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Content

Review Article

Research Article

Short Communication

Case Report


OSANSKI BRDSKI KONJ

REVIEW ARTICLE

Discovery of new antibiotics using bioinformatics and machine learning methods

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ABSTRACT

Antibiotic resistance is a serious global health threat that causes approximately 1.27 million deaths worldwide each year and is expected to reach 10 million by 2050. New antibiotic development is exceptionally challenging, typically requiring 10-15 years and approximately $1.5 billion investment. In this process, genomic and metagenomic analyses play a critical role by revealing the genetic potential of unculturable microorganisms and identifying new antibiotic-producing microorganisms. Additionally, deep learning models analyze molecular structures to identify new compounds with antibacterial activity, and virtual screening techniques analyze large molecular databases to determine potential active compounds. It has been shown that models developed using deep learning can predict antibiotic biosynthesis gene clusters with over 90% accuracy. Alongside these approaches, the identification of antibiotic combinations and the prediction of synergistic effects allow for the development of more effective treatment strategies against multi-drug resistance. These methods contribute to the development of proactive approaches in managing antibiotic resistance and optimize the discovery of new antibiotics and the effective use of existing ones. This review examines the discovery of new antibiotics using bioinformatics and machine learning methods.

Keywords: Bioinformatics, machine learning, new antibiotic discovery

INTRODUCTION

Antibiotic resistance has emerged as one of the most critical global health threats of the 21st century and is defined by the World Health Organization as “one of the greatest threats to global public health, food security, and development” (WHO, 2023). This problem arises from bacteria developing resistance to antibiotics, causing treatable infections to become fatal. Various factors contribute to the rise of antibiotic resistance, including the excessive and inappropriate use of antibiotics, inadequate infection control, and the challenges in developing new antibiotics (Ventola, 2015). According to the World Health Organization (WHO), antimicrobial resistance (AMR) was responsible for an estimated 1.27 million deaths in 2019, based on global statistical modelling across 204 countries (Murray et al., 2022). These estimates include uncertainty intervals (95% UI 0.91–1.71 million deaths) and indicate that, if current trends continue, the annual number of deaths could reach 10 million by 2050. Furthermore, according to World Bank data, global GDP losses of up to 3.8% could occur by 2050 (World Bank, 2017). Addressing this serious threat

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requires a multifaceted approach, with strategies such as promoting the rational use of antibiotics, developing new antibiotics, strengthening global surveillance systems, adopting the One Health approach, and enhancing international cooperation being of paramount importance (Ajulo and Awosile, 2024).

The rapid spread of antibiotic resistance and the decreasing effectiveness of existing antibiotics have made the development of new antibiotics an urgent global health priority (WHO, 2023). However, the development of new antibiotics is a long, costly, and challenging process. Typically, the period from the discovery of a new antibiotic to its market release ranges from 10 to 15 years, with an average cost of approximately 1.5 billion USD (Luepke et al., 2017; Plackett, 2020). This lengthy process includes basic research, preclinical studies, clinical trials, and regulatory approval stages. Moreover, approximately 90% of drug candidates that enter Phase I–III clinical trials ultimately fail to reach approval, largely due to insufficient efficacy and unmanageable toxicity (Sun et al., 2022). The complexity of the new antibiotic development process, combined with its high cost and low return on investment, has reduced the willingness of pharmaceutical companies to invest in this field, leading to a decline in new antibiotic discoveries (Plackett, 2020). Indeed, while the discovery of new antibiotic classes peaked in the 1940s and 1950s, it has shown a significant decline since the 1960s. The numerical distribution of new antibiotic classes discovered between 1900 and 2009 is shown in Figure 1.

The average annual revenue generated after the market launch of a new antibiotic is approximately 46 million USD (Plackett, 2020). This figure is far from covering the development costs and is considerably lower compared to other types of drugs. In addition, the use of new antibiotics is often restricted to prevent the development of resistance, which further reduces sales and profitability. To address this issue, strategies such as promoting academia–industry collaborations, developing new business models, and increasing public funding have been proposed (Theuretzbacher et al., 2020). New financing models, such as the market entry reward, aim to reduce risk and encourage investment by providing substantial rewards to successful antibiotic developers (Årdal et al., 2020). Furthermore, global initiatives such as the Global Antibiotic Research and Development Partnership (CARB-X) and the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (GARDP) are working to accelerate the discovery and development of new antibiotics (Simpkin et al., 2017). Nevertheless, along with the development of new antibiotics, the rational use of existing antibiotics and the prevention of antimicrobial resistance are of great importance (Salam et al., 2023).

Bioinformatics and machine learning methods hold great promise in the fight against antibiotic resistance and have become an important tool in the discovery and development of new antibiotics. These technologies analyze large datasets to rapidly and effectively identify potential antibiotic candidates, offering significant time and cost savings compared to traditional methods (Stokes et al., 2020). Although the applications of bioinformatics

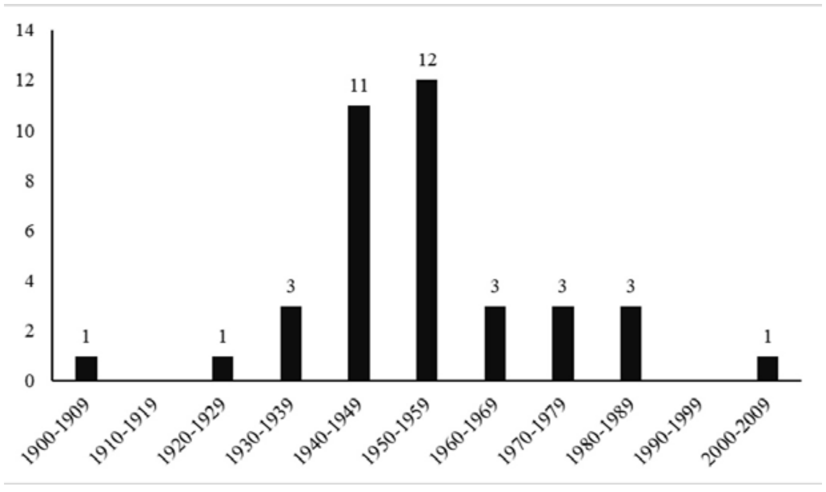

Figure 1 Historical distribution of new antibiotic class discoveries (Stennett et al., 2022)

and machine learning to antibiotic research have been discussed separately in the literature, an integrated perspective that combines both computational fields and their latest methodological advances remains lacking. This narrative review synthesizes recent developments in bioinformatics and machine learning applications for antibiotic discovery. A comprehensive literature search was conducted using PubMed, Web of Science, and Google Scholar databases.

Bioinformatics Methods

Genomic and metagenomic analyses play a crucial role in the discovery and identification of antibiotic-producing microorganisms. These culture-independent approaches are effective in revealing the genetic potential of microorganisms that cannot be cultured (Hover et al., 2018). By overcoming the limitations of traditional culture-based methods, these techniques enable the analysis of genome sequences from microbial communities in natural environments that may contain potential antibiotic-producing organisms. Machine learning algorithms are used to analyze large genomic datasets to predict antibiotic biosynthetic gene clusters (BGCs) and identify new antibiotic candidates (Stokes et al., 2020). Deep learning models can analyze molecular structures to detect compounds with potential antibacterial activity. Compared to conventional high-throughput screening methods, this approach enables the identification of new antibiotic candidates more rapidly and cost-effectively (David et al., 2021). Deep learning tools, such as DeepBGC have demonstrated strong performance in predicting antibiotic biosynthetic gene clusters. This tool achieved an accuracy score of 94.6% AUC in identifying new BGC classes that had not been encountered before (Hannigan et al., 2019). For example, the deep learning model DeepARG, developed by Arango-Argoty et al. (2018), can predict antibiotic resistance genes in metagenomic data with high accuracy. Such models offer higher sensitivity and specificity than traditional approaches. Bioinformatics and machine learning methods are also used for the functional characterization of resistance genes. The Comprehensive Antibiotic Resistance Database (CARD), developed by Alcock et al. (2020), provides a comprehensive catalogue of antibiotic resistance genes and associated phenotypes. This database is used to discover resistance genes present in the genomes of uncultured microorganisms through metagenomic analyses and to predict potential resistance functions of novel genes. A global study conducted by Hendriksen et al. (2019) utilized metagenomic data from wastewater samples to reveal the worldwide distribution and diversity of antibiotic resistance genes. Such studies provide critical information for monitoring and controlling antibiotic resistance at the community level.

The identification of target proteins is a critical step in the discovery of new antibiotics. The deep learning model DeepDrug3D, developed by Pu et al. (2019), can classify drug-binding sites in protein structures with high accuracy by performing three-dimensional analyses. Such models play an important role in identifying and characterizing new antibiotic targets, especially when combined with structural bioinformatics methods. These approaches help identify potential drug-binding regions on proteins, thereby improving the understanding of drug–protein interactions. AlphaFold2, developed by Jumper et al. (2021), enables highly accurate predictions of protein structures, allowing the structural analysis of proteins whose structures have not been determined experimentally. Such tools play a key role in the identification and characterization of potential antibiotic targets. In particular, accurately predicting protein structures is considered a critical step in drug design and in understanding biological processes.

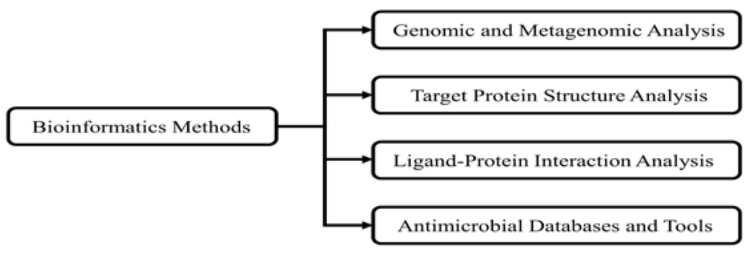

Figure 2 Overview of the Applications of Bioinformatics Methods in Antibiotic Discovery. This schematic illustrates the integrated workflow of bioinformatics approaches in antibiotic research

Modeling ligand–protein interactions is a critical stage in the design and development of new antibiotics, and with the intensive use of bioinformatics and machine learning methods, it has become increasingly sophisticated and effective. For example, molecular docking programs such as AutoDockVina, developed by Trott and Olson (2010), predict possible binding conformations of ligands on proteins, while deep learning models such as DeepDTA, developed by Öztürk et al. (2018), predict drug–target interactions more rapidly and accurately. The GROMACS software, developed by Abraham et al. (2015), offers high performance for conducting molecular dynamics simulations, enabling the investigation of the dynamic behavior of ligand–protein complexes. Pharmacophoremodeling tools such as LigandScout, developed by Wolber and Langer (2005), are used to define the structural features required for the biological activity of ligands through three-dimensional pharmacophore models. These advanced methods make it possible to screen broader chemical spaces more quickly and effectively during the drug discovery process.

Antimicrobial databases, as well as sequence and structure analysis tools, are critical resources in the discovery and development of new antibiotics. These tools provide researchers with access to extensive datasets, facilitating the identification and optimization of potential antibiotic candidates. For example, the Collection of Anti-Microbial Peptides (CAMP) database, introduced by Waghu and Thomas (2020), provides comprehensive information on antimicrobial peptides, enabling the investigation of their structure–activity relationships. Similarly, the Database of Antimicrobial Activity and Structure of Peptides (DBAASP), created by Gogoladze et al. (2014), is used to analyze the structure–activity relationships of antimicrobial peptides. For protein structure analysis, homology modeling tools such as SWISS-MODEL, updated by Waterhouse et al. (2018), help predict protein structures in cases where experimentally determined structures are unavailable. Additionally, molecular visualization and analysis programs such as UCSF Chimera, developed by Pettersen et al. (2004), allow detailed examination of protein structures and ligand–protein interactions. Data mining and text mining techniques are also widely used to extract information about antimicrobial compounds from scientific literature and biological databases. For instance, the DrugBank database, developed by Wishart et al. (2006), provides comprehensive information on approved and experimental drugs, serving as a valuable resource in identifying new antibiotic candidates. These tools and databases accelerate and optimize the process of discovering and developing new antibiotics by providing researchers with a vast pool of information. In the future, the further expansion and integration of these resources will contribute to the development of more effective strategies in combating antibiotic resistance.

Machine Learning Methods

Machine learning methods offer powerful tools for antibiotic discovery in bioinformatics. Supervised and unsupervised learning represent the two main approaches. In supervised learning, models are trained using labeled data; for example, compounds with known antibiotic activity can be used to predict new potential candidates (Stokes et al., 2020). Unsupervised learning, on the other hand, uncovers hidden structures in unlabeled data, which can be important for discovering new classes of antibiotics (Visan and Negut, 2024). Feature selection identifies the most informative features from large molecular datasets, thereby improving model performance and reducing computational load (Saeys et al., 2007). Data preprocessing steps include techniques such as handling missing values, detecting outliers, and eliminating redundant features; these steps enhance the reliability and generalization ability of models (Lee JW, 2022). By enabling the efficient analysis of large-scale biological and chemical datasets, these methods contribute to the faster and more efficient identification of new antibiotic candidates.

Antibiotic activity prediction plays a critical role in the discovery of new and effective antibiotics, with Quantitative Structure–Activity Relationship (QSAR) models and deep learning approaches standing out in this field. QSAR models aim to mathematically describe the relationship between molecular structure and biological activity, offering the ability to rapidly and cost-effectively screen large compound libraries (Cherkasov et al., 2014). These models involve the calculation of molecular descriptors, model construction using statistical or machine learning methods, and model validation. Deep learning approaches, on the other hand, have revolutionized antibiotic activity prediction in recent years, standing out for their ability to learn from complex and large-scale datasets (Stokes et al., 2020). Deep learning models are based on multi-layer artificial neural networks and have the capability to automatically learn complex molecular features. These two approaches play complementary roles: QSAR

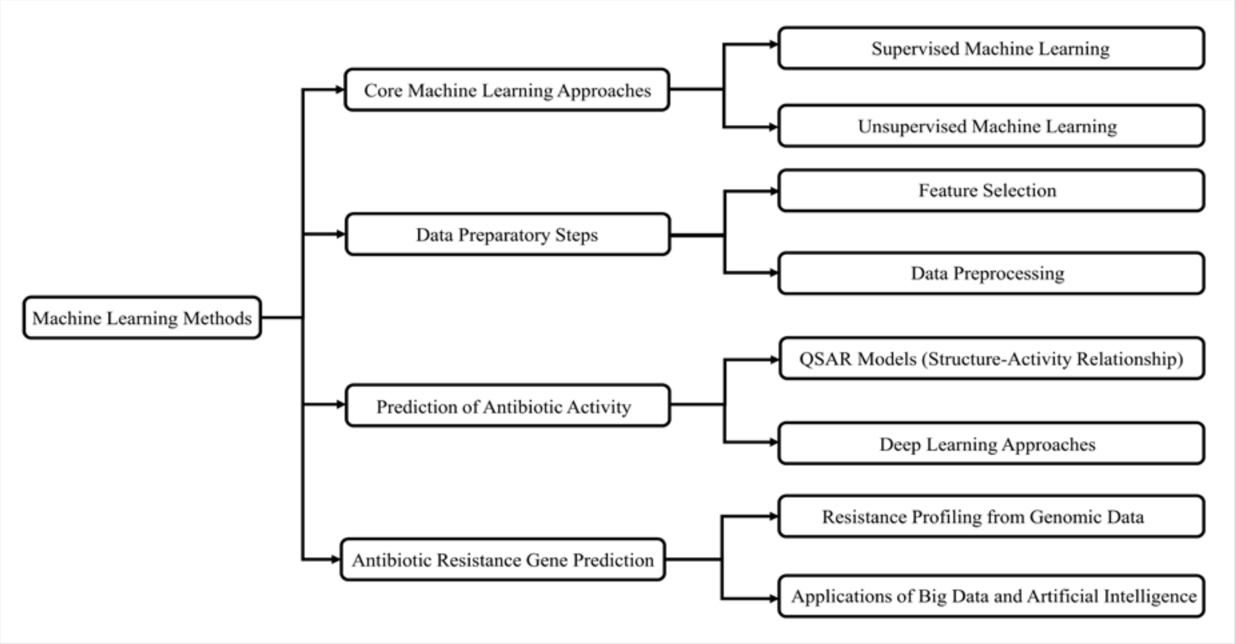

Figure 3 Overview of Machine Learning Applications in Antibiotic Discovery. This diagram depicts the machine learning pipeline for antibiotic research

models are advantageous in terms of interpretability and low computational requirements, whereas deep learning models excel at learning complex patterns and leveraging large datasets.

The prediction of antibiotic resistance genes is of critical importance for developing effective treatment strategies and discovering new antibiotics. In this field, two prominent approaches are the extraction of resistance profiles from genomic data and the application of big data and artificial intelligence.

Resistance profile extraction from genomic data is based on the comprehensive analysis of bacterial genomes, enabling the identification of potential resistance mechanisms. McArthur et al. (2013) developed the Comprehensive Antibiotic Resistance Database (CARD), creating a comprehensive resource that links genomic data with antibiotic resistance. This database provides a powerful tool for identifying and classifying resistance genes. Big data and artificial intelligence applications, on the other hand, integrate genomic data with clinical, epidemiological, and environmental information to produce more comprehensive and accurate predictions. For example, Nguyen et al. (2018) used machine learning algorithms to model the evolution and spread of antibiotic resistance, demonstrating that integrating different data sources can improve prediction accuracy. Similarly, Moradigaravand et al. (2018) applied deep learning methods to predict E. coli antibiotic resistance and showed that this approach achieved higher accuracy than traditional methods. These developments allow for the creation of faster, more accurate, and more comprehensive approaches to predicting antibiotic resistance genes, thereby contributing to the formulation of new strategies in the fight against antimicrobial resistance.

However, despite their high predictive performance, the global generalizability of these models in clinical decision-making and antibiotic discovery remains limited by the current data landscape, which is largely derived from high-income countries. Overcoming this bias will require expanding clinical datasets from diverse geographic and socioeconomic regions and adopting experimental conditions that more closely mimic the biological environment of infection (Nguyen et al., 2018; Peiffer-Smadja et al., 2020; Ayon, 2023).

Applications of Bioinformatics and Machine Learning

Bioinformatics and machine learning methods are driving groundbreaking advancements in the discovery of new antibiotic molecules. In this field, high-throughput screening (HTS), virtual screening techniques, and AI-assisted drug design stand out. HTS enables the rapid and automated testing of large compound libraries, thereby accelerating the experimental evaluation of potential antibiotic candidates (Ayon, 2023). Virtual screening is a technique that systematically screens large molecular databases using in silico methods during the drug discovery process to predict potential active compounds against a specific biological target. This method serves as an effective pre-screening tool, significantly reducing costs and time in the drug development process before moving on to expensive and time-consuming experimental stages (Oliveira et al., 2023). AI-assisted drug design makes it possible to design new molecules faster and more cost-effectively than with traditional methods. For example, in 2020, James Collins and his team used deep learning models to discover halicin, a broad-spectrum antibiotic. This study is a striking example of the potential of AI in antibiotic discovery (Stokes et al., 2020). Similarly, Zhavoronkov et al. (2019) used generative tensorial reinforcement learning (GENTRL) to design new drug-like molecules, demonstrating that this approach is faster than conventional methods. These developments highlight the critical role of bioinformatics and machine learning methods in the discovery of new antibiotic molecules and pave the way for more effective and faster drug discovery processes in the future. The identification of antibiotic combinations plays a crucial role in combating multidrug resistance and developing more effective treatment strategies. Chandrasekaran et al. (2016) conducted a large-scale antibiotic combination screening study using machine learning algorithms and discovered novel combinations with synergistic effects. This approach reduced the number of experimental trials needed, saving both time and cost. Benefo et al. (2024) utilized genomic data and machine learning algorithms to predict the resistance profiles of pathogens to antibiotic combinations. These advancements demonstrate the advantages that bioinformatics and machine learning methods provide in determining antibiotic combinations and open the door to the development of more effective and targeted combination therapies in the future.

Antibiotic resistance management is of critical importance to global health today, with two key approaches standing out: predicting the development of resistance and establishing effective monitoring and control strategies. The prediction of resistance development has been greatly enhanced through the use of bioinformatics and machine learning methods. For example, Nguyen et al. (2018) developed a system that predicts antibiotic resistance from bacterial genomes using machine learning models such as XGBoost. Feretzakis et al. (2020) employed various machine learning methods to model the relationship between antibiotic use and the development of resistance in hospital settings. In their study, they compared the performance of different methods in predicting antibiotic susceptibility and demonstrated that these approaches could be used to support empirical treatment decisions. The results showed that analyzing microbiological data alongside easily accessible information-such as basic patient details-can enable the early prediction of antibiotic resistance. These developments highlight the advantages that bioinformatics and machine learning methods provide in antibiotic resistance management and pave the way for the development of more effective and proactive resistance management strategies in the future.

The application of bioinformatics and machine learning methods in clinical settings brings not only theoretical advantages but also various practical challenges. Peiffer-Smadja et al. (2020) examined the potential of machine learning-based clinical decision support systems (ML-CDSS) in critical areas such as diagnosis, treatment management, and antibiotic selection in infectious diseases. Their study indicates that these systems can play a significant role in combating antimicrobial resistance by optimizing clinical decision-making processes. However, it also emphasizes that challenges, such as data quality, incompatibilities between healthcare systems, and real-time implementation need to be addressed for effective integration of ML-CDSS. Similarly, Rawson et al. (2018), in their evaluation of artificial intelligence applications within antimicrobial stewardship programs, discussed several challenges encountered in clinical practice. Their work highlights issues such as data integration problems between different hospital systems, the necessity of adapting algorithms to local populations, and the importance of healthcare professionals' trust in these systems. Furthermore, they stress that the successful adoption of AI-based systems requires involving end users in the development process and ensuring transparency. These studies demonstrate that, for bioinformatics and machine learning methods to be successfully implemented in clinical environments, technological advancements must be accompanied by organizational and cultural changes.

Table 1 Summary of representative bioinformatics and machine learning methods applied in antibiotic discovery

Method	Primary Application	Reported Performance (as stated in source)	Main Limitations	Reference
DeepARG	Identification of antibiotic resistance genes (ARGs) in metagenomic samples.	Precision ≈ 0.97; Recall ≈ 0.90 (cross-validation).	Data imbalance for underrepresented ARG classes; uncertainty in novel/rare variants.	Arango-Argoty et al., 2018
DeepDrug3D	Classification of protein–ligand binding pockets (nucleotide vs. heme).	AUROC 0.986 (nucleotide), 0.987 (heme); overall accuracy ≈ 95% (TOUGH-C1 dataset).	Limited to nucleotide/heme classes; depends on voxel representation and 3D structural data availability.	Pu et al., 2019
DeepDTA	Drug–target binding affinity prediction (regression).	CI: 0.878 (Davis), 0.863 (KIBA); MSE: 0.261/0.194; model type: CNN/CNN.	Sequence-only input; lacks 3D structural context; dataset-dependent performance.	Öztürk et al., 2018
AlphaFold2	Highly accurate prediction of three-dimensional protein structure.	In the CASP14 assessment, AlphaFold2 achieved near-experimental accuracy across all targets based on GDT_TS score distributions.	Limited accuracy for multi-domain complexes and flexible/disordered regions; ligand and cofactor positions not directly predicted.	Jumper et al., 2021
AutoDock Vina	Molecular docking and binding pose prediction.	According to the original publication, AutoDock Vina is a molecular docking program that is both much faster and more accurate than its predecessor, AutoDock 4.	Limited correlation with absolute affinities; receptor flexibility and solvent effects underrepresented.	Trott & Olson, 2010
GROMACS (MD simulations)	Molecular dynamics analysis and post-docking refinement.	No predictive accuracy metric; performance determined by force field and setup parameters.	High computational cost; results sensitive to force field and simulation timescale.	Abraham et al., 2015
CAMP	Curation and analysis of antimicrobial peptide (AMP) sequences and activities.	No quantitative metric (database-based resource); regularly updated content.	The ability of CAMPSign to identify peptides according to their families is limited by the number of family signatures it is currently trained on.	Waghu and Thomas, 2020
DBAASP	Antimicrobial peptide (AMP) activity and structure database.	Performance metric not applicable (curated dataset).	Experimental heterogeneity; variation in assay conditions.	Gogoladze et al., 2014
LigandScout	Deriving 3-dimensional (3-D) pharmacophores from protein-bound ligands and using these models as virtual screening filters.	It is fast enough to generate pharmacophores in "a few seconds" and selective enough to identify known targets without error.	Requires known ligand–protein complexes; qualitative and template-dependent.	Wolber and Langer, 2005

Method	Primary Application	Reported Performance (as stated in source)	Main Limitations	Reference
QSAR Models	Predicting biological activity, physicochemical, or toxicological properties of compounds from their molecular structure descriptors.	Performance depends on dataset and validation.	Interpretable and data-efficient, but limited generalizability and extrapolation beyond the training set.	Cherkasov et al., 2014
GENTRL	A deep generative model used to design de novo small molecule drugs by optimizing synthetic feasibility, novelty, and biological activity.	Out of the six compounds designed and synthesized by GENTRL, four exhibited activity in biochemical assays, with IC_{50} values of 10 nM, 21 nM, 278 nM, and 1 µM, respectively.	The generated compounds may require further optimization in terms of selectivity, specificity, and other medicinal chemistry properties.	Zhavoronkov et al., 2019

Case Studies

Bioinformatics and machine learning methods have achieved significant successes in the fields of antibiotic discovery and resistance management. One of the most notable antibiotic discovery projects was conducted by James Collins and colleagues, in which researchers used deep learning models to discover halicin, a broad-spectrum antibiotic. Halicin represents a new class of antibiotics shown to be effective against multidrug-resistant bacteria. This study demonstrated the potential of AI-assisted drug discovery, offering a much faster and more cost-effective discovery process compared to traditional methods (Stokes, 2020). Another antibiotic discovered using machine learning methods is Abaucin, developed against the multidrug-resistant Gram-negative pathogen Acinetobacter baumannii. Researchers screened approximately 7,500 small molecules to identify compounds that inhibit the growth of A. baumannii in vitro. They identified nine effective compounds and highlighted the most effective, Abaucin, as a potential therapeutic candidate. Abaucin is only effective against A. baumannii and acts by disrupting lipoprotein transport via the LolE protein (Liu et al., 2023). Moradigaravand et al. (2018) developed a machine learning model that predicts antibiotic resistance in Escherichia coli with high accuracy using whole-genome sequencing data. In their study, data from 1,936 isolates were used to predict resistance profiles for 11 different antibiotics. This approach was able to predict resistance without prior knowledge of resistance mechanisms and provided an important framework for integrating genomic and epidemiological data into clinical diagnosis. Similarly, Pesesky et al. (2016) evaluated the effectiveness of combining whole-genome sequencing data with machine learning and rule-based algorithms to predict the antibiotic resistance profiles of Gram-negative bacilli. The study showed that genotypic, data-driven predictions could be made more rapidly than phenotypic antibiotic susceptibility testing. This method offers significant potential, particularly in clinical settings, to optimize antibiotic selection and support antimicrobial resistance control strategies. These case studies illustrate the substantial advantages provided by bioinformatics and machine learning methods in antibiotic discovery and resistance management, offering hope for the development of more effective antibiotic therapies in the future.

Trends and Challenges

The applications of bioinformatics and machine learning methods in antibiotic discovery and resistance management offer great potential but also bring various challenges and ethical issues. Chief among these challenges is the processing and integration of large and heterogeneous datasets. While integrating genomic data enables a better understanding of complex biological systems, problems such as data quality and standardization remain significant obstacles (de la Lastra et al., 2024). Moreover, the interpretability and explainability of machine learning models are critically important, especially in clinical applications.

In this context, Rudin (2019) addressed the lack of transparency in AI models used for high-stakes decisions, emphasizing that transparent and interpretable models should be preferred. Future research directions include single-cell genomic analyses and AI-assisted drug design. For example, Zhavoronkov et al. (2019) used generative tensorial reinforcement learning (GENTRL) to design novel drug-like molecules and demonstrated that this approach is much faster and more effective than traditional methods. From an ethical and legal standpoint, the privacy and security of personal genomic data are major concerns. Mittos et al. (2019) discussed ethical issues and legal regulations related to the use of genomic data, drawing attention to the difficulties of balancing data sharing with privacy. Furthermore, the regulation of AI-assisted antibiotic discovery and its application poses new challenges for the pharmaceutical industry and healthcare systems. In light of these developments, it is evident that the effective use of bioinformatics and machine learning methods in antibiotic discovery and resistance management will require interdisciplinary collaboration, the establishment of ethical standards, and continuous technological innovation.

CONCLUSION

Antibiotic resistance is one of the most pressing threats to global health security, and addressing this challenge is critical to safeguarding human health. In this context, the opportunities offered by bioinformatics and machine learning in the fields of antibiotic discovery and resistance management go far beyond traditional approaches, representing a paradigm shift. The in-depth analysis of genomic and metagenomic data enables the identification of antibiotic-producing microorganisms and the mapping of resistance genes, with bioinformatics tools driving significant advances in these areas. Machine learning algorithms contribute by analyzing large datasets to predict antibiotic biosynthetic gene clusters and rapidly identify potential antibiotic candidates, while deep learning techniques greatly accelerate the detection of compounds with antibacterial activity through molecular structure analysis. Furthermore, the integration of virtual screening and high-throughput screening methods speeds up the discovery of potential active compounds and optimizes the experimental validation stage. The identification of antibiotic combinations and the prediction of synergistic effects make it possible to develop more effective treatment strategies against multidrug resistance. At the same time, the prediction and monitoring of resistance genes provide a proactive and dynamic approach to antibiotic resistance management, with the potential to shape global health policies. In conclusion, integrating bioinformatics and machine learning into antibiotic discovery and resistance management is not only accelerating research but also reshaping clinical and public health strategies against infectious diseases. Broader implementation of these approaches will be essential for developing new antibiotics, optimizing existing therapies, and strengthening global preparedness for future health challenges. Achieving this vision will require coordinated efforts across technical, regulatory, and collaborative domains.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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Otkrivanje novih antibiotika korištenjem bioinformatike i metoda mašinskog učenja

SAŽETAK

Otpornost na antibiotike predstavlja ozbiljnu globalnu zdravstvenu prijetnju koja svake godine uzrokuje oko 1,27 miliona smrtnih slučajeva, a smatra se da će taj broj do 2050. godine doseći 10 miliona. Razvoj novih antibiotika je izrazito zahtjevan proces koji obično traje 10-15 godina uz investiciju od oko 1,5 milijarde američkih dolara. U ovom procesu genomske i metagenomske analize igraju odlučujuću ulogu u otkrivanju genetskog potencijala mikroorganizama koji se ne mogu kultivirati, kao i u identificiranju novih mikroorganizama koji proizvode antibiotike. Osim toga, modeli dubokog učenja analiziraju molekularne strukture s ciljem identifikacije novih spojeva s antibakterijskom aktivnošću, dok virtualne tehnike skrininga analiziraju velike molekularne baze podataka s ciljem određivanja potencijalno aktivnih spojeva. Dokazano je da modeli razvijeni korištenjem dubokog učenja mogu predvidjeti genske klastere za biosintezu antibiotika s preciznošću od preko 90%. Osim ovakvih pristupa, identifikacija antibiotskih kombinacija i predviđanje sinergističkih učinaka omogućavaju razvoj efektivnijih terapijskih strategija u borbi protiv multirezistentnosti na lijekove. Ovakve metode doprinose razvoju proaktivnih pristupa upravljanju antibiotskom rezistencijom i optimiziranju otkrivanja novih antibiotika i učinkovitijoj primjeni postojećih. Ovaj rad ispituje otkrivanje novih antibiotika korištenjem bioinformatike i metoda mašinskog učenja.

Ključne riječi: Bioinformatika, mašinsko učenje, otkrivanje novih antibiotika

RESEARCH ARTICLE

Computed tomographic (CT) scan study of the anatomy of grasscutter (*Thryonomys swinderianus*, Temminck 1827): Preliminary observations

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ABSTRACT

The grasscutter (*Thryonomys swinderianus*) is an African rodent whose breeding has developed in part due to growing scientific interest**.** This work is to investigate the internal anatomy of grasscutter (*Thryonomys swinderianus*) using computed tomography (CT scan). Six grasscutters with an average age of 345 ± 45.63 days and varying sexes and weights, underwent general anesthesia with a ketamine, xylazine mixture and were then subjected to CT scan examination. Volumetric acquisitions were obtained and reconstructed using specific filters: "thoracic or pulmonary tissue," "abdominal tissue," and "bone tissue." Within the thoracic cavity, structures such as the trachea, bronchi, lungs, esophagus, heart, and aorta were identified. In the abdominal cavity, the liver was observed extending transversely from left to right. The stomach, which contained mineral content, as well as the spleen, were clearly identified. The kidneys were also visible, with the left kidney located more cranially than the right. The urinary bladder was identifiable depending on its degree of filling. These same organs have previously been described in rabbits, lemurs, and guinea pigs through CT imaging, supporting comparative anatomical analyses. However, the ureters could not be visualized on the current images. This study represents the first CT scan–based anatomical investigation of the grasscutter and offers valuable insights for applied research in health of this species.

Keywords: Anatomy, computed tomography, grasscutter, X-ray

INTRODUCTION

The grasscutter (*Thryonomys swinderianus*) is a large rodent of the family Thryonomyidae, widely distributed in sub-Saharan Africa. It is traditionally hunted and increasingly raised in captivity for its meat, which is highly appreciated in West African countries. In the wild, it inhabits wetland environments, including reed beds, herbaceous zones, and riverbanks (Mensah et al., 2007; Yapi, 2013).

Interest in this species has increased significantly, as reflected by the expansion of captive breeding and the growing body of scientific literature on its biology and management (Ananivi et al., 2025; Yapi, 2013). Several

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anatomical studies have investigated its reproductive, nervous, and digestive systems, due to their relevance for understanding its behavior, physiology, and nutritional needs (Broalet et al., 2012; Ibe et al., 2023).

Diagnostic imaging techniques, such as radiography, ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) offer effective, non-invasive means to study the anatomy of small mammals. Among them, CT is particularly useful for providing high-resolution cross-sectional images and three-dimensional reconstructions of both soft tissues and bony structures (Ajayi et al., 2010).

Radiographic assessments of the grasscutter have described the thoracic limb, cardiac anatomy, and gestational development (Ibe et al., 2024; Mpagike and Makungu, 2023; Mustapha et al., 2019). However, conventional radiography is limited to two-dimensional views and lacks depth of detail (Tarbell et Fischetti, 2020). CT scanning addresses these limitations by producing fine anatomical slices that allow detailed visualization of internal organs and pathological changes. Nevertheless, interpreting CT images requires prior knowledge of the normal cross-sectional anatomy (Boussarie, 2014).

CT-based anatomical references have been developed for several domestic and laboratory species, including rabbits, guinea pigs, ferrets, dogs, and cats (Winn, 2006; Zotti et al., 2009; Hoey et al., 2013; Calandra, 2016; Husté, 2016; Müllhaupt et al., 2017; Buch et al., 2022). However, no comparable data are currently available for the grasscutter. Therefore, the purpose of the present study was to use CT to investigate the normal thoracic and abdominal anatomy structures of the grasscutter. Specifically, it aims to provide detailed images to support veterinary anatomists, radiologists, and clinicians working with this species, as well as to establish a foundation for further anatomical, diagnostic, and comparative research.

MATERIAL AND METHODS

Ethical Approval

This study was approved by the Health Research Bioethics Committee (CBRS) of Togo under Opinion No. 056/2023/CBRS dated November 2, 2023. All animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council.

Biological Material

Six clinically healthy adult grasscutters, including three males and three females with a mean age of 345 ± 45.63 days, presented in Table 1, were purchased from local breeders in the Maritime region and included in the study. The average body weight was 4.15 ± 0.55 kg for males and 1.54 ± 0.40 kg for females with the sexual dimorphism reported by Mensah et al. (2007), who reported that males weighed approximately 2.5–4.5 kg and females 2–3 kg at one year of age. The animals were acquired at a young age (2 to 5 months) and fed with panicum and commercially available growing rabbit feed until reaching adulthood (≥ 6 months), at which point anatomical structures are fully developed. Drinking water was provided ad libitum.

Table 1 Characteristics of the grasscutters included in the CT scan study

Sex	Mean Body Weight (kg)	Mean Age (days)
Male	4.15 ± 0.55	373.1 ± 42.77
Female	1.54 ± 0.40	336 ± 48.53

CT Scan Period and Technique

The CT examination was performed in February 2024. The technique described by Boussarie (2014) for rabbits was adapted for this study on the grasscutter, also based on the protocols from Calandra (2016), Huber (2016) and Husté (2016). Imaging was performed using a Neuviz ACE scanner (manufactured in 2020, commissioned in 2021) located at the Human Clinic of Kozah Prefecture. Access to the device was granted exclusively outside of clinic hours.

Preparation and General Anesthesia

Prior to scanning, grasscutters were fasted for 2 to 3 hours. After clinical health assessment and weighing, animals were anesthetized by intramuscular injection

Figure 1 Animal positioning on the CT table: (A) Animal on dorsal recumbency; (B) Animals on lateral recumbency

of a ketamine (0.1 ml/kg body weight) and xylazine (0.5 ml/kg body weight) mixture to ensure complete immobilization during the procedure (Boussarie, 2014; Calandra, 2016; Huber, 2016; Husté, 2016).

Animal Positioning

Four grasscutters were positioned in dorsal recumbency, while two were placed in lateral recumbency shown in Figure 1.

Red laser guides were used to ensure accurate positioning. Animals' bodies were centered along multiple axes prior to image acquisition. The scan parameters are presented in Table 2 and include the following: slice thickness 2 mm, increment 2 mm, 100 kVp, 56 mA, and rotation time of 1 second.

Table 2 CT Scan Parameters

Parameter	Value
Scan Field of View	32 cm
Kilovoltage (kV)	100
mAs	49.7
Milliampere (mA)	56
Number of Images	204
Exposure Duration	29.84 seconds
Slice Thickness	2.00 mm
Slice Increment	2.00 mm
Computed Tomography Dose Index Volume (CTDIvol)	0.0 mGy
Dose Length Product (DLP)	364.8 mGy·cm

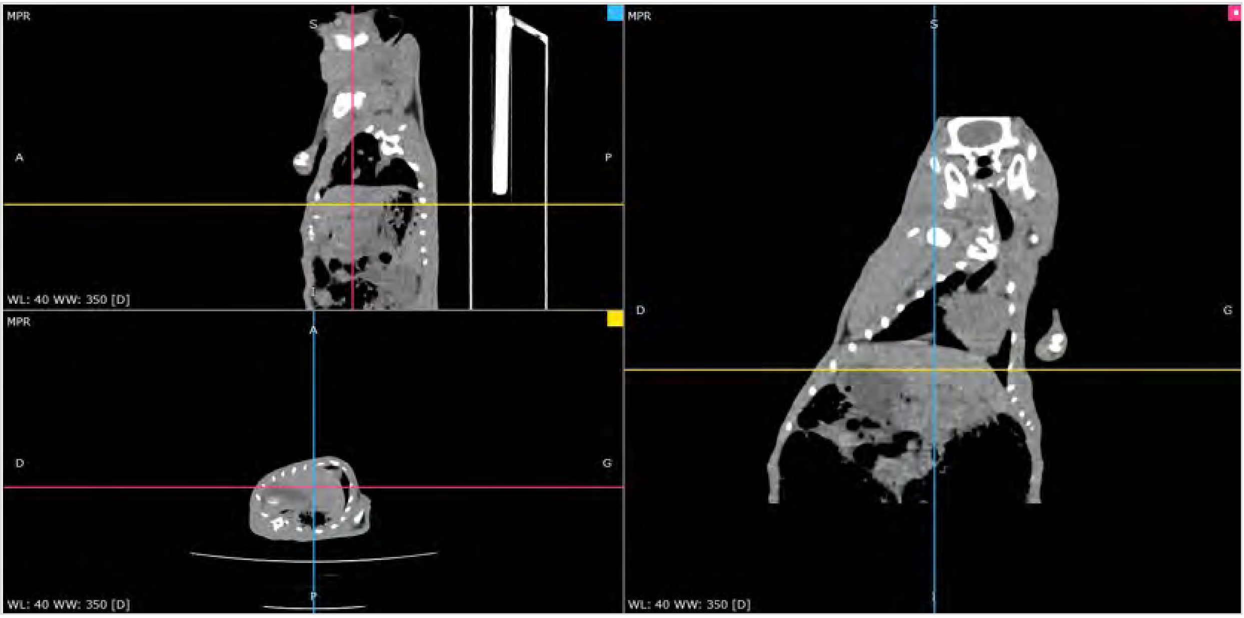

Figure 2 CT image visualization in MPR mode with RADIANT Viewer

Image Processing and Analysis

All volumetric data obtained from the CT examination were visualized using RADIANT Dicom Viewer 2023.1 software, which enabled image reconstruction and identification of anatomical structures. Multiplanar reconstruction (MPR) mode was employed to display transverse, sagittal, dorsal, and coronal planes shown in Figure 2. Additionally, the Minimum Intensity Projection (MinIP) mode facilitated detailed visualization of the respiratory system. Following anatomical recognition, selected transverse and coronal slices were chosen to isolate the greatest number of relevant anatomical structures. Depending on the region, different filters were applied, including « thoracic tissue», « abdominal tissue », « pulmonary tissue », and «bone tissue » filters.

RESULTS

The results of the CT scan examination performed on healthy grasscutters consist of annotated tomographic slice images. The most optimal image quality was obtained when animals were positioned in dorsal recumbency. Tomographic slices obtained using a given reconstruction filter revealed the main anatomical structures, recognizable by their grayscale intensity or anatomical location, and are presented with appropriate legends. On transverse CT sections of the head and neck, the bone filter enabled the identification of osseous structures such as the mandibles, palatine bone, zygomatic arch, and parietal bone (Figure 3).

Between vertebrae C7 and T1, skeletal elements, including the scapula, humerus, radius, ulna, and thoracic and cervical vertebrae, were visualized. Additionally, anatomical passages such as the nasopharynx, tympanic cavity, trachea, and esophagus were clearly delineated. Muscles of the scapular region, notably the infraspinatus and supraspinatus, were also identified on Figure 4.

Using the thoracic soft tissue and pulmonary filters, transverse slices between T7 and T10 allowed clear visualization of the heart, lungs, and main bronchi on Figures 5, 6 and 7. In these sections, the heart occupied a large portion of the mediastinum, while the esophagus and trachea were located dorsally. The two major vessels, the caudal vena cava and the aorta, were also identified.

Observation of the abdominal region with the abdominal soft tissue filter enabled identification of various soft tissue structures. A transverse image at the T10 level revealed the heart, lungs, and liver with distinguishable lobes on both sides. Slices between T10 and T13 shown on Figure 8 allowed identification of the stomach and spleen, with residual mineral contents in the stomach aiding its recognition.

The cecum, clearly visible and voluminous on the left

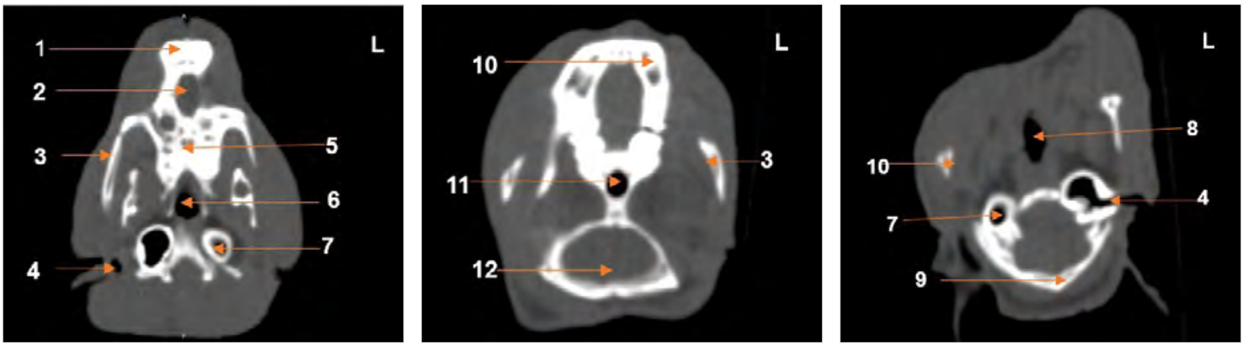

Figure 3 Transverse CT images at the cranial level of a male grasscutter (*Thryonomys swinderianus*)

1. Incisors; 2. Palatine fissure; 3. Zygomatic arch; 4. Tympanic cavity; 5. Palatine bone; 6. Choana; 7. Tympanic bulla; 8. Trachea; 9. Parietal bone; 10. Mandibles; 11. Nasopharynx; 12. Olfactory region

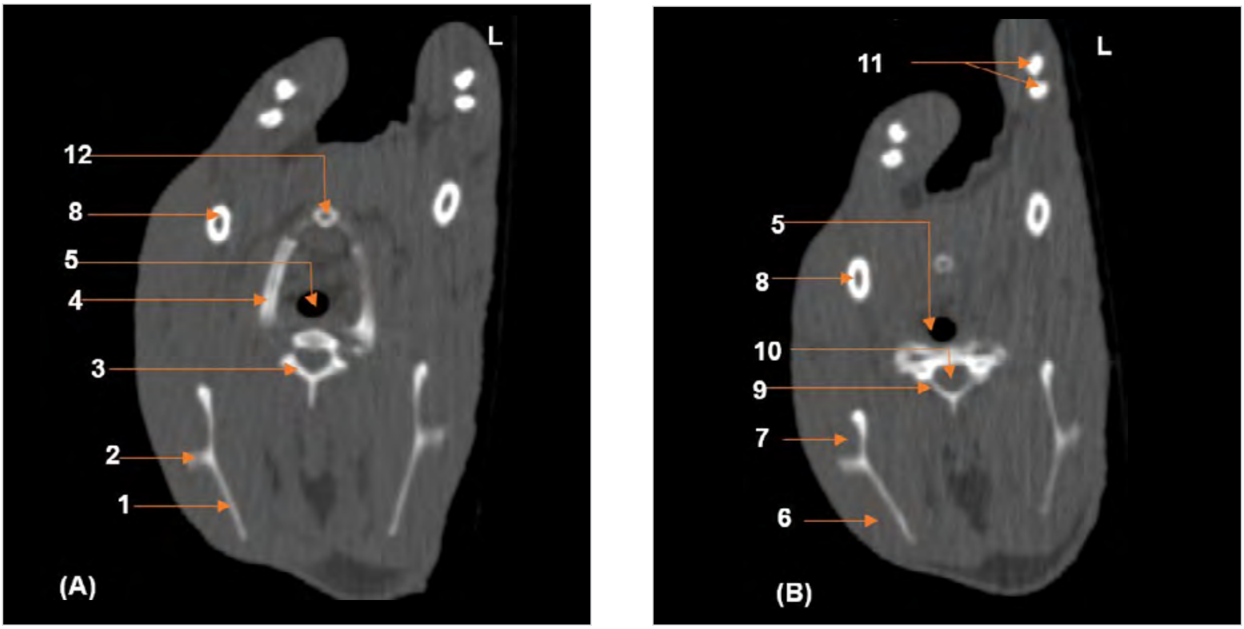

Figure 4 Cross-sectional CT-scans at the level of C7 (A) and T1 (B) vertebrae in the thoracic cavity of male and female grasscutters

1. Scapula; 2. Scapular spine; 3. First thoracic vertebra (T1); 4. First rib; 5. Trachea; 6. Supraspinatus muscle; 7. Infraspinatus muscle; 8. Humerus; 9. Seventh cervical vertebra (C7); 10. Medullary canal; 11. Radius and ulna; 12. Sternum

side, showed the presence of intraluminal gas. The colon was identified through its various segments. Additionally, abdominal and intestinal fat masses were apparent on Figure 9.

In males, the prostate and os penis could not be visualized, whereas in females, the ovaries and uterine horns were not visible. The urinary bladder was clearly delineated on Figure 9 with a homogeneous appearance, and its size varied depending on its content.

As shown on Figure 10, the analysis of CT sections

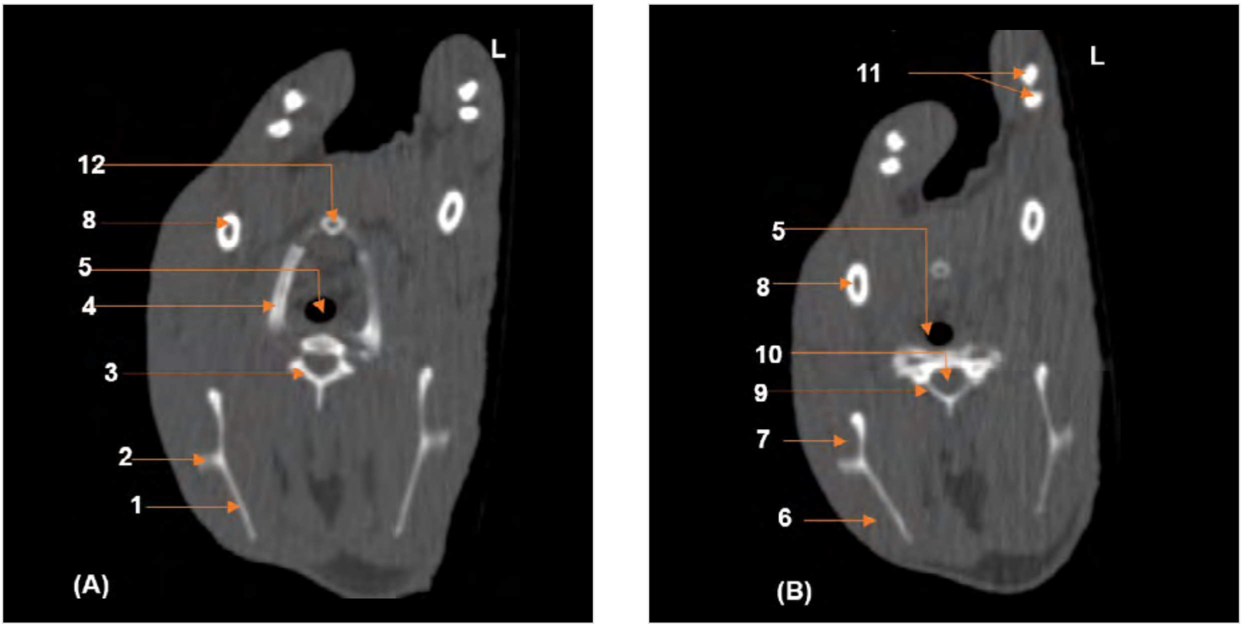

Figure 5 Computed tomography images showing the thoracic cavity using Minimum Intensity Projection (MinIP) (A) and a transverse section at T8 level (B) in a grasscutter

1. Trachea; 2. Right main bronchus; 3. Left main bronchus; 4. Oesophagus; 5. Left lung; 6. Right lung; 7. Heart; 8. Eighth thoracic vertebra (T8); 9. Vena cava; 10. Aorta; 11. Hand

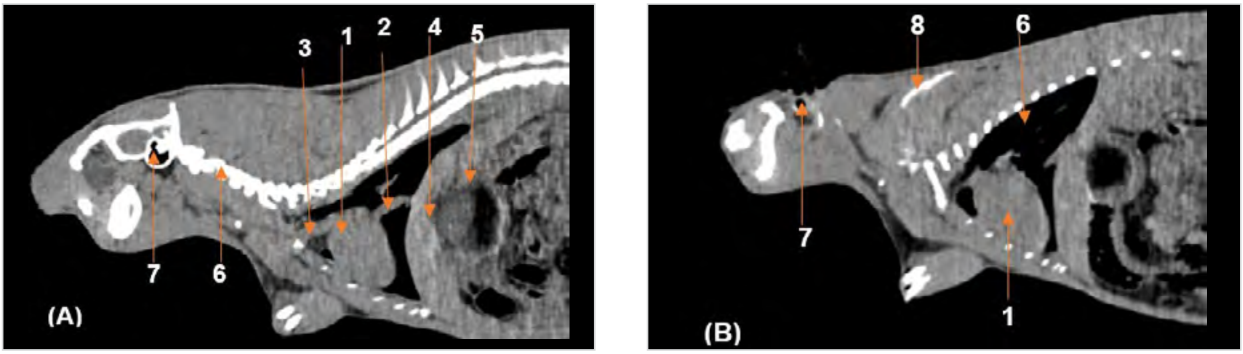

Figure 6 Sagittal CT images of the thoracic cavity in a male grasscutter (A) and a female grasscutter (B)

1. Heart; 2. Caudal vena cava; 3. Cranial vena cava; 4. Liver; 5. Stomach; 6. Left lung; 7. Tympanic bulla; 8. Scapula

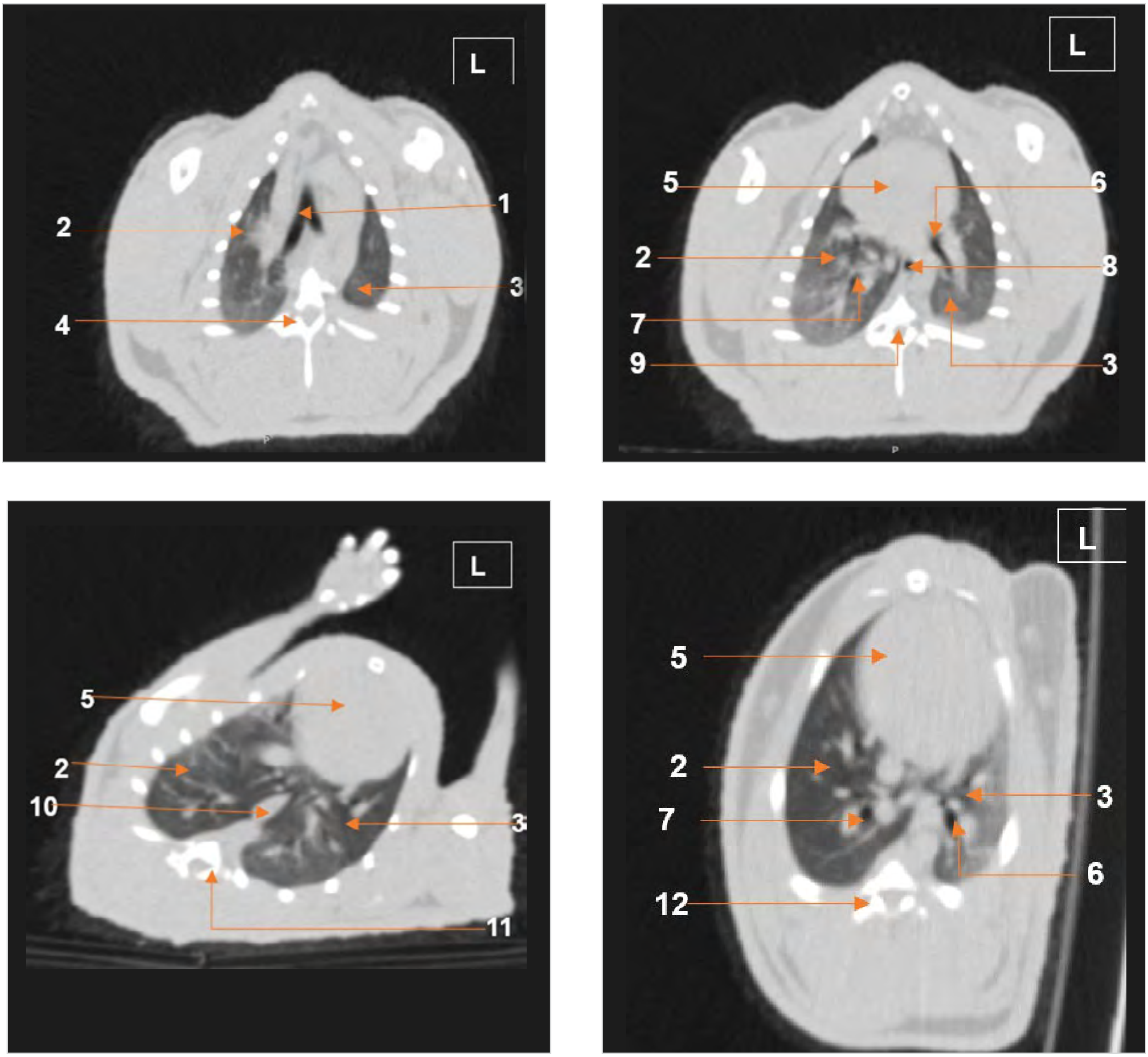

Figure 7 Transverse CT images of the thoracic cavity using a pulmonary window filter

1. Bronchial bifurcation; 2. Right lung; 3. Left lung; 4. Eighth thoracic vertebra (T8); 5. Heart; 6. Left main bronchus; 7. Right main bronchus; 8. Trachea; 9. Ninth thoracic vertebra (T9); 10. Oesophagus; 11. Tenth thoracic vertebra (T10); 12. Seventh thoracic vertebra (T7)

between L2 and L5 revealed both kidneys, with the left kidney showing close anatomical association with the spleen. However, the cortex and medulla could not be differentiated on these images. The ureters were not visualized, although the renal hilum could be identified.

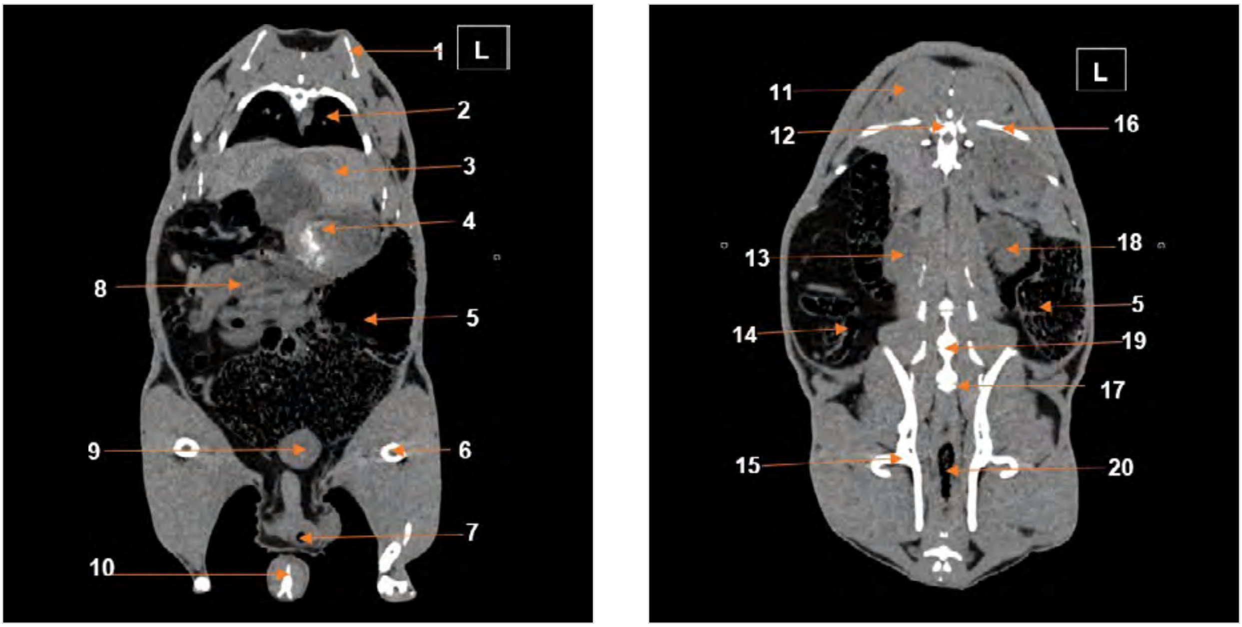

Figure 8 Cross-sectional CT images of the grasscutter's abdomen at the levels of T10 (A) and T13 (B)

1. Liver; 2. Right lung; 3. Heart; 4. Left lung; 5. Tenth thoracic vertebra (T10); 6. Pyloric part of the stomach; 7. Duodenum; 8. Funique part of the stomach; 9. Spleen; 10. Thirteenth thoracic vertebra (T13)

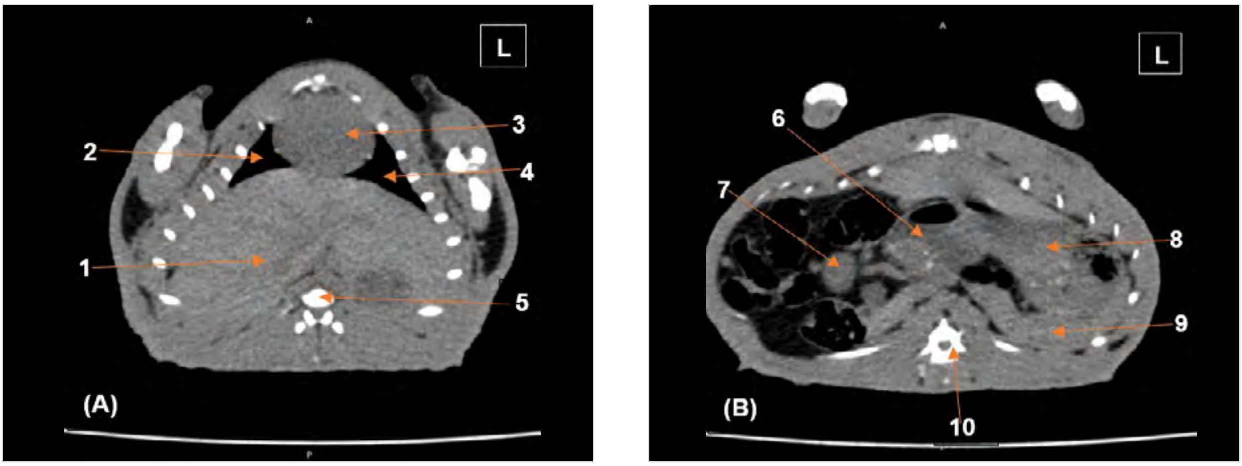

Figure 9 Dorsal and coronal CT-slices of the thoracic and abdominal regions of the grasscutter

1. Scapula; 2. Left lung; 3. Liver; 4. Stomach; 5. Cecum; 6. Femur; 7. Anus; 8. Intestinal mass; 9. Urinary bladder; 10. Coccygeal vertebra; 11. Longissimus thoracis muscle; 12. Twelfth thoracic vertebra (T12); 13. Right kidney; 14. Transverse colon; 15. Coxal bone; 16. Twelfth rib (R12); 17. First sacral vertebra (S1); 18. Left kidney; 19. Sixth lumbar vertebra (L6); 20. Descending colon

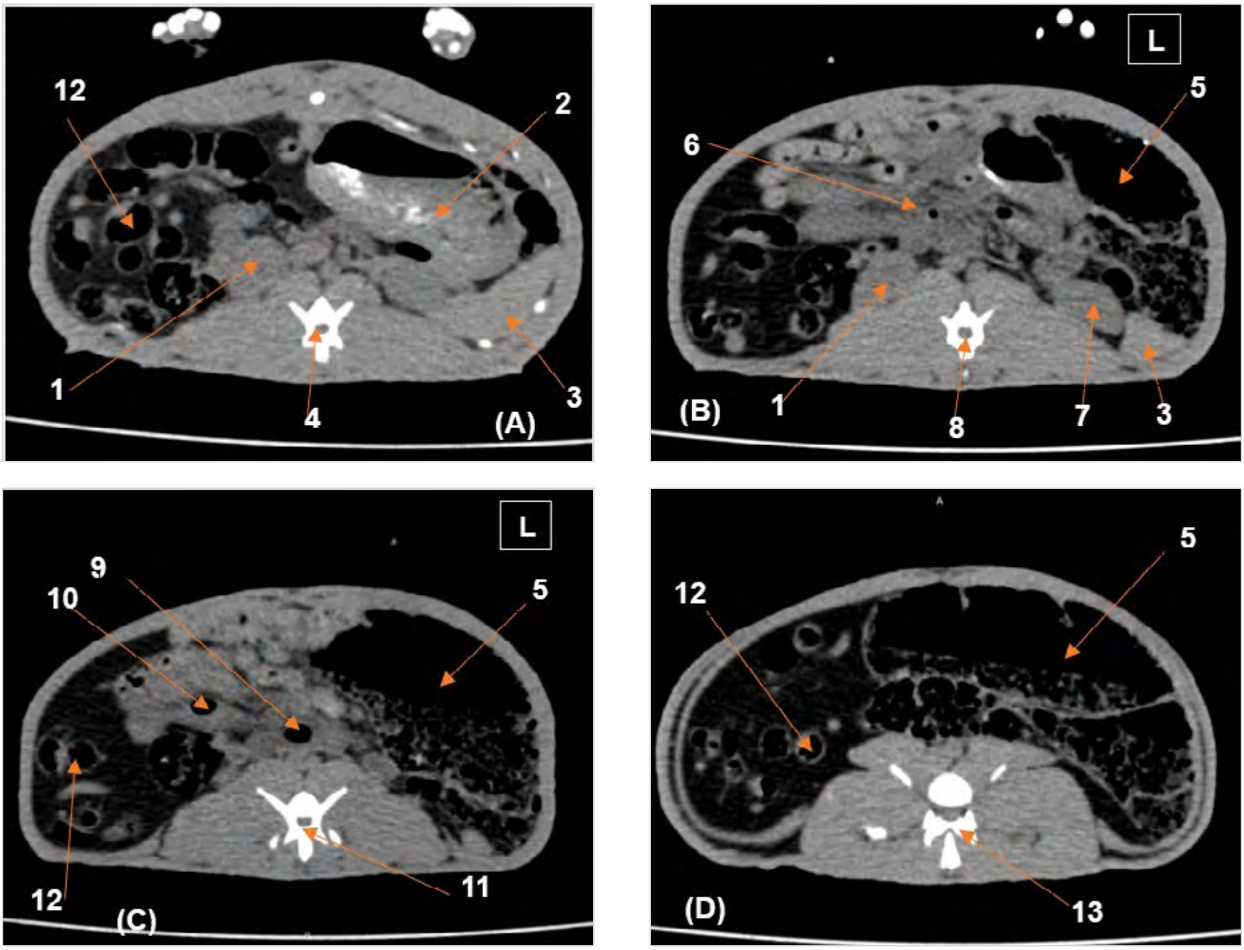

Figure 10 Cross-sectional CT-views of the abdomen at L1, L2, L3, and L5in the grasscutter

1. Right kidney; 2. Stomach; 3. Spleen; 4. First lumbarvertebra (L1); 5. Cecum; 6. Jejunum; 7. Left kidney; 8. Second lumbar vertebra (L2); 9. Descending colon; 10. Ascending colon; 11. Third lumbar vertebra (L3); 12. Transverse colon; 13. Fifth lumbar vertebra (L5)

DISCUSSION AND CONCLUSION

This study, the first to describe the tomodensitometric (CT) features of various internal organs in the greater cane rat or grasscutter (*Thryonomys swinderianus*), focused on comparing the obtained findings with existing data from other mammalian species. On CT images, bones appeared clearly hyperattenuating, and transverse sections of the head and cervical region distinctly depicted the mandibles, tracheal lumen, nasopharynx, and olfactory passages. These results confirm the high spatial resolution of CT imaging for skeletal and dental structures, as previously reported by Boussarie (2014). In clinical practice, CT is commonly employed in this region for detecting rhinitis, vestibular syndromes, and other cranial pathologies (Boussarie, 2014).

The thoracic cavity of the greater cane rat was relatively small, with reduced pulmonary volume, a feature also observed in rabbits (Müllhaupt et al., 2017). The heart occupied a substantial portion of the thoracic cavity, particularly within the cranial and middle mediastinum, as confirmed in this study by CT. These findings mirror those previously described in rabbits using both radiography and CT (Müllhaupt et al., 2017).

Interestingly, the anatomical position of the cardiac apex in the greater cane rat appeared variable across individuals, with a tendency toward a midline orientation rather than the typical left-sided position observed in dogs and cats (Kadja et al., 2007), and occasionally in aulacodes as well (Hoey et al., 2013). A similar variability has also been reported in rabbits (Müllhaupt et al., 2017). The evaluation of the major mediastinal vessels was limited by the absence of intravenous contrast administration, which would have enabled simultaneous opacification of cardiac chambers and vessels, facilitating detailed analysis. Between T2 and T11, the lungs of the greater cane rat demonstrated similar shape and distribution to those of the rabbit (Müllhaupt et al., 2017). On transverse sections, the diaphragm was often indistinct from the liver, likely due to their anatomical proximity, an appearance also noted in dogs and rabbits. The liver, primarily located between T11 and T13, exhibited a lobar arrangement comparable to that described in rabbits (Daggett et al., 2021). In the abdominal cavity, several digestive organs could be readily identified on CT images, although contrast enhancement would have further improved image quality. The stomach, caecum, and colon were clearly distinguishable, consistent with findings in other small mammals. Similarly, the kidneys and urinary bladder were easily identified on tomodensitometric images, as has been previously reported in dogs, cats, rabbits, ferrets, and lemurs (Calandra, 2016; Huber, 2016; Raharison, 2008). In females, the ovaries and uterine horns could not be visualized clearly, likely due to their attenuation values being similar to those of surrounding musculature. The prostate gland could not be identified in male individuals. This may be attributed to its small size and the limited contrast resolution between the gland and surrounding soft tissues on non-enhanced images (Raharison, 2008). Despite the absence of contrast, CT remains a valuable tool for assessing the condition of thoracic and abdominal organs.

Overall, this study was conducted to investigate the normal tomodensitometric anatomy of clinically healthy greater cane rats. Computed tomography is recognized as being superior to conventional radiography in the evaluation of internal diseases in small mammals (Müllhaupt et al., 2017). Due to their anatomical characteristics and physiological similarities to lagomorphs, greater cane rats, like rabbits (James et al., 2016), may serve as potential animal models for experimental research in biomedical sciences (Müllhaupt et al., 2017). Dorsal recumbency provided satisfactory image quality during scanning. However, mechanical limitations of the restraint device occasionally hindered consistent positioning. Lateral recumbency resulted in suboptimal visualization of left-sided structures. Similar positioning challenges have been reported in rabbits, guinea pigs, and lemurs, for whom sternal or ventral recumbency is preferred to optimize anatomical assessment (Boussarie, 2014; Buch et al., 2022; Calandra, 2016; Hoey et al., 2013; Huber, 2016; Husté, 2016; Mackey et al., 2008; Müllhaupt et al., 2017; Raharison, 2008; Zotti et al., 2009). This study provides preliminary species-specific anatomical information on the greater cane rat obtained through computed tomography. The results demonstrate that CT imaging, even without contrast enhancement, allows identification of major digestive reservoirs, the liver, spleen, certain urinary organs, and bony structures. These data are essential to advance research in health, nutrition, and reproduction of this species and may serve as a reference for future anatomical and tomographic studies.

The prospects offered by this study are promising. The introduction of contrast agents in future CT examinations could enable better visualization of organs and soft tissues, thereby providing a more detailed understanding of the greater cane rat's internal anatomy. Correlating CT images with traditional anatomical sections would also help validate and refine the observations, leading to a more precise and comprehensive anatomical mapping.

Furthermore, the anatomical information obtained could be applied to the diagnosis and treatment of diseases in greater cane rats, facilitating more targeted and effective medical interventions. CT images could also serve as educational tools for training veterinarians and researchers in comparative anatomy and medical imaging. Given that the greater cane rat is consumed in sub-Saharan Africa, this work may prove useful for assisting post-mortem inspection. Finally, an improved understanding of the greater cane rat's anatomy could support conservation and captive breeding programs by optimizing the care and living conditions of these animals. By combining various imaging techniques and anatomical approaches, this research paves the way for a rewarding exploration of the greater cane rat's anatomy, with potential applications across diverse scientific and practical fields.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – AA, CG, MT; Design – AA, CG, MT; Supervision – CG, MT; Funding – AA; Materials – AA; Data Collection and Processing – AA; Analysis and Interpretation – AA; Literature Search – AA; Writing Manuscript – AA; Critical Review – CG, MT.

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Kompjuterizovana tomografska (CT) studija anatomije divivskog trstičnog štakora (*Thryonomys swinderianus,* Temminck 1827): Preliminarna opažanja

SAŽETAK

Divovski trstični štakor (*Thryonomys swinderianus*) je afrički glodar čiji se uzgoj djelimično razvio zbog sve većeg naučnog interesa. Ovaj rad ima za cilj istražiti unutrašnju anatomiju divovskog afričkog štakora (*Thryonomys swinderianus*) pomoću kompjuterizovane tomografije (CT). Šest jedinki divovskog štakora, prosječne starosti 345±45.63 dana, različitog spola i težine, podvrgnuto je opštoj anesteziji pomoću mješavine ketamina i ksilazina, nakon čega je izvršeno CT skeniranje. Dobijeni su volumetrijski snimci koji su rekonstruisani korištenjem specifičnih filtera: „torakalno ili plućno tkivo“, „abdominalno tkivo“ i „koštano tkivo“.

Unutar torakalne šupljine identifikovane su strukture kao što su dušnik, bronhiji, pluća, jednjak, srce i aorta. U abdominalnoj šupljini uočena je jetra koja se prostire poprečno s lijeva na desno. Želudac, koji je sadržavao mineralni materijal, kao i slezena, bili su jasno vidljivi. Također su bili uočljivi bubrezi, pri čemu se lijevi bubreg nalazio nešto kranijalnije od desnog. Mokraćna bešika je bila prepoznatljiva, zavisno od stepena njenog punjenja. Isti ovi organi prethodno su opisani kod kunića, lemura i zamoraca pomoću CT snimanja, što podržava komparativne anatomske analize. Međutim, ureteri nisu mogli biti vizualizirani na trenutnim snimcima.

Ovoistraživanje predstavlja prvo anatomsko ispitivanje divovskog afričkog štakora zasnovano na CT snimanju i pruža vrijedne uvide za primijenjena istraživanja o zdravlju ove vrste.

Ključne riječi: Anatomija, kompjuterizovana tomografija, divovski trstični štakor, rentgenski snimak

RESEARCH ARTICLE

Novel atherogenic indices and risk of cardiovascular complications in patients of hypertension

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ABSTRACT

The study was aimed to evaluate the lipid panel parameters in *Hypertension* (HTA) patients along with gender-dependent comparison and *Cardiovascular Disease* (CVD) risk estimated based on Atherogenic Index of Plasma (AIP) values. This study aims to emphasize the importance of the lipid profile in blood in cardiovascular patients with hypertension. Total number of 136 adult HTA patients (85 female/51 male: ≤ 55 years (male:13, female:19); 56 - 70 years (male:23, female:47); > 70 years (male:15, female:19)) were included in this retrospective study. According to CVD risk estimated based on AIP values (Table 2), patients were classified into two groups: HTA patients with low/moderate CVD risk - AIP ≤ 0.21 (n=51) and HTA patients with high CVD risk - AIP >0.21 (n=85). Triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-high-density lipoprotein cholesterol (nHDL-C), total cholesterol (TC), atherogenic coefficient (AC), TG/HDL-C ratio, LDL-C/HDL-C ratio and TC/HDL-C ratio were used for comparison between the groups. Routine lipid parameters were analyzed by standard biochemical methods. For calculation of composite lipid indices, we used reference formulas. HTA patients with high CVD risk had significantly higher levels of TC (p=0.02), TG/HDL-C ratio, TG, AIP, LDL-C/HDL-C ratio, nHDL-cholesterol, TC/HDL-C ratio, and atherogenic coefficient, and significantly lower HDL-C compared to HTA patients with low/moderate CV risk (p<0.001, respectively). Our findings underscore the importance of considering various lipid profile parameters in risk stratification among patients with HTA.

Keywords: Atherogenic dyslipidemia, cardiovascular risk, hypertension

INTRODUCTION

Cardiovascular diseases (CVD) account for above 33% of deaths all around the world and is ranked as a leading cause of mortality. The prevalence of CVD is expected to rise rapidly because of the growing exposure to CVD contributing factors (Niroumand et al., 2015).

Significant predisposing factors for CVD include hypertension and hyperlipidemia. Recent study suggests that lipid profiles analysis commonly

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reported by laboratories may not show significant differences between patients with CVD and healthy individuals. Instead, focusing on the testing of subfractions or subpopulations of individual lipoproteins appears to be more significant and helpful (Chandra and Rohatgi, 2014).

Lipoprotein transport between the periphery and the liver is crucial for lipid metabolism homeostasis. Low-density lipoprotein (LDL) represents a class of lipoproteins that carries the most cholesterol and has the primary function of transporting cholesterol from the liver to peripheral cells. On the other hand, very low-density lipoprotein (VLDL) delivers to periphery, and cholesterol is carried to the liver from peripheral cells by high-density lipoprotein (HDL) (Zanoni et al., 2018; Karathanasis et al., 2017).

Recent studies analyzed blood levels of triglycerides (TG) derived from TG-rich lipoproteins (TRL), including VLDL and chylomicrons (Karathanasiset al., 2017; Mudhaffar, 2013; Parinita, 2012). Large molecules of VLDL and chylomicrons cannot pass through the endothelium of the blood vessel and enter the arterial wall, and in physiological conditions cholesterol is not produced from them. In metabolic disorders, small TRL molecules called residual lipoproteins are synthesized and can pass through the arterial wall. Elevated levels of small dense LDL (sdLDL) are caused by higher concentrations of TG. It is caused by cholesterol ester transfer protein and hepatic lipase activity, which contribute to the formation of sdLDL. The produced sdLDL oxidizes very easily and forms a very harmful abnormal lipoprotein, oxidized LDL cholesterol (oxLDL-C), which is considered a marker of oxidative stress (Kanthe et al., 2012; Borén and Taskinen, 2021).

A number of confirmed etiological factors can disrupt lipid homeostasis, leading to abnormal accumulation of lipids in the peripheral circulation and in the liver. The accumulation of lipid deposits, especially oxLDL-C, on the endothelium of blood vessels leads to the activation of foam cells and the development of atherosclerotic-fatty streaks and systemic inflammation with consequent cardiovascular events.

Disorder of serum lipid concentration is crucial in endothelial dysfunction development, a significant factor in the pathogenesis of conditions such as thrombosis, insulin resistance and hypertension. Various studies have shown that hyperlipidemia and hypertension may have a synergistic effect in atherosclerosis (Albucher et al., 2000).

Hypertension causes the walls of blood vessels to thicken. This occurs as a result of smooth muscle proliferation along with collagen in the central layer of the wall. Consequently, the lumen of blood vessels becomes narrower (Martinez-Quinones et al., 2018). In addition, the high concentration of lipids in the bloodstream makes it difficult to remove them, which results in their deposition within the walls of blood vessels. This deposition eventually leads to the formation of atherosclerotic plaques. Post-mortem studies of human coronary arteries and aortas from different regions of the world have led to the conclusion that atherosclerosis is extensively severe and prevalent in people with hypertension in comparison to those with normal blood pressure (Nasri et al., 2014).

Numerous clinical studies are currently underway in search of a more effective biomarker to assess lipoprotein metabolism, one that can accurately predict CV risk and assess the effectiveness of treatment response (Niroumand et al., 2015; Pirillo et al., 2021; Lopez and Adair, 2019; Fu et al., 2021).

In recent years, lipid indices have been studied for their prognostic values in various metabolic disorders and CVD (Niroumand et al., 2015). Previous studies have proved that to predict the risk of coronary heart disease and atherosclerosis, atherogenic index of plasma (AIP) is a vital biomarker (Carroll et al., 2019; Friedewald et al., 1972), and it is important to use it in combination with other risk markers. For the risk of cardiovascular events, the predictive value of AIP has been demonstrated in T2DM patients. A retrospective study performed on diabetic patients with high risk for CVD indicated an association between AIP and glucose and lipid metabolism abnormality. In addition, the role of AIP as a significant predictor of long-term prognosis in T2DM patients was established (Fu et al., 2021).

This study was destined to evaluate lipid profile parameters in hypertensive patients and compare them in association to gender and CVD risk estimated based on AIP values.

MATERIAL AND METHODS

This single centre study was conducted with the electronic records of 136 patients with hypertension admitted to Primary Health Care Centre in Zenica (Bosnia and Herzegovina) from January to August.

Participants were categorized as hypertensive, who showed systolic blood pressure (SBP) of 140 mmHg or higher, or a diastolic blood pressure (DBP) of 90 mmHg or higher.

Participants were split into two groups: HTA patients with less/moderate CVD - AIP ≤ 0.21 (n=51) and the second group of HTA patients with the high CV risk AIP >0.21 (n=85). Subjects with hypertension who had hematological diseases, malignant diseases or pregnant women were not included in this evaluation.

Sample selection

Demographic, clinical, and laboratory data were collected. We followed these parameters: age, sex, nHDL-C, AC, TC (mmol/L), HDL-cholesterol (mmol/L), triglycerides (mmol/L), LDL-cholesterol (mmol/L), LDL-C/HDL-C ratio, TG/HDL-C ratio, and TC/HDL-C ratio.

Lipid measurements were performed using an XT 1800i hematology autoanalyzer (Sysmex Corporation, Kobe, Japan) and an Olympus AU 480 chemistry analyzer (Beckman Coulter, USA).

Calculation of LDL-C levels was completed using Friedewald formula (Friedewald et al., 1972; Tseng et al., 2023).

HDL-C was subtracted from TC to calculate Non-HDL-C (Zaciragic et al., 2022).

TG was divided by HDL-C, TC by HDL-C, and LDL-C by HDL-C, to calculate TG/HDL-C ratio, TC/HDL-C ratio, and LDL-C/HDL-C ratio, respectively. Ratio of non-HDL-C/HDL-C gave AC values (Namitha et al., 2022).

Logarithm of the ratio between high-density lipoprotein cholesterol and triglycerides was taken to calculate AIP. It is then classified into different CVD risk categories. AIP values below 0.1 were considered low risk, values between 0.1 and 0.24 were considered medium risk, and values equal to or above 0.24 were considered high risk (Lumu et al., 2023).

Statistical analysis

Kolmogorov-Smirnov test was used to test dissemination of quantifiable variables. Standard deviation (SD) and Mean for normally distributed variables was used to express the descriptive statistics for continuous variables. Meanwhile, skewedly distributed variables were expressed as median and interquartile range. Evaluation of significance of mean differences between two groups was performed by independent two-sample Student's t-test. Difference in values of parameters revealed non-normal dispersal pattern when analysed by Mann-Whitney U-test. Chi-square was used to assess the differences between categorical variables. Statistically significant results were considered if the P values less than 0.05.

RESULTS

The initial and clinical characteristics of HTA patients included in the analysis according to gender are shown in Table 1. Overall, 85 (62.5%) of subjects were women. The mean age of the patients was 63.3 ± 10.2 years, with females having an average age of 63.2 ± 8.9 years and males 63.5 ± 12.2 years. No statistically significant variations were observed between the female HTA patients and male HTA patients when comparing age, the prevalence among different age groups, diabetes mellitus type 2 status, cardiovascular events status, or AIP/CVD risk status.

Table 1 Baseline and medical characteristics of HTA patients categorized by gender

	Total	Female	Male	p
n (%)	136 (100)	85 (62.5)	51 (37.5)	
Age (years)	63.3 ± 10.2	63.2 ± 8.9	63.5 ± 12.2	0.855
Age groups, n (%)				
≤ 55 years	32 (23.5)	19 (22.4)	13 (25.5)	0.449
56 - 70 years	70 (51.5)	47 (55.3)	23 (45.1)	
> 70 years	34 (25.0)	19 (22.4)	15 (29.4)	

	Total	Female	Male	p
Diabetes mellitus type 2, n (%)				
Yes	45 (33.1)	29 (34.1)	16 (31.4)	0.742
No	91 (66.9)	56 (65.9)	35 (68.6)	
Cardiovascular event, n (%)				
Yes	54 (39.7)	34 (40.0)	20 (39.2)	0.928
No	82 (60.3)	51 (60.0)	31 (60.8)	
AIP/CVD risk, n (%)				
< 0.1/Low	30 (22.1)	19 (22.4)	11 (21.6)	0.859
0.1-0.21/ Moderate	21 (15.4)	12 (14.1)	9 (17.6)	
> 0.21/High	85 (62.5)	54 (63.5)	31 (60.8)	

Analysis of lipid profile parameters revealed that triglycerides, HDL-cholesterol, serum TC, AIP, TG/HDL-C ratio, LDL-cholesterol, TC/HDL-C ratio, nHDL-C, LDL-C/HDL-C ratio, and AC did not differ significantly between the female and male HTA patients (Table 2).

Table 2 Lipid profile parameters of HTA patients according to gender

	Total (n=136)	Female (n=85)	Male (n=51)	p
Total cholesterol (mmol/L)	6.6 (5.8-7.4)	6.6 (5.9 – 7.3)	6.5 (5.8 – 7.5)	0.919
Triglycerides (mmol/L)	2.2 (1.6-2.8)	2.1 (1.6 – 2.95)	2.2 (1.6 – 2.8)	0.577
HDL-cholesterol (mmol/L)	1.2 (1.0-1.3)	1.1 (1.0 – 1.3)	1.2 (1.1 – 1.3)	0.129
LDL-cholesterol (mmol/L)	4.2 (3.5-5.0)	4.2 (3.4 – 4.85)	4.3 (3.6 – 5.1)	0.412
AIP	0.28 (0.12-0.45)	0.3 (0.12-0.46)	0.26 (0.14-0.43)	0.454
TG/HDL-C ratio	1.9 (1.3-2.8)	2.0 (1.32 – 2.86)	1.83 (1.38 – 2.67)	0.439
LDL-C/HDL-C ratio	3.5 (2.9-4.3)	3.6 (2.96 – 4.23)	3.3 (2.86 – 4.36)	0.770
TC/HDL-C ratio	5.5 (4.8-6.8)	5.75 (4.96 – 6.83)	5.2 (4.57 – 6.82)	0.251
nHDL-C	5.4 (4.6 – 6.2)	5.5 (4.7 – 6.2)	5.3 (4.5 – 6.4)	0.863
AC	4.5 (3.8 – 5.8)	4.8 (4.0 – 5.8)	4.2 (3.6 – 5.8)	0.251

HTA patients with high CVD risk exhibited notably elevated levels of total cholesterol (p=0.02), triglycerides, AIP, AC, TG/HDL-C ratio, LDL-C/HDL-C ratio, TC/HDL-C ratio, nHDL-cholesterol, and significantly lower HDL-C compared to HTA patients with low/moderate CV risk (p<0.001, respectively). However, values of LDL-C reported higher in HTA patients with high CVD risk compared to the HTA patients with low/moderate CV risk, but the change was statistically non-significant (p=0.169) (Table 3).

Table 3 Lipid profile parameters in HTA patients with low/moderate and high CVD risk

Variables	HTA patients with low/moderate CVD risk AIP ≤ 0.21 (n=51)	HTA patients with high CV risk AIP > 0.21 (n=85)	p
Total cholesterol (mmol/L)	6.2 (5.8 – 7.0)	7.0 (5.8 – 7.6)	**0.02**
Triglycerides (mmol/L)	1.5 (1.3 – 1.7)	2.5 (2.2 – 3.2)	**< 0.001**

Variables	HTA patients with low/moderate CVD risk AIP ≤ 0.21 (n=51)	HTA patients with high CV risk AIP > 0.21 (n=85)	p
HDL-C (mmol/L)	1.3 (1.2 – 1.4)	1.1 (1.0 – 1.2)	**< 0.001**
LDL-C (mmol/L)	4.0 (3.4 – 4.6)	4.3 (3.6 – 5.1)	0.169
AIP	0.1 (0.03-0.14)	0.38 (0.29-0.51)	**< 0.001**
AC	3.9 (3.4 – 4.5)	5.1 (4.2 – 6.2)	**< 0.001**
TG/HDL-C Ratio	1.25 (1.08 – 1.38)	2.42 (1.96 – 3.2)	**< 0.001**
LDL-C/HDL-C Ratio	3.23 (2.71 – 3.7)	3.9 (3.24 – 4.67)	**< 0.001**
TC/HDL-C Ratio	4.92 (4.43 – 5.45)	6.08 (5.2 – 7.2)	**< 0.001**
nHDL-cholesterol	4.9 (4.4 – 5.7)	5.9 (5.0 – 6.5)	**< 0.001**

DISCUSSION AND CONCLUSION

Dyslipidaemia hypertension is a very often used term to describe the coexistence of dyslipidaemia and hypertension, which are recognized as two of the majorly significant global CVD risk determinants. This label makes it easier to identify people who have both conditions. The combined impacts of high BP and elevated lipids level in serum are multiple. These metabolic abnormalities are regarded as one of the most crucial determinants for the onset of ischemic heart disease (IHD) or cardiovascular disease (CVD) (Kannel et al., 1971; Dalal et al., 2012; Karadimas TL and Meier HCS, 2024; Omidi et al., 2024).

This study presented differences in routine lipid profile and composite lipid parameters in HTA patients divided into two groups according to CVD risk estimated by AIP values: HTA patients with low/moderate CVD risk and HTA patients with high CV risk.

Results of the study revealed that significantly higher level of total cholesterol (p=0.02), triglycerides, nHDL-cholesterol, and significantly lower level of HDL-C compared to HTA patients with low/moderate CV risk (p<0.001, respectively) were present in HTA participants with greater CVD risk. However, the values of LDL-C were higher in HTA patients with high CVD risk compared to the HTA patients with low/moderate CV risk, but the variation was statistically non-significant (p=0.169).

This finding aligns with the criteria set by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) according to which the highly common disorder in serum lipid profile among HTA patients was noted to be an abnormally high level of LDL-C, followed by high levels of TC and TG. On the other hand, low HDL-C has been found to be the rarest lipid abnormality in HTA patients. It is worth noting that these lipid abnormalities often coexist and do not occur in isolation (Lipsy, 2003).

The findings of the current study appear to be consistent with those of Xie and colleagues who conducted a prospective cohort analysis that included non-hypertensive participants (n=2116). The main objective of the study was to develop a lipid risk score to evaluate the predictive impact of combined lipid profile components on HTA development. The authors concluded that an integrated lipid risk score, which combined LDL-C, non-HDL-C, TC and TG, independently of traditional risk factors, predicted the risk of HTA in people under the age of 55 years (Xie et al., 2022).

The association between high HTA risk and abnormal lipid profiles can be attributed to several pathophysiological mechanisms. One of these mechanisms involves the dysfunction of the vascular endothelium. Abnormal lipid profiles including elevated oxidation of LDL under hypercholesterolemic state, may result in declined production of endothelial nitric oxide synthase (eNOS). Meanwhile, HDL particles may enhance NO production by eNOS reactivity. This endothelial dysfunction characterized by impaired NO production and increased production of vasoconstrictors contributes to increased vascular tone and reduced vasodilation, finally leading to elevated blood pressure (Kawashima et al., 2004; Dąbrowska E and Narkiewicz K., 2023). Dyslipidemia, especially elevated levels of LDL-C, can stimulate the renin-angiotensin-aldosterone system (RAAS). This leads to increased production of angiotensin II, a potent vasoconstrictor. Angiotensin II acts on the smooth muscle cells of blood vessels, causing them to constrict and resulting in an increase in peripheral vascular resistance, ultimately leading to elevated blood pressure. Angiotensin II also promotes sodium and water retention, and oxidative stress, which ultimately affect the maintenance and development of HTA (Ni et al., 2013). Resistance to insulin is another key factor linking abnormal lipid profiles and HTA. Dyslipidaemia, particularly high triglycerides levels and low HDL-C levels, is often linked to insulin resistance. Resistance to insulin impairs the insulin capability to regulate the levels of blood glucose and promotes the inflammatory mediators release (adipokines and cytokines), leading to dysfunction of endothelium and vascular tone elevation, which are potential contributor to HTA (Tsuruta et al., 1996; Alidu et al., 2023). All these physiological mechanisms illustrate the complex interplay between abnormal lipid profiles and HTA, underscoring the significance of effectively managing dyslipidaemia as a potential approach to prevent or control HTA.

The outcomes of our evaluation revealed that values of lipid indices, AIP, AC, LDL-C/HDL-C ratio, TG/HDL-C ratio, and TC/HDL-C ratio were significantly different between HTA patients with high CVD risk and HTA patients with less/moderate CV susceptibility. Namely, the levels of TG/HDL-C ratio, LDL-C/HDL-C ratio, TC/HDL-C ratio, AIP and AC were markedly elevated in HTA patients with a high CVD risk compared to those with low or moderate CV risk ($p<0.001$, respectively).

The AIP and AC have been widely used as predictors of arteriosclerosis in CVD screening and risk stratification. However, the studies focusing on the hypertensive population in relation to these markers are not enough. The results of a study based on 211,833 Chinese adults showed that AIP and AC were elevated in HTA patients in comparison with the normotensive participants, suggesting that a higher proportion of dyslipidaemia has relatedness with HTA. The study indicates the incidence of HTA especially in women, can be readily predicted by AC values (Cheng et al., 2022).

A higher AIP is found to have a positive and crucial association with the risk of hypertension or prehypertension in normoglycemic individuals in Gifu, Japan. This association is predominantly expressed among women, especially those between the ages of 40 and 60 (Tan et al., 2023).

Relationship between TG/HDL-C ratio and HAT was not consistent with the results obtained in the research. A cohort study on men was conducted in Spain, which elaborated that HTA and TG/HDL-C ratio had positive association. The study followed participants for an average of 8.49 years and five equal categories were developed for TG/HDL-C ratio. The risk of HTA was increased up to 90% in the patients with the highest TG/HDL-C ratio compared to the patients with the lowest ratio of TG/HDL-C (Sánchez-Íñigo et al., 2016).

Wu et al. explained in a prospective study that in hypertensive population, arterial stiffness progression had significant association with TG/HDL-C ratio. This was observed during a median follow-up of 4.71 years, but this link was not found in prehypertensive population (Wu et al., 2021).

In the current study, women with HTA were observed to have higher TC, AIP, AC, nHDL-C, LDL/HDL-C, TC/HDL-C, and TG/HDL-C compared to men. However, it is important to note that these differences were not statistically significant. Recent study has shown that non-elderly patients are more likely to have dyslipidaemia compared to the elderly. In comparison to males, higher levels of LDL-C, TG and TC were also observed in female hypertensive patients. Conclusively, authors of the study recommend that the healthcare professionals pay more attention to the lipid profile of HTA patients, especially those who are younger and female (Wu et al., 2022). The variations in hormone levels between men and women contribute to differences in lipid metabolism. Sex hormone concentrations have been found to affect lipoprotein levels, with estrogen playing a significant role in regulating lipid metabolism (Palmisano et al., 2018). In addition, follicle-stimulating hormone (FSH) levels are associated with serum cholesterol levels. However, the influence of progesterone and androgens only partially explains the disparities in serum lipid levels and further research is needed to fully understand their role in lipid metabolism (Wang et al., 2011). Sex differences in the onset and progression of HTA in humans may be attributed to variations in sympathetic nerve activity (SNA) and specific brain regions that express different estrogen receptor subtypes (Sabbatini and Kararigas al., 2020). Gaining insight into the mechanisms by which estrogen affects SNA regulation in key brain regions is important for the development of new, gender-specific treatment for HTA (Hay, 2016).

This investigation has certain constraints that must be recognized. Firstly, the broader applicability of these findings to a wider population is limited because a single centre with a small sample size was used to perform this retrospective study. In addition, detailed patient history and medication use were not ensured, making it difficult to establish a clear association between traditional risk factors (family history, smoking, physical activity, menopause, obesity) and the development of dyslipidaemia and HTA.

However, our findings underscore the importance of considering various lipid profile parameters, including routine parameters and composite lipid indices, in risk stratification among hypertensive patients. The observed differences in lipid profiles between different risk groups highlight the potential utility of these parameters as valuable indicators for cardiovascular risk assessment in this patient population.

Extensive research and advanced studies on a large-scale may be necessary to elaborate the clinical implications of these distinctions and their role in guiding therapeutic interventions for hypertensive patients, at varying levels of CVD risk.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Conception: ED, LČ, ZA, EM, AB, HS; Design: LČ, EM, AB, FK, HS, AF; Supervision: ED, AD, ZA, FK, EL, NŠ AF; Materials: ED, AD, AB, AF; Data Collection and/or Processing: LČ, ZA, FK, EL, HS; Analysis and/or Interpretation: ZA, EM, FK, EL; Literature Search: ED, AD, ZA, EL, NŠ, HS; Writing – Original Draft: ED, LČ. AF; Critical Review: AD, AB, AF.

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Novi aterogeni indeksi i rizik kardiovaskularnih komplikacija kod pacijenata sa hipertenzijom

SAŽETAK

Cilj istraživanja je evaluacija parametara lipidnog panela kod pacijenata s *hipertenzijom* (HTA) te usporedba između spolova, kao i *kardiovaskularnog* rizika (CVD) na osnovu vrijednosti aterogenog indeksa plazme (AIP). Ovo istraživanje ima za cilj naglasiti značaj koji lipidni profil krvi ima kod kardiovaskularnih pacijenata s hipertenzijom. U ovu retrospektivnu studiju je uključeno ukupno 136 odraslih pacijenata s HTA (85 žena/51 muškarac: ≤ 55 godina (muškarci:13, žene:19); 56 - 70 godina (muškarci: 23, žene:47); > 70 godina (muškarci:15, žene:19). Pacijenti su prema CVD riziku procijenjenom na osnovu vrijednosti AIP (Tabela 2) podijeljeni u dvije grupe: HTA pacijenti s niskim/umjerenim CVD rizikom - AIP ≤ 0.21 (n=51) i HTA pacijenti s visokim CVD rizikom - AIP >0.21 (n=85). Trigliceridi (TG), lipoprotein niske gustoće s holesterolom (LDL-C), lipoprotein visoke gustoće s holesterolom (HDL-C), lipoprotein ne-visoke gustoće s holesterolom (nHDL-C), ukupni holesterol (TC), aterogeni koeficijent (AC), omjer TG/HDL-C, omjer LDL-C/HDL-C i omjer TC/HDL-C su korišteni za usporedbu između grupa. Rutinski lipidni parametri su analizirani standardnim biohemijskim metodama. Za izračunavanje ukupnih lipidnih indeksa su korištene referentne formule. Pacijenti sa HTA i visokim CVD rizikom su imali znatno više koncentracije TC (p=0.02), omjer TG/HDL-C, TG, AIP, omjer LDL-C/HDL-C, nHDL-holesterol, omjer TC/HDL-C i aterogeni koeficijent, a znatno nižu koncentraciju HDL-C u odnosu na HTA pacijente s niskim/umjerenim KV rizikom (p<0.001). Naši rezultati naglašavaju značaj razmatranja različitih parametara lipidnog profila kod stratifikacije rizika kod pacijenata s HTA.

Ključne riječi: Aterogena dislipidemija, hipertenzija, kardiovaskularni rizik

RESEARCH ARTICLE

Prevalence and risk factors of feline calicivirus and assessment of knowledge, attitudes and practices among cat owners in Pakistan

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ABSTRACT

Feline calicivirus is an upper respiratory tract infection characterized by mouth ulcers and runny eyes, and one of the commonest viral infections in cats. This study was conducted to determine the prevalence of feline calicivirus and to identify the public's knowledge, risk factors, attitude and practices towards calicivirus and general care of cats in Pakistan. The prevalence was determined based on diagnostic data (pathological observations and serological tests, i.e. CBC) from veterinary clinics in the study area during 2021. A questionnaire was designed to access the sociodemographic, knowledge, attitude and practices regarding feline calicivirus among 298 pet owners. The overall prevalence of feline calicivirus was 27.5%. The majority (62.75%) of the pet owners showed poor overall knowledge about the transmission of the virus and its infectiousness. A poor attitude towards the prevention of feline calicivirus was also observed in 52.68% of respondents. Most pet owners (62.75%) had poor values regarding the cleanliness of their cats. Because most surveys were completed by responders living in urban areas, very few cats were exposed to toxins such as pesticides. Despite not knowing much about the virus itself, responders were keen to keep their feline pets healthy with good hygiene, however, only a minimum ensured it.

Keywords: Islamabad, knowledge, mouth ulcers, prevalence, risk factors

INTRODUCTION

Feline calicivirus is a virulent systemic disease characterized by oral ulcers, edema, alopecia, jaundice (Pesavento et al., 2004), upper respiratory infection, lethargy, nasal and eye discharge, anorexia, sneezing and pyrexia (Wardley and Povey, 1977). The virus is a single-stranded piece of RNA with a length ranging up to approximately 7.7 kb and three open reading frames (Fumian et al., 2018). The particles are 33.5nm in diameter and have scalloped borders and surface indentations. It has strong genetic adaptability, meaning it has a tendency to mutate, which, in turn, increases mortality rates. Mutation could cause resistance to the vaccine administered in cats, which could increase fatality, if infected (Stone et al., 2020). In 2008, a chronic variant

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of feline calicivirus emerged with a higher mortality rate (Hofmann-Lehmann et al., 2022). It was associated with virulent systemic disease leading to ocular lesions, mainly conjunctivitis (Pesavento et al., 2004). Experiments have shown that the clinical symptoms are due to the combination of epithelial (cytolytic) and endothelial injury. Upon closer examination, antigens were found in the necrotic epithelial cells of various tissues. These included the cells of the mucosa, skin follicles and affected alveolar septae and bronchioles. Some of the viral particles were found in the pancreatic exocrine cells but were limited to the necrotic portions (Wardley and Povey, 1977).

The infected cat discharges a large number of oral secretions, which are the primary cause of virus transmission. Although the secretion rate is maximal during the onset of the disease, it progressively reduces. It is rare for an infected cat to shed after 30 days since it contracted feline calicivirus (Radford et al., 2021). The treatment of the virus includes intravenous fluids. In extreme cases where the cat is completely unable to eat, it is important to offer semi-solid food with an intense appealing smell. This is because the cat might be resistant to eat due to the ulcers in the mouth and nasal congestion (Hofmann-Lehmann et al., 2022).

Although calicivirus shows milder symptoms than feline herpesvirus, differentiating between the two viruses is difficult due to the high similarity of clinical symptoms; however, oral ulcers are present in feline calicivirus (Najafi et al., 2014). There are regional variations in the prevalence rate of feline calicivirus, with rates in Iran as low as 2.5% and in Japan as high as 59.1%. Prevalence rates of 17%, 9.2%, 13-36%, and 7.2%, respectively, were found by studies conducted in Pakistan, Europe, California, and Southern Italy. A recent study in Wuhan, China discovered a higher incidence rate of 40% for upper respiratory tract infections, indicating that increasing population density increases the danger of contagion (Afonso et al., 2017; Gao et al., 2023; Coyne and Elwyn, 2006).There is scarcity of literature in Pakistan about feline calicivirus; the present study was aimed to assess the seroprevalence of FCV and assessment of FCV-related knowledge, attitudes, and practices (KAP) among cat owners.

MATERIALS AND METHODS

Study Area

The current study was conducted in Rawalpindi and Islamabad (Twin cities). Both have an increasing population, which across both cities is 1.3 million (Maria and Imran, 2006). Rawalpindi and Islamabad are in the moderate seasonal region, with mild winter temperatures and summer heat and humidity. Due to its yearly temperature range of 21.3°C and precipitation range of 1201 mm, the city has a moderate environment. Islamabad's weather has a distinct seasonal pattern, with spring lasting from March to May, summer beginning in June and lasting until August, autumn, which runs from September to November, and frigid winter, which runs from December to February (Köppen et al., 2011).

Data Collection

Data collection included 02 phases, in the first phase we collected data regarding prevalence of feline calicivirus in cats, in which veterinary clinics were visited in the study area to collect epidemiological (age, gender, breed and color etc.), pathological and diagnostic data. Both private and government veterinary clinics (Hope Pet Clinics, Dr. Rana's pet clinic, Pet Point clinic and the Pets and Vets clinic.) were visited. While in the second phase, the survey was conducted among 298 cat owners in Islamabad and Rawalpindi. Since cats are mainly owned in urban areas, these areas were the focus of the study. Information was collected from cat owners and veterinary clinics about the prevalence of feline calicivirus for the past two years. The survey was conducted from July to December, 2021.

Diagnostic Methods

Physiological and blood tests were carried out to diagnose feline calicivirus (Allison and Little, 2013). Physiological assessments were done by the veterinary doctors, physically examining the felines and looking for symptoms, such as mouth ulcers (the most common symptoms), watery eyes and runny nose. The blood tests involved assessment of a sample for viral identification. Elevated white blood cells in the CBC reports alongside the physical symptoms confirm the diagnosis of feline calicivirus in the specimens.

Study Design and Instruments

A questionnaire was designed to collect data on sociodemographic characteristics as well as knowledge, attitude and practices about feline calicivirus (Mindekem et al., 2018; Potter et al., 2016; Zöldi et al., 2017). A sample size of 385 pet owners was obtained from Islamabad and Rawalpindi. The sample size was determined using the Sample Size Calculator by Raosoft, Inc. The pet owners filled out an online survey and face to face interviews were conducted with a detailed questionnaire. This community-based survey was conducted to study the KAPs. A contrast to be conducted between the different categories of cats as well as their genders and the role vaccinations play in preventing the prevalence of the disease. A questionnaire survey was designed that outlined the important perspectives of the study (Ma et al., 2017). The questionnaire included 48 questions split into four sections: sociodemographic (n = 11), knowledge (n = 12), attitude (n = 9) and practices (n = 22). In this study, the dependent variables were the knowledge, attitude and practices of the pet owners; and the independent variables were their sociodemographic data.

Ethical Approval

The study was approved by the Institutional Review Board (IRB) of the Department of Biosciences, COMSATS University Islamabad, Pakistan.

Data Analysis

Strict data cleaning protocols were used before analysis to guarantee the dataset's completeness and correctness. A database was created by entering data into Microsoft Excel. To prevent bias in the results, duplicate and incomplete data were eliminated. Spreadsheets in Microsoft Excel were updated with the data. The statistical SPSS was used to import, analyze, and arrange the data once it had been gathered in an Excel sheet. Basic frequencies were derived, and the Chi-square method was used to study the correlation between different variables, such as age, gender, vaccination status and domestication status (Maazi et al., 2016). The complete collection of data is shown in tables and narrative form based on responses that were marked as binary (yes/no).

RESULTS

The results of the present study were classified into two sections. The first section has prevalence of feline calicivirus and their risk factors, and the second section has the assessment of knowledge attitudes, and practices of cat owners regarding Ffeline calicivirus.

Prevalence of Feline Calicivirus and Risk Factors

In the study area, the overall prevalence of feline calicivirus was 27.7% (101/364). The gender-based analysis was not statistically significant and male cats (27.45%) had slightly higher (26.09%) FCV prevalence than female. Based on coat color, the most prevalent group was the white (37.04%), followed by black white (31.25%), black brown (33.33%) and other (18.18%), but there was no statistically significant difference observed. Age-wise analysis showed that cats of age range >3–5 years had the highest prevalence (38.89%), followed by 1–3 years old (35.14%) and 0–1 year old. Age was highest in adult cats. Furthermore, breed-wise prevalence had varying levels of infectivity. Stray cats and stray mixed cats had the highest prevalence (33.33%), followed by Persian mixed (22.22%), Persian (20.0%), mix breed (14.29%), but no statistically significant difference was observed. Vaccination status did not vary significantly. Unvaccinated cats had higher(33.33%) prevalence than vaccinated cats (29.41%) (Table 1).

Table 1 Demographics and epidemiological characteristics; pet visited veterinary clinics

Variables	Characteristics	Frequency (N)	Frequency (%)
Species	Feline	74	57.4
	Canine	55	42.6
Gender	Male	63	48.9
	Female	30	23.3
	Not Available	36	27.9
Color	Black	12	9.4
	White	27	20.9
	Black/White	16	12.4
	Black/Brown	9	7.0
	Black/Tan	7	5.4
	Brown	7	5.4
	Grey	10	7.8
	Fawn	4	3.1
	Other	37	27.9

Variables	Characteristics	Frequency (N)	Frequency (%)
Age	0-1yrs	19	14.8
	1-3yrs	18	14
	>3-5yrs	6	4.8
	Not Available	86	66.7
Breed	German Shepherd	23	17.8
	Husky	8	6.2
	Labrador	5	1
	Persian	43	33.3
	Stray	9	7
	Mix Breed	9	7
	Persian Mixed	9	7
	Stray Mixed	3	2.3
	Other	29	22.6
Vaccination	Yes	77	59.7
	No	5	3.9
Diseases	Feline Calicivirus	22	17.1
	Diarrhea	7	4.5
	Infection	17	13.2
	Jaundice	5	3.9
	Parvovirus	12	9.3
	Distemper	3	2.3
	Maggots	7	5.6
	Other	55	44
Diagnostic Method	CBC	16	12.4
	Pathology	78	80.5
	Other	35	27.1
Recovery	Yes	130	100
	No	0	0
Clinics	Private	130	100
	Government	0	0

Assessment of Knowledge, Attitude and Practices Among Cat Owners

Sociodemographic Characteristics of Cat Owners

The current study analyzed the sociodemographic background of the population of Pakistan (sample size=298). Gender-wise, most participants among the surveyed population were female (82.9%) and the male was 17.1%. Age-wise, age range > 30 years wasthe most prevalent (60.7%), followed by 26–30 years (18.5%), 21–25 years (12.4%), and 15–20 years (8.4). With regard to occupation, diverse classes of professions were reported. Among participants, most prevalent were students (60.1%), followed by the employed (31.9%), freelancers (16.1%), medical professionals or healthcare sector workers (15.8%), private business (13.1%), unemployed (7.7%) and workers in the government sectors (6.4%). Additionally, 37% reported were belonging to 'other' occupational category.

Educational qualifications were highly varied,most prevalent were undergraduates (50.3%) in this study, followed by graduates (22.8%), postgraduates (13.4%), and higher secondary education (13.1%). Among the ethnicities, Punjabis were the highest group (63.1%) in the current study, followed by Urdu speaking (17.1%), Pakhtoon (5.7%), Sindhi (4.7%), Balochi (1.3%), and Hazargi (1%). Also, the analysis of residential duration showed 88.9% of participants were living in their residence longer than a year, and 2.7% had moved within the last month. Religion-wise, most prevalent were Muslim cat owners (96.6%), followed by non-religious (2%), Christian (1%), and other religions (0.3%). The household size distribution showed that a majority of the participants (60.1%) lived in houses serviced by 2–5 persons, 31.2% in a house with 6–9 members, 5.4% were in a single member household and 3.4% of more than 10 members. Households containing children showed that 90.3% had 1–3 children, 7.4% had 4–6 children and 2.0% had 7–9 children (Table 2).

Table 2 Prevalence of feline calicivirus with respect to variables

Variables	Characteristics	Frequency (N)	Positive (N)	Prevalence (%)
Gender	Male	51	14	63.63
	Female	23	6	27.27
Color	White	27	10	45
	Black/White	16	5	22.72
	Black/Brown	9	3	13.63
	Others	22	4	18.18
Age	0-1yrs	19	2	9
	1-3yrs	37	13	59
	>3-5yrs	18	7	31
Breed	Stray	9	3	13.6
	Persian	40	8	36
	Mix Breed	5	7	3
	Persian Mixed	9	2	9.0
	Stray Mixed	3	2	9.0
Vaccination	Yes	68	20	99.9
	No	6	2	9.0

Knowledge

The survey showed that 66.1% (n = 197) of respondents gave their cats vaccinations but 25.1% (n = 74) chose not to vaccinate them. The respondents revealed that 6.4% (n=19) among them had vaccinated certain cats, while 1.7% (n=5) were uncertain about their vaccination status. The study revealed that 34.6% (103 respondents) had identified feline calicivirus (FCV) existing previously but 59.4% (177 respondents) were unaware of it, and 5.7% (17 respondents) expressed uncertainty about this virus. Among study participants who owned cats these were distributed as follows: 54% (n = 161) purchased purebred cats, while 21.1% (n = 63) were responsible for stray cats and 23.5% (n = 70) chose mixed-breed cats.

The majority of participants raising cats had 1–3 male cats (42.3%, n=126) along with 1–3 female cats (30.2% total 90), whereas just 6.4% (n = 19) and 4% (n = 12) owned more than three males or females, respectively. Most of the cats in the study belonged to the 1–4 years age group (34.6%, n = 103), whereas 7–12 months and 0–6 months followed closely behind with proportions of 30.9% (n = 92) and 17.4% (n = 52), respectively. With respect to body weight most cats fell within the 2–4 kg range (41.3%, n = 123), while the other categories included 1–2 kg (33.9%, n = 101) and >4 kg (12.1%, n = 36). Research shows that 47.7% (n = 142) of respondents did not perform neutering or spaying procedures on their cats and 36.2% (n = 108) had already done it. The remaining group of 10.7% (n = 32) was uncertain about neutering.

A large proportion of 64.1% (n = 191) kept their cats indoors but 25.2% (n = 75) allowed both indoor and outdoor access, while 9.7% (n = 29) kept them entirely outdoors. Among the respondents 39.9% (n = 119) maintained their cats confined indoors, whereas 34.2% (n = 102) let their cats roam free.

The survey showed that 61.1% of respondents (n = 182) permitted their cats to sleep with family members but 34.6% (n = 103) chose not to grant this freedom. During the research period 37.9% (n = 113) of cat owners who kept their pets inside permitted them to go outside rarely but 34.5% (n = 104) brought their cats outdoors frequently.

Monitoring cat hunting behavior showed 68.1% (n = 206) of respondents did not observe hunting while 27.2% (n = 81) witnessed hunting behavior. The main transmission route for FCV was direct contact transmission which accounted for 57.04% of cases (n = 170), relative to airborne transmission which revealed 21.47% of cases (n = 64), and feces-related transmission which comprised 14.7% of cases (n = 44). The poll results demonstrated that 73% (n = 220) of respondents thought vaccinated cats could not get FCV yet another 22.48% (n = 67) respondents believed they could (Table 3).

Table 3 Sociodemographic backgrounds from general population of Pakistan

Variables	Characteristics	Frequency (N)	Frequency (%)
Gender	Male	51	17.1
	Female	247	82.9
Status	Student	179	60.1
	Unemployed	23	7.7
	Employed	95	31.9
Age	15-20	55	18.5
	21-25	181	60.7
	26-30	37	12.4
	>30	25	8.4
Occupation	Private Business	39	13.1
	Medical or healthcare professional	47	15.8
	Teacher	14	4.7
	Freelancer	48	16.1
	Digital Marketing	18	6
	Farmer	2	0.7
	Government employees	19	6.4
	Others	111	37
Qualification	Higher Secondary	39	13.1
	Undergraduate	150	50.3
	Graduate	68	22.8
	Post-graduate	40	13.4
	Others	1	0.3
Ethnicity	Punjabi	188	63.1
	Sindhi	14	4.7
	Pakhtoon	17	5.7
	Balochi	4	1.3
	Hazargi	3	1
	Urdu Speaking	51	17.1
How long have you been living in this residence	Less than a month	8	2.7
	Less than 6 months	13	3
	7-12 months	12	4
	Over a year	265	88.9
Religion	Islam	288	96.6
	Christianity	3	1
	No religion	6	2
	Other	1	0.3

Variables	Characteristics	Frequency (N)	Frequency (%)
How many people living in your house?	0-1	16	5.4
	2-5	179	60.1
	6-9	93	31.2
	>10	10	3.4
How many children are present in your house	1-3	269	90.3
	4-6	22	7.4
	7-9	6	2

Attitude

Records from this survey revealed that 30.9% of respondents brought their cats to vets regularly but 20.5% sought care rarely, whereas 45% took their cats to the veterinarian only after disease symptoms emerged. A minority (3.7%) indicated alternative practices. Every third person who visited veterinarians infrequently gave time constraints (28.5%), and geographic distance from veterinary services (18.8%) or the unavailability of veterinary hospitals (20.4%) and financial inability to afford care (15.4%) as their primary reasons. Main deterrents to veterinary visits consisted of misunderstandings regarding care needs (11.1%) combined with cats' discomfort (0.3%) and unnecessary vet visits (0.3%).

Survey participants indicated that 72.1% of their cats used the indoor areas for defecation rather than 26.8% who excreted outside facilities, while 1% chose alternative locations. Veterinary service satisfaction was noted by 65.8 percent of respondents but 12.8 percent were dissatisfied and 21.5 percent stayed undecided. The survey revealed different antihelmintic administration schedules where 34.9% dewormed their cats every six months and 29.9% did it yearly while 7.7% used it biennially and 4.4% used it three to four times per year. Only one out of one hundred respondents (0.7%) conducted deworming procedures for their cats every two months.

The survey results showed that 18.8% of respondents never dewormed their cats while 3% provided different answers apart from 0.7% who found deworming unimportant. Assessment of social interactions of cats: responses from 52.3% reported that their cats were in regular contact with other cats and 44.6% did not have such contact. However, a small proportion of 1.3%, 0.7%, or other (1%) of respondents reported occasional, or uncertainty, or other. And 80.2 percent of owners checked infrequent illness in their cats as contrasted with 10.1 percent who felt that their cats were frequently ill, 8.7 percent who were undecided and 1 percent who answered other. One hundred and thirty seven out of 196 respondents (69.5%) sought veterinary intervention when their cats appeared to have become ill, while, respectively, contacted a veterinary service within a week (13.1%), within a month (4%), and only when their cat's condition had become severe (12.4%).

The dietary habits were also examined in which 29.2% of the respondents stated that their cats had eaten raw meat inside or outside the household, whereas 59.7% informed that their cats did not eat raw meat. Another large percentage, 10.1%, were uncertain and 1% voted for other responses. There were differences in hygiene practice: 75.1% used to clean their cat's litter box daily, 18.4 every three days and 6.3 weekly. Likewise, daily cleaning of a cat's' feeding bowl was done by 73.8%, 19.7% – 3 days later, and 4.6% weekly. About 91.6 % of the participants stated that they had cats in their households, 3.7 % did not own cats, and 4.3 % had cats occasionally. Cat owners who reported exposure of their animal to environmental toxins were 94 percent and 1 percent, respectively. In addition, 3.3 % were included in the occasional exposure group, 0.6 % responded as other. These findings are in turn valuable for understanding feline management, public attitudes towards veterinary care, hygiene and possible zoonotic risks (Table 4).

Table 4 Knowledge of participants from general population of Pakistan

Variables	Characteristics	Frequency(N)	Frequency(%)
Did you vaccinate your cats?	Yes	199	66.8
	No	68	22.8
	Some	19	6.4
	Maybe	5	1.7
	Other	7	2.3
Have you heard of feline calicivirus before?	Yes	103	34.6
	No	177	59.4
	Maybe	17	5.7
Is your cat a breed or stray?	Breed	161	54
	Stray	63	21.1
	Mix	70	23.5
	Others	4	1.3
Please specify the gender(s) of your cat(s)	1 male, 1 female	15	5
	1 male and 2 female	3	1
	1 male and 3 females	2	0.7
	1-3 females	90	30.2
	1-3 males	126	42.3
	2 male and 1 female	15	5
	5 males and 5 females	5	1.7
	>3 females	12	4
	>3 males	19	6.4
	Other	11	3.7
Age of (all) of your cat(s)	0-6months	52	17.4
	7-12months	92	30.9
	1-4years	103	34.6
	5-7years	24	8.1
	8-10years	4	1.3
	>10	9	3
	Different ages	5	1.7
	Others	9	3
What is the average weight of your cat(s)? Please specify all if applicable.	1-2kg	101	33.9
	2-4kg	123	41.3
	5-6kg	1	0.3
	<1kg	21	7
	>4kg	36	12.1
	Others	16	5.4
Are you cat(s) neutered/spayed?	Yes	108	36.2
	No	142	47.7
	Maybe	32	10.7
	Some	16	5.3
Where do you keep your cat(s)?	Indoors	191	64.1
	Outdoors	29	9.7
	Both	75	25.2
	Others	3	1

Variables	Characteristics	Frequency(N)	Frequency(%)
How often do your cat(s) leave the premises of your house?	Once or twice a day	5	1.7
	Once or twice a week	69	23.2
	Whenever they please	102	34.2
	Never	119	39.9
	Other	3	1
Do your cat(s) sleep with any household member?	Yes	182	61.1
	No	103	34.6
	Maybe	3	1
	Sometimes	5	1.7
	Other	5	1.7
If your cat(s) live indoors, how many times do you take your cat(s) out yourself?	Rarely	113	37.9
	Often	104	34.5
	All the time	30	10.1
	Never	48	16.1
	Others	3	1
Does your cat(s) hunt?	Yes	81	27.2
	No	206	68.1
	Some of them	8	2.7
	Others	3	1
Have you heard of feline calicivirus before?	Yes	103	34.6
	No	177	59.4
	Maybe	17	5.7
	Other	1	0.3
What, in your opinion, is the mode of transmission of the disease?	Through contact	170	57.04
	Through air	64	21.47
	Through the touch of faeces	44	14.7
	Through bite	20	6.7
Do you think vaccinated cats can get feline calicivirus?	Yes	67	22.48
	No	220	73
	Maybe	8	2.6
	I don’t know	3	1

Practices

The practices of participants to the general population of Pakistan as to feline calicivirus (FCV) information is presented in Table 5. When asked about the mode of transmission of the disease, majority (56%) believed the disease was spread by respiratory mode, followed by other modes of transmission (24.49%) and blood borne (19.5%). Regarding previous diagnoses of their cats for FCV, 74.8% believed their cats were diagnosed with FCV, 11.8% stated no, 13.1% were unsure and 1.3% chose other responses. Among infected cats their age distribution also indicated that 32.2% were 0–6 months, followed by7–12 months (13.8%), 1–3 years (10.1%), 4–7 years (3%), and 8–10 years (0.7%). The method of FCV diagnosis was varied. Among them, pathologically diagnosed were (41%), PCR (11.4%), ELISA (4.7%), and other methods (9.1%), while 33.2% didn’t have any records.

In infected cats, conjunction with at least one other symptom was themajority of cases (18.1%), fever (9.1%), lethargy (8.7%), lack of appetite (5.7%), ulcers of the mouth (4.0%), runny eyes (2.3%), and respiratory distress (8.7%). A major portion of respondents chose not applicable and other symptoms (21.1%). Differential symptoms used for diagnosis include fever (22.5%), lethargy(18.8%), ulcers to the mouth (17.4%), and diarrhea (0.3%), while vomiting and bloody stool (0.3%) were least. With respect to upper respiratory symptoms, 19.5% of cats did have such symptoms, 41.3% did not have any, and 31.9% of owner could not know.

Prevalence of feline calicivirus (FCV) was assessed in surveyed cats. Most cases were (79.2%) not diagnosed, (5.7%) diagnosed, (12.8%) uncertain and (2.3%) untreated responses. Of the respondents, 18.1% reported that they were told by their veterinarian of possible FCV infections on examining them, and (55%) said that their veterinarian did not suspect FCV, (20.8%) were unsure, (1.3%) said that they didn't apply and (5.7%) responded 'other'. Of the cat owners, (79.5%) preferred using private veterinary services, (12.1%) used government-provided services, (2.7%) used both and (5.7%) did not have responses. The efficacy of home remedies was also tested and deemed successful (13.8%), (60.7%) identified as ineffective, (22.5%) unsure, and (3%) chose other.

Regarding the time taken to get to the veterinarian after a symptom, the incidence of 24.8% was reportedfor within a day, 1.7%, within a 2–4-day interval, 21.8% within five days, 8.1% two weeks, 4% a month, and 0.3% after three months. The responses for never sought veterinary care were 17.1%, not applicable 8.1% and unavailable 14.4%. In 22.5% of cases the affected cats had to be hospitalized, 58.4% did not, and 16.4% were uncertain. Patient waiting time to confirm FCV diagnosis depended on how long it took, 27.2% was confirmed within a day, 16.4% within five days, 12.1% within a week, 2.7% within two weeks, and 5.4% within a month. Of the total number of the respondents, 11.1% chose not applicable, and 25.2% provided other response. Results from recovery outcome also reported that 31.2% of cats recovered, 18.5% of cats did not, 14.4% stated most of the cats recovered, and 7.4% of the cats stated not most of the cats recovered. In addition, not applicable was chosen by 25.5%, and 10.84% were unavailable.

Antivirals were the 1st choice of treatment which included 46.6%, 12.1% home remedies, 2% antibiotics, 1.7% antipyretics and 15.4% chose 'not applicable'. Finally, 1.3% selected 'do not know' and 20.8% gave other responses. As for the improvement after treatment, 35.2% said they were better, 17.4% said unchanged, 17.3% were unsure, 10% chose not applicable and 19.5% other. Veterinary knowledge was assessed (35.2% agreed with a vet having adequate knowledge, 17.4% disagreed with a vet's FCV knowledge, 17.8% did not know, 19.5% not available, 1.3% not applicable, and 8.7% had never had their cat checked for FCV). FCV diagnosis temporally to symptom onset was also explored and FCV was diagnosed within one week in 27.5%, within two weeks in 16.4%, within a month in 6.7%, and within two months in 2.7%. An overall total of 19.5% said 'never,' 7.3% did not apply, and 18.8% other. 33% of respondents reported that the virus was transmitted by their cat to others (23.5%), did not (26.2%), couldn't say (4%), not applicable (7%), or other (16.8%) (Table 5).

Table 5 Attitude of participants from general population of Pakistan

Variables	Characteristics	Frequency(N)	Frequency (%)
How often do get your cat(s) checked up?	Often	92	30.9
	Rarely	61	20.5
	Upon Distress	134	45
	Other	11	3.7
If you rarely take them to the vet, what is the reason?	Cat panics	1	0.3
	Cost	46	15.4
	Distance	56	18.8
	Lack of knowledge	33	11.1
	Lack of need	1	0.3
	Lack of proper veterinary hospital	3	1
	Time	85	28.5
	Upon Distress	12	4
	Not Available	61	20.4
Where does your cat(s) defecate?	Indoors	215	72.1
	Outdoors	80	26.8
	Other	3	1

Variables	Characteristics	Frequency(N)	Frequency (%)
Are you satisfied with the veterinary center that you go to?	Yes	196	65.8
	No	38	12.8
	Maybe	64	21.5
How often do you deworm your cat(s)>	Every 2 months	2	0.7
	Every 3-4 months	13	4.4
	Every 6 months	104	34.9
	Once a year	89	29.9
	Once every two years	23	7.7
	Never	56	18.8
	Not applicable	2	0.7
	Other	9	3
Does your cat(s) get in contact with other cats?	Yes	156	52.3
	No	133	44.6
	Maybe	2	0.7
	Sometimes	4	1.3
	Other	3	1
Does your cat(s) get sick often?	Yes	30	10.1
	No	239	80.2
	Maybe	26	8.7
	Other	3	1
When do you seek veterinary help when your cat(s) is sick?	Immediately	207	69.5
	In a week	39	13.1
	In a month	12	4
	When it gets crucial	37	12.4
	Other	3	1
Does your cat(s) eat raw meat inside/outside of the house?	Yes	87	29.2
	No	178	59.7
	Maybe	30	10.1
	Other	3	1
How often do you clean your cat's litter box?	Every day	224	75.1
	Every 3 days	55	18.4
	Every week	19	6.3
How often do you clean your cat's eating bowl?	Every day	220	73.8
	Every 3 days	59	19.7
	Every week	14	4.69
Do you have cats over at your house?	Yes	273	91.6
	No	11	3.7
	Sometimes	13	4.3
	Others	1	0.3
Are you cats ever exposed to toxins such as pesticides?	Yes	3	1
	No	283	94
	Sometimes	10	3.3
	Other	2	0.6

Association of knowledge, attitude, and practices with sociodemographic factors

The current study had an analysis of the association between various risk factors and knowledge, attitude, and practices (KAP) status, with P-values indicating statistical significance. For gender, there was no significant association with knowledge or attitude. However, a significant association existed between gender and practices, with females having better practices compared to males. Employment status was significantly associated with knowledge and practices, but not with attitude. Students demonstrated better knowledge and practices compared to unemployed individuals and employed individuals. Education level showed a strong association with both knowledge and practices, but not with attitude. Graduates had the highest levels of knowledge and practices, while individuals with primary education show the lowest levels (Table 6).

Table 6 Practices of participants from general population of Pakistan

Variables	Characteristics	Frequency(N)	Frequency (%)
What, in your opinion, is the mode of transmission of the disease?	Blood Borne	58	19.5
	Respiratory	167	56
	Other	73	24.49
Did your cat(s) ever get diagnosed with the feline calicivirus?	Yes	32	10.7
	No	223	74.8
	Maybe	39	13.1
	Other	4	1.3
How many of your cats have ever gotten infected with the virus?	0-1	251	84.2
	2-4	30	10.1
	>4	11	3.7
	Other	6	2
What was the age of your cat(s) that got infected? (Please answer in the other if the number is more than and for each)	0-6months	96	32.2
	7-12months	41	13.8
	1-3years	30	10.1
	4-7years	9	3
	8-10years	2	0.7
	Not applicable	71	23.8
	Other	49	16.4
What was the method of diagnosis?	ELISA	14	4.7
	PCR	34	11.4
	Pathologically	124	41
	Not recorded	99	33.2
	Other	27	9.1
If your cat(s) was ever diagnosed with calicivirus, what were the symptoms?	Ulcers in the mouth	12	4
	Runny eyes	7	2.3
	Respiratory distress	26	8.7
	Laziness	26	8.7
	Lack of consumption of food	17	5.7
	Fever	27	9.1
	All of the above	54	18.1
	Not applicable	66	22.1
	Other	63	21.1
If your cat was diagnosed with calicivirus, what was the differential symptom on which you or the vet based the diagnoses? (Add the test name in other if applies)	Bloody stool	1	0.3
	Diarrhea and vomiting	1	0.3
	Fever	67	22.5
	Laziness	56	18.8
	Ulcers in the mouth	52	17.4
	Not applicable	47	15.8
	Other	74	24.8

Variables	Characteristics	Frequency(N)	Frequency (%)
Did your cat(s) have upper respiratory symptoms or a respiratory disorder?	Yes	58	19.5
	No	123	41.3
	I don't know	95	31.9
	Not applicable	8	2.7
	Others	14	4.7
Has your cat(s) ever been diagnosed with feline herpes virus (FHV)?	Yes	17	5.7
	No	236	79.2
	Maybe	38	12.8
	Not available	7	2.3
Did your vet ever suggest the possibility of your cat having calicivirus upon observation?	Yes	54	18.1
	No	164	55
	I don't know	59	19.8
	Not applicable	4	1.3
	Others	17	5.7
Do you take your cat(s) to a private or government veterinarian?	Government	36	12.1
	Private	237	79.5
	Both	8	2.7
	Not available	17	5.7
If your cat was ever diagnosed with the virus, did home remedies work in treating it?	Yes	41	13.8
	No	181	60.7
	Maybe	67	22.5
	Other	9	3
How long after the symptoms did you take your cat(s) to the vet?	A day	74	24.8
	2-4 days	5	1.7
	<5 days	64	21.8
	After two weeks	24	8.1
	After a month	12	4
	After 3 months	1	0.3
	Never	51	17.1
	Not applicable	24	8.1
	Not available	43	14.4
If your cat was diagnosed with the virus, did you have to hospitalize your cat(s)?	Yes	67	22.5
	No	174	58.4
	Maybe	49	16.4
	Not available	8	2.7
If your cat(s) was diagnosed with the virus, how long did it take to confirm the diagnosis?	A day	81	27.2
	< 5 days	49	16.4
	< a week	36	12.1
	<Two weeks	8	2.7
	A month	16	5.4
	Not applicable	33	11.1
	Other	75	25.2
If your cat(s) had the virus, did your cat(s) ever recover?	Yes	93	31.2
	No	55	18.5
	Most of them	43	14.4
	Not most of them	22	7.4
	Not applicable	76	25.5
	Not available	32	10.84

Variables	Characteristics	Frequency(N)	Frequency (%)
In case of your cat being diagnosed with the calicivirus, what were the treatment methodologies?	Antibiotics	6	2
	Antipretics	5	1.7
	Antivirals	139	46.6
	Home remedies	36	12.1
	Not applicable	46	15.4
	Do not know	4	1.3
	Other	62	20.8
If your cat was diagnosed with the virus, how long did it take for your cat(s) to show signs of improvement?	Yes	125	35.2
	No	52	17.4
	Maybe	53	17.3
	Not Applicable	30	10
	Other	58	19.5
If your cat(s) has been diagnosed with the virus, did the vet seem to have the proper knowledge to treat it?	Yes	105	35.2
	No	52	17.4
	Maybe	53	17.8
	Not Available	58	19.5
	Not applicable	4	1.3
	Never got them checked for calicivirus	26	8.7
If your cat has ever been diagnosed with the virus, how long after the symptoms started showing?	Within a week	82	27.5
	Within two weeks	52	16.4
	Within a month	20	6.7
	Within two months	8	2.7
	Never	58	19.5
	Not applicable	22	7.3
	Other	56	18.8
If your cat(s) ever had the virus, was the virus contagious to other cats?	Yes	70	23.5
	No	78	26.2
	Maybe	67	22.5
	Can’t say	12	4
	Not applicable	21	7
	Other	50	16.8

DISCUSSION AND CONCLUSION

This study characterizes the first evaluation of knowledges, attitudes and practices of cat owners about feline calicivirus and feline management in general in Pakistan. Of the 298 participants, 82.9% were females, having a higher percentage among the participants of the age group 21–25. Due to the distribution of questionnaire among university students, 50.3% of the participants were recorded as undergraduate students but mostly having jobs with good income. Similar sociodemographics were observed in other related studies (Alrukban et al., 2022).

Over half of the pet owners did not have any knowledge about the disease. 57.4% of the pet owners responded with contact being the primary source of transmission of virus, which was consistent with a previous study (Tamiru et al., 2022). The owners make efforts to improve their knowledge of the hygiene of their pets, which includes being aware of the diseases their pets can catch, which is consistent with the previous studies, as the participants had some knowledge of the proper hygiene and care of their pets even if they had little knowledge of feline calicivirus (San Jose et al., 2020). 45% of the respondents brought their cats right away to the veterinarian, and always dewormed their cats, and mentioned their cats as healthy; and majority of the respondents had an indoor litter box for their cats to defecate. More than half of the cats were in contact with other feline companions, a finding that is consistent with the results from a previous study on the attitudes of the pet owners (San Jose et al., 2020), which talked about the knowledge, attitude, and practices with pets in the Philippines. The pet owners had a similar attitude towards the care of their pets and not having adequate knowledge and attitude towards the proper care of their pets.

Most of the respondents used private clinics, where 41% of the cats were diagnosed mainly based on the oral ulceration, which is typical of feline calicivirus infection. However, many owners felt that veterinarians did not have adequate knowledge of FCV. Approximately half of the affected cats were treated with antiviral therapy, which was linked to high recovery rates and low mortality rates, and 35.2% of cats fully recovered, while the rest showed mixed results. Our investigation finds an important rarity of antiviral data, i.e., the individual agents, the dosage regimen, and the duration of therapy involved in the compiled data. The observed prevalence of antiviral utilization can be taken as an affirmative indication of deliberate therapeutic deployment and predilection towards proactive viral disease control, as opposed to a sole reliance on antibacterial agents or traditional home remedies. This can be an issue in contexts where limited resource availability is the rule, and the term “antivirals” may be used in its widest sense to include all modalities of action towards viral inhibition and immune modulation. Accordingly, the current observation is likely to be more of a reflection of local linguistic conventions and perceptions of therapy, rather than an example of clinical hyper-utilization of a specific treatment. For these reasons, we recommend the elaboration and stratification of drug treatment schemes as well as the establishment of robust drug classification systems to be included in future epidemiological research.

While there is a lack of direct empirical data from Pakistan at present, a synthesis of the existing literature from around the world and regional surveillance reports suggest that many small animal veterinary clinics working in low-resource settings are primarily using clinical necropsy observations (such as the characteristic oral ulcerations, repetitive sneezing, and nasal exudates) as provisional diagnostic criteria used for feline calicivirus, rather than using rigorous molecular diagnostics (Vijay et al., 2021). Consequently, there is a dire need for future research efforts to be conducted in Pakistan to carry out a comprehensive evaluation of the diagnostic infrastructure, specifically to map the existence and functioning of polymerase chain reaction assays and virus isolation facilities in veterinary practices and to guide the creation of diagnostic procedures based on available evidence.

A statistically significant relationship was found between the female respondents aged between 21-24 and high knowledge levels. While formal education, overall, did not have a statistically significant impact, the subset of undergraduates had superior awareness. In urban areas, the respondents showed comparatively unfavorable attitude towards preventive measures for FCV, while as other sociodemographic variables, they showed no significant effect. The domain of practices showed a marginal but statistically significant relationship between women 21-24 years of age living in urban milieus, especially those who were employed undergraduates, who displayed their more regular hygiene practices and showed greater responsibility in relation to care. The results highlight positive trends which were consistent with the previous studies (Bordicchia et al., 2021; Radford et al., 2009). However, the virus can spread more quickly in places with poor immunization rates (Radford et al., 2021).The danger of feline calicivirus transmission increases with the number of cats living close to one another. This is due to the fact that the virus is mainly transmitted by contact with the saliva or respiratory secretions of infected cats (Wang and Lin, 2024). Management techniques, including sharing food and water dishes or litter boxes, can help feline calicivirus spread. The regularity with which cats’ environments are cleaned and sanitized can also affect the likelihood of transmission (Möstl et al., 2013).

Although feline calicivirus is not zoonotic, the practices that have been described (feeding raw meat, poor disinfection, and close human-cat contact) are indirect zoonotic risks. These practices highlight the need for the integration of feline health into the broader One Health framework. Improving the cooperation between veterinary experts, public health agencies and local governmental bodies is necessary to reduce the risks of disease at the human-animal-environment interface (Mohammed and Ahmed, 2024).The limitations of the study include reliance on self-reported information, which may introduce recall bias, both of veterinary experts and owners, and an urban skewed sampling frame, which may fail to adequately reflect rural populations. Future studies should include on-site clinical verification, sampling in rural areas, genomic screening of FCV strains to test molecular epidemiology and vaccine coverage gaps.

In conclusion, the prevalence of feline calicivirus was low and cat owners did not have proper or adequate knowledge of feline calicivirus. They also showed poor practices and a relatively poor attitude towards the proper hygiene and care of their cats. The study mostly targeted university students and an urban population. In consequence, their literacy rate and age mean they are aware of feline calicivirus practices and inclined towards adopting a positive attitude and having good knowledge. Female students showed more positive results compared with other populations, although there could have been a bias here due to more females filling in the survey. In conclusion, people did not have significant knowledge regarding feline calicivirus and also showed a lack of knowledge and implementation of proper hygiene practices for the health of their pets.

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CONFLICTS OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CONTRIBUTIONS

Conception: MH, MSA; Design: MH; Supervision: JC; Materials: KI, ED; Data Collection and/or Processing: MAG, MH; Analysis and/or Interpretation of the Data: HA, JC; Literature Review: NAS, AA, HA, JC; Writing: JZ,JC, HA; Critical Review: HA, ED

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Prevalenca i rizični faktori mačjeg kalicivirusa i procjena znanja, stavova i praksi vlasnika mačaka u Pakistanu

SAŽETAK

Mačji kalicivirus predstavlja infekciju gornjeg respiratornog trakta koju karakteriziraju ulceracije usne šupljine i iscjedak iz očiju, a jedna je od najčešćih virusnih infekcija mačaka. Cilj ovog istraživanja jeste određivanje prevalence mačjeg kalicivirusa i identifikacija znanja, rizičnih faktora, stavova i praksi vezanih za kalicivirus, kao i opće brige za mačke u Pakistanu. Prevalenca je određena na osnovu dijagnostičkih podataka (patološki nalazi i serološki testovi, npr. KKS) prikupljenih na veterinarskim klinikama u području istraživanja u 2021. godini. Kreirali smo upitnik kojim smo procijenili sociodemografiju, znanja, stavove i prakse vezane za mačji kalicivirus kod 298 vlasnika mačaka. Ukupna prevalenca mačjeg felicivirusa je iznosila 27.5%. Većina (62.75%) vlasnika kućnih ljubimaca je pokazala slabo opće znanje o prenosu virusa i njegovoj infektivnosti. Kod 52.68% anketiranih su uočeni neadekvatni stavovi o prevenciji mačjeg kalicivirusa. Većina vlasnika kućnih ljubimaca (62.75%) su slabo održavali čistoću svojih mačaka. Obzirom da većina ispitanika živi u urbanim područjima, mali broj mačaka je bio izložen toksinima poput pesticida. Uprkos nedovoljnom poznavanju samog virusa, ispitanici su bili voljni održavati svoje mačke zdravim, u dobrim higijenskim uvjetima, ali je samo mali broj to postigao.

Ključne riječi: Islamabad, prevalenca, rizični faktori, ulceracije usne šupljine, znanje

RESEARCH ARTICLE

Sexual dimorphism of central midface skeleton: Geometric morphometrics approach on 3D models of human skull

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ABSTRACT

Morphological differences of the skeleton between the genders have long attracted the attention of disciplines concerned with sex determination, leading to the development of various approaches for gender assessment. Geometric morphometrics has recently been introduced as a method for identifying sex based on skeletal features through the application of statistical and mathematical tools. The study was conducted on 3D models of 210 human skulls (139 male and 71 female). Within the central midface skeleton, nine landmarks were selected and marked in the Landmark Editor software. After marking the landmarks, their spatial coordinates were extracted and then imported into the MorphoJ program to analyze shape and size variation between sexes. Principal component analysis, covariance matrix generation, and discriminant function analysis were carried out with sex as the classification variable. The findings indicated that sex estimation based on the size and shape of the central midface skeleton reached an accuracy of 73% for males and 70% for females. The shape of the central midface skeleton exhibited statistically significant sexual dimorphism independent of size. After excluding the influence of facial size and considering only shape variables, geometric morphometrics achieved predictive accuracy of 73% for males and 72% for females.

Keywords: Central midface skeleton, geometric morphometrics, sexual dimorphism, 3D models of human skulls

INTRODUCTION

Morphological differences in skeletal remains between males and females have consistently drawn the interest of scientific fields focused on sex determination, leading to the development of various approaches for establishing biological sex (Sarač-Hadžihalilović et al., 2020). Alongside conventional methods, geometric morphometrics has more recently been adopted for this purpose, relying on skeletal material to improve accuracy. Geometric morphometrics is a technique applied to quantify and evaluate the shape of biological structures, including skeletal elements, through advanced statistical and mathematical procedures (Ajanović et al., 2023a).

The application of geometric morphometrics gained momentum in the latter

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half of the 20th century, when researchers began to recognize the limitations of conventional measuring techniques. Traditional approaches did not allow for a detailed exploration of complex morphological variation, as they were unable to precisely capture subtle shape differences. Unlike these earlier methods, geometric morphometrics provides a framework that can accurately describe shape variability, which is essential for understanding processes such as evolution, development, aging, and sexual dimorphism (Ivanović and Kalezić, 2013).

Geometric morphometrics introduced a novel framework where, instead of analyzing individual linear measurements, the spatial coordinates of landmarks are used to define shape with high precision (Hadžiomerović et al., 2023).

A landmark-based system is applied by marking specific, anatomically recognizable points on skeletal structures. Through multidimensional statistical techniques, this approach allows detailed shape assessment, supporting more accurate classification and evaluation of variation both across populations and at the level of individuals (Ajanović et al, 2023).

Geometric morphometrics relies on methods such as Generalized Procrustes Analysis, applied to two- or three-dimensional models. Techniques including Procrustes superimposition, Principal Component Analysis (PCA), and Euclidean Distance Matrix Analysis (EDMA) enable researchers to examine morphological differences related to age, sex, and population groups, independent of scale or orientation. Over time, geometric morphometrics has become an essential methodology in areas such as evolutionary biology and forensic anthropology (Ivanović and Kalezić, 2013).

The concept of digitizing skeletal remains to create three-dimensional models dates back to the early 1980s, but only in recent decades has it been more widely adopted, with a growing number of studies addressing these issues globally (Guzel et al. 2025; Hadžiomerović et al., 2025; Ocumura and Araujo, 2019).

Because of the crucial importance of accurate sex determination, many authors worldwide focus not only on analyzing individual skeletal regions but also on identifying methodological approaches that maximize accuracy in biological sex estimation.

In order to identify which regions of the viscerocranium are most affected by sexual dimorphism, a study was performed on a dataset of 340 computed tomography (CT) scans of adult Bulgarians, including 156 males and 184 females (Toneva et al., 2022). The main outcomes indicated that male viscerocrania were significantly larger than those of females, with the most pronounced differences observed in the nasal, maxillary, and zygomatic areas. Among the regions, the orbital area displayed the greatest shape variation, while the nasal region showed the least. The accuracy of sex determination based solely on size reached 81.8%, whereas shape alone yielded an accuracy of 60–70% (depending on the region). The highest accuracy, 89.5%, was achieved when both shape and size were combined.

The pyriform aperture represents the anterior bony opening of the nasal cavity and plays a key role in facial anatomy and the respiratory system. Sarač-Hadžihalilović et al. (2022) assessed the accuracy of sex estimation using the pyriform aperture. Their study included 211 skulls. The analysis showed that males were correctly classified in 64% of cases, and females in 70%, when both shape and size parameters were considered.

When size was excluded as a variable, classification accuracy decreased to 59.7% in males and 62.5% in females. In general, the male nasal aperture is taller and narrower, with a deeper nasal base, whereas in females it tends to be wider and bordered by straighter nasal bones.

Asghar et al. (2016), working on a sample of 40 skulls of unknown age and sex, aimed to broaden the understanding of sexual dimorphism in the morphology of the nasal opening. Their findings indicated that males exhibited significantly larger dimensions of the pyriform aperture compared to females, including height, width, and the pyriform index (calculated as width/height × 100). However, no statistically significant differences were detected in the size of the nasal bones between sexes. The most common shape of the pyriform aperture was described as triangular-oval, observed in 83.5% of cases.

The objective of our research was to investigate sex-related variation in the central midface skeleton, using geometric morphometrics applied to three-dimensional skull models from the Bosnian-Herzegovinian population.

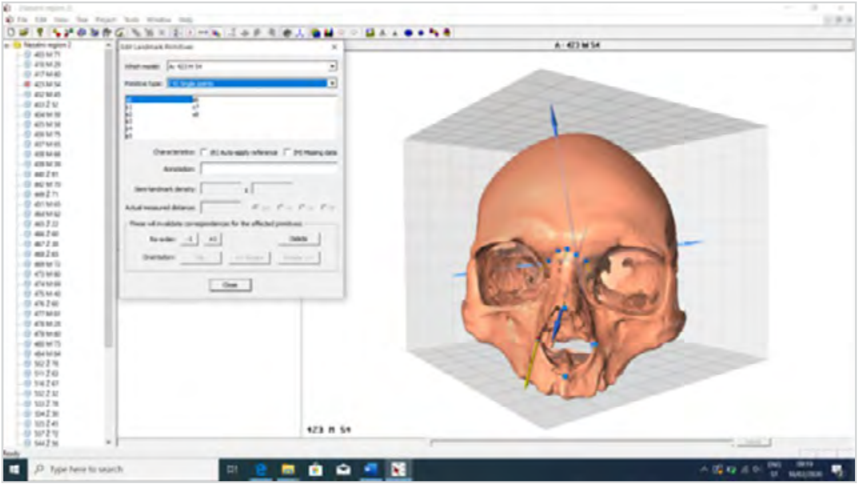

Figure 1 Landmarks marked on 3D models on central midface skeleton

MATERIAL AND METHODS

The research was conducted on three-dimensional models of 210 human skulls from Osteological Collection at Department of Human Anatomy, University of Sarajevo, Faculty of Medicine. All skulls were of known sex and age (139 male and 71 female). The 3D models were generated using a David Pro SLS 2 laser scanner.

On the reconstructed 3D models, nine landmarks were identified in the central midface skeleton using the Landmark Editor software. Each sample was marked with a total of nine points—three paired and three unpaired (Figure1).The paired landmarks were: apertion- most lateral point on pyriform aperture, maxillonasofrontale- cross section of frontonasal, frontomaxillar and nasomaxillar sutures, and maxillofrontale- cross section of frontomaxillar suture and medial border of orbit.The unpaired landmarks were: akanthion-on the anterior nasal spine, rhinion on the top of pyriform aperture, and nasion- on cross-section of frontonasal and internasal sutures.

After the specific landmarks were identified, their spatial coordinates (x, y, z) within the coordinate system were exported as NTSYS files. These datasets were then imported into the MorphoJ software to perform analyses of shape and size differences between sexes (Klingenberg, 2011).

MorphoJ is a statistical platform that enables the evaluation of shape and size of specific regions. Within the program, Procrustes analysis is applied and principal component (PC) scores are recalculated to determine whether statistically significant differences between sexes exist, using geometric morphometrics techniques. Geometric morphometrics provides detailed information about the shape of the studied structures, allowing the detection of morphological variation among them. For these purposes, the method relies on Generalized Procrustes Analysis (GPA). GPA functions by removing the effects of size, position, and orientation of the studied structures. Once these effects are excluded, only shape-related information remains, which can then be used for subsequent comparative analysis.

The effect of size is eliminated by scaling the configuration of landmarks to a unit centroid size, representing the fundamental geometric measure of the structure under investigation (Ivanović and Kalezić, 2013).

RESULTS

For analysis of sexual differences of central midface skeleton on 3D models of human skulls were used nine landmarks marked on 3D models represented on Figure 2.

In MorphoJ, Procrustes distances were calculated, after which sex was introduced as a classification variable to perform shape and size comparisons. A covariance matrix was then generated, and Principal Component Analysis (PCA) was applied. The results showed that the first two principal components (PC1 and PC2) accounted for 56.920% of the total variability in the central midface skeleton when both shape and size were considered (Table 1). Distribution of the examined skull sample based on the shape and size of the central midface skeleton in morphological space (skull variability by shape) defined by the first two principal components is presented on Figure 3.

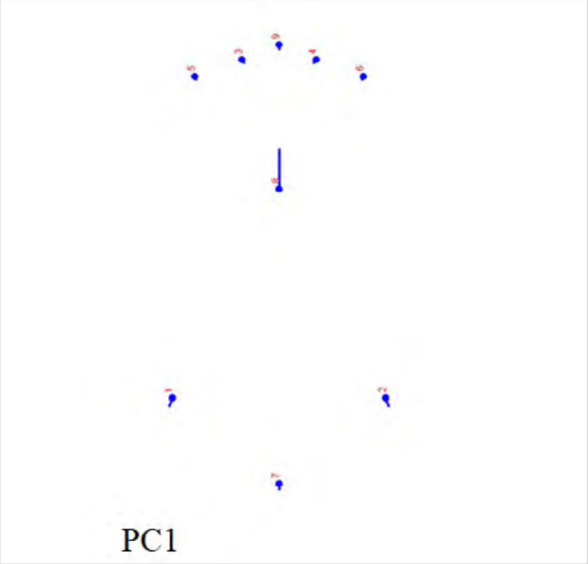

Figure 2 Patterns of shape variation in the central midface skeleton described by Principal Component 1. Blue circles represent the mean distribution of specific landmarks.

Blue lines indicate the direction and magnitude of changes in the mean positions of landmarks 1 and 2 – apertion, 3 and 4 – maxillonasofrontale, 5 and 6 – maxillofrontale, 7 – akanthion, 8 – rhinion, 9 – nasion

Table 1 Eigenvalues and percentage of shape and size variability of the central midface skeleton explained by eigenvalues obtained through Principal Component Analysis (PCA*)*

No of PCs	Eigenvalues	% Variance	Cumulative %
1.	0.00286803	35.755	35.755
2.	0.00169771	21.165	56.920
3.	0.00094943	11.836	68.756
4.	0.00079373	9.895	78.651
5.	0.00052518	6.547	85.198
6.	0.00039906	4.975	90.173
7.	0.00031326	3.905	94.079
8.	0.00025471	3.175	97.254
9.	0.00016515	2.059	99.313
10.	0.00003723	0.464	99.777

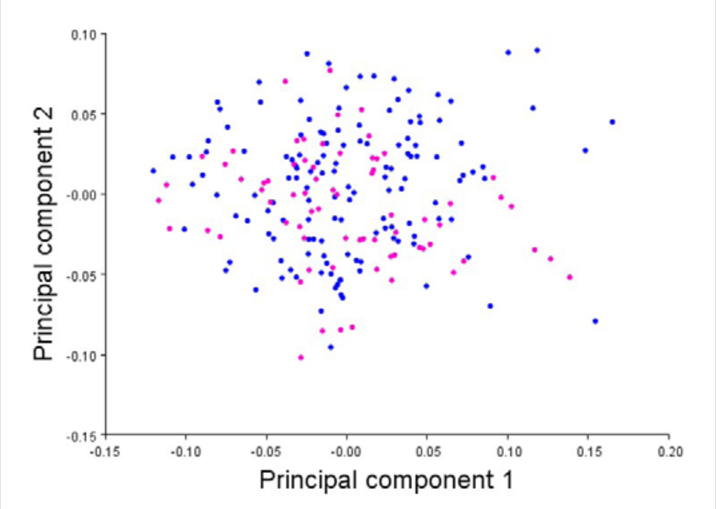

Figure 3 Distribution of the examined skull sample based on the shape and size of the central midface skeleton in morphological space (skull variability by shape) defined by the first two principal components

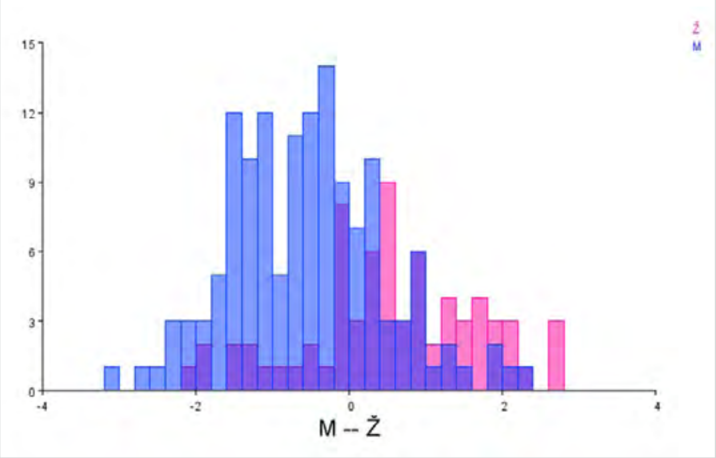

Figure 4 Discriminant functional analysis of the influence of shape and size of the central midface skeleton on sexual dimorphism (M- male, Ž- female)

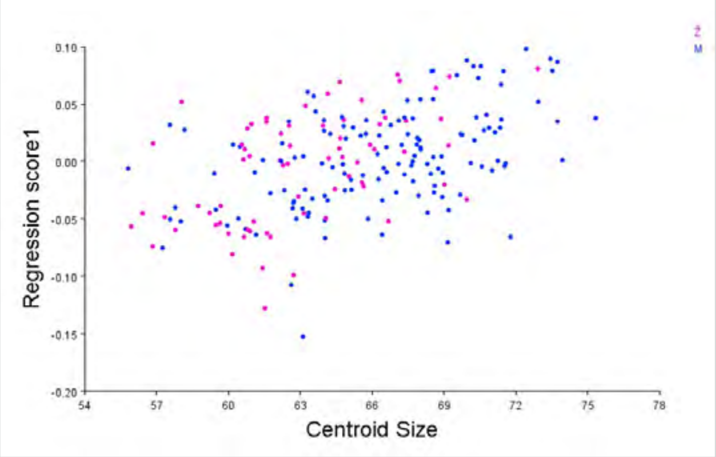

Figure 5 Effect of central midface skeleton size on sexual dimorphism of central midface skeleton shape

Discriminant functional analysis was used to compare the two groups. The analysis was performed by calculating the mean values for both groups using Procrustes and Mahalanobis distances. To determine whether statistically significant sex-related differences existed in the shape and size of the central midface skeleton, a classification accuracy test combined with discriminant functional analysis was conducted.The computed Procrustes distance was 0.0220, and the permutation test (with 1000 permutations) produced a p-value of less than 0.0001, confirming a statistically significant sexual dimorphism in both shape and size of the central midface skeleton which is presented on Figure 4.

Following the classification accuracy test, a regression analysis was conducted in the MorphoJ software to evaluate the impact of the central midface skeleton size on its shape. The mean values of central midface skeleton size, expressed as centroid size, indicated that size contributed 6% to shape variation. This effect was statistically significant ($p < 0.0001$, based on 10,000 permutations).

The influence of central midface skeleton size on overall shape, as well as the distribution of skulls in morphological space conditioned by central midface skeleton size, is illustrated in Figure 5.

After excluding the influence of size on the shape of the central midface skeleton, principal component analysis was recalculated. The results showed that the first two principal components accounted for 54.4% of the total shape variability in the central midface skeleton. (Table 2).

Table 2. Eigenvalues and percentage of shape variability of the central midface skeleton explained by eigenvalues obtained through Principal Component Analysis (PCA)

No of PCs	Eigenvalues	% Variance	Cumulative %
1.	0.00272333	36.883	36.883
2.	0.00129337	17.516	54.399
3.	0.00091176	12.348	66.747
4.	0.00078860	10.680	77.428
5.	0.00049098	6.649	84.077
6.	0.00039502	5.350	89.427
7.	0.00031078	4.209	93.636
8.	0.00025224	3.416	97.052
9.	0.00016250	2.201	99.253
10.	0.00003722	0.504	99.757

An analysis of sexual differences in the shape of the central midface skeleton, excluding the effect of size, was performed using the classification accuracy test, discriminant functional analysis. The difference between group means, expressed through Procrustes distance, was 0.0021. The permutation test with 1000 iterations yielded a p-value of less than 0.0001, confirming a statistically significant sex-related difference in the shape of the central midface skeleton independent of size.

The classification accuracy test showed that, out of 139 male skulls, 101 were correctly identified as male, corresponding to an accuracy rate of 73% for the male group. For the 71 female skulls, 51 were classified as female, resulting in an accuracy of 71% for the female group (Table 3).

Table 3 Predictive accuracy of sex determination based on the shape of the central midface skeleton of the skull

			Predictability of sex	Total
Sex	Male	**101**	38	139
	Female	20	**51**	71
	Total	*121*	*89*	*210*

The results of the discriminant functional analysis evaluating the influence of shape of central midface skeleton on cranial sexual dimorphism in the examined sample are presented in Figure 6, while the interval of shape variation in the central midface skeleton is illustrated in Figure 7.

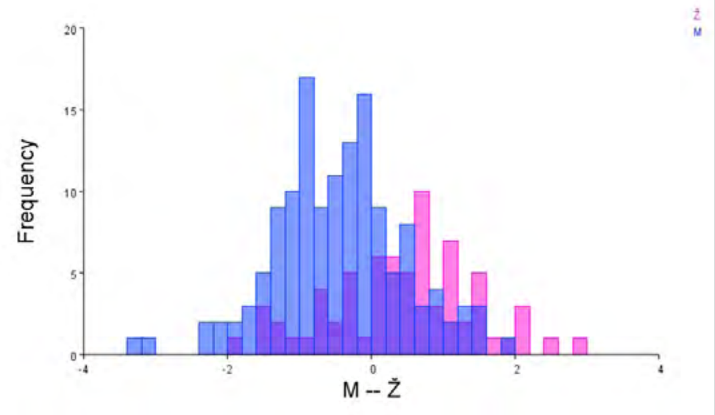

Figure 6 Discriminant functional analysis of the influence of central midface skeleton shape on cranial sexual dimorphism (M- male, Ž- female)

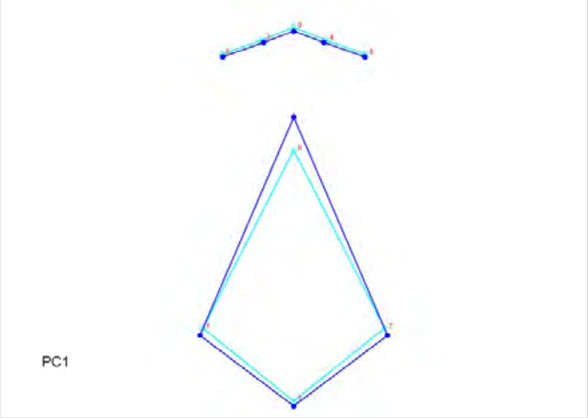

Figure 7 Range of shape variation of the central midface skeleton in the examined skulls

DISCUSSION AND CONCLUSION

The investigation of sexual dimorphism in the human skull is of great relevance for anthropology, evolutionary studies, and osteology. By reviewing previous studies dealing with sexual dimorphism of the midface skeleton, differences can be observed in the reported results, which may be population-specific. Moreover, authors employ various methodological approaches in their investigations, contributing to variability in findings.

In the study provided by Del Bove et al. (2023), a landmark-based technique was developed to enable automated assessment of dimorphic traits on the cranium. The research sample consisted of 228 individuals of known sex from various geographic backgrounds. The analysis revealed that the glabellar and supraciliary regions, the mastoid process, and the nasal area exhibited the strongest dimorphic differences. The accuracy of sex estimation using these regions was 73%, and when they were analyzed together, the accuracy increased to 77%.

In the study provided by Milella et al. (2021), the findings indicate that, consistent with Wainer's rule, male crania display greater overall variability in both size and shape, although statistical significance was confirmed only for total cranial size. The analysis showed that sexual differences were primarily explained by size, while shape contributes to a much smaller extent. Shape alone accounts for only a minor portion of the observed variability, and the cranial base revealed virtually no sexual dimorphism. Among the examined variables, the facial Procrustes form emerged as the most reliable indicator for determining skeletal sex, providing the highest classification accuracy.

In the study provided by Holton et al. (2016), they analyzed 290 cephalometric radiographs from 38 individuals (20 males and 18 females) across nine age groups. The focus was on sex-related differences in nasal shape in relation to body growth, patterns of non-allometric variation, and the degree of integration between the nasal region and other parts of the facial skeleton. The results showed that both sexes shared similar patterns of variation, but males exhibited a disproportionately greater increase in nasal height with body growth compared to females. In addition, the male nasal region was found to be less integrated with adjacent facial structures than in females. It was concluded that developmental differences in the nasal region between males and females were linked to sex-specific differences in energy requirements (Holton et al, 2014).

The aim of the study of Cantin et al. (2009) was to assess the presence of sexual dimorphism in the dimensions of the pyriform aperture and to explore its possible association with skin color. Ninety skulls from the UNIFESP collection were examined, with available data on sex, age, and skin color. Measurements included the height, upper width, and lower width of the pyriform aperture. Male skulls showed larger values across all parameters, although statistical significance was confirmed only for height. When the sample was divided by skin color, the analysis revealed that male individuals consistently had significantly greater aperture height across all three groups (white, black, and brown). Among black individuals, significant differences were also observed in the upper width. Overall, aperture height emerged as the most reliable indicator of dimorphism, while the effect of skin color appeared minimal. These findings differ in part from previously published reports and highlight the need to reconsider traditional markers used for sex determination in specific populations.

The nasal index (NI) is recognized as a sensitive anthropometric indicator. It reflects sexual variation and has significant applications in forensic medicine and reconstructive surgery, serving as a valuable tool for sex estimation when identity is uncertain. In the study provided by Sharma et al. (2023), the aim was to evaluate nasal height (NH), nasal breadth (NB), and the nasal index (NI) in both sexes, using clinical and radiographic methods, and to explore their role in sexual dimorphism. They concluded that nasal measurements were important anthropometric parameters for distinguishing between sexes. They provided reliable support for the assessment of sexual dimorphism, having practical implications in forensic medicine, anthropology, and nasal reconstructive procedures.

In the study provided by Cappella et al. (2022), they concluded that while cranial morphology–based sex estimation methods were widely applied in forensic anthropology, their reliability had not been consistently validated across populations. Unlike craniometric techniques, which are better established, morphological approaches still require further investigation due to the influence of population variability on sexual dimorphism. In their research, the accuracy of existing regression models was assessed in a contemporary Italian population, and new logistic regression models were developed. These new models demonstrated improved precision and specificity compared to earlier approaches. The findings contribute to updating reference standards for this geographical area and emphasize the necessity of using validated methods when constructing biological profiles in forensic contexts.

In the study on the sample from Bosnian population (Sarač-Hadžihalilović et al., 2022), determination of gender based on form (size and shape) of pyriform aperture was of 64.03% accuracy for male and 70.83% accuracy for female gender, but based on the shape of pyriform aperture excluding effect of size, sex determination was possible with 59.71% accuracy for male and 62.5% accuracy for female.

Compared with this study, our results showed a higher percentage of correct gender determination based on the central midface skeleton.

The analysis of sexual dimorphism in the central midface skeleton confirms that this anatomical area represents a valuable marker for distinguishing between males and females. Parameters of central midface skeleton consistently demonstrate sex-related differences, although the degree of dimorphism varies depending on the population studied and the methodological approach applied. These findings support the existence of sexual dimorphism but also highlight the importance of population-specific variability, emphasizing the need for reference standards tailored to specific geographic and ethnic groups. The application of modern techniques, including 3D morphometrics, logistic regression models, and validation of existing formulas, provides greater accuracy and reduces the risk of misclassification in forensic practice. Consequently, the central midface skeleton emerges as a reliable parameter in anthropometric, clinical, and forensic investigations, although its diagnostic value depends on appropriate methodological application and the consideration of population-specific variation.

In our study, the application of geometric morphometrics for sex assessment based on the central midface skeleton demonstrated predictive accuracy ranging from 70% to 73%.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ZA, ED; Design – ZA, ED; Supervision – ZA, ED; Funding – ZA; Materials – ZA, UA; Data Collection and Processing – ZA, UA, FZ; Analysis and Interpretation – ZA, UA; Literature Search– MŠ, KĐ, GB, NK; Writing Manuscript – MŠ, KĐ, GB, NK; Critical Review– FZ, ED.

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Seksualni dimorfizam centralnog dijela skeleta sredine lica: Pristup geometrijske morfometrije na 3D modelima ljudske lobanje

SAŽETAK

Morfološke razlike skeleta između spolova već dugo privlače pažnju disciplina koje se bave određivanjem spola, što je dovelo do razvoja različitih pristupa za procjenu spola. Geometrijska morfometrija je nedavno uvedena kao metoda za identifikaciju spola na osnovu skeletnih obilježja putem primjene statističkih i matematičkih alata. Istraživanje je provedeno na 3D modelima 210 ljudskih lobanja (139 muških i 71 ženskih). Unutar centralnog dijela sredine lica odabrano je devet tačaka (landmarka) koje su označene u softveru Landmark Editor. Nakon označavanja tačaka, njihove prostorne koordinate su izdvojene i unesene u program MorphoJ radi analize varijacija oblika i veličine između spolova. Provedene su analiza osnovnih komponenti, generisanje kovarijacione matrice i diskriminantna analiza funkcija, pri čemu je spol korišten kao klasifikacijska varijabla. Rezultati su pokazali da je procjena spola na osnovu veličine i oblika centralnog dijela sredine lica postigla tačnost od 73% za muškarce i 70% za žene. Oblik centralnog dijela skeleta sredine lica pokazao je statistički značajan seksualni dimorfizam, nezavisan od veličine. Nakon isključivanja uticaja veličine lica i uzimanja u obzir samo varijabli oblika, geometrijska morfometrija je postigla tačnost predikcije od 73% za muškarce i 72% za žene.

Ključne riječi: Centralni dio skeleta sredine lica, geometrijska morfometrija, seksualni dimorfizam, 3D modeli ljudskih lobanja

SHORT COMMUNICATION

Assessment of the efficacy of stable liquid chlorine dioxide (CLO_2) in disinfection of stored table eggs

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ABSTRACT

Microbiological contamination of table egg shells poses an important public health and technological challenge, particularly in the European Union, where washing and disinfection of Class A eggs are prohibited due to adverse effects associated with previously used sanitising agents. This study evaluated the efficacy of stable liquid chlorine dioxide (ClO_2) in reducing microbial load on eggshells during storage under commercial conditions. A total of 200 eggs were divided into two groups: a control group (untreated) and an experimental group treated with a fine aerosol of 50 ppm ClO_2. Microbiological cleanliness was assessed using total aerobic mesophilic bacterial count (UBAMB) and luminometry at predetermined intervals.Immediately after treatment (day 0), the experimental group showed a pronounced microbiological reduction, with median UBAMB values decreasing from 35,000 CFU in the control group to 70 CFU in the treated group ($p = 0.029$). Significant differences in luminometry values between the groups were observed on days 0 and 14 ($p = 0.028$; $p = 0.025$), indicating sustained antimicrobial activity during storage. Within the experimental group, luminometry values showed significant temporal variation ($p = 0.015$), characterised by an increase by day 7, followed by stabilisation by day 14, when values returned to levels comparable to those observed immediately after disinfection ($p = 0.980$). No significant temporal changes were detected in the control group ($p = 0.276$). Stable liquid chlorine dioxide proved to be an effective and technologically acceptable disinfectant, achieving significant microbial reduction without compromising egg integrity. These findings highlight the potential applicability of ClO_2 as a modern, non-destructive sanitising option that addresses microbiological safety concerns while meeting the hygienic and regulatory requirements related to table egg production. Further research is recommended to assess the internal quality of eggs and to explore broader microbiological indicators under varying storage and environmental conditions.

Keywords: Disinfection, eggshell, egg quality, EU legislation, stable liquid chlorine dioxide

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INTRODUCTION

Eggs intended for human consumption are typically not washed or disinfected. To ensure the quality and safety of eggs, which are a highly nutritious food source, it is crucial to strictly adhere to proper technological procedures during animal management and egg collection on farms (Hutchison, 2003). Despite these measures, eggs can pose significant health risks to humans due to the presence of dangerous pathogens, such as Salmonella, Pseudomonas, and Escherichia. While table eggs are generally left unwashed, there is a growing need for a simple yet effective method to enhance the hygienic treatment of their shells (Abdulwahid, 2020). After an egg is laid, its external temperature is lower than that of the hen's body, causing the internal structures of the egg to contract. This contraction can draw surface particles through the shell's pores into the egg itself, allowing microorganisms, including various pathogens and fungi, to penetrate (Gagić, 1999). Public health considerations emphasize controlling microorganisms during the intensive production and distribution of both hatching and table eggs. Proper hygienic management of these egg categories is essential to prevent economic losses in poultry production. Foodborne illness outbreaks linked to contaminated table eggs pose a global public health concern. In contrast to table eggs, breeding eggs are often subjected to collection, washing, and routine disinfection. Controlling pathogenic and conditionally pathogenic microorganisms is fundamental to effective disinfection practices (Bermúdez-Aguirre, 2025). Research conducted in the past decade has demonstrated the successful application of disinfectants from the DioxyActiv Supra line, which utilizes stable liquid chlorine dioxide for various disinfection needs (Gagić et al., 2013a; Gagić et al., 2013b; Ališah et al., 2023; Hansung et al., 2018., Ališah, 2020), Compared to other disinfectants, especially in their gaseous forms (Kustura et al., 2012., TurtoriandBorda, 2014; Morouj et al., 2016; Rrahimi, 2021), stable liquid chlorine dioxide offers a broad spectrum of action, straightforward application, and minimal toxicity to living tissue. Additionally, it functions effectively at lower concentrations than chlorine and its compounds, which can negatively impact disinfecting efficacy and lead to harmful by-products. Unlike other recommended disinfectants, chlorine dioxide is neither toxic nor environmentally hazardous, unlike chlorine, while offering equal efficacy and greater stability compared to ozone. Furthermore, it does not leave undesirable residues that could compromise the hygienic status of treated surfaces or materials (Gagić et al., 2013a).The European Union's regulatory framework regarding the treatment of class A table eggs is based on Regulation (EC) No. 589/2008 and Delegated Regulation (EU) 2023/2465, which state that eggs for direct consumption must not be washed or disinfected before being placed on the market. This prohibition stemmed from the assumption that washing and chemical treatments could damage the shell's cuticle, disrupt the microstructure of the pores, and, therefore, increase the risk of microorganisms penetrating the interior of the egg (EFSA 2005; FSAI 2019). Historically, the most commonly used agents for disinfection included chlorine, formaldehyde, and hydrogen peroxide, whose aggressive properties could adversely affect the organoleptic properties and stability of the egg. Consequently, the European Union established a ban that remains in force today (Reg. 853/2004, Annex III, Sect. X).Recent research suggests that the prohibition on washing and disinfection no longer reflects current technological possibilities. Stable liquid chlorine dioxide (ClO_2) has emerged as one of the most promising disinfectants due to its wide spectrum of action, high stability, and minimal toxicity. Unlike traditional chlorine compounds, ClO_2 operates effectively at low concentrations and does not produce toxic by-products (Gagić et al., 2013b). Stable liquid chlorine dioxide demonstrates selective action by effectively removing pathogenic microflora while preserving beneficial saprophytic bacteria without negatively impacting product quality (Ališah et al. 2025).

There are no published studies that have addressed the issue of disinfecting stored table eggs using stable liquid chlorine dioxide. Accordingly, the aim of our research was to evaluate the effectiveness of stable liquid chlorine dioxide (ClO_2) in reducing the microbial load on eggshells during storage.

MATERIAL AND METHODS

A total of 200 table eggs (52 – 64 grams) were taken in the sorting plant of a large farm for the production of table eggs. The eggs are divided into two groups, each containing 100 pieces. The control group of eggs was not disinfected. A few hours after grading, the experimental group of eggs was returned to a sorting conveyor belt consisting of rotating roast beads and passed through a fine aerosol, prepared from 100 ml of 50 per mille solution of disinfectant based on stable liquid chlorine dioxide (ClO_2). After treatment of the experimental eggs, both groups were stored in a cold chamber at the fridge temperature and a relative humidity (RH) of 55% to 65% for the remainder of the experiment.The number and reduction of the total number of aerobic mesophilic bacteria (UBAMB), and the intensity of luminance in phentomols (fm), were performed in the programmatically provided control terms, namely UBAMB and zero-day luminance one (1) hour after storing both groups of eggs in the refrigerator.Then, we determined only the luminance on the seventh (7) and 14th day. During the controls, we prepared six (6) aggregate samples from each egg group. One aggregate sample consisted of five (5) eggpieces.

Microbiological Method

Each swab was immersed in 10 cc of peptone water in a test tube, and the first dilution was prepared after 30 minutes at room temperature. Later, 0.1 mL of the diluted content was applied to the UBAMB medium in appropriate Petri plates and incubated at 37°C for 24 hours, followed by a reading of the results.

Luminometry Method

Luminometry only shows the degree of microbiological contamination of the controlled surface or medium by the use of ATP luminators and appropriate swabs. The criteria for determining an acceptable sanitary-hygienic cleanliness zone in accordance with USA standards are for surfaces, up to 25000 RLU, and for drinking water zero (0) RLU (Relative Light Units)

Statistical Analysis

Descriptive statistics were calculated as median, mean and interquartile range for continuous variables. Comparisons between experimental and control groups were performed using the Mann–Whitney U test on log10 (value + 1) - transformed data due to non-normal distributions. Changes over time within each group for LUM data were tested using the Kruskal – Wallis test. When significant differences were detected, pairwise post-hoc comparisons were performed using Dunn's test with Bonferroni correction for multiple testing. All analyses were conducted in R, version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria). A value of $p<0.05$ was considered statistically significant.

RESULTS

Descriptive statistics are presented in Table 1. At baseline (day 0), the control group showed higher laboratory (LAB) values (median 35000) compared with the experimental group (median 70), whereas luminometry (LUM) values were more comparable between groups (Table 1). On pairwise comparison between experimental and control groups using the Mann–Whitney U test on log10(value + 1)-transformed data, LAB values at day 0 ($W = 0$, $p = 0.029$) and LUM values at day 0 and 14 ($W = 0$, $p = 0.028$, $W=0$, $p=0.025$) were statistically significant (Table 2).

Over time, within-group for LUM data comparisons using the Kruskal–Wallis test indicated a significant change in the experimental group ($\chi^2 = 8.35$, $p = 0.015$), while in the control group remained stable ($\chi^2 = 2.58$, $p = 0.276$).

Post-hoc pairwise comparisons using Dunn's test, corrected for multiple testing, showed that in the experimental group, LUM values decreased significantly from day 0 to day 7 ($p = 0.013$), while no significant differences were observed between day 0 and day 14 ($p = 0.980$) or between day 7 and day 14 ($p = 0.187$). LUM measurements in the control group did not show significant differences between any time points.

Table 1 Descriptive statistics for control and experimental groups over a 14-days period. Values represent the mean, standard deviation (SD), median, interquartile range (IQR), and range (min-max) for each group and method at days 0, 7, and 14

Group	Method	Day	Mean	SD	Median	IQR	Min	Max
Control	Lab	0	40000	31622.8	35000	400000	10000	80000
	Lum	0	21474	14899.8	16458	13563	10059	42921
	Lum	7	66817.8	60462.4	50383.5	58505.21	14834	151670
	Lum	14	39377.8	27516.4	29480	15923.8	18622	79929

Group	Method	Day	Mean	SD	Median	IQR	Min	Max
Experimental	Lab	0	65	55.1	70	75	0	120
	Lum	0	6268	778.9	6596.5	635.5	5120	6759
	Lum	7	16655	4794.3	17794	4483.5	9987	21045
	Lum	14	8114.5	2022.4	8889	1299.5	5122	9558

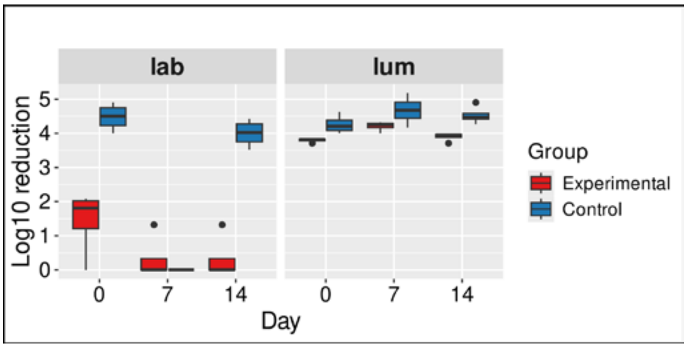

Figure 1 Boxplot distribution of log 10 (value+1) -transformed measurement values at days 0, 7, and 14 for the experimental and control groups. Data are presented separately for each method (lab and lum). For data set, whiskers present the full range of variations (minimum-maximum), box present 25th - 75th percentiles and horizontal lines in box present median values

Table 2 Results of the Mann–Whitney U test for comparing changes between the two study groups(p<0.05)

	Day	W	p-value
Lab	Zero day	0	**0.029**
Lum	Zero day	0	**0.028**
	7th day	3	0.211
	14th day	0	**0.025**

DISCUSSION AND CONCLUSION

According to the available literature, we did not find any published studies that directly examined the impact of stable liquid chlorine dioxide on the hygienic quality of the eggshell. This limits the possibility of a detailed comparison with similar research. However, previous studies investigating chlorine dioxide–based disinfectants-primarily in gaseous or liquid forms-have reported notable antimicrobial efficacy (Gagić et al., 2013b; Hansung et al., 2018; Ališah et al., 2025), supporting the outcomes observed in our study. Also, Turtoi and Borda (2014) demonstrated the effectiveness of UV-based shell decontamination, while Morouj et al. (2016) and Rrahimi (2021) reported reductions in Salmonella contamination using hydrogen peroxide and sodium carbonate. These findings collectively suggest that ClO_2-based disinfection offers a technologically feasible and microbiologically effective approach to improving shell hygiene.

Results of this study clearly demonstrate that the application of stable liquid chlorine dioxide (Dioxy Activ Supra) at 50 ppm significantly improves the microbiological hygiene of table egg shells. Immediately after treatment (day 0), the experimental group exhibited a substantial reduction in microbiological load, as confirmed by significantly lower LAB values compared with the control group (p = 0.029). This rapid antimicrobial effect is consistent with the known oxidative modes of action of chlorine dioxide, which target essential bacterial cellular components and lead to swift inactivation of microorganisms.

The LUM data provide additional insight into the temporal dynamics of microbial contamination during storage. Significant differences between the experimental and control groups were detected on days 0 and 14, confirming that the antimicrobial advantage of chlorine dioxide persists beyond the immediate post-treatment period. Within-group comparisons further revealed a significant temporal change in the experimental group (p = 0.015), characterised by an initial increase in LUM values between day 0 and day 7 ($p = 0.013$), followed by stabilisation between days 7 and 14. Importantly, no significant difference was observed between day 0 and day 14 ($p = 0.980$), which suggests that despite natural recolonisation occurring during storage, the microbial load returns to levels comparable to those measured immediately after disinfection.

By contrast, the control group exhibited no significant changes over time ($p = 0.276$), and consistently maintained higher levels of microbial contamination. These findings underscore the value of chlorine dioxide treatment in reducing initial contamination and maintaining favourable hygienic conditions throughout storage.

Overall, chlorine dioxide treatment proved to be a simple, cost-effective, and efficient method to reduce microbial contamination of table egg shells. The ability of stable liquid chlorine dioxide to maintain reduced microbial levels up to 14 days highlights its potential for use in commercial egg production systems, particularly those with extended storage periods.

This study has several limitations. Although the results confirm the reduction of bacterial load on the external eggshell surface, the internal structure of the egg (albumen, yolk, and membranes) was not examined. Therefore, it cannot be concluded with certainty that stable liquid chlorine dioxide has no toxicological or microbiological impact on internal egg components. Previous literature (e.g., Gagić et al., 2013a) provides supporting evidence, but does not offer comprehensive toxicological evidence to validate this assumption for stored table eggs fully. This gap highlights the need for future studies focusing on the internal microbiological and toxicological safety profile of eggs treated with stable liquid chlorine dioxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTION

Conception: AA, AG; Design: AA, AG; Supervision: EŠ, AG; Materials: AA, AG; Data Collection and/or Processing: AA, AG; Analysis and/or Interpretation of the Data: AA, AG; Literature Review: AA, EŠ; Writing: AA; Critical Review: AG, EŠ

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Procjena efikasnosti stabilnog tečnog hlor-dioksida (CLO_2) u dezinfekciji skladištenih konzumnih jaja

SAŽETAK

Mikrobiološka kontaminacija ljuski konzumnih jaja predstavlja značajan javnozdravstveni i tehnološki izazov, osobito u Europskoj Uniji, u kojoj su pranje i dezinfekcija jaja A klase zabranjeni zbog nuspojava povezanih sa ranije korištenim sredstvima za dezinfekciju. Ovo istraživanje evaluira efikasnost stabilnog tečnog hlor dioksida (ClO2) u smanjenju mikrobiološkog optrećenja ljuski jaja za vrijeme skladištenja u komercijalnim uvjetima. Ukupno 200 jaja je podijeljeno u dvije grupe, kontrolnu (netretiranu) i eksperimentalnu koja je tretirana sa finim aerosolom od 50 ppm ClO2. Mikrobiološka čistoća je procijenjena ukupnim brojem aerobnih mezofilnih bakterija (UBAMB) i luminometrijom rađenom u prethodno određenim intervalima. Neposredno nakon tretmana (Dan 0), eksperimentalna grupa je pokazala izraženu mikrobnu redukciju sa srednjim UBAMB vrijednostima koje su se smanjile sa 35,000 CFU u kontrolnoj grupi na 70 CFU u tretiranoj grupi ($p = 0.029$). Signifikantna razlika u luminometrijskim vrijednostima između grupa je uočena u Danima 0 i 14 ($p = 0.028$; $p = 0.025$), što ukazuje na perzistirajuću antimikrobnu aktivnost za vrijeme skladištenja. Luminometrijske vrijednosti su u eksperimentalnoj grupi pokazale signifikantnu temporalnu varijaciju ($p = 0.015$) karakteriziranu rastom da dana 7, nakon čega je do Dana 14 nastupila stabilizacija, pri čemu su se vrijednosti vratile na vrijednosti zabilježene neposredno nakon dezinfekcije ($p = 0.980$). U kontrolnoj grupi nisu otkrivene nikakve temporalne promjene ($p = 0.276$). Stabilni tečni hlor dioksid se dokazao kao učinkovit i tehnološki prihvatljiv dezinficijens kojim je postignuta značajna redukcija broja mikroba, bez uticaja na integritet jaja. Ovakvi razultati naglašavaju mogućnost primjene ClO2 kao suvremene, neškodljive opcije dezinfekcije koja uzima u obzir mikrobiološke sigurnosne mjere dok zadovoljava higijenske i zakonske zahtjeve povezane sa proizvodnjom konzumnih jaja. Preporučuju se daljnja istraživanja sa ciljem procjene unutrašnje kvalitete jaja, kao i šire ispitivanje mikrobioloških indikatora u različitim uvjetima skladištenja i okoliša.

Ključne riječi: Dezinfekcija, EU zakonodavstvo, kvalitet jaja, ljuska jaja, stabilni tečni hlor dioksid

CASE REPORT

Pseudoacetabulum formation on the femur following untreated femoral neck fracture in a juvenile cat: Surgical management and outcome

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ABSTRACT

Femoral neck fractures are relatively common in cats and often occur after falls from significant heights. If not treated appropriately, these injuries may lead to long-term complications, such as pseudoarthrosis, joint instability, and impaired limb function. This report presents a rare case of pseudoacetabulum formation secondary to an untreated femoral neck fracture in a four-month-old kitten. Following a fall, conservative management was chosen over surgery. Over the next months, the cat developed progressive lameness and hip pain. At seven months, advanced imaging revealed separation of the femoral head from the acetabulum and the development of a pseudoarticulation between the femoral head and metaphysis, consistent with pseudoacetabulum. Surgical excision of the femoral head and neck led to satisfactory functional recovery. This case emphasizes the importance of timely surgical intervention in feline femoral neck fractures and documents pseudoacetabulum formation as a rare but clinically relevant complication of delayed or insufficient treatment.

Keywords: Animal, conservative treatment, extremities, lameness, pseudarthrosis

INTRODUCTION

Proximal femoral fractures can involve the femoral head, neck, greater trochanter, or subtrochanteric region in various forms. Although fractures occurring in this region are relatively rare, the literature contains limited information regarding their clinical outcomes (Roberts and Meeson, 2022). Management of proximal femoral fractures may involve either conservative methods or surgical intervention. The conservative treatment approach relies on the body's intrinsic biological mechanisms for fracture healing. This method may be considered a viable option, particularly in young cats with acute femoral neck fractures provided that bone resorption or the remodelling process has not yet commenced. However, if radiographic findings raise suspicion of metaphyseal osteopathy, clinical improvement is unlikely to be achieved through conservative management alone (Lafuente, 2011).

Pseudoacetabulum formation, a compensatory articulation typically described in human patients with chronic hip instability or post-arthroplasty, has not been well characterized in veterinary literature. Its development as a secondary complication of femoral neck fracture has not been previously documented in feline cases to the authors' best knowledge.

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The purpose of this case report is to describe the formation of both pseudoarthrosis and a pseudoacetabulum following non-surgical management of a femoral neck fracture in a juvenile cat. The case is presented with emphasis on clinical signs, diagnostic imaging, surgical approach, and functional recovery. This case emphasizes the potential for rare yet clinically significant complications resulting from delayed or suboptimal management of proximal femoral fractures in cats and highlights the critical importance of timely surgical intervention.

CASE DESCRIPTION

A four-month-old male domestic cat was presented to Ankara University, Faculty of Veterinary Medicine, Animal Hospital Surgery Clinic following a fall from height, exhibiting non-weight-bearing lameness of the right hindlimb. Orthopedic examination revealed a femoral neck fracture on the right side, and surgical repair was recommended. However, due to concerns regarding the risks associated with general anesthesia, the owner declined operative treatment. Consequently, conservative management consisting of cage rest and a seven-day course of non-steroidal anti-inflammatory drugs (meloxicam, 0.2 mg/kg SC as an initial (loading) dose on day 1, followed by 0.05 mg/kg SC, q24h) and analgesics (butorphanol, 0.4 mg/kg SC as an initial dose on day 1, followed by 0.2 mg/kg SC, q8h) was initiated without surgical intervention. The animal was not brought in for follow-up evaluation, as the owner perceived clinical improvement. However, seven months later, the cat was re-presented with progressive lameness, pain, and limited mobility in the affected limb. Anamnesis confirmed the absence of any treatment during the interim.

Initial clinical examination was performed in accordance with Barnhart's lameness scoring system (Table 1). The assessment revealed moderate lameness consistent with Grade 2 (intermittent non-weight bearing lameness), characterized by marked muscle atrophy of the right pelvic limb, restricted range of motion in the hip joint, pain elicited during passive flexion and extension, palpable crepitus, and mild subluxation. The cat exhibited minimal weight-bearing on the affected limb and frequently displayed unilateral limb-carrying behavior.

Table 1 Five-point lameness scoring system used for visual assessment before and following surgery (Barnhart, 2016)

None	No lameness observed	0
Mild	Weight-bearing lameness	1
Moderate	Weight-bearing lameness with intermittent non-weight bearing	2
Severe	Non-weight-bearing lameness with brief intermittent weight- bearing	3
Non-weight bearing	Lameness at all times	4

Computed tomography (CT) evaluation demonstrated malunion of the femoral neck fracture (Figure 1), with 180° rotational displacement of the femoral head, which had rearticulated ectopically (Figure 2). The femoral head was no longer positioned within the anatomical acetabulum and had developed an abnormal articulation with the proximal metaphysis of the femur. Based on these findings, a diagnosis of pseudarthrosis and pseudoacetabulum formation was established. Surgical intervention in the form of femoral head and neck excision arthroplasty was planned to restore function and alleviate pain.

An Informed Consent Form was obtained from the animal's owner prior to the examination and surgical procedure. All procedures in this case report were conducted in accordance with national animal welfare regulations and no additional experimental interventions were performed.

A multimodal anesthesia protocol was used. Premedication included medetomidine (10–20 µg/kg, IM, Pfizer, Finland) and butorphanol (0.3 mg/kg, SC, Richter Pharma, Austria). Anesthesia was induced with ketamine (5 mg/kg, IM, Richter Pharma, Austria) after initiating prophylactic cefazolin (25 mg/kg, IV, Tümekip Med, Türkiye). Maintenance was achieved with isoflurane (1.5–2.5%, Piramal Critical Care, USA). Intraoperative monitoring, lactated Ringer's infusion (Koçak Farma, Türkiye), and thermal support were provided.

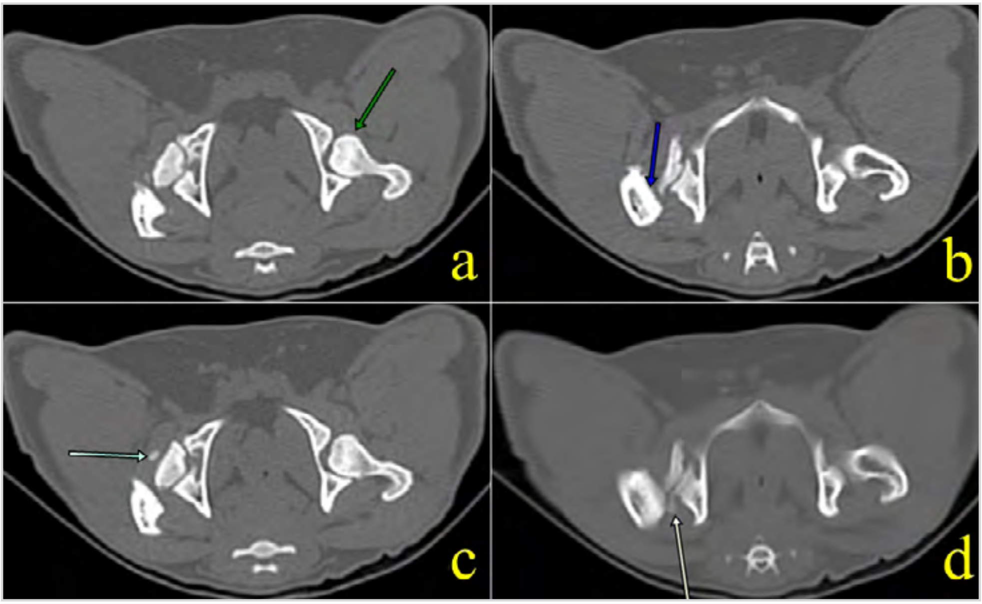

Figure 1 Transverse plane CT images of the coxofemoral joints a) Normal anatomical configuration of the left coxofemoral joint (green arrow). b) Pseudoacetabular cavity formation in the proximal region of the right femur secondary to displacement of the fractured femoral neck together with the intact femoral head (blue arrow) c) Residual bone fragment within the right coxofemoral joint, associated with fractures of the femoral neck and head (light blue arrow) d) Pseudoarthrosis formation between the right acetabular cavity and the femur, resulting from residual fragments of the femoral head (white arrow)

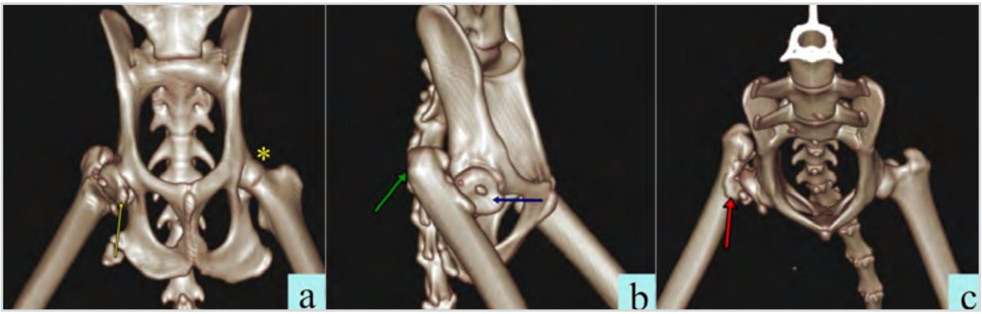

Figure 2 Three-dimensional reconstruction of pelvic CT images a) Coronal CT view showing the pseudoacetabulum observed in the right coxofemoral joint (yellow arrow) and the contralateral normal coxofemoral joint (asterisk) b) Sagittal CT view of the right coxofemoral joint demonstrating the femoral head within the acetabular cavity (blue arrow) and rest of the femur is displaced cranioposteriorly (green arrow) c) Coronal CT view demonstrating the pseudoacetabulum together with the remaining femoral head, while the rest of the femur is displaced cranioposteriorly (red arrow)

Figure 3 Postoperative day 14 ventrodorsal pelvic radiograph demonstrating the separation between the proximal femur and the irregular acetabulum following excision arthroplasty (arrow)

A craniolateral surgical approach to the right hip was performed, with the animal positioned in left lateral recumbency. The *M. tensor fasciae latae* and *M. biceps femoris* were retracted, and the *M. gluteus medius* and *M. gluteus profundus* were displaced ventrally and dorsally, respectively. When the surgical site reached the fibrotic and irregular joint capsule, the femoral head fusion in an inverted position to the proximal metaphysis, without anatomical articulation with the acetabulum was observed. The abnormal bony structure was excised using a bone rongeur. The fractured part of collum femoris was removed using a sagittal saw and after that smoothed with bone rasp (Figure 3), and anatomical reconstruction of surrounding musculature was performed prior to routine closure of the surgical site. The total duration of the surgical procedure, measured from the initial skin incision to extubation was 55 minutes.

Postoperative analgesia was achieved using meloxicam (0.1 mg/kg, SC, q24h for 5 days, Bavet, Türkiye) and butorphanol (0.2 mg/kg, SC, q12h for 3 days). Prophylactic antibiotic therapy with cefazolin (25 mg/kg, IV, q12h, Tümekip Med, Türkiye) was initiated preoperatively and continued for 7 days. Postoperative pain was effectively managed, and the surgical site healed uneventfully, without any complications.

Passive range of motion exercises and controlled mobilization were initiated on the 10th postoperative day. Ambulation on irregular surfaces, with owner assistance, was incorporated to prevent muscle atrophy and to maintain joint flexibility. Throughout the recovery process, the cat's ambulation and comfort levels were monitored daily. Over the course of two months, the patient exhibited improved limb use and marked reduction in lameness. The lameness score decreased to grade 1 postoperatively and eventually approached grade 0.

DISCUSSION AND CONCLUSION

In feline orthopedic practice, fractures involving the proximal physis and femoral neck are relatively common, with traumatic femoral neck fractures being more frequently documented in the literature (DeCamp et al., 2016). While growth plate injuries are often considered the weakest anatomical points for fractures in juvenile animals, it has been reported that in kittens under six months of age, femoral neck fractures occur more frequently than physeal separations (Perez-Aparicio and Fjeld, 1993). The present case is noteworthy as it illustrates the consequences of non-operative management of a femoral neck fracture in a 4-month-old cat following a fall, which ultimately led to the rare formation of both pseudarthrosis and a pseudoacetabulum.

In young cats presenting with hindlimb lameness or weakness, femoral head and neck fractures should always be included in the differential diagnosis, regardless of trauma history. These injuries often result from high-energy trauma such as vehicular accidents or falls. Pain elicited during manipulation of the hip joint, including restricted motion, crepitus, or subluxation, may provide clues to the underlying pathology (Lafuente, 2011). In the present case, a definitive diagnosis of a femoral neck fracture was established through clinical and tomographic evaluation.

Pseudoacetabulum formation, characterized by abnormal articulation of the femoral head with the ilium in chronic hip dislocations, has been well-documented in human medical literature—an observation notably emphasized by Odak (2012) in his radiological evaluation. However, reports of pseudoacetabulum formation in veterinary patients remain exceedingly rare. Jones et al. (2019) documented seven canine cases in which chronic hip dislocation resulted in pseudoacetabulum development near the true acetabulum. Witsberger et al. (2007) described pseudoarthrosis-like structures where the femoral head articulated with the iliac wing. To the authors' knowledge, the formation of a pseudoacetabulum directly on the femur has not been previously described in either dogs or cats. In this respect, the presented case is remarkable, as it represents an unprecedented clinical report in the veterinary literature of pseudoacetabulum formation originating from the femur. In light of this, it should also be noted that the main limitation of this report is its single-case nature, which restricts the generalizability of the findings; thus, the conclusions should be interpreted with caution and validated in future studies involving larger cohorts.

Both conservative and surgical options are available for managing proximal femoral fractures. Surgical treatment is typically favored to prevent complications such as nonunion and loss of function (Lafuente, 2011). In feline patients, femoral neck fractures are often managed surgically through Kirschner wire fixation or femoral head and neck excision (FHNE), particularly in cases of physeal fractures (Borak et al., 2017; McNicholas et al., 2002; Fischer et al., 2004). Overall, surgical management is generally preferred, as it offers better prospects for functional recovery and reduces the risk of complications (Fischer et al., 2004).

Femoral head and neck excision (FHNE) is considered a salvage procedure that can yield favorable functional outcomes in cats. This technique, along with total hip replacement (THR), may be indicated when primary surgical management of coxofemoral joint arthropathy is not feasible. Such situations may arise due to patient-related factors, such as inadequate bone stock for implant placement or client-related considerations, including financial limitations or difficulties in ensuring postoperative activity restriction (Yap et al., 2015).

In the present case, excision arthroplasty was selected due to the chronic nature of the fracture and the abnormal positioning of the femoral neck, which had fused to the acetabular region in an inverted orientation. Although advanced surgical interventions such as total hip replacement might have been considered, financial constraints led the owner to opt for femoral head and neck excision. The irregular and thinned morphology of the femoral neck also rendered anatomical repositioning unfeasible. Imaging studies, particularly CT, supported the decision for excision as the most appropriate treatment to relieve pain and restore mobility.

Intraoperatively, the femoral head and neck were confirmed to be malunited in an inverted position and were excised carefully. Closure of the surgical site preserved the anatomical integrity of surrounding tissues. Postoperatively, the patient exhibited progressive, pain-free mobility in the affected limb. At the 60-day follow-up, the cat demonstrated notable improvement in gait and absence of clinical signs of pain.

Femoral fractures in cats are typically not well suited to conservative management, and in most cases require some form of internal fixation to achieve successful healing (Beale, 2004), as delayed intervention or absence of treatment may result in fibrous nonunion and resorption of the femoral neck, ultimately leading to degenerative alterations within the joint (Aithal et al., 2023), as observed in this case.

In this case, conservative treatment was initially chosen due to concerns regarding the risks of general anesthesia. However, the formation of a pseudoacetabulum due to malunion underscored the limitations and potential severity of this approach. Surgical intervention ultimately became unavoidable due to the progression of clinical symptoms.

This report provides a detailed account of pseudoacetabulum formation originating from the proximal femur in a feline patient—a condition that, to the authors' knowledge, has been rarely documented in the veterinary literature. Following a comprehensive evaluation of treatment options and consideration of financial constraints, femoral head and neck excision was selected. Surgical removal of the abnormally fused femoral structures resulted in improved mobility and significant clinical recovery. This case highlights pseudoacetabulum formation as an uncommon but clinically relevant complication of untreated femoral neck fractures and contributes valuable insights to the field of veterinary orthopedic surgery.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Aİ.: Literature review, writing. REE: Conception, design, writing. ST.: Materials. IE.: Supervision, writing, critical review.

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Formiranje pseudoacetabuluma nakon neliječene frakture vrata femura kod mačeta: Operativni tretman i ishod

SAŽETAK

Kod mačaka su frakture vrata femura relativno česte, obično uzrokovane padom s velikih visina. Ako se adekvatno ne liječe, ovakve povrede mogu izazvati dugotrajne komplikacije kao što su pseudoartroza, nestabilnost zgloba i oštećenje funkcije ekstremiteta. Ovo je prikaz rijetkog slučaja formiranja pseudoacetabuluma uslijed neliječene frakture vrata femura kod četveromjesečnog mačeta. Mače je nakon pada liječeno konzervativno umjesto operativno, a u narednim mjesecima je došlo do razvoja progresivnog šepanja praćenog bolom u kuku. Za sedam mjeseci, napredno je snimanje pokazalo separaciju glave femura od acetabuluma i razvoj pseudoartroze između glave femura i metafize, što odgovara nastanku pseudoacetabuluma. Operativna ekscizija glave i vrata femura je dovela do zadovoljavajućeg funkcionalnog oporavka. Ovaj slučaj naglašava značaj pravovremene operativne intervencije kod fraktura vrata femura kod mačaka i dokazuje formiranje pseudoacetabuluma kao rijetke, ali klinički relevantne komplikacije odloženog ili nedovoljnog tretmana.

Kjjučne riječi: Ekstremiteti, konzervativna terapija, pseudoartroza životinja, šepanje

CASE REPORT

Management of a mixed enterococcal and streptococcal infection in a three-year old bull mastiff bitch: A case report

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ABSTRACT

Infertility in bitches poses a great concern to dog owners and breeders who intend to uplift their economic gain from sale of puppies. This case report represents a successful management of a mixed infection of *Enterococcus* and *Streptococcus* in a bull mastiff bitch, presented following repeated still birth in two consecutive pregnancies. Vital parameters were normal, but haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC) and total leukocyte count (WBC) were below the reference range on the day of presentation, indicative of anaemia and leukopenia. Also, vaginal cytology indicated estrus. Bacterial culture obtained from the vaginal swab revealed *Enterococcus* and *Streptococcus* species, which were highly susceptible to erythromycin and resistant to amoxicillin and ciprofloxacin. Since serum agglutination test returned negative for brucellosis, a confirmatory diagnosis of a mixed infection of *Enterococcus* and *Streptococcus* was made. The bitch was successfully treated with erythromycin. Post-treatment culture was negative, which allowed breeding at the next estrus post treatment; the hematological parameters demonstrated improvement, indicating a positive therapeutic response. This case report underscored the importance of laboratory diagnostics and management of systemic infections to sustain fertility.

Keywords: Bitch, *Enterococcus*, *Streptococcus*, vaginal cytology

INTRODUCTION

There is a wide range of causes of reproductive disorders in bitches. The most frequent are abnormal embryonic development due to genetic defects or competition between placental spaces, uterine abnormalities (uterine torsion, cystic endometrial hyperplasia, inflammation, etc), nutritional deficiencies, environmental stressors, hormonal imbalances (including hypoluteodism and hypothyroidism) and infections (Mantziaras and Zakosek, 2025). Given the complexity of these cases, accurate diagnosis often requires a comprehensive, multi-faceted approach to identify the underlying causes. Bacterial infections are the most common clinical presentations, which are associated with reproductive disorders in bitches. Infections can be caused by single bacterial line or in combination of several bacteria. *Enterococci* are Gram- positive facultative anaerobic cocci in short and medium chains. They were recognized as a separate genus from streptococci by DNA hybridization in 1984 (Said et al., 2024). *Enterococci* infection is commonly implicated in stillbirth and inflammation of the placenta, foetal membrane or umbilical cord (Gabrielli et

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al., 2025). Also, they can be residents of intestinal flora, occasionally causing genital tract inflammation and urinary tract infections (Stepien-Pysniak et al., 2021).

Streptococci are Gram-positive, non-motile, non-spore-forming, catalase-negative cocci occurring in pairs or chains. Most streptococci are facultative anaerobes, while some are obligates anaerobes (Patterson, 1996). Streptococci invade tissues opportunistically, when there is a bridge in normal host barriers, resulting in disease manifestations, such as pneumonia, endocarditis and foetal death (Tsutsumi, 2025).

In a 10-year study of the trends in reproductive disorders in animals presented at a veterinary hospital in Enugu state by Ibe et al. (2025), the highest prevalence of reproductive disorders was in the canine species. This may be attributed to the fact that dogs are the most important companion domestic species to man, thus, the increased demand for their maintenance appropriate for specific purposes, such as company, sport, hunting and/or guarding (Costa et al., 2019). In another retrospective survey on 203 bitches with genital disorders, such as infertility, vaginitis, pyometra and puppy death by Bjurstrom (1993), majority of the infertile bitches gave rise to mixed cultures involving streptococci. Mixed infection has also been reported as a common cause of infertility in bitches (Antunes et al., 2016).

This case report reveals infertility in a bitch caused by mixed infection by the rare combination of the *Enterococci* and *Streptococci.* The systematic diagnosis, management and good prognosis of the case instigated its report in the literature.

CASE PRESENTATION

A 3-year-old Bull Mastiff bitch weighing 46.0 kg was presented to the Small Animal Clinics Unit of the Veterinary Teaching Hospital, University of Nigeria, Nsukka, on the 20th of May, 2024, with the chief complaint of ascertaining if the bitch was in estrus. It was revealed that the bitch previously had aborted in two consecutive pregnancies, after mating with two different dogs, and that no diagnostic testing was conducted to ascertain the cause of the abortions. There was no other dog in the compound. The vaccination history was up to date.

Diagnostic Plan

Physical Examination

Physical examination revealed a normal mucous membrane and uninflamed mandibular and prescapular lymph nodes. There was a mild tick infestation. The rectal temperature, respiratory rate, pulse rate and heart beat were all within the normal range (Table 1).

Table 1 Vital parameters of the patient on the day of presentation

Vital Parameter	Patient's Value	Normal Range in Dogs
Rectal temperature	39.3°C	37.9°C - 39.9°C
Respiratory rate	36 cycles/minute	15-40 cycles/minute
Pulse rate	82 beats/minute	70 - 120 beats/minute
Heart rate	90 beats/minute	70 - 120 beats/minute
Capillary refill time	<2 seconds	

Laboratory Screening

Laboratory analyses included vaginal cytology, *Brucella* screening, haematology and microbial culture and sensitivity tests. Vaginal cytology was conducted using a sterile vaginal mucosal swab pre-soaked in normal saline and inserted from an angle of 45^0 on the vaginal mucous membrane. A smear of the swab on an albuminized slide preceded air-drying and methanol fixation for about 20 minutes. The fixed slides were rinsed with running water and stained with Giemsa. Stained slides were examined and photomicrographs were obtained at different magnifications, using a digital eyepiece (Scopetek®, DCM500, Hangzhou, Zhejiang Province, China; Resolution: 5M pixels) attached to a light microscope (OLYMPUS®, Model XSZ107BN, Hamburg, Germany).

Brucella screening was conducted using 3 ml of cephalic blood into a plain sample bottle. Serum was obtained from the sample after keeping the sample in a slanting position for 12 hours at room temperature to allow for blood clotting and separation of the serum. Serum was aspirated into labeled 2 ml serum vials, using a Pasteur pipette and maintained on ice. The sera samples were thawed and used for *Brucella* screening, using the serum agglutination method as described by Brown et al. (1981). For haematological analysis, cephalic blood sample was aseptically collected using a 2 ml sterile syringe and 23-gauge needle into a sterile ethylenediaminetetraacetic acid (EDTA) sample bottle. Red blood cell (RBC) and total leukocyte count (WBC) were determined by haemocytometer method, packed cell volume (PCV) was determined by microhaematocrit centrifugation method, while haemoglobin concentration (HC) was determined by cyanomethaemoglobin method (Kachmar, 1970).

Vaginal swab, collected as already described, was used for microbial culture and sensitivity test. The swab sample was enriched by inoculating on an enrichment media containing nutrient broth and peptone water at 42°C for 48 hours. A colony was taken from the enrichment media, using a sterile metal loop, and sub-cultured onto a blood agar, Eosin Methylene Blue, Mannitol Salt Agar, Salmonella Shigella Agar, McConkey Agar, by streaking. These were incubated at 37°C for 24 hours aerobically. Growing colonies were examined with Gram-staining techniques. Suspicious colonies were identified using biochemical tests. The antibiotic sensitivity test for the identified bacteria was applied with multi-discs containing ceftriaxone (10 µg), azithromycin (20 µg), amoxicillin (30 µg), ampicillin (30 µg), levofloxacin (10 µg), chloramphenicol (20 µg), ciprofloxacin (10 µg), gentamycin (10 µg) and streptomycin (30 µg). The antibiotics sensitivity test was performed according to the Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020).

Management

Based on the tick infestation, management commenced on the day of presentation, prior to laboratory results. Injection of ivermectin at dosage of 0.4 mg/kg was given subcutaneously and repeated after 2 weeks.

Tentative Diagnosis

These included Streptococcal infection and Brucellosis.

Laboratory Findings

Vaginal cytology revealed very scarce parabasal cells and many anucleated superficial cells. This was indicative for the estrus stage of estrous cycle.

Serum agglutination test for Brucellosis was negative. Heamatology test revealed low values of PCV, Hb, RBC, WBC and neutrophils (Table 2)

Table 2 Haematological parameters of the patient on the day of presentation

Haematological value	**SI Unit**	**Patient's Value**	**Normal Range in Dogs (**Khan et al., 2011**)**
PCV	%	30.0	35.0-57.0
Hb	g/µl	9.89	11.9 - 18.9
RBC	x10^6	3.90	4.95- 7.87
WBC	x10^3µ/L	3.2	5.0– 14.0
Lymphocyte	%	23.6%	8.0-38.0
Neutrophils	%	76.0	51.0-84.0
Monocyte	%	0.4	1.0-9.0
Eosinophils	%	0.0	0.0-9.0
Basophils	%	0.0	0.0-1.0

Haemo-analyzer used was ABAXIS Zoetis Vetscan (VS2 HM5) blood analyzer

The microbial culture result showed heavy growth of *Enterococci* and *Streptococci* (Figure 1).

The microbes were very sensitive (+++) to streptomycin, azithromycin and erythromycin, mildly sensitive to levofloxacin (++), and resistant to ceftazidime, rifampicin, ceftriaxone, amoxicillin and ciprofloxacin (Figure 2).

Confirmatory Diagnosis

Mixed infection of *Enterococci* and *Streptococci*.

Figure 1 Result of bacterial culture, showing heavy growth of *Enterococci* and *Streptococci*

Figure 2 Result of bacterial sensitivity test

Continuation of Management

Following confirmation and antimicrobial sensitivity tests, treatment continued with administration of azithromycin at dosage of 10 mg/kg I.M. for seven days; vitamin B complex was also administered at dose of 2 ml I.M., x 5/7.

The patient was re-examined 7 days post- treatment. Physical examination revealed that the dog continued to weigh 46 kg and maintained a normal mucous membrane. The mandibular and prescapular lymph nodes were normal. There was no tick infestation. The rectal temperature, respiratory rate, pulse rate and heart beat were all within the normal range in dogs (Table 2).

Table 3 Vital parameters of the patient on the day 7 post-treatment

Vital Parameter	Patient’s Value	Normal Range in Dogs
Rectal temperature	38.9°C	37.9°C - 39.9°C
Respiratory rate	34 cycles/minute	15-40 cycles/minute
Pulse rate	84beats/minute	70 - 120 beats/minute
Heart rate	89 beats/minute	70 - 120 beats/minute
Capillary refill time	<2 seconds	

Analysis of blood sample collected 7 days post-treatment also revealed normal values of PCV, Hb concentration, RBC, WBC and neutrophils (Table 4)

Table 4 Haematological values of the patient on the day 7 post-treatment

Haematological value	SI Unit	Patient’s Value	Normal Range in Dogs (Khan et al., 2011)
PCV	%	38.0	35.0-57.0
Hb	g/µl	12.3	11.9 - 18.9
RBC	x10^6	5.10	4.95- 7.87

Haematological value	SI Unit	Patient's Value	Normal Range in Dogs (Khan et al., 2011)
WBC	x 10^3 u/L	3.5	5.0 – 14.0
Lymphocyte	%	30.5	8.0-38.0
Neutrophils	%	68.8	51.0-84.0
Monocyte	%	0.7	1.0-9.0
Eosinophils	%	0.0	0.0-9.0
Basophils	%	0.0	0.0-1.0

Haemo-analyzer used was ABAXIS Zoetis Vetscan (VS2 HM5) blood analyzer

Repeated bacterial culture result did not yield any bacterial growth.

Recommendations to the owner of the bull mastiff bitch

The owner was advised not to breed the bitch, until the next estrus/heat. This was to enable completion of management of the Enterococcal and Streptococcal infections. The client was also advised to present the dog intended for breeding to the hospital prior to breeding. This was to screen the dog for any sexually transmitted diseases before breeding.

Prognosis

The bitch was bred on the next heat after treatment (6 months post-treatment). She whelped successfully.

DISCUSSION AND CONCLUSION

Laboratory confirmation of Streptococcus species in the present study is similar to other reports (DeWinter et al., 1999; Okpara et al., 2018), in which abortions and stillbirths in dogs were linked to streptococcal infections. *Streptococcus canis* is an opportunistic pathogen of dogs. However, the Lancefield group G of *Streptococcus canis* has been incriminated in infertility in bitches. Although Lamm et al. (2010) opined that confirmation of the diagnosis requires histopathologic examination and routine aerobic cultures from aborted material, the present case had no access to the aborted placenta or fetus. However, isolation of streptococci from maternal vaginal swabs was also indicative of a likely cause of the abortions; similar findings have been reported by other authors mentioned above.

This case underscores the importance of holistic laboratory examination in the management of potential infective causes of infertility in bitches. It is a known fact that *Brucella canis* is the most common cause of abortion and stillbirths in bitches (Buhmann et al., 2019). However, negative isolation of *Brucella* species should not rule out other bacterial causes of abortion, as the present case has revealed. Viral, bacterial, fungal and protozoal infections have been implicated in canine abortions. The progression of foetal development may be affected by the direct action of microorganisms such as Streptococci, which either degenerates the placenta or causes the release of placental toxins by inflammatory processes leading to hyperthermia, hypoxia and endotoxemia, leading to abortion (Antunes et al., 2016). In a study conducted by Shambulingappa et al. (2010), Streptococci ranked the second highest frequently occurring causes of canine abortions after *Escherichia coli*. Haematology revealed anaemia and leukopenia on the day of presentation (PCV, Hb, RBC and WBC were below their reference range). Studies have reported an association between severe anaemia and bacteraemia in dogs (Miller et al., 2004), although more often anaemia is due to inflammatory disease and cancer-related anaemia (Chervieret al. 2012; Num-Adom and Amali, 2024). Bacteraemia can cause anaemia in dogs through the immune response, which leads to the destruction of red blood cells. This immune-mediated damage, driven by pro-inflammatory cytokines, is the primary cause, not a direct effect of the bacteria themselves (Miller et al., 2004). These haematological values returned to normal post- treatment, underscoring the importance of early therapeutic intervention of infections. Leukopenia was recorded in the present study, similar to what was reported in a pit bull terrier cross breed with bacterial infection (Hanekom et al., 2020). In the present study, there was an improvement in the total white blood cell count on day 7 compared to day 1 (although the result was still below the normal range), likely due to the therapeutic control of the bacterial infection.

Vaginal cytology has many practical applications in the evaluation of both the normal and abnormal estrous cycles in bitches. It was employed in the present case because it is a simple, quick, non-invasive and inexpensive procedure used to identify the stage of the estrous cycle in dogs and to diagnose some pathologic conditions of the genital tract. There are different vaginal cell types viz. parabasal, intermediate, superficial and anuclear cells. It is generally accepted that vaginal cell types in the smear are related to the stage of the estrous cycle, which makes the vaginal exfoliative cytology a valuable add-on test in reproductive clinical diagnostics. Although the vaginal mucosal swab of a clinically healthy dog is not expected to be sterile, bacterial species isolated from bitches with reproductive disorders do not differ significantly from those found in healthy bitches (Groppetti et al., 2012). However, specific microorganisms within the vaginal mucosa, such as *Enterococci* and *Streptococci*, may cause infertility. The few parabasal and anucleated cells observed in the present case were indicative of the estrous stage of estrous cycle.

This case report is a re-emphasis on the need for a holistic laboratory examination, beyond the primary complaint of a client. It has demonstrated that mixed infection of *Enterococcus* and *Streptococcus* can cause reproductive disorders in dogs, and the use of the correct antibiotic therapy can cure the condition. It is hoped that this report will be useful to small animal clinicians.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ICC, AKO, TEY;Design – ICC, TEY; Supervision – AKO; Fundings – ICC; Materials – AEA; Literature review - ICC; Analysis and interpretation of data - ICC; Writing – ICC, AKO, TEY, AEA; Critical review - AEA

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Liječenje miješane enterokokne i streptokokne infekcije kod trogodišnje kuje pasmine bull mastiff: Prikaz slučaja

SAŽETAK

Neplodnost kod kuja izaziva značajnu zabrinutost kod vlasnika pasa i uzgajivača koji nastoje ostvariti ekonomsku korist prodajom štenaca. Ovaj prikaz predstavlja uspješan slučaj liječenja miješane *Enterococcus* i *Streptococcus* infekcije kod kuje pasmine bulmastif, koja je dovedena nakon ponovljenih mrtvorođenih štenaca u dvije uzastopne trudnoće. Vitalni parametri su bili uredni, ali su hematološki parametri poput zapremine pakovanih ćelija (PCV), koncentracije hemoglobina (Hb), broja crvenih krvnih zrnaca (RBC) i ukupnog broja leukocita (WBC) bili ispod referentnih vrijednosti na dan pregleda, što ukazuje na anemiju i leukopeniju. Vaginalna citologija je pokazala da je kuja bila u estrusu. Bakterijska kultura uzeta iz vaginalnog brisa otkrila je prisustvo *Enterococcus* i *Streptococcus* vrsta koje su veoma osjetljive na eritromicin, a otporne na amoksicilin i ciprofloksacin. Pošto je serološki test aglutinacije bio negativan na brucelozu, postavljena je konačna dijagnoza miješane *Enterococcus* i *Streptococcus* infekcije. Kuja je uspješno liječena eritromicinom. Kultura posijana nakon provedenog tretmana je bila negativna, što je omogućilo parenje u narednom estrusu nakon liječenja; hematološki parametri su pokazali poboljšanje, što ukazuje na pozitivan terapijski odgovor. Ovaj prikaz slučaja naglašava važnost laboratorijske dijagnostike i liječenja sistemskih infekcija radi očuvanja plodnosti.

Ključne riječi: *Enterococcus,* kuja*, Streptococcus*, vaginalna citologija

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7. Discussion and Conclusion,
8. Acknowledgements,
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Harvey N, Kingsley M, Terek F. 2019. Anatomical aspect of the red fox lung. Veterinaria, 64(3), 20-5.

Include the doi number if one exists

When more than a six authors:

Catanese G, Grau A, Valencia JM, Garcia-March JR, Vázquez-Luis M, Alvarez E, et al. 2018. Haplosporidium pinnae sp. nov., a haplosporidian parasite associated with mass mortalities of the fan mussel, Pinna nobilis in the Western Mediterranean Sea. J Invertebr Pathol, 157, 9-24.

Books:

Brown C, Laland K, Krause J. 2011. Fish Cognition and Behavior. 2nd ed. Oxford, UK: Wiley-Blackwell.

Chapters in books:

King AS. 1993. Apparatus urogenitalis. In Baumel JJ, King AS, Breazile JE, et al (Eds), Handbook of Avian Anatomy: Nomina Anatomica Avium (2nd ed., pp. 329-90). Cambridge, USA: Nuttall Ornithological Club.

Conference proceedings:

Bregoli M, Dediasi K, Pasolli C. 2006. Antibiotic resistant E. Coli in free-ranging alpine wild ruminants, in: VII Conference of the European Section of the Wildlife Diseases Association. p. 78.

Web Adresses:

Papazoglou LG, Basdani E. 2015. Exploratory laparotomy in the dog and cat. https://www.cliniciansbrief.com/article/exploratory-laparotomy-dog-cat (accessed 4.18.17).

Theses:

Donker SA. 2010. Arctic ground squirrels in the Southwest Yukon Territory: evidence for habitat specific demography and source-sink dynamics. MSc, University of British Columbia, Vancouver, Canada.

Watts AJR. 2012. Nutritional status and trophic dynamics of the Norway lobster Nephrops norvegicus (L.). PhD, University of Glasgow, Glasgow, UK.

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