginal fistula were sutured in accordance with the technique. Follow-up visits indicated normal defecation and urination, with the wound lips healing without complications.

INTRODUCTION

Various anomalies can manifest in domestic animal species, particularly ruminants. While the precise pathogenesis of many of these anomalies remains unclear, genetic and environmental factors, individually or in combination, are suggested contributors. Environmental factors such as the consumption of teratogenic poisonous plants in proximity to dams and maternal exposure to viral diseases during the fetal period are identified as key causative elements. The distribution of these anomalies among body systems has been documented according to various sources, approximately 24% in the musculoskeletal system, 13% in the digestive and respiratory systems, 22% in the central nervous system, 4% in the abdominal wall and urogenital system, 3% in the cardiovascular system, 4% in the cardiovascular system. 20% are other anomalies (Bademkıran et al., 2009; Kılıç and Sarıerler 2004; Kılıç et al., 2005).

Within these systems, anomalies associated with the urogenital system in calves have been documented, encompassing conditions such as atresia ani, atresia recti, intestinal atresia, rectovaginal fistula, and atresia vulva (Oral et al., 2013). These anomalies may manifest individually or concurrently in calves. Atresia ani and intestinal atresia are commonly observed conditions in calves. While rectovaginal fistula may occasionally accompany these anomalies, the association with atresia vulva is infrequent and considered a rare anomaly.

In the context of atresia cases, intestinal atresia denotes the absence or termination of a segment of the intestine as a blind pouch, atresia recti involves the rectum terminating blindly under the skin in the anal region, and atresia ani is characterized by the closure of the anus. Intestinal atresia is categorized into four main types: Type I involves the closure of the intestinal lumen with a membranous curtain, Type II is characterized by the termination of the last part of the colon as a structure without peristalsis, with or without a lumen, Type III is defined as the termination of the colon with a blind end in the abdominal cavity, and Type IV presents as the blind sorghum-like termination of the colon, resembling Type III. Additionally, the simultaneous occurrence of multiple intestinal atresia is

termed multiple intestinal atresia, and the coexistence of intestinal atresia with a closed anus is termed atresia ani et recti (Bademkıran et al., 2009).

Calves with atresia ani typically exhibit a normal appearance after birth, suckling like healthy counterparts for initial days, and may appear unaffected. However, restlessness emerges within a few days when owners, upon inspection, discover the absence of an anus opening. Key clinical signs in affected calves include bilateral abdominal distension, restlessness, depression, inability to strain and defecate, and swelling in the anal area. The conclusive diagnosis is established through clinical examinations and radiography revealing the extension of the intestinal segment to the absent anus opening (Rahal et al., 2007).

Rectovaginal fistula is characterized by the development of a fistula between the dorsal wall of the vagina and the ventral wall of the rectum, leading to complications such as vaginal irritation, cystitis, reduced conception rates, and megacolon in animals as feces pass into the vaginal cavity and urine into the rectum (Mainau and Mantece, 2011). Conversely, vulvar atresia is defined by the absence of the normal opening of the vagina, with fused outer labia covering the typical canal. The simultaneous occurrence of these anomalies in calves is uncommon, and in cases of atresia, operative intervention is reported as a feasible treatment option when the condition affects an organ or system and is not overly complex (Özaydın, 1996).

This case report discusses a rare anomaly in a calf, specifically focusing on atresia ani et vulva with an associated rectovaginal fistula.

MATERIALS AND METHODS

The subject of this case report was a 5-day-old female Jersey crossbred calf weighing 11 kg, presented to Siirt University Faculty of Veterinary Medicine Animal Hospital Surgery clinic with the chief complaint of a closed anus and vulva. Initial routine clinical examinations, including assessments of mucous membranes, heart rate, respiratory rate, capillary refill time (CRT), peripheral pulse rate and quality, and thoracic auscultation, did not indicate any abnormalities. Nevertheless, specific examination findings disclosed an orange-sized swelling in the perianal region with a fluctuant consistency. Both the anus and vulva lips were observed to be underdeveloped, accompanied by bilateral abdominal distension. The calf exhibited persistent straining and assumed a defecation position without the ability to defecate and urinate (Figure 1). Radiologic and ultrasonographic examinations unveiled the progression of a fluid, presumed to be meconium, to the terminal part of the rectum. The external swelling in the perianal region was found to contain a liquid-like content. Consequently, the patient was diagnosed with atresia ani et vulva, prompting the decision for operative intervention.

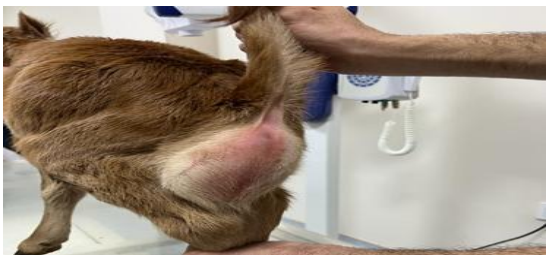

Figure 1. Preoprative perianal swelling

For the operative procedure under general anesthesia, the patient received a sedative dose of xylazine HCL (Xylazinbio 2%, Intermed, Ankara) at 0.2 mg/kg for sedation and an induction dose of ketamine HCL (Ketasol 10%, Interhas, Ankara) at 10 mg/kg, administered intramuscularly. Following the shaving of the operative area, the patient was positioned on the operation table in the sternoabdominal posture and prepared for the operative intervention following the principles of aseptic surgery. Initially, a puncture was executed from the ventral part of the bulge using a scalpel. The opened hole was enlarged by approximately 1 to 2 cm with Metzenbaum scissors, allowing for the complete evacuation of meconium. Following the thorough evacuation of meconium, the area was irrigated with a 0.9% saline solution. Subsequently, an elliptical incision, approximately matching the size of the anus entrance, was made in the region where the anus should be located, resembling the procedure in cases of atresia ani, to establish the anus opening. The incision line, comprising the skin and rectal mucosa, was sutured with simple separate sutures utilizing polyglycolic acid (USP: 2/0, Alcasorb®, Katsan) thread material. Delicate manipulation of the area exposed an opening between the dorsal wall of the vagina and the ventral wall of the rectum, leading to the additional diagnosis of a rectovaginal fistula in the patient. Subsequently, the fistula canal between the rectum and vaginal walls was repaired using an X suture technique applied to the vaginal wall. For vaginoplasty, the incision was extended dorsally in an oval shape from the line of the initial puncture. A suitable piece of skin was excised, facilitating the creation of the vulva opening (Figure 2). The wound incision was sutured using polyglycolic acid (USP: 2/0, Alcasorb®, Katsan) suture material employing a simple interrupted suture technique. Before concluding the operative intervention to mitigate potential complications, a two-way rubber feeding catheter, suitable for the anatomy to avoid irritation, was introduced into the vagina. This facilitated the complete discharge of meconium mixed with urine from the vagina. The vaginal cavity was thoroughly irrigated with a 0.9% saline solution to ensure the absence of fecal particles. Subsequently, the presence of the orificium urethra externa opening in the ventral wall of the vagina was assessed, confirming its appropriate location. To verify the patient's ability to urinate normally, gradual pressure was applied to the caudal abdomen from the ventral side by a non-sterile assistant, resulting in the observation of the patient passing urine comfortably.

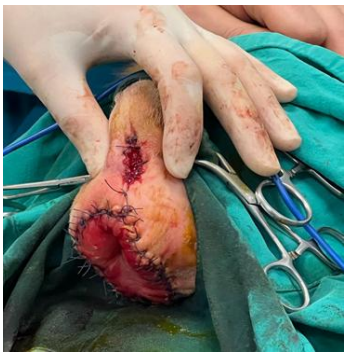

Figure 2. Perioperative appearance of the anus and vulva

Postoperatively, the patient received a single subcutaneous dose of 0,3mg/kg meloxicam (Meloxicam, Bavet, Istanbul) and intramuscular administration of

22.000 IU /kg penicillin G potassium for seven days (Vetimycin, Vetas, Istanbul). As part of the postoperative care, it was advised to apply 10% povidone iodine solution to the suture line twice daily. On the 5th and 15th postoperative days, the patient demonstrated normal stool and urination, and the wound area continued to heal without complications (Figure 3). On the 45th postoperative day, the owner was informed via phone that the patient was successfully passing normal feces and urine. Upon reviewing photographs of the surgical site sent by the owner, it was confirmed that the incision line had completely healed (Figure 4).

Figure 3. Postoperative day 15

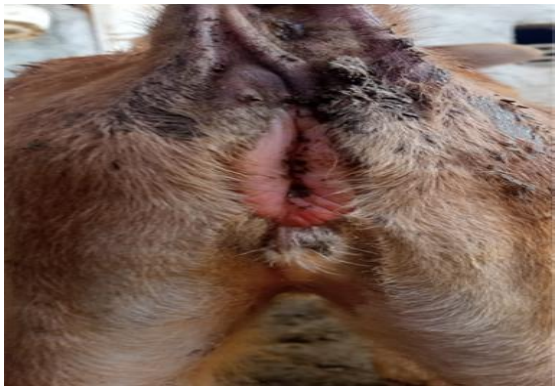

Figure 4. Postoperative day 45

RESULTS AND DISCUSSION

Genetic or non-genetic congenital anomalies can impact a single organ or an entire bodily system (Tyagi and Singh, 1999). While the pathogenesis of non-genetic congenital anomalies remains incompletely understood, these anomalies have been attributed to various factors such as mutations, viral agents, nutritional influences, and traumatic effects on the amniotic sac during early pregnancy. Genetic congenital anomalies are reported to be inherited through autosomal recessive genes, and in instances where this gene transfer is disrupted, the cells responsible for forming organ openings during fetal development may not invaginate, leading to a lack of ruptures necessary for opening formation, ultimately resulting in agenesis or atresia in living organisms (Timurkan and Mert, 1987; Newman et al., 1999; Noh et al., 2003). In the present case, the etiological cause of the anomaly observed remains uncertain, as the anamnesis from the owner and clinical examinations were insufficient to unveil whether the anomaly is of genetic or environmental origin.

In domestic animals, particularly in calves and lambs, anomalies such as atresia ani, atresia recti, rectovaginal fistula, vaginourethral agenesis, or vulvar atresia have been documented (Shetty et al., 1978). While these anomalies typically manifest individually, encountering multiple anomalies in the same animal is uncommon. The most prevalent anomaly in calves is atresia ani, occasionally accompanied by a rectovaginal fistula, but cases involving vulvar atresia are infrequently reported (Tyagi and Singh, 1999). In this particular case, a rare occurrence was noted where three distinct anomalies coexisted in a single calf: atresia ani, atresia vulva, and rectovaginal fistula. It is noteworthy that this is an uncommon scenario, and the fact that the animal continued to live adds further rarity to the case.

It has been noted that operative intervention is the sole treatment option in cases of atresia, with the prognosis influenced by the number of systems affected by the anomaly (Noden and de Lahunta, 1985; Özaydın 1996). In this specific case, the prognosis was initially deemed poor due to the concurrent presence of multiple anomalies. Nevertheless, despite this challenging scenario, the decision to proceed with the operation resulted in a surprisingly favorable prognosis during the animal's recovery process, contrary to initial expectations.

The operative intervention involves the removal of an oval-shaped piece of tissue suitable for the vulvar opening, creating a permanent urogenital opening during the surgical procedure (Yılmaz et al., 2014). Careful consideration of the anatomical structure guided the removal of excess skin, ensuring that an appropriate piece was excised without compromising the vulvar entrance. Subsequently, the position of the orificium uretra externa was evaluated, confirming the successful occurrence of urine outflow through this opening.

In cases of rectovaginal fistula, the passage of feces into the vaginal cavity and urine into the rectum has been reported to lead to pathologies such as vaginal irritation, cystitis, decreased conception rate, and megacolon in animals (Kumar et al., 2020). Notably, vaginal irrigation was not mentioned in some case reports addressing the surgical repair of atresia ani and congenital rectovaginal fistula in calves (Shakoor et al., 2012). In another case report involving surgical treatment of atresia ani complicated with a rectovaginal fistula in a Sahiwal breed calf, vaginal irrigation was also not performed (Kumar et al., 2020). When the case report of Kumar et al is examined, it becomes clear that irrigation is not applied consistently in cases of atresia sudden and rectovaginal fistula to prevent possible complications that may arise from the passage of feces into the vaginal canal. The assumption may be that the feces will flow directly into the external environment. However, in this case, considering the increased risk of complications due to the closed vulva, irrigation of the vaginal canal was deemed necessary during the surgery. Therefore, in cases of sudden atresia with rectovaginal fistula, it is recommended to consider vaginal irrigation due to possible complications.

In conclusion, the presentation of atresia ani et vulva accompanied by a rectovaginal fistula in a calf is a rare occurrence in veterinary medicine. Despite reports in the literature suggesting a poor prognosis with an increasing number of anomalies in the same animal, the desired improvement was achieved following operative intervention in this particular case. We believe that sharing

this case will be valuable for our colleagues practicing large animal medicine.

Acknowledgement

-

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: M.B.A., Design: M.B.A., S.K., Data Collection or Processing: M.B.A., K.S., B.E., Analysis or Interpretation: M.B.A., S.K., A.G., K.S., Literature Search: M.B.A., S.K., A.G., B.E., Writing: M.B.A., S.K., K.S., B.E.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Bademkıran S, İçen H, Kurt D. 2009. Congenital recto vaginal fistula with atresia ani in a heifer: A case report. YYÜ Veteriner Fakültesi Dergisi, 20(1): 61-64.

Gangwar AK, Devi KS, Singh AK, Katiyar N, Patel G, Srivastava S. 2014. Congenital anomalies in ruminants and their surgical correction. Advances in Animal and Veterinary Sciences, 2(7): 369-376.

Kılıç E, Baran V, Yayla S, Özen, H. 2015. Atresia ani, atresia vulva and rectovaginal fistula in a tuj breed lamb. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 12(3): 219-222.

Kılıç N, Sarıerler M. 2004. Congenital intestinal atresia in calves: 61 cases. Revue Medicine Veterinary, 155(7): 381-384.

Kumar G, Arora N, Tiwari DK, Shamar S, Kaushik D. 2020. Surgical management of atresia ani complicated with rectovaginal fistula in a sahiwal calf. Journal of Entomology and Zoology Studies, 8(3): 714-716.

Mainau E, Manteca X. 2011. Pain and discomfort caused by parturition in cows and sows. Applied Animal Behaviour Science, 135(3): 241-251.

Newman SJ, Bailey TL, Jones JC, DiGrassie WA, Whittier WD. 1999. Multiple congenital anomalies in a calf. Journal of Veterinary Diagnostic Investigation, 11: 368- 371.

Noden DM, deLahunta A, 1985. The Embryology of Domestic Animals, Williams and Wilkins Co., Baltimore, USA.

Noh DH, Jeong WI, Lee CS, Yung CY, Chung JY, Lee YH, Do SH, An MY, Kwon OD, Williams BH, Jeong KS. 2003. Multiple congenital malformation in a holstein calf. College of Veterinary Medicine, 129: 313-315.

Oral H, Ozaydin I, Kaya S, Kuru M. 2013. The case of atypical vulvar atresia in a brown swiss calf. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19(7): 221-222.

Özaydın İ. 1996. A case of atresia ani, vulva hypoplasia and rectovaginal fistula in a calf. Veterinary Surgery Journal, 2: 37-39.

Rahal SC, Vicente CS, Mortari AC, Mamprim MJ, Caporalli EH. 2007. Rectovaginal fistula with anal atresia in 5 dogs. The Canadian Veterinary Journal, 48(8): 827.

Shakoor A, Muhammad SA, Younus M, Kashif M. 2012. Surgical repair of congenital recto-vaginal fistula with atresia ani in a cow calf. Pakistan Veterinary Journal, 32(2): 298-300.

Shetty BR, Nigam JM, Singh GR. 1978. Surgical correction of vagino-urethral and anal agenesis in a calf. Indian Veterinary Journal, 55: 234-236.

Timurkan H, Mert N. 1987. Embryophathia in domestic animals-causes of congenital anomaly and abortion. Elazığ Bölgesi Veteriner Hekimleri Odası Dergisi, 2: 59-69.

Tyagi RP, Singh SJ. 1999. Ruminant Surgery, CBS Publishers, New Delhi, India. 222p. Veterinaire, 155, (7): 381-384.

Yılmaz O, Yazıcı E, Uçar M, Bidane, MK. 2014. Treatment of congenitally developed fused vulva labia in a brown-swiss heifer. Turkish Journal of Veterinary and Animal Sciences, 38(1): 116-119.

IJVAR

International Journal of Veterinary and Animal Research

Journal homepage: https://ijvar.org/index.php/ijvar

Intersection of Toxicology and Archaeology Sciences

Doga Can Nalbantoglu ✉, Mustafa Yipel

Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Hatay, Turkiye

ARTICLE INFO

Received: 16/01/2025
Accepted: 25/03/2025

DOI: 10.5281/zenodo.15109919

✉**Corresponding Author:** 2092112501@ogr.mku.edu.tr

Keywords

Archaeology
Toxicology
Ancient Biomarkers
Poisons
Paleo-ecotoxicology
Science

Cite this article as: *Nalbantoglu D.C and Yipel M. 2025. Intersection of Toxicology and Archaeology Sciences. International Journal of Veterinary and Animal Research, 8(1): 28-31. DOI: 10.5281/zenodo.15109919.*

ABSTRACT

Toxicology is a science that dates to Ancient Egypt. Animal and herbal poisons were classified according to their source, and cases were categorized based on symptoms, with comments about prognosis, were included at texts were written Ancient Egypt period. In the texts written by the ancient writer Strabo, it is understood that communities with mastery on snakes lived in Anatolia. There is also information that plant-derived poisons were used in hunting and war tools in ancient time. It is seen that poisons were used quite widely in the Roman Period. There is also the extensive use of lead, especially in Roman plumbing, caused researchers to think about whether there was mass chronic poisoning.
The aim of this study is to determine the current situation in order to use the Toxicology discipline in the field of Archaeology and to shed light on the period by using animals that met needs such as food sources, mounts and protection in the ancient period as biomarkers.

INTRODUCTION

Toxicology, deals with the physicochemical properties of toxic substances, classification, effects on living organisms (human, animal, etc.), treatment and prevention procedures, analysis methods (qualitative and quantitative; chromatographic, molecular, in silico, new aprroches, in vivo, in vitro, experimental, screening, etc.) in different matrices for diagnosis/treatment and scientific researchs, as well as future predictions in order to take/develop measures, etc. (Langman and Kapur, 2006; Yipel and Altınok-Yipel, 2025). Toxicology, which is called the science of poisons and while dates back to Ancient Egypt, starts scientificaly with Paracelsus and Orfila.

Although there are many legends claiming that the entrance to Tust's Tomb, which is known to date back to 3000 BC, is indeed cursed as described in the hieroglyphs, an extraordinary article has suggested that there may be a scientific explanation for the tomb's curse. According to this article, the tomb's remains were found to contain much higher levels of radiation than would be expected from limestone, a common building material, suggesting an artificial source. In addition, Egyptologists working in the tomb reported unusual deaths that coincided with the symptoms of radiation sickness (Fellowes, 2024).

One of the oldest known texts on toxicology is the Ebers Papyri, dated to around 1550 BC. The Ebers Papyri are thought to be copies of texts by Imhotep, who lived in the early 3rd millennium BC. Discovered in 1872, the papyri contain more than 900 prescriptions. The text contains passages on opium, aconitine, arsenic trioxide, cyanogenic glycosides or physostigmine isolated from Calabar beans (Nepovimova and Kuca, 2019).

One of the oldest known written texts on toxicology is the Brooklyn Papyrus (600-525 BC), written in Ancient Egypt. In these texts, besides the description of venomous snakes; three prognoses defined as certain death, uncertain and survival were mentioned. In making these distinctions, not only the type of snake but also its adulthood was taken as a criterion; in addition, the prognosis was described according to the severity of the poisoning according to the symptoms (Wexler, 2014). This has great similarities with the methods used in modern toxicology to characterize the clinical picture. It shows a very similar understanding of the use of criteria such as factors related to the toxin and

its source, dose-related factors and patient-related factors. It is possible to see in the Ebers papyrus that they treat mental illnesses as they do in the modern world without separating them from physical illnesses.

The Berlin Medical Papyrus, written in approximately 1200 BC, contains treatments used for scorpion bites, while the Edwin Smith Papyrus describes tetanus, which is said to be caused by open wounds, and explains that treatment is not possible (Wexler, 2014).

In the West, probably the best known case of poisoning is that of Socrates, who ended his life by drinking hemlock (*Conium maculatum, Apiaceae*) poison. Hippocrates (460-377 BC) almost never mentioned poisons in his *Corpus hippocraticum*, one of the main sources of medicine. This was probably because he condemned the use of poisons in murder. However, he did mention the symptoms of poisoning (Nepovimova and Kuca, 2019).

In the section where the ancient writer Strabo mentions the ancient city of Parion, which is located in the Biga District of Çanakkale today, he mentions a community called Ophiogen, which he states that they belong to the Snake Tribe. Strabo attributed the Ophiogenes to the Psyls, the ancient Libyans, and stated that they had the same abilities (Strabon, 2000).

The ancient Libyan Ophiogenes were immune to snake venom, knew how to treat various poisonings, and the Romans held them in high esteem because of this (Jones-Lewis, 2016).

Nikandros of Colophon, who lived in the 2nd century BC, has two writings on poisons and antidotes, Alexipharmaca and Theriaca. It is known that Nikandros recommends flaxseed tea as an emetic as a general antidote and recommends the removal of the poison from the tissues in poisonous animal bites and stings by sucking the poison with the mouth (Nepovimova and Kuca, 2019).

The term mithridatism has its origins in a legend about the King of Pontus Mithridates VI (132-63 BC). The king's fear of dying by poisoning was so great that he spent his entire life trying to develop a single, marvelous antidote, which would be called “mithridaticum” by the Romans. According to legend, at the end of his life, the king wanted to end his life by poisoning himself, but he was unsuccessful in his suicide attempt because he had developed a tolerance to poisons by constantly poisoning himself in small doses. This is why the term 'mithridatism' is used today, meaning becoming resistant to poisons through exposure to small doses (Nepovimova and Kuca, 2019).

It is known that poisons were widely used in Ancient Rome. Although the Romans had knowledge of poisons of plant, animal and mineral origin, it is reported that they generally used plant-based poisons. Especially during the reigns of Julius Caesar and Cladius, the use of poisons became very widespread and many specific and general antidotes were widely used (Cilliers and Retief, 2000; Nepovimova and Kuca, 2019).

It is said that the Romans threw angry bees at the enemy with slingshots, and that Mithridates, while fighting the Romans, drilled holes in the tunnels dug by the Romans and sent wasps into the tunnels. Other ancient sources mention the existence of winged scorpions in Ancient Egypt and India. Scorpion venom was said to be more effective than snake venom. For a long time in both Egypt and Rome, scorpions were associated with cursed or evil spirits that were feared, but later they became the symbol of Rome's elite military units and even became the name of a kind of instrument used in sieges because of its power (Arıkan and Akcicek, 2022).

However, the use of lead metals was extremely widespread in Ancient Rome. Lead oxide was used in construction, lead carbonate in medicine, and lead acetate in wine and food flavorings and condoms. Lead and tin alloys were frequently used to coat containers for storing food and drink. In ancient Rome, lead was used so extensively in the transportation and storage of drinking and potable water that the word 'plumbing' was derived from the Latin word 'plumbum' meaning lead. For these reasons, there are opinions that lead exposure indirectly accelerated the decline of the Roman Empire by causing a decrease in the aristocratic population due to its effect on fertility. However, the amount of lead found in the analysis of bones from the Roman period is reported to be less than half of that of today's European populations (Cilliers and Retief, 2019).

Analytical Studies Conducted on The Topic

A study was conducted to identify herbal poisons that may have been used in arrows and similar hunting weapons used on game animals, and then to compare their standards with archaeological samples. Then, in order not to damage the precious artifacts, samples were prepared by rubbing the surfaces of the artifacts with pure water and cotton and sent to the laboratory. In the laboratory, the samples were first enriched by removing solvents with nitrogen and then derivatized. Then they were analyzed by LC-Q Orbitrap and GC- MS. The analysis confirmed the presence of aconite in the Chinese ceramic vessel containing aconite. An iron arrow, a wooden dart and a bone spearhead were found to contain compounds that can be considered as evidence for the presence of Antiaris toxicara, and an iron arrow and a spatula were found to contain compounds that can be considered as evidence for the presence of *Strychnos* species plants. It was also mentioned that the artifacts from which the samples for these analyzes were obtained were found in European museums after being obtained from the Asian and African continents and that the sampling was done from the museum inventory (Borgia et al., 2017).

In this study, the fact that the finds were sampled perhaps years after they were unearthed, the lack of enlightening records about their fate during this period, and the difficulty of obtaining standards are important handicaps. However, the use of a double quadropole device for more precise determination of the compounds sought would have provided more satisfactory results, at least on the gas chromatography side.

In another study, LC-MS/MS was used in skull bone tissues obtained from burial chambers containing the remains of people who were treated and died during the 17th century in Milan, and active components of Erythroxylum spp. including cocaine were found. Exposure was thought to be due to the therapeutic use of cocaine at the time (Giordano et al., 2024).

In another study, human bones obtained from excavations in a necropolis in the Lombardy region of Italy and dated from the Neolithic to the Bronze Age (9000-1000 BC) were sampled and trace elements were determined using ICP-MS. Lead isotopes were used to detect post-mortem contamination. The study provided insight into the diet of the period, and the low Cu and Zn values led to the conclusion that they were fed a low-protein diet. Furthermore, no significant differences were observed between different bone tissues of the same

individuals and between genders. In addition, the importance of a multidisciplinary approach with chemical, statistical and archaeological perspectives is emphasized. Analysis of trace elements in bone tissue has been shown to provide a well-preserved archive of nutrition and therefore lifestyle (Corti et al., 2013).

Trace elements are elements that can be both essential and toxic depending on their concentration, presence and some other factors, and have an impact on environmental problems, human, plant and animal health (Swaine, 2000).

In another study conducted in Cartagena, Spain, 30 bones and 8 recent bone tissues covering a period from the Neolithic Period to the Byzantine Period were studied. As a result of the study, an increase in trace element levels was detected starting from the Neolithic period and it was noted that different trace element levels increased in different periods (Martínez-García, et al., 2005).

A comparison of pre-Hispanic and modern bones from El Hierro Island revealed that Pb and Cd levels were lower in pre-Hispanic remains and that Pb and Cd levels in El Hierro Island were lower than in the other Canary Islands. Considering the presence of volcanic activities on El Hierro Island, it should be considered that the distribution of heavy metal residues from ancient periods may be significantly different due to non-anthropogenic factors (González-Reimers, et al., 2005).

Although archaeology and toxicology seem to be two unrelated disciplines, the relatively recent introduction of instruments such as LC-MS/MS, LA ICP-MS, LC-Q Orbitrap HRMS may have made this field difficult to recognize. Although the number of studies on the presence of trace elements is higher than others, it is far from being a routine to investigate the presence of trace elements in valuable bone remains from archaeological excavations. On the other hand, the study to detect the presence of herbal poisons on archaeological hunting tools is one of the very rare examples in this field (Borgia et al., 2017).

CONCLUSION

Information on toxicology (toxic substances, individual and social cases, effects of toxicology on political and cultural history, etc.) before Paracelus (1493-1541) and especially before ancient Egypt is very limited. The historical implications of potential toxicological events have become more important, especially since toxic substances have caused deaths and illnesses since antiquity, have been used in political and other crimes, and toxicological methods have played an important role in wars in the past century. On the other hand, with the discoveries and developments in the fields of forensic toxicology, archaeology and anthropology etc. with chromatographic analysis methods (mass spectroscopy etc.) and forensic toxicology, working hypotheses involving the analysis of archaeological findings with modern toxicological methods have become popular research topics.

As mentioned by the researchers, the fact that the hunting implements were sampled from the inventories of museums in other countries long after they were obtained from archaeological excavations, and that no records of the treatments applied to the hunting implements and the conditions in which they were found were not kept during this time, although these were major disadvantages for the study, promising results for the future could be obtained.

In the future, with the advancement of technology in the field of analytical chemistry, the presence of toxicologists in the scientific committees of archaeological excavations, and the protection of data on the remains, just as the protection of ancient artifacts, may enable the disciplines of archaeology and toxicology (both medicine, veterinary, and environmental sciences fields) to obtain important outputs regarding the past and the future.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: D.C.N., M.Y., Design: D.C.N., M.Y., Data Collection or Processing: D.C.N., M.Y., Literature Search: D.C.N., M.Y., Writing: D.C.N., M.Y.

Financial Support

This research received no grant from any funding agency/sector.

REFERENCES

Borgia, V., Carlin, M. G., and Crezzini, J. 2017. Poison, plants and Palaeolithic hunters. An analytical method to investigate the presence of plant poison on archaeological artefacts. Quaternary International, 427: 94-103.

Cilliers, L., and Retief, F. 2019. Lead poisoning and the downfall of Rome: Reality or myth?. In Toxicology in Antiquity, Academic Press, 221-229.

Cilliers, L., and Retief, F. P. 2000. Poisons, poisoning and the drug trade in ancient Rome. Akroterion, 45(1): 88-100.

Corti, C., Rampazzi, L., Ravedoni, C., and Giussani, B. 2013. On the use of trace elements in ancient necropolis studies: Overview and ICP-MS application to the case study of Valdaro site, Italy. Microchemical Journal, 110: 614-623.

Fellowes, R. 2024. The Pharaoh's Curse: New Evidence of Unusual Deaths Associated With Ancient Egyptian Tombs. Journal of Scientific Exploration, 38(1): 41-60.

Giordano, G., Mattia, M., Biehler-Gomez, L., Boracchi, M., Porro, A., Sardanelli, F., ... and Cattaneo, C. 2024. Forensic toxicology backdates the use of coca plant (Erythroxylum spp.) in Europe to the early 1600s. Journal of Archaeological Science, 170: 106040.

González-Reimers, E., Arnay-De-La-Rosa, M., Velasco-Vázquez, J., Galindo-Martín, L., and Santolaria-Fernández, F. 2005. Bone cadmium and lead in the ancient population from El Hierro, Canary Islands. Biological Trace Element Research, 105: 37-51.

Arıkan and Akçiçek, Scorpious “Living Fossils” Ege Üniversitesi Yayınları, 2022.

Jones-Lewis, M. 2016. The case of the Psylloi. The Routledge Handbook of Identity and the Environment in the Classical and Medieval Worlds, 192.

Langman, L. J., and Kapur, B. M. 2006. Toxicology: then and now. Clinical Biochemistry, 39(5): 498-510.

Martínez-García, M. J., Moreno, J. M., Moreno-Clavel, J., Vergara, N., García-Sánchez, A., Guillamón, A., ... and Moreno-Grau, S. 2005. Heavy metals in human bones in different historical epochs. Science of the Total Environment, 348(1-3): 51-72.

Nepovimova, E., and Kuca, K. 2019. *The history of poisoning: from ancient times until modern ERA*. Archives of Toxicology, 93(1). 11-24.

Strabon, G. A. A. C. K. 2000. XII-XIII-XIV, Adnan Pekman (Eds.), Arkeoloji ve Sanat Yayınları, 4th ed., İstanbul, 96.

Swaine, D. J. 2000. Why trace elements are important. Fuel Processing Technology, 65: 21-33.

Gonzalo M. sanchez and W. Benson Harer 2014. Toxicology in Ancient Egypt, in Philip Wexler (Eds.) History of Toxicology and Environmental Healt Toxicology in Antiquity, Volume :1 Academic Press is an imprint of Elsevier 225 Wyman Street, Waltham, MA 02451, USA, 1-8.

Yipel ve Altınok-Yipel. 2025. Aquatic Animals in Experimental Studies. In Laboratory Animal Medicine, Güneştıp Kitapevi.