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Content


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EDITORIAL

Dear colleagues,

A general opinion that veterinary science studies animal health, public health and biomedicine has "silently" distanced veterinarians from ecology. Given the complexity of life on Earth, delicate laws of ecology and so evident human impact on the biosphere, we must take a more cholistic approach.

World Health Organization One Health Initiative is an attempt to face these issues as efficiently as possible. Such concept represents a global strategy for strenghtening interdisciplinary cooperation and communication in all aspects of human, animal and environmental welfare. Health of individuals, populations and ecosystems is interconnected to such an extent that it makes it almost impossible to consider them separately if we want to get adequate answers and solutions. Achieved synergism should improve care in the 21st century onwards by expanding biomedical research, increasing efficiency of public health, expeditively enlarging the body of scientific knowledge and improving medical education and clinical care. Once properly implemented, it will become helpful in protection and salvage of myriads of lives of present-day and future generations.

Key objectives of WHO One Health Initiative (OHI) are:

1. Revitalize One Health approach in order to reduce human vulnerability in emergencies and provide better health and conditions;
2. Facilitate access in order to adjoin the environmental issues (e.g. land use conversion and more urban ecosystems) to human-animal ones and consider the relations between human health, animal health and ecosystems;
3. By applying One Health approach, to undertake the activities toward lowering risks and mitigating the impacts of the future outbreaks of zoonoses and vector diseases, endemic and breaking out infectious and non-infectious diseases with the focus on the breaking out zoonoses with a potential to become epidemics or pandemics.

One Health Initiative Team lists, among other, the steps to be undertaken, out of which we've decided to implement the following two:

1. Cooperation between the humanistic schools of veterinary medicine, schools of public health and schools of ecology and environmental protection;
2. Joint efforts in publishing, attending conferences and networking through the similar health networks;

In this regard, starting from this number of "Veterinaria" on, the articles selected for publishing will cover a wider range of topics, including ecology and environmental protection.

If we consider the global climate changes, anthropogenic influences on the entire planet, regional and local unreadiness and rigidity to find new modalities in health and environment management, we consider an imperative to expand the horizons of veterinarians to be, both countrywise and wider so that we could readily embrace the challenges ahead of us all.

Best regards,
Editor in Chief, Veterinaria
Muhamed Katica, DVM, Ms, PhD
Full Professor

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REVIEW ARTICLE

ADVANCING VETERINARY EPIDEMIOLOGY BY INTEGRATION OF MACHINE LEARNING: CURRENT STATUS AND FUTURE PERSPECTIVES

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ABSTRACT

The integration of machine learning (ML) in veterinary epidemiology offers transformative potential for data analysis and disease management, a significant shift from traditional statistical methods. This review explores the burgeoning role of ML, emphasizing its capacity to handle complex, high-dimensional data and uncover nonlinear relationships, which are pivotal in epidemiology. Key ML methodologies, including supervised, unsupervised, and reinforcement learning, provide robust frameworks for predictive modeling, pattern recognition, and decision-making processes. Applications in veterinary medicine are already evident in diagnostic imaging and animal behavior monitoring, showcasing ML's ability to enhance diagnostic accuracy and welfare monitoring.

Despite these advancements, the field faces challenges such as imbalanced datasets, data quality issues, and the need for interdisciplinary collaboration. Strategies like Synthetic Minority Over-sampling Technique and ensemble methods help address class imbalance, while robust preprocessing techniques mitigate data noise. Future advancements in natural language processing and reinforced learning promise further integration, optimizing disease surveillance and intervention strategies.

The review highlights the transformative potential of ML in veterinary epidemiology, advocating for continued research and collaboration to overcome existing hurdles. By leveraging ML's capabilities, veterinary professionals can improve disease prediction, develop targeted preventive programs, and enhance overall animal health and food security, marking a significant advancement in veterinary science.

Keywords: Animal health, artificial intelligence, disease surveillance, predictive modeling, veterinary medicine

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INTRODUCTION

Historically, data analysis and inferences made by applying analytical and statistical methods were epidemiological research's predominant roles and outcomes. However, the fast and rapid development of data science and artificial intelligence (AI) tools seen in the past couple of years, mainly in the domain of integration of powerful machine analysis of big data aggregation, opened a new window of opportunities for all biomedical disciplines including epidemiology. Although there has been increasing evidence of the use of machine learning (ML) as one of the growing tools under the AI umbrella in veterinary medicine, significant clinical and research potential and opportunities involving ML remain largely untapped, putting veterinary science behind other biomedical research fields.

This review does not attempt to offer an exhaustive elaboration of examples and progress in the integration of these new tools in the analysis of data collected through traditional surveillance approaches. Instead, it offers a glimpse into basic terms, principles, and current ML practices and their potential use within epidemiology and broader scope of veterinary medicine issues. Several comprehensive review articles delve deeper into AI and ML strategies in human medicine and data science. However, we recognised the need to explore the current status of integration of AI methods in the analysis of biological data in the animal domain, which might be very complex considering relations in the traditional epidemiological triangle of agent – host – environment. We were aware of the fact that certain levels of scepticism and ignorance exist yet in the scientific and professional community related to fast and progressive game-changing process we face today with AI and ML. This review is aimed at unpacking potential of ML methods in integration with veterinary medicine, and more specifically, with its data analysis discipline – epidemiology. We believe this can serve to research community in better understanding of the ongoing and future processes by exploring the transformative potential of ML in veterinary epidemiology. To prove this, the aim of this manuscript was to elaborate and highlight the advantages, challenges, and prospects of integrating advanced ML techniques into this field, offering a basic comparison with the most common traditional statistical methods.

Statistics and Machine Learning in the Domain of Epidemiological Researches

Frequentist inference is the predominant tool in epidemiological research. These methods, which might be complex and hardly understandable for non-statisticians, are grounded in hypothesis testing and probability calculations to support or refuse research thesis (Rothman et al., 2008). Common practices include basic statistical tests and multivariable regression models that are pivotal in identifying associations or treatment effects between variables. In epidemiology, traditional regression models serve dual functions: they are utilized either to predict a dependent variable from multiple independent variables (predictive models) or to measure the effect or association of specific independent variables on a dependent variable (explanatory models). These modeling strategies provide essential insights for both researchers and decision makers, and rely on several data assumptions such as linearity, absence of multicollinearity, and proportional risks/odds/hazards over time, familiar to epidemiologists (Vittinghoff et al., 2012). Data challenges stemming from inconsistencies, imbalances, or weak connections can impede model performance (Molenberghs and Kenward, 2007). To address these challenges, incorporating advanced data analysis has significant advantages, allowing the model to autonomously learn from the data's structure and features. This approach prioritizes predictive capabilities over explanatory or causal inference, showing potential for enhancing disease management strategies. ML is particularly suited for this role, offering distinct advantages in predictive accuracy (James et al., 2013).

ML is a subset of AI dedicated to developing algorithms that enable computers to glean

insights and make predictions or decisions from data. While ML and statistics are interconnected disciplines centered around data analysis, they diverge significantly in their methodologies, objectives, and focal points. ML aims to develop algorithms capable of identifying patterns and making informed predictions or decisions, emphasizing predictive precision and the ability to generalize. This contrasts with conventional programming paradigms, where tasks are defined by explicit instructions, and ML algorithms autonomously discern and refine patterns from data, adapting their performance iteratively to diverse scenarios (Hastie et al., 2009). Conversely, statistics is focused on the comprehension and interpretation of underlying data structures and relationships, primarily for inference, hypothesis testing, and parameter estimation (Casella and Berger, 2002). ML algorithms are designed to optimize performance across specific tasks such as classification, regression, clustering, or reinforcement learning, whereas statistical methodologies aim to draw population inferences from sample data and estimate probability distribution parameters. ML techniques emphasize algorithmic intricacy and scalability, facilitating the handling of diverse data types through feature engineering and preprocessing mechanisms, contrasting with statistics, which predominantly deals with structured datasets, drawing upon specific distributional assumptions, and emphasizing theoretical rigor and interpretive insights (Bishop, 2006).

Over the past decade, ML techniques have gathered considerable attention within biomedical research, although their integration into population health remains somewhat limited. ML methods provide a flexible alternative to traditional statistical approaches by handling complex, high-dimensional data and capturing nonlinear relationships prevalent in epidemiological studies. Unlike conventional regression models, ML models can adaptively learn from the data, proving invaluable when data structures are intricate or unknown. Despite their advantages, ML models also present several challenges. They often require large datasets to perform effectively, which may not always be available in epidemiological research, especially in the application of active data collection systems/surveillance with limited resources. Moreover, the interpretability of ML models can be problematic. Unlike traditional statistical methods that offer clear and interpretable coefficients, ML models can be perceived as "black boxes" that provide limited insights into the underlying mechanisms driving the predictions. This lack of transparency can hinder their acceptance in epidemiology, where understanding causality and mechanisms is crucial.

Nevertheless, the advantages of ML over traditional statistical methods in epidemiological research are substantial. ML excels in processing large volumes of data swiftly, a critical capability in a field where datasets can be extensive and heterogeneous. By automating feature extraction and model selection, ML reduces the manual effort and time typically required for complex data preprocessing and model building. This efficiency not only accelerates the pace of research but also conserves valuable resources, allowing epidemiologists to focus more on interpreting results and translating findings into actionable insights. Furthermore, ML's ability to handle nonlinear relationships and complex interactions among variables enhances its predictive accuracy compared to traditional regression models, offering promising avenues for improving disease management strategies and public health interventions. These advantages underscore ML's transformative potential in advancing epidemiological research beyond the constraints of traditional statistical methodologies.

Exploring the Triad of ML Methodologies

While exhibiting similarities, ML introduces terminology distinct from that commonly encountered in the realm of statistics. A knowledge of these unique terms is imperative for the effective utilization and interpretation of ML algorithms and models. As presented by Wiemken and Kelley (2019), the terms are explained in Table 1.

Table 1 Comparison of terminology between biostatistics/epidemiology and machine learning

Term in biostatistics and epidemiology	Term in Machine Learning	Explanation
Dependent variable	Label/class	Main factor of interest in a study
Independent variable	Feature	Factors that are thought to influence the dependent variable
Contingency table	Confusion matrix	Displays the counts of true positive, true negative, false positive, and false negative outcomes of a classification algorithm
Number of variables	Dimensions	Refers to the number of measurable properties or characteristics of each data point in a dataset (e.g. age, weight, breed, and vaccination status of an animal)
Sensitivity	Recall	Measures the proportion of true positives (animals correctly identified as having the disease) among all animals that actually have the disease
Positive predictive value	Precision	Measures the proportion of true positives among all positive test results
Outcome group with the highest frequency	Majority class	Refers to the most prevalent category within the dataset (e.g. the most common diagnostic test result observed in a population of animals)
Outcome group with the lowest frequency	Minority class	Refers to the least prevalent category within the dataset (e.g. rare condition or diseases that occur infrequently observed in a population of animals)
Proportion of cases in each category of the outcome variable (when outcome is categorical)	Class balance	Distribution of different categories or classes within the outcome variable.

ML encompasses three primary methodologies: supervised learning, unsupervised learning, and reinforcement learning. Each of these approaches has distinct methods for handling data and is tailored to achieve different objectives, yet they can be integrated to address complex datasets comprehensively. The compiled brief explanations and prominent examples of essential ML methods obtained from relevant scientific literature are provided in Table 2.

Table 2 Overview of supervised, unsupervised, and reinforcement learning techniques with corresponding algorithms and references.

Methodology	Explanation	Example algorithms	References
Supervised Learning	Involves training a model on a labeled dataset, where the input data is paired with the correct output. The goal is for the model to learn a mapping from inputs to outputs, allowing it to make predictions on new, unseen data.	Linear Regression, Support Vector Machines, Neural Networks, Random Forests	Goodfellow et al. (2016), Kotsiantis et al. (2007), Bishop (2006)
Unsupervised Learning	Involves training a model on data that has no labels or predefined outcomes. The goal is to discover underlying patterns or structures within the data.	K-means Clustering, Hierarchical Clustering, Principal Component Analysis, t-Distributed Stochastic Neighbor Embedding	Murphy (2012), Jain (2010), Hastie et al. (2009)
Reinforcement Learning	Involves training a model through interactions with an environment, where the model receives feedback in the form of rewards or penalties. The goal is to learn a policy that maximizes cumulative rewards over time.	Q-Learning, Deep Q-Networks, Policy Gradient Methods, Actor-Critic Methods	Sutton and Barto (2018), Mnih et al. (2015), Kaelbling et al. (1996)

Supervised learning is characterized by its use of labeled datasets, where each input is associated with a specific output. This method aims to learn the mapping from inputs to outputs, enabling the model to make accurate predictions on new, unseen data. Supervised learning is particularly effective for tasks that involve classification, where the objective is to categorize inputs into predefined classes, and regression, which focuses on predicting continuous outcomes. This approach is underpinned by a variety of algorithms, such as linear and logistic regression, support vector machines, neural networks, and random forests, each offering unique advantages in different scenarios (Bishop, 2006; Kotsiantis et al., 2007; Goodfellow et al., 2016). One of the earliest applications of supervised learning in the medical field was for the diagnosis of diseases. In the 1960s, researchers began using linear regression models to predict the presence of certain diseases based on patient data (Bishop, 2006). The development of neural networks in the 1980s further revolutionized medical diagnostics, enabling more accurate and complex pattern recognition (Albahra et al., 2023).

In epidemiology, supervised learning has been used to predict disease outbreaks and understand the spread of infectious diseases. One of the pioneering studies in this field used logistic regression to model the risk factors associated with the spread of malaria in populations (Kuang et al., 2009).

In contrast, unsupervised learning deals with data that lack labeled outputs, aiming to uncover hidden patterns, structures, or relationships within the data. This approach is instrumental for exploratory data analysis and is essential when the goal is to identify inherent groupings or reduce data dimensionality. Common applications include clustering, where similar data points are grouped together; dimensionality reduction, which simplifies data while retaining essential information; and anomaly detection, which identifies outliers within the data. Key algorithms in unsupervised learning include k-means clustering, hierarchical clustering, principal component analysis (PCA), and t-distributed stochastic neighbor embedding (t-SNE) (Hastie et al., 2009; Jain, 2010; Murphy, 2012). Unsupervised learning was first applied in the medical field to cluster patient data into meaningful groups. In the 1970s, clustering algorithms were used to group patients based on symptoms and laboratory results, aiding in the identification of disease subtypes (Jain, 2010). These early applications helped in understanding the heterogeneity of diseases and improving personalized medicine approaches. In epidemiology, unsupervised learning was first used to identify patterns in disease incidence data. For example, clustering algorithms were applied to group geographic regions based on the similarity of disease outbreak patterns, which helped in identifying hotspots and potential sources of outbreaks (De Silva, 2007). This approach has been essential in monitoring and controlling infectious diseases.

Reinforcement learning (RL) stands apart by focusing on learning through interaction with an environment. In RL, an agent learns to make decisions by performing actions and receiving feedback in the form of rewards or penalties. The primary goal is to develop a policy that maximizes cumulative rewards over time. This approach is highly applicable to scenarios that require sequential decision-making and adaptability, such as robotics, game playing, and autonomous systems. Notable algorithms in reinforcement learning include Q-learning, which focuses on learning the value of action-reward pairs; deep Q-networks, which utilize neural networks to approximate Q-values; policy gradient methods, which optimize the policy directly; and actor-critic methods, which combine value and policy learning to enhance performance (Kaelbling et al., 1996; Mnih et al., 2015; Sutton and Barto, 2018). RL has more recently been applied in the medical field, particularly in personalized treatment planning. In the 2009, researchers used RL algorithms to develop adaptive treatment strategies for blood glucose control in diabetic patients, optimizing the timing and dosage of interventions based on patient responses (Yasini et al., 2009). In epidemiology, RL has been explored to optimize public health interventions. One of the most

important applications was in the development of strategies for vaccination campaigns, where the goal was to determine the most effective allocation of limited vaccine supplies to minimize disease spread (Wei et al., 2021; Lu et al., 2023; Rey et al., 2023), which effectiveness was evident during COVID-19 pandemic (Beigi et al., 2021; Hao et al., 2022). Unfortunately, in the field of veterinary epidemiology no studies explored and utilized potential of RL on disease control strategies.

These three approaches to ML-supervised, unsupervised, and reinforcement learning — provide robust frameworks for analyzing and interpreting complex data. By leveraging the strengths of each method, researchers and practitioners can develop more effective and accurate models tailored to a wide range of applications, from predictive analytics to automated decision-making systems.

Use of ML in Veterinary Medicine

Significant applications of ML in veterinary medicine are already evident in diagnostic imaging, where ML algorithms, particularly deep learning models, have demonstrated considerable promises. These models analyze images from ultrasounds, MRIs, and X-rays to detect abnormalities such as tumors or fractures (Currie and Rohren, 2022; Tahghighi et al., 2023). In veterinary medicine, convolutional neural networks have been employed to diagnose conditions in pets and livestock with high accuracy, occasionally surpassing that of human experts (McEvoy, 2015). This application not only accelerates the diagnostic process but also enhances its accuracy, providing substantial support to veterinary professionals. Additionally, ML is utilized to monitor animal behavior and welfare. Sensors and cameras gather data on animal movements and behaviors, which ML algorithms analyze to detect signs of illness or stress. This application is particularly crucial in large-scale farming operations where manual monitoring is impractical. By providing continuous, automated monitoring, ML tools help ensure the well-being of large animal populations, highlighting unusual behaviors that might indicate health issues (Bidder et al., 2014).

ML emerged as a game-changing force in veterinary epidemiology, offering new tools to better understand zoonotic diseases spillover and enhance surveillance, detection response and recovery. The current applications of ML span a broad spectrum, from predictive modeling to pattern recognition, fundamentally altering how epidemiological data are analyzed and interpreted. Looking forward, ML is poised to further integrate into this field, addressing complex epidemiological challenges with increasingly sophisticated algorithms. At present, one of the primary applications of ML in veterinary epidemiology is in the development of predictive models. These models utilize historical data to forecast disease outbreaks, allowing for timely preventive measures. Supervised learning techniques, such as logistic regression and random forests, have been particularly effective in identifying the likelihood of disease occurrences based on various risk factors. For instance, models have been developed to enhance surveillance (Hepworth et al., 2012; Fountain-Jones et al., 2019; Bollig et al., 2020; Hyde et al., 2020; Zhang et al., 2021), aid in decision-making (Romero et al., 2020), and identify risk factors for animal diseases (Silva et al., 2019; Ghafoor and Sitkowska, 2021). These capabilities are crucial for allocating resources effectively and mitigating potential outbreaks before they become widespread.

The primary obstacle to the increased utilization of ML in veterinary epidemiology is likely the frequent occurrence of imbalanced class distributions in the data, where one class is significantly underrepresented compared to others. As it is often the case, especially in countries with diversity of production systems and lack of population data, the sample size, although considered representative of the population, is not deemed large enough to produce robust predictive models for diseases. These obstructions are extremely discouraging for researchers tackling rare diseases and/or small populations in building adequate predictive models for diseases. However, in the initial phases of applied ML, Weiss (2004) highlighted the challenge of imbalanced datasets, which can be classified into two categories:

relative rarity (where imbalance occurs within a large sample size) and absolute rarity (where the sample size is small and imbalanced). In many practical examples, a problem that commonly occurs concerning imbalanced datasets is that the cost of misclassifying a minority class example is significantly higher than that of misclassifying a majority class example. Since the development of control measures in epidemiology heavily relies on the use of statistical data calculations to construct disease models, it is crucial to develop classifiers that minimize overall misclassification costs, as stated by Weiss et al. (2007). This approach typically improves performance on the minority class compared to treating all misclassification costs equally. It also prevents the classifier from always predicting the majority class in highly imbalanced scenarios.

Several strategies are effective for handling skewed class distributions with unequal misclassification costs (He and Garcia, 2009). The most straightforward approach involves using a learning algorithm that inherently accounts for these costs when constructing the classifier (Weiss et al., 2007). Another approach for addressing skewed data with varying misclassification costs is to use sampling techniques to modify the class distribution of the training data. Two primary sampling methods can be employed for this purpose: oversampling and undersampling (Shelke et al., 2017; Mohammed et al., 2020). Oversampling involves replicating existent or creating new synthetic minority-class examples (Zheng et al., 2015), whereas undersampling entails removing examples from the majority class (Gosain and Sardana, 2017), thus eliminating potential bias due to misclassification (He and Garcia, 2009; Mohammed et al., 2020).

Among the oversampling techniques, Synthetic Minority Oversampling Technique (SMOTE) stands out as a particularly influential method for addressing imbalanced datasets in veterinary epidemiology. Introduced by Chawla et al. (2002), SMOTE generates synthetic samples from the minority class instead of creating exact copies. This method involves selecting examples that are close in the feature space, drawing a line between the minority samples in the space and generating new samples along that line. This approach helps create a more generalizable decision boundary for the minority class, effectively enhancing the performance of ML models (Chawla et al., 2002). In addition to SMOTE, advanced dimensionality reduction techniques are pivotal when dealing with high-dimensional data, which is typical in biological data relevant to veterinary epidemiology. Dimension reduction techniques like PCA and t-SNE are utilized to simplify the complexity of data while retaining its most significant features. PCA, for example, reduces the dimensionality of the data by transforming it into a new set of variables, which are linear combinations of the original variables and capture the maximum variance within the data (Jolliffe, 2002). t-SNE, on the other hand, is particularly well-suited for the visualization of high-dimensional datasets and can help identify clusters of similar data points, which is valuable for understanding the underlying structures of disease outbreaks (van der Maaten and Hinton, 2008). Another corrective method in the arsenal for tackling imbalanced datasets is the use of ensemble methods, such as Random Forests and Boosted Trees, which combine multiple weak classifiers to form a robust classifier. These methods can be particularly effective because they naturally handle imbalance by constructing multiple decision trees and aggregating their predictions, thereby providing a balanced perspective on the classes (Breiman, 2001; Schapire, 2003).

To implement these strategies effectively, it is essential for researchers in veterinary epidemiology to apply these techniques, but also rigorously validate their models. This includes cross-validation techniques and stratified sampling to ensure that the models are robust and perform well across different subsets of data (Kohavi, 1995). Moreover, ongoing research into novel ML techniques that can address the specific challenges of imbalanced data in veterinary contexts is crucial. Additionally, the construction of successful ML models can be significantly hindered by various data quality issues, commonly referred to as “noise”.

Noise in ML is defined as random or irrelevant data within a dataset that does not contribute to the pattern or structure the model is designed to learn (Gupta and Gupta, 2019). This can obscure the true relationships between features and the target variable, thereby complicating the model's ability to generalize effectively to new, unseen data (Saseendran et al., 2019). One prevalent source of noise is measurement errors, which occur due to inaccuracies or inconsistencies in data collection methods or instruments used in field studies or clinical environments. These errors can include everything from error-prone measurement tools to biological variability in samples, which can skew data and mislead analytical models (Greenland et al., 2016). Label noise also poses a significant challenge, particularly when diagnostic criteria are subjective or when multiple evaluators are involved without sufficient calibration. Incorrect or inconsistent labeling of training data can lead to misclassifications and reduce the overall effectiveness of predictive modeling (Garcia et al., 2015).

Furthermore, veterinary epidemiological datasets often include irrelevant features-data points that do not have a meaningful relationship with the target variable. This can happen due to the inclusion of extensive and diverse data sources, which may dilute the predictive power of the model (Van der Waal et al., 2017). Environmental factors introduce another layer of complexity: external conditions such as temperature or humidity at the time of sample collection can introduce variability that is not relevant to the study's objectives, but can affect the data collected (Weatherhead et al., 1998). Lastly, the inherent randomness in the data generation process, which is not captured by the model, contributes to noise. This includes biological variability among subjects or uncontrolled environmental influences during data collection, adding further uncertainty to the data analysis process (Louppe, 2014). The presence of such noise in the dataset can lead to several complications such as overfitting, where the model erroneously learns to capture the noise as if it were a true signal, thereby harming the model's performance on new data. It can also reduce the overall accuracy and increase the complexity of the model, resulting in higher computational costs and inefficient learning processes (Domingos, 2012).

To mitigate these issues, it is crucial for researchers in veterinary epidemiology to employ robust data preprocessing techniques to clean and normalize data, utilize feature selection algorithms to reduce dimensionality and discard irrelevant features, and apply advanced ML techniques that are inherently more resistant to noise, such as ensemble methods or regularized regression models (Kumar and Minz, 2014). These strategies help ensure that the predictive models developed are both accurate and robust, capable of providing reliable insights for disease management and control.

Future of ML

Looking to the future, the integration of ML in veterinary epidemiology is expected to expand further with the advancement of technologies such as natural language processing (NLP) and reinforced learning (RL). NLP can be utilized to shift through vast amounts of unstructured data from veterinary records, social media, and literature to identify disease trends and outbreaks. This capability could be particularly transformative in managing zoonotic diseases, where early detection and rapid response are critical (Clark et al., 2020).

RL, on the other hand, offers potential for optimizing decision-making processes in epidemiology. For example, RL could be used to develop dynamic vaccination strategies, adjusting in real-time based on feedback from ongoing disease surveillance data. This approach could maximize the effectiveness of interventions by tailoring them to the specifics of an outbreak scenario, thereby minimizing the spread and impact of diseases (Libin et al., 2021).

Despite these advances, the application of ML in veterinary epidemiology is not without challenges. Issues such as data quality, privacy concerns, and the need for robust computational infrastructure pose significant hurdles. Additionally, there is a

need for interdisciplinary collaboration between veterinarians, epidemiologists, and data scientists to ensure that ML models are effectively integrated into veterinary practices and that they address practical, real-world problems (Van der Waal et al., 2017).

In summary, based on our review and current understanding, we propose that the incorporation of ML into veterinary epidemiology heralds a potential paradigm shift. This integration could significantly improve disease surveillance, detection, and management throughout the value chain. By leveraging ML's predictive accuracy and ability to handle complex data, veterinary professionals can improve hazard recognition and detection, develop better "tailor made" preventive programs and improve health outcomes for animal populations and food safety and security trends. However, overcoming challenges such as data quality, privacy concerns, and interdisciplinary collaboration is crucial. Continued research and development in ML applications will undoubtedly pave the way for more robust and effective epidemiological practices, ultimately advancing veterinary science and animal health on a global scale.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept and Design – AM, NF; Supervision – NF; Literature review, analysis and interpretation of data - AM, NF; Writing and Critical review - AM, NF

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UNAPREĐENJE VETERINARSKE EPIDEMIOLOGIJE INTEGRACIJOM MAŠINSKOG UČENJA: TRENUTNO STANJE I PERSPEKTIVE

SAŽETAK

Integracija mašinskog učenja (MU) u veterinarsku epidemiologiju ima transformativni potencijal u polju analize podataka i menadžmenta, što predstavlja značajan zaokret u odnosu na tradicionalne statističke metode. U ovom Pregledu istražujemo rastuću ulogu MU sa naglaskom na njegov kapacitet u upravljanju složenim, višedimenzionalnim podacima i otkrivanju nelinearnih odnosa koji su od presudnog značaja u epidemiologiji. Ključne metodologije MU, uključujući *supervised, unsupervised* i *reinforcement learning* osiguravaju robustne okvire za kreiranje prediktivnih modela, obrasce prepoznavanja i procese donošenja odluka. Već se primjenjuju u veterinarskoj medicini u oblasti dijagnostičkog snimanja i monitoringu životinjskog ponašanja, pokazujući sposobnost MU da poveća dijagnostičku preciznost i ojača zdravstveni monitoring.

Uprkos ovakvom napredovanju, praktični izazovi uključuju neizbalansirane skupove podataka, probleme kvalitete podataka te potrebu za interdisciplinarnom suradnjom. Strategije poput *Synthetic Minority Oversampling Technique* i ansambl metode su od pomoći kod neuravnotežene distribucije klasa, dok robustne tehnike prethodne obrade ublažavaju podatkovne šumove. Budući razvoj u obradi prirodnog jezika i pojačanog učenja obećava daljnju integraciju, optimiziranje praćenja bolesti i interventne strategije.

Ovaj Pregled naglašava transformativni potencijal MU u veterinarskoj epidemiologiji promovirajući kontinuirano istraživanje i suradnju sa ciljem prevazilaženja postojećih problema. Iskorištavanjem mogućnosti MU, veterinari mogu unaprijediti predviđanje bolesti, razviti ciljane preventivne programe i u cjelosti unaprijediti zdravlje životinja i ispravnost hrane, dajući značajan doprinos razvoju veterinarske znanosti.

Ključne riječi: Kreiranje prediktivnih modela, praćenje bolesti, veterinarska medicina, vještačka inteligencija, zdravlje životinja

REVIEW ARTICLE

SUDDEN DEATH RELATED TO ACUTE IATROGENIC CONDITIONS IN DOMESTIC RUMINANTS AND HORSES: A REVIEW

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ABSTRACT

Drug poisoning in animals is generally due to adverse drug reactions (ADRs), drug-drug interactions (DDIs) or iatrogenesis. No drug is exempt from the risk of adverse reactions or side effects. Acute drug poisoning can lead to unexpected animals death, with anaphylaxis being the most common and harmful pathophysiological phenomenon among ADRs. Antibiotics are primarily involved, either through an individual effect or through interaction with another drug. Furthermore, self-medication, a widespread practice in veterinary medicine, amplifies the risk of iatrogenesis. ADRs, DDIs, and iatrogenesis are all potentially serious risks that can lead to sudden death (SD) in ruminants and horses. In such situations, forensic medicine is needed to determine the cause of death, and veterinary forensic analyses may be necessary in spite of the lack of toxicological drugs references in veterinary toxicology laboratories. Thus, it seems appropriate to consider iatrogenic factors as a hypothetical cause in the differential diagnosis of sudden death syndrome (SDS).

Keywords: Adverse drug reaction, drug-drug interactions, iatrogenesis, sudden death, ruminants, horse

INTRODUCTION

The complexity of veterinary pharmacology and toxicology lies in the multiplicity of animal species, as well as diversity among breed, age, sex, and pathophysiological state, all of which may react differently to certain drugs (Anadon, 2016). In addition, a lack of information about the safe and appropriate use of veterinary medicines is a major cause of erroneous and dangerous medication (Wallis et al., 2019; Van der Riet, 2021). Drug poisoning in animals usually occurs due to off-label use, incorrect dosage, negligence, accidental ingestion, contaminated drugs, serious adverse reactions, and dangerous drug interactions (Brown et al.,1988; Uppal, 2000; Siroka and Svobodova, 2013). Butself - medication practices remain the principal cause of iatrogenesis in veterinary practice (Ruiz, 2010; Fainzang, 2014; Sala et al., 2019). Sudden death is a complex multifactorial syndrome defined as the sudden unexpected death of an apparently healthy animal with no obvious clinical signs of disease. Published studies on SD in various species of ruminants is more extensive (Benchohra et al., 2024) than that conducted on equines, with the majority of it focused on racehorses (Navas de Solis et al 2018; Morales-Briceno, 2020). However, the literature does not include iatrogenic factors, which can directly cause certain acute deaths in horses or ruminants, among the wide range of SDS causes (Diab et al., 2017; Bennet and Parkin, 2022; Benchohra et al., 2024). The aim of this review is to highlight the importance of iatrogenic factors as potential causative agents of unexpected death in ruminants and horses and their consequent relevance to forensic investigations.

ADVERSE DRUG REACTIONS

Maddison (1992) defines ADR as any undesirable or unintended response by the animal to a drug administered to aid in the diagnosis, treatment, or prevention of disease, which may be related to the drug itself or the excipients contained in the formulation.

In practice, no drug is free from the risk of adverse or side effects, some of which can be life-threatening or even fatal (Fresnay, 2017; Zhao et al., 2023). Several groups of drugs are involved in producing adverse reactions in animals, and it is estimated that about 20% occurs in bovine (Uppal, 2000) and equine (Fresnay, 2017) (Table 1). Some drugs have a very narrow safety margin; consequently, a small variation in the administered dose or in the sensitivity of a particular animal can easily produce an adverse reaction (Keen and Levingston, 1983).

In bovine and equine practice, when several drugs are administered simultaneously, it is difficult to assess with certainty whether an adverse reaction is caused by one of those drugs or by a drug-drug interaction. According to Fresnay (2017), in horses, adverse reactions linked to antiparasitic and nervous system drugs, and vaccines are mostly mild ; whereas, for antibiotics and nonsteroidal anti-inflamatory drugs (NSAIDs), the majority of cases are severe. However, in other species, nervous system drugs and vaccines are generally responsible for relatively severe reactions (Fresnay, 2017).

Anaphylaxis

Anaphylaxis is the most harmful pathophysiological phenomenon among ADRs, leading to fatal outcomes (Siroka and Svobodova, 2013; Khusro et al., 2021). It is defined as a severe and potentially fatal systemic allergic reaction that occurs suddenly after contact with an allergen (Sampson et al., 2006). The anaphylactic reaction usually manifests within minutes after the administration of a sensitizing substance with which had previously been treated (Madisson, 1992), but sometimes it can be delayed for several hours (Liebhart and Panaszek, 2007). In animals, lethal anaphylaxis is characterized by severe airway constriction and/or intense hypotension leading to shock (Uppal, 2000).

The involvement of procaine penicillin in anaphylactic shock and acute death in horses has long been well known (Brown et al., 1988; Madisson, 1992). In some cases, the reaction to

procaine benzylpenicillin may be attributed to the procaine rather than the penicillin itself (Keen and Levingston, 1983); procaine toxicity may account for 90% of adverse events related to procaine penicillin use (Lopez et al., 2020). To sum up, reactions to procaine penicillin may be due to procaine toxicity, allergic reaction to penicillin itself, or an accidental intravenous administration (Madisson, 1992).

Omidi (2009) reports an anaphylactic reaction that occured immediately following an injection of penicillin-streptomycin into a Holstein-Friesian cow; similarly, penicillin induced fatal shock in calf (Tjalve, 1997). Sulfonamide administration causes hypersensitivity reactions and IgE-type anaphylactic reactions in horses, the clinical manifestations being hypotension and collapse (Khusro et al., 2021).

Oxytetracycline is an antibacterial drug widely used in ruminants, but it is not totally safe; a few cases of fatal anaphylactic shock have been reported following its administration in cattle (BVD, 1992). Enrofloxacin can also present serious risks, with one case reported of a cow collapsing following treatment with this antibiotic (Tjalve, 1997). Similarly, a case of SD was reported in a calf one minute after an injection of ceftiofur sodium, and a necropsy exam confirmed an anaphylactic shock (BVD, 1992).

In food animal practice, vitamin therapy is frequently used for fattening, therapeutic, or preventive purposes. However, anaphylactic shock has been linked to the injection of vitamin A, D, and E complexes (Uppal, 2000). There have also been reported a number of SD cases in cattle (BVD, 1992) and horses (Brown et al., 1988) after the administration of vitamin B12 plus minerals. Vaccination is not without risk, and in a small proportion of the vaccinated population, the possibility of a potentially fatal allergic reaction exists (Gershwin, 2018). In horses, 28% of post-vaccination responses have been considered important and manifest within 30 minutes of vaccination (Rougier and Laurentie, 2020). Anaphylactic vaccine reactions can cause acute lung edema, with the lungs being the primary site of lesions and collapse and death being the sequelae (Underwood et al., 2015). Tjalve (1997) reported the death of cows following administration of live vaccine of *Trichophyton verrucosum*.

Antibiotics

Antibacterials appear to be the most implicated therapeutic group for adverse reactions in cattle (Tjalve, 1997; Siroka and Svobodova, 2013). However, in horses, analgesics and antiparasitics are more involved in ADRs than antibiotics (Khusro et al., 2021). Procaine penicillin toxicity is low in equine practice, but ADRs can occur at any given time and in a variety of clinical settings (Lopez et al., 2020; Khusro et al., 2021).

Antibiotics, principally ionophores, are often added to ruminant food because of their anticoccidial effects (Plumlee, 2003) and their ability to improve the efficiency and performance of beef and dairy animals (Marques and Cooke, 2021). Their principal property is electrolyte imbalances and changes in K+, Na+, and especially Ca2+ concentrations in cells, leading to disturbances in muscle contractility (Siroka and Svobodova, 2013) and involving acute cardiac rhabdomyocyte degeneration and necrosis (Miller and Gal, 2017).

Excessive ingestion of monensin, maduramicin, and salinomycin causes intoxication in sheep and cows, with symptoms appearing a few hours later (Plumlee, 2003). Myocardial failure results from monensin's specific toxicity to the mitochondria of the heart (Keen and Levingston, 1983). Omidi et al. (2010) observed that calves died of intoxication due to a high dose of salinomycin added to the diet (70 g/kg of concentrate) within 10 hours of ingestion.

Derived from oxytetracycline, doxycycline is a semi-synthetic antibiotic with bacteriostatic activity, a broad spectrum of action, and a higher therapeutic efficacy than other tetracyclines (Turk et al. 2020). Side effects include renal damage, cardiomyopathy, and necrotic myopathy (Brihoum et al., 2011). Doxycycline overdose toxicity in calves has been documented by a number of authors, including cases of acute death (Chiers et al., 2004; Brihoum et al., 2010; Karapinar et al., 2019).

Tilmicosin phosphate is a macrolide antibiotic, approved for treating specific pneumonia in cattle and sheep. The cardiotoxicity of this drug is thought to be mostly due to calcium channel blockade (Lust et al., 2011). Papich (2016) states that tilmicosin administered intravenously in any species can cause acute death. Likewise, when injected by the intramuscular route into young lambs, tilmicosin can lead to death by heart failure. Lastly, sulfabromethazine administered intravenously to calves has been shown to induce ataxia and collapse (Uppal, 2000).

Antiparasitics

Levamizole is used both as an anthelmintic and as an immunomodulator, and it has a number of possible adverse and toxic effects. Levamisol acts on nicotinic acetylcholine receptors, leading to respiratory muscle paralysis and asphyxia, but neurotoxicity is the main clinical manifestation (Siroka and Svobodova, 2013). In young animals, whose blood-brain barrier is still developing, the drug can reach the brain and cause neurotoxicity (Keen and Levingston, 1983). Suffocation due to respiratory failure is the main cause of death in cases of lethal levamisole poisoning (Hsu, 1980). In sheep and heifers, clinical symptoms such as dyspnea, ptyalism, and oculopalpebral edema appear one hour after levamisole administration (Uppal, 2000). According to Müller and Dwyer (2016), levamisole treatment resulted in the death of calves 12 weeks old.

Ivermectin is a macrocyclic lactone antiparasitic drug commonly used in ruminants that is effective on both endoparasites and ectoparasites. This drug can enter the central nervous system and have adverse neurological effects due to the increased permeability of the blood-brain barrier (Siroka and Svobodova, 2013). Vermeulen et al. (2016) reported concerns about a fatal oral ivermectin intoxication in calves aged two to four weeks, probably intentional, with a dose eight times higher than the recommended therapeutic dose.

Closantel is a salicylanilide antihelmintic used against several developmental stages of bloodsucking nematodes and trematodes in ruminants and sheep oestrosis. It acts on parasites by interfering with the synthesis of adenosine triphosphate (ATP) by cellular oxidative phosphorylation which disrupts the liquid and ion transport mechanisms in the parasite's membranes and impairs parasite motility (Venkatesh et al., 2019). Ecco et al. (2006) reported accidental poisoning in kid goats weighing 14kg after administration of closantel 10% at a dose 8 to 10 times higher than the manufacturer's recommendation. However, it seems that young lambs were more sensitive to closantel toxicosis with lethal doses begin at two times the recommended dose (Rivero et al., 2015). Clinical orientation signs include nervous disturbances and blindness one to two days after administration of the drug (Ecco et al., 2006).

Nitroxinil is an anthelmintic belonging to the phenolic substitutes group, used for its fasciolicidal, nematodicidal, and estricidal properties. Nitroxinil toxicity occurs in various species, most often as a result of overdosing (Lovatt et al., 2014). A high incidence of lethality has been reported in kids and adult goats, occurring five hours after subcutaneous overdosing (Brito Junior et al., 2021). Frequently reported clinical signs of acute intoxication include respiratory distress, decubitus, and death (Lovatt et al., 2014; Brito Junior et al., 2021).

Vitamins and minerals

Selenium (Se) is a trace element that plays an important role in animal health and production (Mehdi and Dufrasne, 2016). Given the relatively narrow range between recommended feed concentrations and the toxicity limit, a minor mixing error can be responsible for a toxic or even fatal incident (Raisbeck, 2020). Manifestation of Se toxicity includes myocardial necrosis leading to severe pulmonary edema, severe respiratory distress, and death within hours (Amini et al., 2011; McKenzie and Al-Dissi, 2017). Sudden deaths of newborn kids (Amini et al., 2011) and young lambs (McKenzie and Al-Dissi, 2017) have been reported after parenteral Se therapy due toacute selenosis.

In cattle, one case of death was reported following an IM injection of vitamin E and Se by the owner; the other cows treated at the same time showed transient anxiety and dyspnea (BVD, 1992).

DRUG-DRUG INTERACTIONS

Drugs used in combination may interact rather than act independently, which is a major factor in DDIs (Table 1). Physical and/or chemical incompatibility between drugs is common (Hsu, 2008), which can lead to metabolic interactions when one drug inhibits the metabolism of another. The drug's toxicity is increased as its plasma concentration increases due to inhibit metabolism (Wang et al., 2021). Sudden death is a potential consequence of DDIs if phenylbutazone and other NSAIDs are combined (Uppal, 2000). Similarly, the combination of NSAIDs or corticosteroids with certain antibiotics could be harmful. Acute mortality in calves has been reported following treatment with flunexin meglumine and gentamicin sulfate (BVD, 1992), as well as infusion of prednisolone, which is a corticosteroid hormone, with chloramphenicol (Uppal, 2000). In addition to its own ADRs, levamisole can potentially interact with chloramphenicol, with fatal outcomes (Plumb, 2011). Local treatment of mastitis is the most common form of treatment for dairy cows and is commonly practiced by owners without the need for veterinary support. However, anaphylactic shock has been reported following the infusion of a preparation of colistin, streptomycin, and prednisolone for a cow suffering from mastitis (Uppal, 2000).

Table 1 Summary of ADRs and DDIs that may cause acute death in ruminants and horses, with clinical courses and toxicity-determining factors.

Drug	Animals	Route of administration	Clinical course	Toxicity-determining factor	Authors
Antibiotics					
Benzylpenicillin procaine	Cattle	IM	Anaphylactic shock and death.	Procaine toxicity, Accidental IV injection, Pulmonary embolisme.	Dechant (2021) Tjalve (1997) Keen and Levingston (1983)
	Horse	IM, IV	Seizures, collapse, anaphylactic shock, death within few minutes.	Accidental IV injection, A high dose at a single injection site.	Brown et al. (1988) Lopez et al. (2020) Khusro et al. (2021)
Potassium benzylpenicillin	Horse	IV	Allergic anaphylactic reaction.	Allergy to penicillin.	Tjalve (1997)
Penicillin-streptomycin	Cattle	IM	Severe dyspnea, incoordination, anxiety, salivation, lacrimation, rhinitis, and shock.	Allergy to penicillin.	Tjalve (1997) Omidi (2009)

Sulphabromethazine	Calf	IV	Ataxia and collapse.	Unspecified	Uppal (2000)
Trimethoprim-sulfadiazine	Horse	IV, PO	Hypersensitivity, collapse, and death in few minutes. Anaphylaxis, cardio-vascular collapse and cerebral infarction.	Hypersensitivity, collapse, and death in few minutes. Anaphylaxis, cardio-vascular collapse and cerebral infarction.	Tjalve (1997) Desjardins (2010) Khusro et al. (2021)
Trimethoprim	Horse	IV	Acute anaphylactic shock.	5.5 mg/kg dissolved in aqueous lactic acid (50 mg/mL)	Lopez et al. (2020)
Ionophores	All ruminants	In feed	Disturbances in muscle contractility, acute cardiac rhabdomyocyte degeneration and necrosis.	Overdosage	Keen and Levingston (1983) Siroka and Svobodova (2013) Miller and Gal (2017)
	Horse	In feed	Dyspnea, tachycardia, ataxia, myocardial failure and dead.	Off-label use Consumption of ruminant feed.	Hunchak et al. (2014) Keen and Levingston (1983)
Monensin	Horse	In feed	Severe colic, collapse, recumbency. Even death during exercise. Myocardial necrosis.	10 mg/kg Unspisified	Lopez et al. (2020) Desjardins (2010)
Salinomycin	Calf	PO	Death occurs in 10h.	Wrong dosage	Omidi et al. (2010)
Narasin	Horse	In feed	Restlessness, polyuria, dyspnea, sweating, progressive ataxia, recumbency and death.	Off-label use	Lopez et al. (2020)

Oxytetracycline	Cattle	Unknow	Anaphylactic shock.	Unspecified	BVD 1992
	Horse	IM	Fatal colitis.	Off-label use	Keen and Levingston (1983) Lopez et al. (2020)
		IV	Adverse cardiovascular effects and acute renal failure, hypotension and collapse.	Rapid IV administration (Propylene glycol and 2-pyrrolidone excipient).	Lopez et al. (2020) Desjardins (2010)
Doxycycline	Calf	IM	Cardiomyopathy, necrotic myopathy, and renal damages.	Overdose	Brihoum et al. (2011) Karapinar et al. (2019)
	Horse	PO IV	Agitation, anxiety, depression, tachycardia, tachypnea, hypertention, collapse and cardiac arrest.	0.18 to 10 mg/kg IV injection is not recommended	Lopez et al. (2020) Desjardins (2010)
Tilmicosin	All species, Young lamb	IV IM	Cardiotoxicity due to calcium channel blockade. Death by heart failure.	Particularly hypertoxic drug IV route is fatal	Lust et al. (2011) Christodoulopoulos (2009)
Ceftiofur sodium	Calf	SC	Anaphylactic shock, death 1 min after injection.	Unspecified	BVD (1992)
Antiparasitics					
Levamisole	Sheep, heifer	Unknow	Neurologic signs. Vomiting, increased salivation, ataxia, clonic convulsions, respiratory paralysis, and cardiovascular collapse.	Similar to nicotinic effects	Hsu (1980) Uppal (2000) Siroka and Svobodova (2013)
	Young animals	IM	Drugs may enter the brain to exert neurotoxic effects.	Unknow	Keen and Levingston (1983)

Levamisole + Oxyclosanide	Cattle	PO	Anaphylactic shock. Death within one houre from onset of clinical signs.	Unspecified	Jadhav et al. (2017)
Ivermectin	Goat kids, young lambs	PO	Nervous signs, blindness, and death.	8 to 10 times recommended dose. Two times the recommended dose in lambs.	Ecco et al. (2006) Siroka and Svobodova (2013) Rivero et al. (2015)
	Horse	SC, IM	Depression, mydriasis, ataxia, muscle fasciculation, and death may occur.	Overdose	Brown et al. (1988) Siroka and Svobodova (2013)
		IV	Anaphylactic shock and sudden death.	Off-label use	Lopez et al. (2020)
Moxidectin	Horse	Injection	Signs similar to ivermectintoxicity.	Overdose	Hunchak et al (2014)
Nitroxynil	Sheep, cattle, goat	PO, SC	Respiratory distress, recumbency and death.	Overdose	Lovatt et al. (2014) Brito Junior et al. (2021)
Diaminazen (babesicidal)	Cattle	IM	Sudden death.	Unknow	Tjalve (1997)
Imidocarb dipropionate (babesicidal)	Horse	IM	Salivation, diarrhea, abdominal pain, and death in some cases.	Anticholinesterase activity at therapeutic dose (2.4mg/kg)	Abutarbush et al. (2013)
Vitamins and minerals					
Selenium	Kids	PO	Cardiac faillur leading to severe respiratory distress.	Overdose	Amini et al. (2011)
	Young lamb	IM	Death within few hours.	1.2 mg/kg of BW	McKenzie and Al-Dissi (2017)
	Sheep	IM	Death may occur.	Overdose 0.2-0.5 mg/kg of BW	Daunoras (2012)
	Cattle	Unknow	Death may occur.	Overdose 1.2 mg/kg of BW	Reis et al. (2010)

Selenium + Vitamin E	Cattle	IM	Acute anaphylaxis.	Unspecified	BVD (1992)
Analgesics and anesthetics					
Droperidol-fentanyl (neuroleptanalgesia)	Ruminants Horse	Unknow	Bradycardia and respiratory depression within 10 min of injection.	Unspicified	Uppal (2000)
Xylazine	Heifer	IM	Vomiting and death.	Unspecified	Tjalve (1997)
Xylazine + Butorphanol + Tolazoline	Horse	IV IV slowly	Agitated, then agonal and death.	100 mg 5 mg 1.000 mg	Scofield et al. (2010)
Xylazine + Detomidine + Yohimbine	Horse	IV	Recumbency and death within 4 min.	600 mg 5 mg 25mg (Yohimbine is off-label use in horses)	Scofield et al. (2010)
Xylazine + Butorphanol +Yohimbine	Horse	IV	Death.	100 mg 5 mg Off-label use	Scofield et al. (2010)
Detomidine + Yohimbine Lidocaine (used as antiarrhythmic)	Horse Horse	IV Slow IV	Anxiety, tremors, seizure and collapse.	Overdose	Mitchel (2017)
Other drugs					
Dinoprost (cloprostenol)	Cow	IV, IM	Local reaction to injection, systemic illness and death.	Unspecified	Tjalve (1997) BVD (1992)
Furosemide (strong diuretic)	Horse	IM	Fatal arrhythmogenesis.	Unspecified Horses racing on furosemide are 62% more at risk of sudden death.	Bennet and Parkin (2022)

NSAIDs	Horse	Unknow	GI toxicity such as GI hemorrhage and ulceration, and fatal outcome.	Unspecified	Modi et al. (2012)
Clenbuterol (bronchodilator)	Horse	PO	Fatal outcome due to acute renal failure, rhabdomyolysis, and cardiomyopathy.	Overdose	Thompson et al. (2011)
Quinidine (antiarrhythmic)	Horse	IV	Exacerbation of heart failure, cardiovascular collapse and sudden death.	Unspecified	Navas de Solis (2020)
Magnesium Sulfate (antiarrhythmic)	Horse	IV diluted in 0.9% NaCl	Neuromuscular blockade with respiratory depression and cardiac arrest.	Overdose	Mitchel (2017)
Flecainide (antiarrhythmic)	Horse	IV	Sudden death after cardiac arrest.	Two consecutive infusions	Carstensen et al. (2018) Navas de Solis (2020)

SELF-MEDICATION AND IATROGENESIS

The use of medicines by individuals to treat self-recognized or self-diagnosed conditions or symptoms is known as self-medication (Ruiz, 2010). It represents a major public health problem due to the threat of ADRs, DDIs, disease masking, and increased morbidity (Baracaldo-Santamaría et al., 2022). This practice is growing among breeders and people unaware of the toxic risks of certain veterinary drugs, which are generally not subjected to any official control in developing countries (Kisaka and Tumwebaze, 2023). Also, in developed countries, self-medication is increasingly identified as the main iatrogenic threat in bovine medicine (Sala et al., 2019). The high incidence of drug poisoning in animals is attributed to off-label use, incorrect dosing, and inadvertent administration (Siroka and Svobodova, 2013). It is expected that drug intoxications constitute 10 to 30% of poisonings in animals, and some routine therapies carried out by farmers result in severe or fatal events (Siroka and Svobodova, 2013; Sala et al., 2019). In this regard, it is crucial to make a difference between iatrogenesis, which refers to damage induced to a patient through medical ignorance or negligence, and ADRs, which can be induced by almost any medication (Lopez et al., 2020).

Sala et al. (2019) found in their retrospective study that the most frequent problem was incorrect administration of medication in 43% of cases. This includes cases where drugs administration

does not comply with the instructions in the leaflet, particularly regarding dosage and route of administration, as well as cases where injections are carried out by untrained staff (Sala et al., 2019). Using a drug against the manufacturer's instructions can significantly increase its adverse effects. In fact, Morales-Briceno et al. (2018) report that hypersensitivity reactions caused by illicit drug use are the cause of 5% of horses' unexpected deaths.

Ramsay et al. (2005) reported unexpected sudden death two hours after intravenous injection of the vaccine. Similarly, tetracycline administered intravenously to cattle can cause a profound drop in blood pressure and serious cardiac disorders (Keen and Levingston, 1983). Also reported was the sudden death of a calf following an IV injection of sulbactam and ampicillin (BVD, 1992). Cases of convulsions and pulmonary embolism have been reported when procaine benzylpenicillin has been given intravenously (Keen and Levingston, 1983). Because of the risk of embolism, insoluble suspensions must not be given intravenously. Intravenous injection of cloprostenol has also been reported to be fatal in cows (BVD, 1992). Clenbuterol is a β(2)-adrenergic receptor agonist authorized for veterinary use as a bronchodilator and sometimes used in racehorses to improve breathing capacity. Fatal adverse events have been reported following a deliberate overdose of clenbuterol in two racehorses (Thompson et al., 2011).

Drug self-making is a fairly rare practice, which has very harmful consequences. Thus, Genetzky et al. (1994) reported the loss of calves after 30 minutes of intravenous treatment with spectinomycin prepared from a water-soluble powder. Furthermore, the risks associated with inappropriate use will be further complicated by the deliberate administration of drugs with an expired date (Lopez et al., 2020; Kisaka and Tumwebaze, 2023).

Drug toxicity or drug reactions can lead to the death of one or more animals. The situation becomes even more complicated if self-medication is denied, making the elucidation of the context of death even more problematic. The owner's attitude can be explained by feelings of remorse or guilt, or by a desire to conceal the treatment history for insurance purposes or for forensic concerns.

VETERINARY FORENSIC AND IATROGENIC THREATS

Veterinary forensics is a relatively new and important discipline, which is experiencing a significant increase in the application of forensic medicine to investigations centered on crimes against animals (Parry andStoll, 2019). Additionally, veterinary malpractice and insurance claims are among its growing areas of interest (Parry and Stoll, 2019). However, veterinary forensics could be necessary, especially in cases of unexpected animal deaths (Cooper and Cooper, 2008).

The fundamental principles of any veterinary forensic investigation must involve objectivity, meticulous recordkeeping, and maintaining the chain of evidence (Newbery and Munro, 2011); thus, toxic materials and drugs constitute evidence at a hypothetical crime scene (Parry and Stoll, 2019). However, drug-related deaths are difficult to prove at postmortem examination, particularly if the forensic pathologist has not been informed of the nature of the drugs administered prior to death (Elliot, 1975). Unfortunately, the reference range of toxic products established by veterinary toxicology laboratories is fairly limited and generally does not include any potentially toxic drugs. This list consists of heavy metals and minerals, certain rodenticides, plant toxins, and mycotoxins, which leads to perplexity with regard to drug intoxications, for which no reference value exists (Gwaltney-Brant, 2016).

CONCLUSION

The differences between domestic animal species, as well as variations in body weight, age, sex, and physiological and pathological conditions within the same species, are all factors responsible for

different reactions to certain drugs. However, self-medication is by far the greatest cause of drug off-label use, amplifying the risks of adverse reactions and making them potentially fatal. Furthermore, in veterinary practice and forensic medicine, the sudden loss of one or more animals as a result of medical treatment can interfere with the SDS. The situation is further complicated if the practitioner has not been informed of this medical act, whether intentionally or inadvertently. This review, therefore, demonstrates, on the one hand, that knowledge of the adverse effects of certain drugs intended for ruminants and horses, combined with analysis of clinical data, can provide answers to many cases of death whose cause is iatrogenic, and on the other hand, it provides a guiding tool for medico-legal investigations, where it may be an act of negligence, malpractice, or even deliberate intent to kill an animal using drugs. In light of this, it seems appropriate to consider iatrogenic factors as a hypothetical cause in the differential diagnosis of SDS.

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CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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IZNENADNA SMRT POVEZANA S AKUTNIM JATROGENIM STANJIMA KOD DOMAĆIH PREŽIVARA I KONJA: PREGLED

SAŽETAK

Trovanje lijekovima kod životinja generalno nastaje usljed neželjenih djelovanja (ADR), interakcija među lijekovima (DDI) ili jatrogenezom. Svaki lijek može imati rizik od neželjenh djelovanja ili nuspojava. Akutno trovanje lijekovima ili ADR može uzrokovati neočekivanu smrt životinje, s tim da anafilaksa predstavlja najštetniji patofiziološki fenomen. Uzročnici anafilakse su prvenstveno antibiotici, bilo pojedinačno ili interakcijom sa drugim lijekovima.

Nadalje, samoinicijativno davanje lijekova koje je široko rasprostranjena praksa u veterinarskoj medicini pojačava rizik jatrogeneze. ADR, DDI i jatrogeneza predstavljaju potencijalno ozbiljne rizike koji mogu izazvati iznenadnu smrt (SD) kod preživara i konja. U ovakvim situacijama je potrebna forenzična medicina kako bi se odredio uzrok smrti, a veterinarske forenzične analize mogu postati neophodne uprkos nepostojanju referentnih toksikoloških vrijednosti u veterinarskim toksikološkim laboratorijama. Na taj način se čini odgovarajućim razmotriti jatrogene faktore kao potencijalni uzrok u diferencijalnoj dijagnozi sindroma iznenadne smrti (SDS).

Ključne riječi: Interakcije među lijekovima, jatrogeneza, iznenadna smrt, konj, neželjeno djelovanje lijekova, preživari

RESEARCH ARTICLE

WHAT IS THE FUTURE OF CRNO LAKE ON TRESKAVICA MT. IN BOSNIA AND HERZEGOVINA?

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ABSTRACT

Crno jezero (1675 m a.s.l.) is an alpine lake on Treskavica Mt. in Bosnia and Herzegovina (BiH). We investigated the distribution and abundance of aquatic macrophytes in the lake after the standard BAS EN 15460:2009 and orthophotos that were taken by a drone at different heights (124, 80, 50, 20 m). Aquatic macrophytes were sampled on both the longitudinal and vertical profile of the lake, whereby the depth and temperature of water were measured by the Mares Quad dive computer. For the assessment of its ecological status, we used Macrophyte Nutrient Index for Ponds (M-NIP) and QGIS ver. 3.22.13. The most abundant macrophytes were: *Equisetum fluviatile, Potamogeton alpinus, P. perfoliatus and Carex rostrata*. By comparison of our results with the previous ones originating from 1954, we made a predictive model for the succession dynamics of macrophytic vegetation around the lake. In spite of that, calculated M-NIP classified the ecological status of Crno jezero as good. This research represents an original approach in the investigation of alpine lakes in BiH with the potential to be applied on a national scale as a contribution to global monitoring programs.

Keywords: Aquatic macrophytes, alpine lakes, Bosnia and Herzegovina, global changes, monitoring

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INTRODUCTION

Mountain ecosystems are proven sentinels of climate changes (Pepin et al. 2015; Zamora et al. 2016; Kang et al., 2019; Peñas et al., 2023), whereby the most apparent responses to changes in environmental parameters, such as acidification or nitrogen dynamics, are detectable in alpine lakes (Catalan et al., 1994; Camarero et al., 1995; Mosello et al., 2002; Beniston and Stoffel, 2013; Pulido et al., 2015; Peñas et al., 2023). However, the greatest ecological challenge for these delicate ecosystems is global warming (Schindler et al., 1996; Sommaruga-Wograth et al., 1997; Catalan et al., 2006; Trevisan et al., 2010; Pulido et al., 2015; Schmeller et al., 2018; Moser et al., 2019; Pastorino and Prearo, 2020; Gnjato et al., 2022; Lind et al., 2022). Since the rate of air warming rises with altitude (Pepin et al., 2015; Preston et al., 2016; Obertegger and Flaim, 2021), alpine lakes manifest steeper surface warming in comparison to the low-land lakes (Sadro et al., 2019; Moser et al., 2019; Obertegger and Flaim, 2021). This affects aquatic biota mainly through accentuated water stratification and periodicity of ice cover (Adrian et al., 2009; Moser et al., 2019; Obertegger and Flaim, 2021).

Due to their low accessibility, alpine lakes are less impacted by direct human activities (Kollmair et al., 2005; Peñas et al., 2023), which makes them a good reference point for monitoring of the ecological status of lentic ecosystems in general. Indirect human impact, however, relates mainly to the changes in biogeochemical cycles (Galloway et al., 2008; Kang et al., 2019) which alter their chemistry, nutrient supplies, and consequently, a bioproduction level (Saros et al., 2005; Vinnå et al., 2021; Oberteggerand Flaim, 2021; Gnjato et al., 2022). On the other hand, remote locations of alpine lakes led to poor knowledge of their biodiversity and ecological processes (Sommaruga, 2001; Tolotti et al., 2006). In Bosnia and Herzegovina (BiH) alpine lakes are many, but almost completely unexplored in terms of their macrophyte-based ecological status. By now, the objective of the research was the diversity of macrophytic communities or genera of special conservation interest (Milanović, 1954; Mišić, 1984; Redžić et al., 2013; Milanović et al., 2014). Our study aims to develop and test methods for monitoring of alpine lakes in BiH by selecting Lake Crno jezero as a case study.

MATERIALS AND METHODS

Lake Crno jezero is situated in a depression between the peaks Ljeljen (1974 m) and Ilijaš (1879 m) on Treskavica Mt. at an altitude of 1675 m a.s.l. (Fig. 1). The geological foundation here is mainly limestone, except for the lake's southern section where clastic formations - quartz sandstones occur (Hrvatović, 2006). The ratio between the mean and maximum depth for Crno jezero is 0.32, which is the threshold value between the funnel and convex-shaped basin of the lake. Indeed, the lake is funnel-shaped along its W-S-N axis and convex in the E, which has its consequences in terms of siltation dynamics, substrate type, water depth, and vegetation. Around the lake occurs rendzine, calcomelanosol, and ilimerized types of soil (Ćirić, 1980). According to some authors (Cvijić, 1900; Milojević, 1934; Bušatlija, 1977), this lake is glacial by its origin. However, Spahić (1984) stated the hypothesis that the origin of the lake was polygenetic. The lake is fed by precipitation and springs which are under the peaks Ljeljen and Ilijaš.

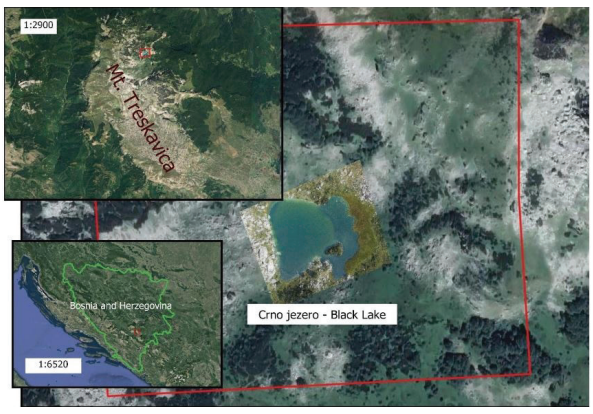

Figure 1 Physico-geographical position of Crno jezero in Bosnia and Herzegovina.

The climate at the lake is alpine with precipitation maximum in November and December (Fig. 2). Due to large quantities of snow in winter and low temperatures in general, the snow cover lasts until May, and the water level of the lake oscillates about 30 cm (Spahić, 1984). According to historical records, the depth of the lake has dropped from 5.3 m (Protić, 1926) to 3.7 m (Spahić, 1984). The succession of open water into the wetland, running from the East to the West, was noted already decades ago (Spahić, 1984).

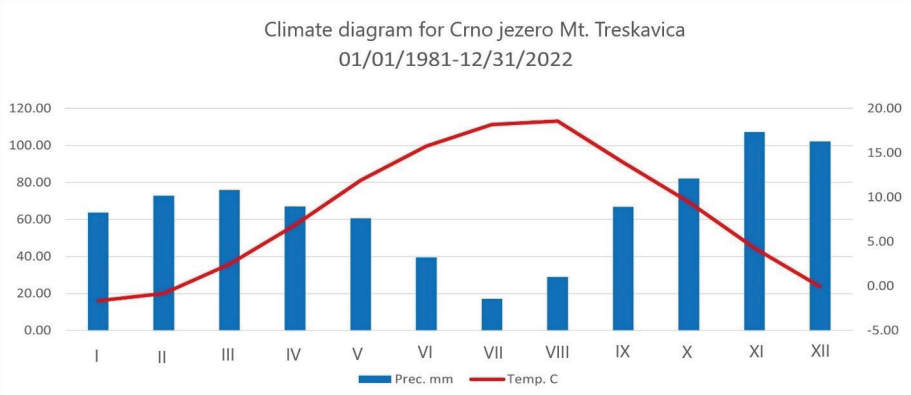

Figure 2 Climate diagram for Treskavica Mt.

The fieldwork was carried out in 2022 (October) and 2023 (June and August). Sampling, analysis, and processing of macrophytes was conducted according to the standards BAS EN 15460: 2009. The sampling of macrophytes was based on the obtained orthophotos of the lake that showed distinct vegetation belts. The abundance of submersed macrophytes was calculated based on orthophotos that were taken at 124, 80, 50, and 20 m by angle of -90 □MP 1 /2.0 FOV 84 F 2 8. The high-altitude diving was performed to delineate the thermal gradient of the lake using the Mares Quad dive computer. All methods were modified and adjusted to UAVs (Moreno, 2022). The ecological status of the lake was assessed according to The Macrophyte Nutrient Index for Ponds (Sager and Lachavanne, 2009). For spatial analysis, we used QGIS ver. 3. 2213 within the coordinate reference system ED50 / UTM zone 34N. The bathymetric map (Spahić, 2011) was overlaid with obtained orthophotos and processed in QGIS, with depths that were measured during diving and integrated into a digitized map. We used the available data from the literature and descriptive statistics to calculate standard deviation (σ) and Confidence Interval (CI) using the formulas: $\sigma = \sum\sqrt{(X-\bar{X})/N}$; $CI = \bar{X} \pm Z \times (\sigma/\sqrt{N})$, Z value fit CL 95% = 1.960. For the prediction of sedimentation dynamics, we used the linear function $Y = mX + b$ where the annual sedimentation rate was m-coefficient to the independent variable, and gross sedimentation was the dependent variable Y.

RESULTS

The temperature of the upper layers of water on a sunny summer day was +19°C. The reaction of the water in the lake was alkaline (pH = 8.7), electro-conductivity amounted 129.3 μS, and transparency of the lake was fully up to 3.7 m. However, macrophytes occurrence coincide with the thermocline at 3.2 m depth (+14°C). Here is noteworthy Lake Trnovačko in Montenegro (1517 m a.s.l.), which at 4 m depth had a temperature of +17.5°C (Blaženčić & Blaženčić, 2005), and the bottom was covered with Chara carpets. The fact that temperature is a limiting factor for macrophytes growth in shallow and fully transparent lakes was noticed earlier (Dale, 1986; Søndergaard et al., 2013).

According to the results, the most abundant macrophytes in Lake Crno jezero were *Equisetum fluviatile* L., *Potamogeton alpinus Hegetschw., P. perfoliatus* L. and *Carexrostrata*, which coincides with a list of the most common species in Dinaric lakes (Blaženčić & Blaženčić, 2005). In the riparian zone of Crno jezero occur: *C. goodenowü* Gay, *C. paniculata* L., *C. muricata* L., *C. flava* L., *C. oederi* Retz, *C. vesicaria* L., *Juncus articulatus* L., *J. alpinoarticulatus* Chaix, *Alchemilla xanthochlora* Rothm., *Geumrivale* L., *Filipendula ulmaria* (L.) Maxim., *Dactylorhiza cordigera* subsp. *bosniaca* Beck Soó and *Mentha longifolia* (L.) L. (Fig. 3).

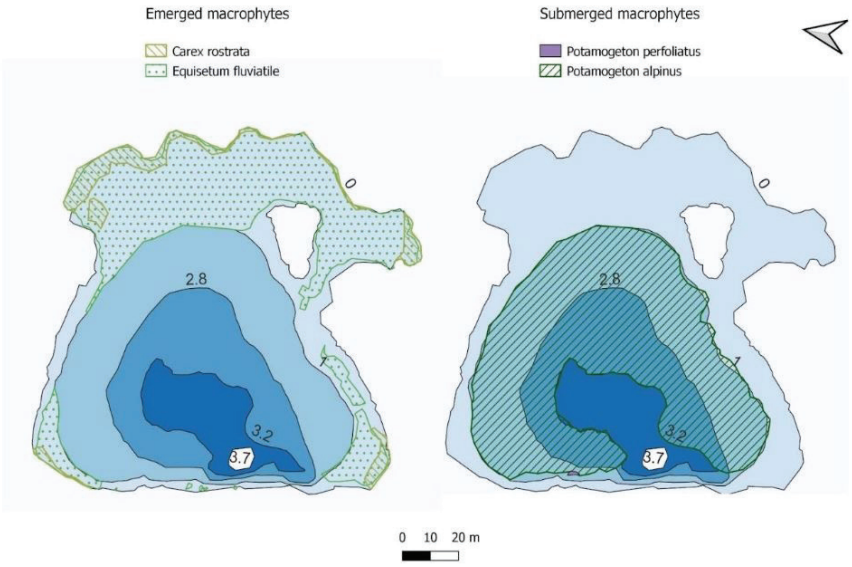

Figure 3
Distribution of macrophytes in Crno jezero

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DISCUSSION AND CONCLUSIONS

This kind of low species richness was found to be common for oligotrophic alpine lakes in the Dinarides with rather specialized species and aquatic plant communities (Radulović et al., 2010). Vegetation belts in and around the lake are clearly distinguished from each other and follow the ecological gradient of the water level and lake temperature (Fig. 4).

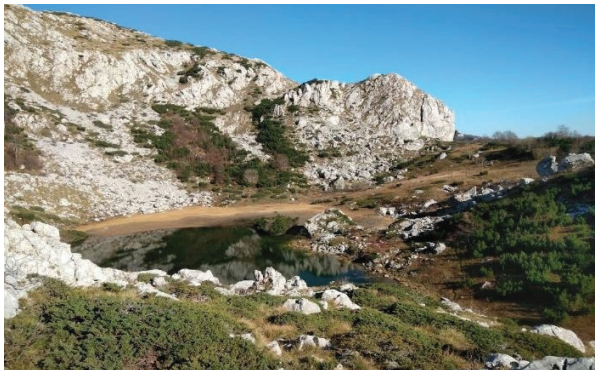

Figure 4 Lake Crno jezero in autumn (2022)

In 1954, Milanović reported that the Equisetum belt was 7 to 8 m wide, whereas today, according to our results, it has expanded to 19 m. The succession runs from the most shallow and convex section of the lake, in the East and continues to the West (Figure 5). In the SW, this process has led to formation of small peat bog fragments. The observed changes in the surface of the lake affect the ratio between volume and surface (Hutchinson, 1957; Obertegger and Flaim, 2021) which plays a crucial role in the dynamics of the warming (Poff, 2002; Lacoul and Freedman, 2006a; Lind *et al*., 2022).

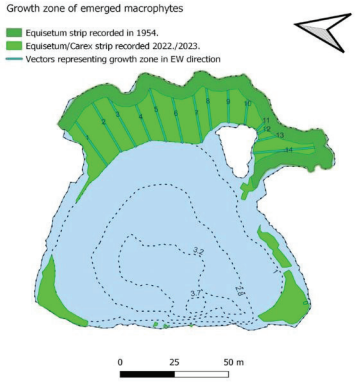

Figure 5 Widening of *Equisetum* belt through time

Studies have shown that the distribution of macrophytes is directly affected by lake bathymetry, due to the availability of light and the extent of littoral which is more prone to colonization of emergent plants (Horppila and Nurminen, 2003; Boon et al., 2019). Since emergent macrophytes increase the siltation rate and temperature in the shallows, the positive feedback loop establishes, and expansion of the emergent vegetation accelerates.

The sedimentation rate in alpine lakes is affected by parental rocks, climate, size and ecological status of the lake, bioproduction, catchment size and anthropogenic impacts. It varies between 100 µm and 23 mm per year (Anderson et al., 2011; Arnaud et al., 2016; Miller, 2023). In alpine lakes at Triglav Mt. (Slovenia), which is most similar to Crno jezero and for which we have available results, sedimentation rate was 0.45 ± 0.11 cm/year (Šmuc et al., 2013). This kind of sedimentation rate in Crno jezero would take 42.26 ± 14.80 (±35.0%) (p=0.05, confidence level 95 %) years for equalization/leveling of current isobaths 1 and 2.8 to 1 m. For isobaths 2.8 - 3.2 m, we expect this process to take 51.65 ± 18.09 (±35.0%) (p=0.05) years, and for isobaths 3.2 - 3.7 it would take 63.39 ± 22.20 (±35.0%) (p=0.05) years. Increasing the zone of the lake that is 1 meter deep or less, is

a prerequisite for the spread of Equisetum zone. Current Equisetum zone (from 0-1 m depth) would probably convert to peat bog in the next 30.52 ±10.68 (CI 35 %) (p=0.05).

Moreover, we have analyzed changes of maximum depth of Crno jezero that can be traced back to 1926 when it was measured 5.3 m (Protić), while in 1984 it was measured 3.7 m (Spahić). In 2023, however, we measured a maximum depth to be 3.7 m. Since in the period between the last two measurements bathymetry of the lake has not been changed, we state the hypothesis that geological events in the past might have led to lake's perimeter reduction and expansion of an emergent macrophyte belt. According to Spahić (1984), the basin of Crno jezero is encircled by two faults. First one runs from the peak Veliki Lopoč (1776 m) to the SE shoreline of Crno jezero, while the second one runs from the peak Oblik (1877 m) to its SW shoreline.

It is noted that the earthquakes, landslides or avalanches can cause strong and sudden sediment input in small lake basins (Wilhelm et al., 2016; Brisset et al., 2017; Fouinat et al., 2018; Rapuc et al., 2018; Rapuc et al., 2022), or change its hydrological discharge without additional sedimentation. In the local language, Treskavica Mt. stands for the "shaking mountain" due to a high seismic activity in the area. As a matter of fact, this area represents one of nine seismogenic zones identified in Bosnia and Herzegovina (Ćatić, 2023). In 1962, an earthquake struck this area with a magnitude Mw= 5.9 and a focal depth at 15 km (Isik et al., 2022). We strongly believe that this kind of event might have created cracks in the parental rocks or fault sliding and lowered the water table of Crno jezero. We hypothesize, therefore, that the bathymetric changes noted in the period 1926-1984 were rather the result of a tectonic activity than of the sedimentation process.

However, according to the macrophytes assemblage, current ecological status of Crno jezero is assessed to be GOOD (Table 1).

Table 1 Assessment of ecological status for Crno jezero

Species	Indicator value	Weighting factor	Q - plant quantity index	IV*W*Q
Carex rostrata	1.79	1	8	14.32
Equisetum fluviatile	1.96	1	64	125.44
Potamogeton perfoliatus	0.67	8	1	5.36
Potamogeton alpinus	2.25	1	64	144
M-NIP Index		Class		EQR
2.01		II - Mesotrophic		0.34 (GOOD)

Small mountain lakes without a direct anthropogenic impact offer the possibility of the more reliable conclusions and understanding of changes in the ecosystem (EC, 2000; EEB, 2014), but the ongoing climate changes on a global scale might jeopardize their natural structure and processes in many ways. For example, increased temperature of water can "open the door" for the introduction of alien species (Holzapfel and Vinebrooke, 2005; Obertegger and Flaim, 2021). Such was the case with the previously non-vegetated and remote Himalayan lakes that were lately invaded by Ranunculus trichophyllus (Lacoul and Freedman, 2006b; Lind et al., 2022). Radulović et al. (2010) have shown that the natural climate stressors in alpine lakes can either select or deselect particular species. Hellmann et al. (2008) believe that a climate change will challenge the definition of invasive species itself, because some previously non-invasive species might become invasive (Lind et al., 2022). These findings were confirmed by our study, for we have, for the first

time, recorded Potamogeton perfoliatus in Crno jezero (NW section) and associated its occurrence with a couple of ducks which have most probably introduced it from the 0.76 km distant Veliko jezero (Great Lake). Here, it co-dominates with P. alpinus in the middle of the lake.

Currently, overgrowth of the littoral zone in Crno jezero by Equisetum belt has reached 1-meter isobath, which represents 11 m widening compared to 1954. However, there is no data on its depth in 1954, making it impossible to predict the succession trend for the entire lake, but indicating that continuous monitoring in future is required.

Furthermore, our study represents the initial step toward incorporation of the small alpine lakes into the national legislation of Bosnia and Herzegovina in terms of the assessment of ecological status. This is a critical moment, considering the fact how fast the climate changes take their toll and how little we know about the biodiversity supported by the alpine lakes in the Dinarides.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept and Design: MČ, ST; Supervision:MČ, ST, MG; Fundings: MČ; Materials: MČ, ST, MG; Data collectionand Processing:MČ, ST, ESM, MG; Analysis and Interpretation: MČ, ST, ESM, AH; Literature review: MČ, ST, ESM; Writing and Critical review: MČ, ST, ESM, AH.

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KAKVA JE BUDUĆNOST CRNOG JEZERA NA PLANINI TRESKAVICI U BOSNI I HERCEGOVINI?

SAŽETAK

Crno jezero (1675 m) je alpsko jezero na planini Treskavici u Bosni i Hercegovini (BiH). U radu je analizirana distribucija i abundanca akvatičnih makrofita u Crnom jezeru prema standardu BAS EN 15460:2009 te na osnovu orto-fotografija snimljenih pomoću drona pri različitim visinama (124, 80, 50, 20 m). Uzorkovanje akvatičnih makrofita u jezeru je realizirano na longitudinalnom i vertikalnom transektu, prilikom čega je, pomoću ronilačkog uređaja Mares Quad, izmjerena dubina i temperatura vode. Ekološki status Crnog jezera je procjenjen na osnovu indeksa - Macrophyte Nutrient Index for Ponds (M-NIP) i primjenom QGIS ver. 3.22.13. Najzastupljenije vrste akvatičnih makrofita u jezeru su: *Equisetum fluviatile, Potamogeton alpinus, P. perfoliatus* and *Carex rostrata*. Usporedbom naših sa rezultatima ranijih istraživanja iz 1954. godine, izradili smo prediktivni model sukcesijske dinamike za makrofitsku vegetaciju oko Crnog jezera. Uprkos tome, izračunata vrijednosti M-NIP indeksa klasificira Crno jezero kao jezero dobrog ekološkog statusa. Prikazano istraživanje predstavlja originalni multidisciplinarni pristup istraživanjima alpskih jezera BiH sa potencijalnom mogućnošću primjene na nacionalnom nivou, a kao doprinos programima globalnog monitoringa.

Ključne riječi: Monitoring, globalne promjene, vodeni makrofiti, alpska jezera, Bosna i Hercegovina

RESEARCH ARTICLE

SUBCLINICAL MASTITIS OF LACTATING SAHEL GOATS FROM URBAN SMALLHOLDER HERDS IN MAIDUGURI, NIGERIA: PREVALENCE AND INTRA-MAMMARY INFECTIONS

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ABSTRACT

Risk of chronic clinical mastitis could be increased by insidious subclinical mastitis (SCM) in urban smallholder goat herds. This study was a cross-sectional survey of apparently healthy lactating Sahel goats (February-March, 2023) for prevalence of SCM using California mastitis test (CMT) and bacterial culture of raw milk in Maiduguri, Nigeria. Positive CMT was graded as +1 (mild), +2 (moderate), or +3 (severe). Standard procedures for cultural isolation and identification of bacteria were followed. Prevalence of SCM was 38% (95% CI, 26.9-52.2) from 100 goats, with the parity-specific prevalence (18.7-75.0%) decreasing ($p < 0.05$) from 50.0% at 1st parity to 18.7% at 3rd parity and increasing ($p < 0.05$) to 75.0% at 6th parity. Parity was not significantly ($P > 0.05$) associated with the prevalence. Most of the cases (71.1%, 27/38) were mild. The CMT diagnosis was significantly ($p < 0.0001$) associated with bacterial isolation from the milk samples. Isolation rate was 92.1% (35/38) in SCM cases, but CMT negative milk samples had isolation rate of 4.8% (3/62). *Staphylococcus spp* and *Streptococcus spp* were isolated in pure culture in 26.3% and 10.5% of 100 samples, respectively. Both *Staphylococcus spp* and *Streptococcus spp* were isolated together in 63.2% of the samples. Out of 34 isolates of Staphylococcus spp, 24 (70.6%) were coagulase-positive. Staphylococci (89.5%, 34/38) and streptococci (73.7%, 28/38) isolation rates did not differ ($\chi2 = 3.118$; $p < 0.077$). Therefore, prevalent population of SCM cases among healthy lactating Sahel goats with intra-mammary infections should be controlled to prevent escalation to clinical mastitis.

Keywords: Subclinical mastitis, Sahel goat, *Streptococcus*, *Staphylococcus*, prevalence

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INTRODUCTION

Goats in Nigeria have a daily milk yield of 0.4-1.3 kg during a lactation period of 100-126 days (Akpa et al 2002), and improved goat milk production has been advocated (Egwu et al 1995), but the incidence of mastitis is among diseases limiting this goal (Abba et al 2013, 2014). Clinical mastitis affects about 8-17% of goats (Ameh et al 1993; Ameh and Tari, 1999; Danmallam and Pimenov, 2019) where clinical signs were identified with abnormalities in the produced milk. However, subclinical mastitis is usually not associated with visible abnormality in the udder or milk, but milk production could decrease, and raw milk secreted contains elevated number of somatic cells (Koop et al 2012). The diagnostic approach to identifying SCM involves somatic cell count in milk and bacterial culture of the milk, but increased somatic cell count may not be accompanied by bacterial culture. Therefore, somatic cell count and bacterial culture are done together for effective monitoring of SCM in goats (Koop et al, 2012). California mastitis test (CMT) is a screening test which detects the amount of deoxyribonucleic acid (DNA) in milk, correlating with the quantity of somatic cells in the milk, and could predict somatic cell count (Persson and Olofsson, 2011; Souza et al, 2012), which is helpful in detecting SCM with intra-mammary infection (McDougall et al, 2010).

In northern Nigeria, SCM of Sahelian goats was previously detected solely by bacterial culture of raw milk from apparently healthy udders, and the prevalence was 35-47% (Ameh et al, 1993; Ameh and Tari, 1999; Alawa et al, 2000); but CMT was used recently in the diagnosis of caprine SCM, and the prevalence was 23-60% in Kaduna, Bauchi and Plateau States (Udo et al, 2019; Danmallam and Pimenov, 2019). The reduction in milk production could lead to malnutrition of the kids causing poor growth and kid mortality due to starvation (Fthenakis and Jones, 1990). The mortality of goat kids was reported to be 41.4% and associated with diarrhea and starvation (Ameh et al., 2000). The infected milk could also be a source of infection to the kids resulting in gastroenteritis and diarrhea with subsequent mortality. The prevalence of SCM appears to be higher than clinical mastitis (Koop et al, 2012) and could persist in the herd without regular monitoring and adoption of preventive and control strategies. In Maiduguri (Borno State), Nigeria, prospective survey of SCM in lactating Sahel goats has not been reported recently and the surveillance seems to be inactive for the epidemiological monitoring and control of the disease. The objective of the observational study was to conduct cross-sectional survey of apparently healthy lactating Sahel goats for prevalence of SCM using CMT and bacterial culture of raw milk.

MATERIALS AND METHODS

Study design

The study was a non-randomized cross-sectional survey of apparently healthy lactating Sahel goats in smallholder farms in an urban area with the purpose of diagnosing SCM using CMT and bacterial culture of raw milk samples collected from clinically healthy mammary glands (udders) without gross evidence of inflammation. In order to exclude clinical mastitis during the survey, the selected goat was expected to have normal vital parameters and no abnormality after physical examination of the body and udder, and milk was considered normal when it had no gross abnormality in color, odor or consistency.

Selection of Sahel goats

The Sahel goats were selected by convenience sampling and snowball technique from 28 smallholder goat herds located within the township environment of Maiduguri (11.83^{o}N, 13.15^{o}E), Nigeria, during the months of February-March, 2023. The herds were housed within fenced premises and managed semi-intensively. The goats were fed cowpea husks, groundnut hay, sorghum and wheat offal, and offered water in their pens, but allowed to move around the vicinity of the homestead to browse on any available feedstuff (Figure 1). Physical examination was conducted to ascertain health status. The goats were aged by

dental examination (2.0-5.0 years), and were not weighed, but were estimated to be in good body condition. Rectal temperature was measured with digital thermometer and confirmed to be normal (37.5-39.0° C). The history of kidding was taken to find out parity and number of kids per kidding. Each doe had a recent kidding of 1-3 kids and was lactating.

Figure 1 An urban herd of Sahel goats in Maiduguri, Nigeria

Milk sample collection

Udder of the doe was disinfected using 70% alcohol and 2 - 3 squirts of milk were manually milked off and discarded, after which another 2-3 squirts were collected in a gloved hand to check the physical quality of the milk in terms of the color, consistency and odor. The raw milk was collected aseptically for CMT and bacterial culture in a sterile vial, and transported to the laboratory on a cold-chain facility.

California mastitis test (CMT)

Qualitative estimation of somatic cells infiltrated into the milk was determined using CMT (Leach et al, 2008). Briefly, the milk was collected into the paddle and mixed with equal amount of CMT liquid (BOVI-VET, KRUUSE, Denmark) by gentle movement of the hand. There was no gel formation in negative test, but positive (+1 [mild], +2 [moderate], +3 [severe]) tests were based on the intensity of gel formation. The paddle was washed and the procedure was repeated with another collected milk sample.

Cultural isolation and identification of bacteria from milk

Milk sample was inoculated by direct streaking onto 5% sheep blood agar, mannitol salt agar, and eosin methylene blue agar and incubated at 37 °C for 48 hr for the growth of bacterial colonies. The morphological characteristics of bacterial growth and hemolytic effect, the nature of Gram staining, and coagulase and catalase tests guided the identification of the bacterial isolate (NMC, 2004; Quinn, 2011).

Statistical analysis

The data were summarized descriptively as counted numbers and their proportions of the population tested. The overall prevalence was calculated as the proportion of the population testing CMT positive in either half or both halves of the udder (goat level), and the 95% confidence interval (CI) for the prevalence was calculated. The parity-specific prevalence was calculated as the positive proportion at each parity level; the comparison of parity prevalence (1-6 parity) was done with one-way chi-squared test. The two-way chi-squared test for trend was calculated by entering CMT negative and positive categories in one classification and parity in the second classification to determine relationship between parity and prevalence. Two-sided chi-squared with Yates correction was carried out to determine the association of CMT positive tests with bacterial isolation. All calculations and inferences were done with statistical software (www.medcalc.org/calc/ version 22.017; accessed February 2, 2024 or GraphPad InStat, GraphPad Software Inc., CA., USA, www.graphpad.com).

RESULTS

The overall prevalence of SCM, based on CMT, in Sahel goat population (n =100) was 38% (95% CI, 26.9-52.2%), with the parity-specific prevalence (18.7-75.0%) decreasing ($p < 0.05$) from 50.0% at 1st parity to 18.7% at 3rd parity and increasing ($p < 0.05$) to 75.0% at 6th parity (Table 1). The SCM was not observed at 7-9th parity. Parity was not significantly ($P > 0.05$) associated with the prevalence. Most of the cases of SCM (71.1%, 27/38) were mild, while moderate and severe SCM occurred in 18.4% (7/38) and 10.5% (4/38) of the prevalent cases, respectively. The CMT diagnosis of SCM was significantly ($p < 0.0001$) associated with the isolation of intra-mammary bacteria from the milk samples, with positive and negative predictive values of 92.1% and 95.2%, respectively (Table 2). The isolation rate was 92.1% (35/38) in CMT positive SCM cases; whereas CMT negative milk samples had isolation rate of 4.8% (3/62) of staphylococci. *Staphylococcus spp* and *Streptococcus spp* were isolated in pure culture in 26.3% and 10.5% of 100 samples, respectively. Both *Staphylococcus spp* and *Streptococcus spp* were isolated together in other 63.2% of the samples. Out of the 34 isolates of Staphylococcus spp, 24 (70.6%) were Coagulase-positive and identified phenotypically as S. aureus. The frequency of isolation of staphylococci (89.5%, 34/38) and streptococci (73.7%, 28/38) in SCM cases did not differ ($\chi 2 = 3.118$; $p < 0.077$). There was no *E. coli* isolation in any sample.

Table 1 Prevalence of subclinical mastitis in lactating goats based on California mastitis test

		Positive				
Parity	Negative, N	+1	+2	+3	N (%)	Total (N)
1	9	4	2	3	9 (50.0)*	18
2	25	17	2	0	19 (43.2)	44
3	13	2	1	0	3 (18.7)	16
4	4	0	0	1	1 (20.0)	5
5	5	1	2	0	3 (37.5)	8
6	1	3	0	0	3 (75.0)	4
7	2	0	0	0	0	2
8	2	0	0	0	0	2
9	1	0	0	0	0	1
	62	27	7	4	38 (38, 26.9-52.2)	100

N, number of goats; Prevalence (%) in parenthesis

*Significant ($p < 0.0001$) variation in parity-specific prevalence (one-way chi-squared)

Table 2 Association of subclinical mastitis diagnosed by California mastitis test (CMT) with bacterial isolation from milk samples

	Isolate	No isolate	Total
CMT positive	35	3	38
CMT negative	3	59	62
Total	38	62	100

Two-sided chi-squared (with Yates correction) = 72.5; $p < 0.0001$; OR = 229.4 (95% Confidence Interval = 43.9-1200.0)

Table 3 Frequency of isolation of bacteria from the milk samples in subclinical mastitis of Sahel goats

Bacterial species	Number	%
Staphylococcus	10	26.3*
Streptococcus	4	10.5
Staphococcus and Streptococcus	24	63.2
Escherichia coli	0	0

*Significant ($p < 0.0001$) variation in values (one-way chi-squared test)

DISCUSSION AND CONCLUSION

The results of the study revealed that SCM, based on CMT, in lactating Sahel goats located in Maiduguri (Borno State, northeastern Nigeria) was endemic at a goat-level prevalence of 38%, comparable to the recent prevalence (23-60%) reported in Sahelian goats in other parts (Kaduna, Kebbi and Kaduna States) of northern Nigeria (Udo et al 2019; Danmallam and Pimenov, 2019). The parity of the goats influenced the prevalence without significant trend or association. The primiparous goats had higher prevalence of SCM than those in the 2nd and 3rd parity, but further parity tended to increase the prevalence until 6th parity when the prevalence was beyond primiparous level (75%). This may be related to husbandry practices in the local herds where manual milking of goats for household consumption is rarely done and milk letdown often occurs with the kid suckling the dam. It is presumed that teat injury may be more common in the primipara that is also immunologically naïve to the pathogens associated with SCM, and may not experience spontaneous recovery from teat infections that took place during parturition (McDougall et al, 2002). Parity was similarly reported not to be associated with CMT score (Boscos et al, 1996) and the prevalence of SCM (Hafty et al, 2016; Mishra et al, 2018). However, SCM increased in older dams (Hafty et al, 2016) that were possibly having higher number of parity, and CMT-based prevalence increased with increasing parity (Mahlangu et al, 2018). Parity seemed to have an impact on milk somatic cell count, which could be detected by CMT in goats, with somatic cell count increasing with parity, but the count was higher in primipara than multipara in sheep (Paape et al, 2007).

The SCM was mostly (71.1%, 27/38) mild and rarely severe (10.5%, 4/38) based on CMT score. Severity of SCM was diagnosed by CMT which qualitatively estimated the amount of DNA of inflammatory cells infiltrating the milk in the

mammary gland (McDougall et al, 2010; Persson and Olofsson, 2011; Souza et al, 2012). SCM was significantly associated with intra-mammary bacterial infections which were isolated in the milk samples from 92.1% of cases. The CMT-negative cases with bacterial isolation of staphylococci in milk could be in early infection with undetectable somatic cell count using CMT. The bacteria isolated were either Staphylococcus spp, Streptococcus spp, or both. These infections elicit pro-inflammatory cytokines which cause inflammatory cells to migrate into mammary glandular tissues (Albenzio et al, 2016; Bochniarz et al, 2017; Ruiz-Romero et al, 2020). Most of the reported intra-mammary infections in SCM were staphylococci (Contreras et al, 1995; Marogna et al, 2012; Akter et al, 2020), and they were frequently more of Coagulase-negative than positive strains (Zhao et al, 2015; Dore et al., 2016; Gelasakis et al, 2016). However, this study indicated the involvement of Coagulase-positive strains among 70.6% of the staphylococcal isolates. In northern Nigeria, Coagulase-positive *S. aureus* were reported as the most commonly isolated bacteria from mastitic goats (Ameh and Tari, 1999; Danmallam and Pimenov, 2019). These Coagulase-positive strains cause more losses in milk production than Coagulase-negative stains, and can lead to clinical form with gangrenous lesion (Gelasakis et al., 2016). Additionally, streptococci played contributory role as pathogen of SCM, but *E coli*, an environmental contaminant, was not associated with any case, perhaps due to good hygiene in the herds. Previous reports in the country causally associated SCM with streptococci and *E. coli* (Ameh et al, 1993, 1994; Ameh and Tari, 1999; Danmallam and Pimenov, 2019). These were also bacteria that caused clinical mastitis when the infections persisted in the goat herd.

The major limitation of the prevalence data was the low total number of goats and the low number at strata of parity, especially the higher parity. The goat populations were not large in the area of study, and older goats might have been culled because of unidentified reasons. Nevertheless, this study is considered a pilot non-randomized study to identify the preliminary outlook on SCM existing in the population. As a survey, the study has identified the occurrence of an insidious disease condition which exists without clinical indicators, but could have the potential of decreasing milk yield and quality (Leitner et al, 2004), increasing kid mortality by milk underfeeding and infective enterotoxicity (Contreras et al, 2003; Silanikove et al, 2014), spreading of latent intra-mammary infections that could escalate to clinical mastitis (Koop et al, 2013), and undermining the development of commercial goat dairy production.

In conclusion, SCM of lactating Sahel goats associated with intra-mammary infections by staphylococci and streptococci was prevalent, and the diagnosis of SCM at various levels of parity by CMT was strongly associated with isolation of the bacteria in the milk.

CONTRIBUTIONS

DTY: 1, 2, 3, 4, 5, 6 7, 9 ; DLM: 1, 2, 4, 7, 9; NAI: 7, 8, 9, 10 ; MNJ: 4, 5, 6, 7 ; IOI: 1, 2,3, 4, 6, 7, 8, 9, 10

(1)Conception; (2) Design; (3) Supervision; (4) Fundings; (5) Materials; (6) Data Collection and/or Processing; (7) Analysis and/or Interpretation of the Data; (8) Literature Review; (9) Writing; (10) Critical Review

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest.

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SUBKLINIČKI MASTITIS SAHEL KOZA U LAKTACIJI U MALIM STADIMA U URBANIM SREDINAMA U MAIDUGURIJU, NIGERIJA: PREVALENCA I INTRAMAMARNE INFEKCIJE

SAŽETAK

Rizik od hroničnog kliničkog mastitisa može biti povećan zahvaljujući prisustvu neprepoznatog subkliničkog mastitisa (SCM) u malim stadima koza u urbanim sredinama. Naše istraživanje predstavlja presječni pregled naizgled zdravih Sahel koza u laktaciji (februar-mart, 2023.) na prevalencu SCM korištenjem California mastitis testa (CMT) i bakterijskih kultura sirovog mlijeka u Maiduguriju, Nigerija.

Pozitivan CMT je označen kao +1 (blagi), +2 (umjereni) ili +3 (teški). Za izolaciju bakterija su primijenjene standardne procedure izolacije kultura. Prevalenca SCM je iznosila 38% (95% CI, 26.9-52.2) na 100 koza, pri čemu je bila ovisna o paritetu (18.7-75.0%) na način da je opadala ($p < 0.05$) s 50.0% u prvom paritetu na 18.7% u trećem, a potom rasla ($p < 0.05$) na 75.0% u šestom paritetu. Paritet nije statistički signifikantno ($P > 0.05$) povezan sa prevalencom. Većina slučajeva (71.1%, 27/38) je bila blaga. Dijagnosticiranje CMT testom je statisitički signifikantno ($p < 0.0001$) povezano s izolacijom bakterija u uzorcima mlijeka. Stopa izolacije je iznosila 92.1% (35/38) u slučajevima SCM-a, s tim da su CMT negativni uzorci mlijeka imali stopu izolacije od 4.8% (3/62). *Staphylococcus spp* i *Streptococcus spp* su kao čista kultura izolirani u 26.3%, odnosno 10.5% od 100 uzoraka. *Staphylococcus spp.* i *Streptococcus spp.* su zajedno izolirani u 63.2% uzoraka. Od ukupno 34 izolata Staphylococcus spp, 24 (70.6%) su bili koagulaza pozitivni. Stopa izolacije stafilokoka (89.5%, 34/38) i streptokoka (73.7%, 28/38) se nisu razlikovale ($\chi 2 = 3.118$; $p < 0.077$). Stoga je neophodno kontrolirati prevalentnu populaciju sa SCM među zdravim Sahel kozama u laktaciji koje imaju intramamarne infekcije sa ciljem prevencije eskalacije do kliničkog mastitisa.

Ključne riječi: Prevalenca, Sahel koza, *Staphylococcus*, *Streptococcus*, subklinički mastitis

RESEARCH ARTICLE

EVALUATION OF HEMATOBIOCHEMICAL AND OXIDATIVE STRESS PARAMETERS IN NATURAL BOVINE *TRYPANOSOMA BRUCEI* INFECTION

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ABSTRACT

This study aimed to evaluate the hematobiochemical and oxidative stress levels and some antioxidant enzyme activities in cattle naturally infected with T. *brucei* compared to the healthy controls. Blood and fecal samples were obtained from 120 cattle from selected farms and grouped based on infection status, Group 1 (n=32, T. *brucei* and helminthosis co-infection), Group 2 (n=41, T. *brucei* only), Group 3 (n=20, helminthosis only), and Group 4 (n=27, healthy control) to assess the association between the disease conditions and co-infection. Among the animals examined, 20 test samples and 6 healthy controls were selected from Group 2 and Group 4, respectively. Groups 1 and 3, as well as animals with a history of infection, inflammation, stress, and serum samples showing hemolysis, were excluded from the study. Hematological indices, serum biochemistry, oxidative stress biomarkers (MDA, SOD, CAT, GSH), and trace elements (Zn, Cu, Fe) were analyzed using standard assays. Infected cattle showed significant decreases ($p<0.05$) in PCV, Hb, RBCs, TLC, and platelets compared to controls, along with a significant increase ($p<0.05$) in eosinophils. Lymphocytes and monocytes also significantly decrease ($p<0.05$), while MCV, MCH and neutrophils significantly increase ($p<0.05$). Total protein, albumin, globulin, potassium and phosphorus decreased significantly ($p<0.05$). AST, ALT, ALP, GGT and creatinine also decreased significantly ($p<0.05$). MDA increased significantly ($p<0.05$), while SOD and GSH decreased significantly ($p<0.05$), indicating elevated oxidative stress. Zn also decreased significantly ($p<0.05$) in infected cattle. T. *brucei* infection caused significant hematological and biochemical alterations, disrupted antioxidant status, and decreased Zn, reflective of pathogenic effects. This study demonstrates T. *brucei* induces oxidative stress exceeding the antioxidant capacity, contributing to disease.

Keywords: Cattle, hematology, oxidative stress biomarkers, serum biochemistry, *Trypanosoma brucei*

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INTRODUCTION

African animal trypanosomosis is a blood protozoan disease caused by various trypanosome parasites (Muhanguzi et al., 2017). Among these, *Trypanosoma brucei* (T. *brucei*), is very important because it is responsible for sleeping sickness in humans and nagana in cattle (Possart et al., 2021) with devastating impacts in sub-Saharan Africa. The tissue and body organ activities of T.*brucei* can induce marked hematological, pathological, and serum biochemical derangements in different species (Ohaeri and Eluwa, 2011). The severity of these conditions can be influenced by the virulence of the infecting trypanosome, infective dose, and the host's immune status (Dagnachew et al., 2015).

Evaluating hematological values is crucial for assessing animal health, especially in cases of bovine trypanosomosis which commonly causes anemia, reflected by decreased packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) counts, along with severe leukopenia (Silva et al., 1999), with leukopenia characterised by neutropenia, eosinopenia and lymphopenia in cattle infected with T. *congolense* (Biryomumaisho et al., 2007). Tissue damage, indicated by changes in serum enzyme levels, has been reported in animal trypanosomosis. Significant increases in AST, ALP, and ALT levels have been reported in experimental infection with T. *brucei* (Orhue et al., 2005). Other reported biochemical alterations include hypoglycemia, and increased plasma bilirubin in T. *brucei* infected animals (Omotainse et al., 1994; Gow et al., 2007). Elevated serum urea levels in rats infected with T. *brucei* have also been reported (Egbe-Nwiyi et al., 2005).

Infection with trypanosome results in the increased production of reactive oxygen species (ROS) which act as cytotoxic agents (Paiva et al., 2018), and play a significant role in the pathophysiologic mechanism of trypanosomosis. Evaluating the role of oxidative stress parameters in trypanosomosis can illuminate the cellular damage inflicted by the parasite and the host's inflammatory response, with effective treatment and management strategies that can mitigate the impact of this debilitating condition.

Since oxidative stress plays a key role in tissue damage and disease severity (Abubakar and Nado, 2023), targeting this pathway rather than only eliminating the parasite could offer additional therapeutic benefits. By identifying key biomarkers in the oxidative stress response, improved therapeutic strategies through antioxidant interventions can aim at mitigating free radical damage and improving clinical outcomes.

Microminerals, including trace elements, mediate vital biochemical reactions by acting as cofactors for many enzymes, as well as acting as centers for stabilizing structures of enzymes and proteins (Cedeño et al., 2020). Emerging research has shown the importance of trace elements in the pathogenesis of regenerative mechanisms, the body's response to oxidative stress, and the maintenance of immunity against pathogens (Wada, 2004; Bonaventura et al., 2015). Microminerals such as zinc (Zn), copper (Cu), Iron (Fe), and selenium are integral parts of the antioxidant defence system. They are utilized to synthesize antioxidant enzymes that protect tissues from free radical-induced damage (Evans and Halliwell, 2001). Cu and Zn play an important catalytic role in the enzymatic activity of superoxide dismutase (SOD). Cu–Zn-SOD enzyme complex catalyzes the conversion of superoxide anion ($O2^{-}$) into hydrogen peroxide (H2O2) and water, thereby effectively neutralizing large amounts of oxidants (Das et al., 2022). Some metallic ions, such as iron and copper, participate in oxidation-reduction reactions in energy metabolism. Iron, as a constituent of hemoglobin and myoglobin, also plays a vital role in the transport of oxygen. Therefore, measuring Zn and Cu concentration in serum can indicate the micromineral status as well as represent their coordinated antioxidant roles accompanied by antioxidant enzyme activities. Assessment of serum iron levels can also be beneficial in clarifying the oxidant/antioxidant status (Cavdar et al., 2003).

The relationship between oxidative stress and changes in antioxidant enzymes is well established. However, its documentation in natural T. *brucei* infection in cattle remains uncertain, with limited local data. Hence, our study aimed to evaluate the hematobiochemical parameters, oxidative stress level, and some antioxidant enzyme activities in cattle naturally infected with T. *brucei*.

MATERIALS AND METHODS

Sample population and design

Blood and fecal samples were collected from 120 White Fulani cattle of both sexes and various ages from farms in Ibadan. The cattle were categorized into four groups: Group 1 (n=32; co-infection of T. *brucei* and helminthosis), Group 2 (n=41; T. *brucei* infection without helminthosis, test samples), Group 3 (n=20; helminthosis with no parasitemia), and Group 4 (n=27; absence of parasitemia and helminth parasites, healthy control). Among the animals examined, 20 test samples, and 6 healthy controls were selected from Groups 2 and 4, respectively. Groups 1 and 3, as well as animals witha history of infection, inflammation, stress, and serum samples showing hemolysis, were excluded from the study.

Two blood samples (5 ml each) were collected via the jugular vein from each animal into Sarstedt S-monovette tubes. The samples were divided into EDTA bottles (3 ml) for hematological analysis and plain bottles (7 ml) for serum chemistry. Clotted blood samples were centrifuged at 4000 revolutions per minute for 10 minutes. Clear serum was separated and stored at -20°C until needed.

Hematological assay

These utilized standard protocols. The packed cell volume (PCV) was determined through the microhematocrit centrifugation technique (Thrall and Weiser, 2002). The hemoglobin concentration (Hb) was measured using the cyanomethemoglobin colorimetric method (Higgins et al., 2008). Red blood cells (RBCs), white blood cells (WBCs) and differential WBC counts were performed using microscopic examination and Giemsa staining. The platelet count (PC) was determined following the Rees and Ecker counting technique, as described by Ihedioha and Agina (2014).

Serum biochemical analysis

Blood serum was analyzed to obtain energy, nitrogen, liver, kidney and mineral profiles using spectrophotometric methods and commercial kits Randox® assay kit (Randox Laboratories, Ltd., UK) and Teco® assay kit (Teco diagnostics, USA) for some of the parameters of the mineral profile (sodium, potassium, phosphorus and chlorides) following the manufacturer's instructions. The low-density lipoprotein (LDL) concentration was calculated using the Friedewald equation (Mukhtar et al., 2021). Total Protein (g/dL)/TP245; Albumin (g/dL)/AB362; Albumin/globulin ratio; AST (IU/L)/AS 101; ALT (IU/L)/AL 146; ALP (IU/L)/AP 542; GGT (IU/L)/GT 2750; Creatine kinase (u/L)/CK1296; BUN (mg/dL)/UR1068; Creatinine (mg/dL)/CR510; Glucose (mg/dL)/GL 364; Cholesterol (mg/dL)/CH 200; Triglyceride (mg/dL)/TR 210; HDL (mg/dL)/CH 203; Calcium (mmol/L)/CA590; LDH (u/L); Globulin (g/dL).

Determination of parasitemia

This involved a wet mount, stained thin blood smear and buffy coat as described by Cheesbrough, (1998). Hemoparasites were identified at the species level based on structural and morphometric criteria, as described by Gibson (2023).

Fecal examination

Fecal samples were screened for helminthsusing sedimentation, flotation, and modified McMaster techniques (Jeremiah and Banwo, 2018).

Serum analysis measured: superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), malondialdehyde (MDA) levels and the trace elements Zn, Cu, and Fe.

Determination of SOD activity

The activity of the SOD enzyme was assessed using the nitrobluetetrazolium (NBT) reduction assay, as described by Amar et al. (2019). This assay leverages SOD's ability to catalyze the dismutation of superoxide radicals generated by xanthine and xanthine oxidase, thereby preventing NBT reduction to blue formazan dye. The degree of NBT reduction, quantified spectrophotometrically at 560 nm, inversely correlates with SOD activity. One unit of SOD activity is defined as the amount causing a 50% inhibition of NBT reduction.

Determination of GSH activity

GSH levels in serum were evaluated with the adapted DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) method following Amar et al., (2019) with some modifications to suit cell-free serum. Proteins lacking sulfhydryl groups were precipitated, leaving only GSH to react with DTNB, forming a yellow product. The absorbance of this product at 412 nm was directly proportional to the GSH concentration, determined by a standard curve comparison.

Determination of catalase (CAT) activity

This was evaluated using a modified version of Claiborne's method as described by Amar et al. (2019). The reaction mix contained 30% hydrogen peroxide (H2O2), phosphate buffer, and serum samples. Catalase catalyzes H2O2 decomposition, decreasing its concentration over time. This change was monitored spectrophotometrically at 240 nm, as proposed by Molina and Guillen (2017). Absorbance readings at 30-second intervals tracked the rate of H2O2decomposition, proportional to catalase activity.

Determination of MDA level

Quantification of MDA, a marker of lipid peroxidation, is achieved using the thiobarbituric acid reactive substances (TBARS) assay, following a modified protocol byVarshney and Kale (1990). Proteins were precipitated with trichloroacetic acid (TCA), and MDA reacted with thiobarbituric (TBA) under acidic conditions, forming a pink MDA-TBA complex, measured optically at 532 nm. The intensity of color, determined spectrophotometrically, was directly proportional to the MDA concentration.The standard used in this modified TBARS assay is a precursor of MDA, 1,1,3,3-tetraethoxypropane (TEP), used to generate MDA standards with known concentrations, and standard curve from which the MDA levels in the serum samples are quantified. C = F x 6.41 x A (C- Concentration, F- Dilution factor, and A- Absorbance).

Trace element analysis

Serum was prepared through an acid digestion processusing nitric acid (69%), and hydrogen peroxide (33% w/v). Initially, 0.5ml of serum was combined with 1ml of concentrated nitric acid (HNO3) and 0.5ml of H2O2 in propylene tubes. This mix was heated at 60 °C for two hours to facilitate digestion. After digestion, the mix was diluted by adding 2.5ml of ultrapure water. The resulting solutions were centrifuged at 2000 rpm for five minutes. The quantification of trace elements such as Zn, Cu, and Fe in the supernatant was performed using an ICP-MS (Perkin Elmer Ultima 8000, USA) according to the protocols outlined by Luna et al. (2019). The ICP-MS calibration standard for these elements (Zn, Cu, and Fe) is 0.01-1mg/L. The analytical masses monitored included 63Cu, 66Zn, and 56Fe. Nitric acid and hydrogen peroxide were considered blank in the analytical method. To quantify the concentrations of elements, 1.2+25 (vs+vd) dilution protocol was used,vs-volume of the sample, and vd-volume of diluent water. The calibration curves were performed with multielement solutions from Specsol Brand Standards traceable by the National Institute of Standards and Technology (NIST) to ensure the quality and accuracy of the ICP-MS analysis.

Statistical analysis

All statistical analyses of the data were completed using IBM SPSS software package version 21

and GraphPad PRISM 10.2.1 (San Diego, CA, USA). Data were expressed as mean and standard deviation (SD). A confidence level of 95% and p-values< 0.05 were considered statistically significant. The distribution pattern of data was assessed by Kolmogorov-Smirnov test (p>0.05) to confirm normal distribution. Comparisons between means were made by using unpaired t-tests. The data were summarised in tables using descriptive statistics including frequencies and percentages. (F-test) was used to test associations between the four groups based on the presence or absence of Trypanosomosis and helminthosis.

RESULTS

Association between trypanosomosis and helminth-infected animals

Table 1 shows no significant association between cases of trypanosomosis and helminth. Out of the 120 cattle sampled, 41 were infected with only trypanosomosis, while 27 served as the healthy control, free from both disease conditions and any other infections that could act as confounding factors in the study.

Table 1 Trypanosomosis and helminth co-infection

Trypanosomosis and Helminth Co-infection?	Trypanosomosis Positive	Trypanosomosis Negative	Total (N)	(F. Exact Sig)	Odd Ratio	df
Helminth Positive	32 (61.5%)	20 (38.5%)	52 (43.3%)	P>0.99	1.05	1
Helminth Negative	41 (60.3%)	27 (39.7%)	68 (56.7%)			
Total	73	47	120 (100%)			

Hematological values in bovine trypanosomosis

There wasa significant decrease in the mean PCV, RBC, and hemoglobin, and a non-significant difference in MCV, MCH and MCHC (Table 2a) between the infected group and the healthy control. There is a statistically significant decrease in total leukocyte count and a significant increase in eosinophils (Table 2b). Also, there is increased neutrophil, though not significant, showing variable differences in lymphocyte, monocyte and platelet counts.

Table 2a Erythrogram values (mean ± SD) in bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
PCV (%)	30.21±1.36[a]	19.8±1.36[b]
Hb (g/dL)	26.2±1.83[a]	6.26±0.48[b]
RBC (x 106/µL)	4.82±0.22[a]	2.92±0.29[b]
MCV (fL)	62.73±0.73	69.03±4.50
MCH (pg)	20.16±0.22	21.77±1.38
MCHC (g/dL)	32.17±0.23	31.55±0.32

SD = standard deviation, MCV= mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, MCH= Mean corpuscular hemoglobin. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Table 2b Leukocyte values (mean±SD) in bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
TLC (x103µL)	4.16±1.89[a]	2.72±1.72[b]
Lymph (%)	60.90±1.18	60.1±3.12
Neut (%)	33.2±1.23	33.3±3.17
Eos (%)	2.4±0.4[a]	4.28±0.24[b]
Mono (%)	3.67±0.29	2.46±0.55
Platelets (µL)	98.28±2.46	84.6±2.61

TLC= Total leukocyte count, lymph= lymphocyte, neut= neutrophil, mono= monocyte, and eos= eosinophil. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Influence of trypanosomosis on serum biochemical indices of Cattle

There is a significant decrease in the mean total protein, albumin, globulin, potassium and phosphorus, with a significant decrease in AST, ALT, ALP, GGT and creatinine (Table 3). The Table also reflected a non-significant decrease in BUN, glucose, cholesterol, triglyceride, HDL, LDH, sodium, chloride and calcium, with a non-significant increase in albumin/globulin ratio and creatinine kinase.

Table 3 Influence of trypanosomosis on serum biochemical indices of cattle

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
Total Protein (g/dL)	7.8 ± 2.34[a]	3.7 ± 1.35[b]
Albumin (g/dL)	3.5 ± 1.29[a]	1.5 ± 0.67[b]
Globulin (g/dL)	4.3 ± 2.33[a]	2.2 ± 1.12[b]
Albumin/globulin ratio	0.81	0.68
AST (IU/L)	128 ± 10.31[a]	297 ± 12.43[b]
ALT (IU/L)	8 ± 1.43[a]	19 ± 2.13[b]
ALP (IU/L)	108 ± 4.52[a]	197 ± 6.21[b]
GGT (IU/L)	4 ± 1.56[a]	10 ± 2.78[b]
Creatine kinase (u/L)	59 ± 4.12	61 ± 3.29
BUN (mg/dL)	15.7 ± 3.54	14.5 ± 3.65
Creatinine (mg/dL)	0.4 ± 0.41[a]	0.7 ± 0.11[b]
Glucose (mg/dL)	62 ± 6.82	50 ± 11.13
Cholesterol (mg/dL)	50 ± 14.51	42 ± 9.27
Triglyceride (mg/dL)	11 ± 2.41	8.2 ± 2.13
HDL (mg/dL)	27 ± 11.45	26 ± 6.52
LDH (u/L)	116 ± 13.47	100 ± 13.38
Sodium (mmol/L)	124± 8.45	117 ± 6.38
Potassium (mmol/L)	2.9 ± 1.17[a]	1.6 ± 1.12[b]

Chloride (mmol/L)	100 ± 2.31	94 ± 4.38
Phosphorus (mmol/L)	1.9 ± 0.65[a]	1.0 ± 0.26[b]
Calcium (mmol/L)	9.5 ± 3.63	8.7 ± 4.98

AST= aspartate transaminase, ALT= alanine transaminase, ALP= alkaline phosphatase, GGT= gamma-glutamyltranspeptidase, BUN= blood urea nitrogen, HDL= high-density lipoprotein, LDH= lactate dehydrogenase, LDL= low-density lipoprotein. The means bearing different superscripts (a, b) differ significantly (P<0.05) between the groups.

Oxidative stress parameters in the two groups

Comparative evaluation of oxidative stress parameters (Mean ± SD) between healthy control and bovine trypanosomosis shows a significant increase in MDA, a significant decrease in SOD, and GSH, and Zn with a non-significant decrease in catalase, Fe, Cu (Table 4).

Table 4 Comparative evaluation of oxidative stress parameters (Mean ± SD) between healthy control and bovine trypanosomosis

Parameters	Healthy control (n=6)	Bovine trypanosomosis (n=20)
SOD (u/ml)	50.26±0.0705[a]	30.43±0.1603[b]
MDA (nmol/ml)	29.83±3.357[a]	41.54±0.2287[b]
GSH (umol/l)	38.04±1.478[a]	29.17±0.5967[b]
Catalase (u/ml)	188.0±2.169	177.70±6.602
Fe (mg/l)	0.71±0.00278	0.64±0.048
Cu (mg/l)	0.45±0.0645	0.37±0.047
Zn (mg/l)	0.70±0.5354[a]	0.25±0.06455[b]

Malondialdehyde (MDA), Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH). The means bearing different superscripts (a,b) differ significantly (P<0.05) between the groups.

DISCUSSION AND CONCLUSION

Serum hemato-biochemical changes are characteristics of trypanosome infection. The characteristic changes are often determined by the pathophysiological effect of the disease on different body organs, the strain of the infecting trypanosome and the host (Awekew et al., 2017). In the present study, we reported a decrease in Hb and PCV, which may result from direct physical damage to red blood cells due to parasite whipping movement and hemolytic activity of the hemolysins that directly rupture red blood cells, causing hemoglobin release into the bloodstream. Furthermore, the decreased mean PCV, RBC and TLC concentration in trypanosomosis-affected group agrees with the work of Sivajothi et al. (2014) and several other findings (Sulaiman and Adeyemi, 2010; Hussain et al., 2016).

As observed by Pays et al. (2006), the significant decrease in TLC in cattle infected with T.*brucei* may be due to the immunosuppressive action of trypanosomes as well as the exhaustion of the immune system, leading to a lower overall output of leukocytes. Besides this, leucopenia is always seen at the terminal stage of the infection (Sivajothi et al., 2015). The eosinophilia in infected cattle agrees with the previous report of Sivajothi et al. (2014) in *T. evansi*- infected cattle, as a feature of parasitic infections which may be associated with immediate-type hypersensitivity reactions.

In the present study, we reported a significant decrease in serum albumin, total protein and globulin compared to that of the healthy control. This agrees with the works of Megahed et al., 2012 and Dagnachew et al., 2014 that hypoalbuminemia in trypanosomosis may be due to increased hepatocellular damage arising in trypanosomosis infection.

The elevated levels of AST and ALT in the infected cattle are in line with the findings of several other studies (Yusuf et al, 2012; Hussain et al., 2016) as with increased ALP in both clinical and sub-clinical trypanosome infections (Takeet and Fagbemi, 2009; Adeyemi and Sulaiman, 2012) and GGT. This suggests a probable invasion of the vital body organs like the liver, heart and brain. The increased levels of creatinine in this study could be associated with renal malfunction, as reported by Abenga and Anosa (2005).

The significant decrease in potassium levels may be due to the rapid replication and metabolism of the parasites, which may interfere with cellular uptake and homeostasis of this electrolyte. This is in line with previous reports of reduction in the level of potassium in an experimental infection with *T. brucei* and *T. evansi* (Ogunsanmi et al., 1994; Da Silva et al., 2011).This can equally be associated with inappetence in clinical infection. Some of the clinical implications of hypokalemia and hypophosphatemia are muscle weakness and lethargy exacerbating disease severity. Awekew et al. (2017) reported trypanosomes require lipoproteins for them to multiply under axenic culture. Thus, the lowering of the serum lipids and cholesterol, as observed in the present and previous studies (Biryomumaisho et al., 2003; Adamu et al., 2008) could, partly, be the result of trypanosomal utilization of the molecules.

The non-significant decrease in serum glucose in this study might be due to excessive utilization of blood glucose by trypanosomes for their metabolism through their glycolytic pathway, as reported by Kadima et al. (2000), where serum glucose levels were significantly low on Days 3, 4, and 5 post infections of cattle with T. vivax, corresponding with the first parasitemia build up.

In this study, bovine trypanosomosis was associated with a significant increase in oxidative stress markers in infected cattle compared to the control. This is supported by several studies (Hussain et al., 2018; Eljalii et al., 2015), which reported a significant rise in MDA levels, a common marker of lipid peroxidation. SOD levels decreased significantly in the infected group compared to the control group, due to the impact of oxidative stress. This agrees with the findings of Saleh et al. (2009), where a decrease in serum SOD was observed in the *T. evansi*-infected camel compared to the healthy control. Ranjithkumar et al. (2011) also reported a decrease in horses

naturally infected with *T. evansi*. The drop in SOD activity may be due to its high demand as a free radical scavenger throughout the oxidative process in the naturally occurring *T. brucei* infection.

The catalase results in this study showed an insignificant lower mean catalase concentration in T. *bruce*-infected cattle compared to the healthy control. This is closely related to a study by Mishra et al. (2017) where trypanosomosis-infected cattle showed a significant decrease in erythrocytic catalase concentration compared to that of the healthy control. Wang et al. (1999) also reported a decline in serum catalase concentration in the trypanosome-infected Cape buffalo. The serum GSH from this study shows a significant decrease in the infected group compared to the healthy control. This result agrees with the findings of Abubakar and Dabo (2023) and Saleh et al. (2009) who reported a significant reduction in serum GSH for infected compared to the control group. The infection could be impairing the mechanisms responsible for GSH synthesis, limiting its availability.

The decrease in iron (Fe) level in this study is comparable with the work of Neils et al. (2007), which reported a significant decrease in serum iron in sheep infected with *T. congolense*. This decrease in iron could be linked to anemia, a common symptom of trypanosomosis, due to the importance of iron in red blood cell production. A significant decrease in Zn levels in infected cattle might be related to impaired immune function, as Zn is crucial for immune cell development and response against parasitic infections (Dardenne, 2002).

In conclusion, T. *brucei* infection caused significant hematological and biochemical alterations, disrupted antioxidant status, and decreased Zn, reflective of pathogenic effects. This study demonstrates the parasite induces oxidative stress exceeding antioxidant capacity, contributing to disease. Further research is warranted to better characterize oxidative alterations over time and their implications on pathogenesis.

CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conceptualization, writing – review and editing – OGB; Data collection and processing – DOP; Analysis and interpretation – JA; Materials and supervision – OTJ.

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EVALUACIJA HEMATOLOŠKIH, BIOHEMIJSKIH PARAMETARA I PARAMETARA OKSIDATIVNOG STRESA KOD PRIRODNE BOVINE TRYPANOSOMA BRUCEI INFEKCIJE

SAŽETAK

Cilj našeg istraživanja je evaluacija hematobiohemijskih parametara i razine oksidativnog stresa i aktivnosti određenih antioksidativnih enzima kod goveda prirodno inficiranih sa *T. brucei* u odnosu na zdrave kontrole. Uzeti su uzorci krvi i fecesa od 120 goveda sa selektiranih farmi i podijeljeni u grupe na osnovu statusa infekcije: Grupa 1 (n=32, koinfekcija *T. brucei* i helmintoza), Grupa 2 (n=41, samo *T. brucei*), Grupa 3 (n=20, samo helmintoza) i Grupa 4 (n=27, zdrave kontrole) kako bi procijenili vezu između stanja bolesti i koinfekcije. Od svih ispitanih životinja, selektirano je 20 testnih uzoraka i 6 zdravih koontrolnih jedinki iz Grupe 2 i Grupe 4. Grupe 1 i 3, kao i životinje sa anamnezom prethodne infekcije i inflamacije i one čiji su uzorci seruma pokazivali hemolizu su isključene iz istraživanja. Hematološki indeksi, biohemijska analiza, biomarkeri oksidativnog stresa (MDA, SOD, CAT, GSH) i elementi u tragovima (Zn, Cu, Fe) su analizirani korištenjem standardnih eseja. Inficirana goveda su pokazala signifikantan pad ($p<0.05$) PCV, Hb, RBC, TLC i trombocita u odnosu na kontrolne jedinke, kao i signifikantan porast ($p<0.05$) eozinofila. Limfociti i monociti su također pokazali signifikantan pad ($p<0.05$), dok su MCV, MCH i neutrofili signifikantno porasli ($p<0.05$). Ukupni protein, albumini, globulini, kalij i fosfor su signifikantno sniženi ($p<0.05$). AST, ALT, ALP, GGT i kreatinin su također signifikantno sniženi ($p<0.05$). MDA je signifikantno povišen ($p<0.05$), dok su SOD i GSH signifikantno sniženi ($p<0.05$) ukazujući na povišen oksidativni stress Zn je kod inficiranih goveda također signifikantno snižen ($p<0.05$). Infekcija sa *T. brucei* je uzrokovala signifikantne hematološke i biohemijske promjene, poremećen antioksidativni status i snižen Zn, kao posljedice patogenog učinka. Naše istraživanje je pokazalo da *T. brucei* inducira oksidativni stres koji prevazilazi antioksidativni kapacitet i doprinosi nastanku bolesti.

Ključne riječi: Goveda, hematologija, biohemijska analiza seruma, biomarkeri oksidativnog stresa, *Trypanosoma brucei*

RESEARCH ARTICLE

EVALUATION OF ANALGESIC EFFECT OF XYLAZINE, TRAMADOL AND LIGNOCAINE ON PROPOFOL ANAESTHESIA IN WEST AFRICAN DWARF (WAD) GOAT

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ABSTRACT

Twenty clinically healthy matured male West African Dwarf (WAD) goats aged 10 to 12 months with a mean weight (±SEM) of 17.5 kg were randomly assigned to four groups A, B, C, and D of five goats per group. The groups A, B, C and D received normal saline (2 ml IV), xylazine (0.05 mg/kg body weight [b/w] intravenously [IV]), tramadol (2 mg/kg b/w IV) and lignocaine (2.5 mg/kg b/w, subcutaneous infiltration at surgical area [SC]), respectively. Each goat in all the groups was anaesthetized with propofol at 9 mg/kg body weight (b/w) and maintained at 6 mg/kg b/w bolus administration. Laparotomy was performed on each goat in each group. Anesthetic indices and vital parameters were measured. Propofol alone (group A), and propofol/tramadol combination (group C) provided a short duration of analgesia despite high maintenance doses and quantity of propofol used in the groups. However, propofol/ xylazine combination (group B) provided analgesic duration enough to perform laparotomy, while propofol/ lignocaine combination (group C) provided the longest duration of action. Both lignocaine and xylazine combinations provided enough analgesia for the surgical procedure in this study. However, xylazine combination was associated more with prolonged sleeping, recumbent and recovery time, cardiac and respiratory depression, which are undesirable in ruminants due to the resultant cardiopulmonary and gastrointestinal complications. Lignocaine has more stabilizing effect on cardiac functions in addition to moderate sleeping, recumbent and recovery time. Hence, lignocaine with the long duration of analgesia, hemodynamic effects, and moderate anesthetic indices may be preferred among other selected drugs as a better combination agent with propofol for induction of anesthesia prior to apainful surgical procedure in West African dwarf (WAD) goat.

Keywords: Analgesia, lignocain, propofol, tramadol, WAD goat, xylazine

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INTRODUCTION

General anesthesia in ruminants, especially in sheep and goats, using propofol anesthesia has been advocated (Dzikitas et al., 2013; Ukwueze et al 2014). Propofol-induced anesthesia has desirable qualities, including fast recovery and non-cumulative effects upon repeated administration (Hall, et al., 2001; Dzikitas, 2013). However, the use of propofol as a sole drug to induce anesthesia is not sufficient, especially for noxious surgical procedure, due to its poor analgesic property (Ospina et al., 2024). This deficiency can be supplemented through drug combinations to produce balanced anesthesia. Balanced anesthesia can be achieved with the use of more than one anesthetic drug or with premedication (Hall, et al., 2001).

Premedication is a practice of administration of some drugs prior to induction of anesthesia with the aim of reducing the dose, complementing the effects and counteracting the side effects of the induction agents (Geel, 1991, Sheen et al., 2014). This improves the quality of anesthesia and facilitates the achievement of balanced anesthesia (Marviya et al., 2020; Moore et al., 2024). The use of different class of drugs such as opioids, alpha-2-adenoreceptor agonists, local anesthetics in premedication, especially during propofol induced anesthesia, has been documented (Abrahamsen, 2008; Sheen et al., 2014). Opioids such as Pentazocine and Tramadol provide analgesia for moderate and severe pain by actions in the central nervous system (Sameer and Neeran, 2011). Xylazine, an alpha-2-adenoreceptor agonist produces analgesic, sedative and muscle relaxant effects (Fereidoon et al., 2005; Ukwueze et al., 2014), while lignocaine provide regional or local analgesia and has antiarrhythmic effects (Yalamuru et al., 2022; Silva et al., 2023).

Propofol is known to combine well with different drugs. The combination of propofol with xylazine, tramadol, ketamine and lignocaine has been reported in dogs, cats, and goats (Geel, 1991; Ajadi et al., 2012; Tanaka et al., 2015; Weinberg et al., 2015; Gupta and Bhalotra, 2019; Hammadet al., 2020). However, determination of the most effective drug combination is imperative to ensure potent analgesia and fewer side effects.

The main objective of this study was to evaluate the efficacy of xylazine, tramadol, and lignocaine as premedicants during propofol anesthesia in West African Dwarf goats (WAD).

MATERIALS AND METHODS

Animals used

Twenty (n = 20) healthy male WAD goats with average age (±SEM) 12 months, and average weight (±SEM) 17.5 kilograms were used for this study. The animals were acclimatized for three weeks during which they were evaluated and stabilized for surgery prior to the beginning of the study. When the animals arrived to the college facilities, they were thoroughly examined for apparent signs and symptoms of illness. Blood samples were collected and examined for the presence of blood parasites. Feacal samples were collected from each animal and examined for helminth ova. The animals were dewormed with levamisol injection (Hebei Huarun Pharmacy ltd China) and also treated with antibiotic, oxytetracycline (Chongqing Bull Animal Pharmaceutical Co., Ltd) 10% short acting for 3 consecutive days at 1 ml per 10kg intramuscularly to safeguard against bacterial infections. They were also vaccinated against Peste des Petits ruminatum (PPR) virus using PPR vaccine from National Veterinary Research Institute, Vom. Jos, Plateau State, Nigeria. The animals were kept in well-ventilated, concrete floor and aluminum-roofed animal house at the college facilities. The wall of the house was constructed with blocks up to one quarter, and the remaining height with wire gauze allowing the light period of 12 hours. The goats were kept in clean pens with bedding. They were fed with grasses and feed concentrates, and water was provided *ad libitum*.

Ethical approval

Ethical approval was granted by the College of Veterinary Medicine Research Ethics Committee, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (MOUAU/CVM/REC/202410). Animal welfare was ensured in accordance with the Helsinki Declaration of 1975 and its revised edition from 1983, while also respecting the International Statute on Experiments Conducted on Humans and Animals.

Drugs used

Drugs used in the study were propofol (Pofol, 1% Dongkook Pharmaceuticals, Korea), xylazine (Kepro Ltd., Holland), tramadol (Makcur Laboratory Ltd. Gajarat, India) and lignocaine (Nama Pharmaceutical drug, Mumbai).

Criteria for premedicant drug selection

The drugs were selected based on the properties of the individual drugs as follows:

1. Xylazine (sedative): analgesic, muscle relaxant and CNS depressant.
2. Tramadol (opioid): analgesic, CNS depressant.
3. Lignocaine: (local anesthetic): analgesic, antiarrhythmic

Experimental design

The healthy male goats were randomly assigned into four groups A, B, C, and D of five goats each. Group A served as control in all the experiments.

The groups A, B, C and D received normal saline (2 ml, IV), xylazine (0.05 mg/kg body weight IV), tramadol (2 mg / kg IV), lignocaine (2.5 mg / kg SC), respectively, as premedicants. Each goat in all the groups was anaesthetized with propofol at 9 mg/kg body weight (b/w), and anesthesia was maintained at 6 mg / kg b/w bolus administration. Laparotomy was performed on each goat.

Surgical procedure (Laparotomy)

The animal were fasted prior to surgery. Food was withheld for 18 hours and water for 12hours. The goat in each group were prepared for normal aseptic surgery. The hairs around left flank region of the goats were thoroughly clipped. The proposed surgical site was scrubbed with chlorhexidine gluconate B. P. 0.3% W/V (Purit®, Saro Lifecare Limited, Lagos, Nigeria).

Each goat was placed on right lateral recumbency to expose the left flank. Laparotomy was done according to a standard procedure described by (Ames, 2007; Hendrickson, 2007).

The surgical incision was closed routinely from inside out; peritoneum and muscle layers were closed with atraumatic needle and chromic catgut, size 1 (Agary Pharmaceuticals Ltd, Xinghuai, China) using simple continuous suture pattern. The subcutaneous layer was closed with chromic catgut, size 2 and atraumatic 1/2 circle taper point needle (Agary Pharmaceuticals Ltd, Xinghuai, China) using simple continuous suture pattern. The skin was closed using interrupted horizontal mattress suture pattern with nylon (Agary Pharmaceuticals Ltd, Xinghuai, China).

Post-operative analgesic was given to all the animals used in the study irrespective of the group it belonged. Diclofenac sodium 2.5 mg/kg, body weight was administered intramuscularly.

Anesthetic indices

Modified method of determination of anesthetic indices (Adetunji et al., 2002) was used. The onset of anesthesia, analgesic duration, sleeping time, recumbent time and standing time were recorded following administration of the anesthetics using a modified method of determination of anesthetic indices (Adetunji et al., 2002).

Analgesic duration:

This was determined by recording the time of disappearance of flank twitch as sign of loss of pain using a needle prick before the beginning of the surgery and also recording the time of appearance of pain using pain responses: muscle twitch, body movement and vocalization during the surgery.

The difference between the time of disappearance (TD) and the time of reappearance (TA) of flank twitch reflex gives the analgesic duration. TA – TD = AD

Physiologic parameters

The following physiological parameters were monitored: heart rate, respiratory rate and rectal temperature.The parameters were recorded before the induction of anesthesia at time zero (0 min) and, subsequently, at every ten minutes (10 min) interval post induction throughout the period of anesthesia. The parameters were observed and recorded manually as well as electronically using Veterinary multi-parameter patient monitor, ARI-800C (ARI medical equipment Co, Ltd, Zing, Hong Kong)

Data analysis

All the data obtained in these experiments were analyzed using one-way Analysis of Variance and presented as mean ± standard error of the mean. The variant means were separated by the least significant difference of the different groups. Significance was accepted at the level of $P < 0.05$.

RESULTS

Anesthetic indices

The time of onset of propofol was less than 0.60 mins (1 min) in all the groups. It was 0.58 ± 0.01 min (58 seconds) in propofol alone, 0.54 ± 0.02 min (54 seconds) in xylazine/propofol group, 0.59 ± 0.01min (59 seconds) in tramadol/propofol group and 0.59 ± 0.00 min (59 seconds) in lignocaine/propofol group. The onset was significantly ($p < 0.05$) shorter in xylazine/propofol group when compared to the control and other groups (Figure 1). The mean onset of propofol anesthesia was the same in all the groups except in group B, which was significantly shorter when compared to the control group A and other groups.

The mean analgesic period recorded in group D was significantly ($p < 0.05$) longer followed by group B, when compared to the control group A and other groups (Figure 2).

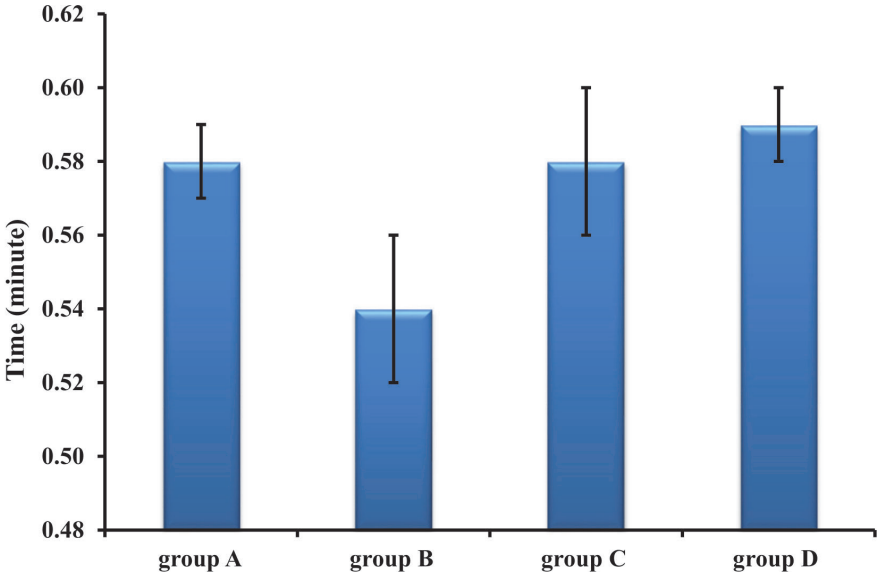
The mean sleeping time was significantly ($p < 0.05$) longer in groups B and D, but shorter in group C when compared to the control and other groups (Figure 3).

The mean recumbent time was significantly ($p < 0.05$) longer in group B, but shorter in group C when compared to the control and other groups (Figure 4).

The mean standing time was significantly ($p < 0.05$) long in group B when compared to the control group A and other groups (Figure 5)

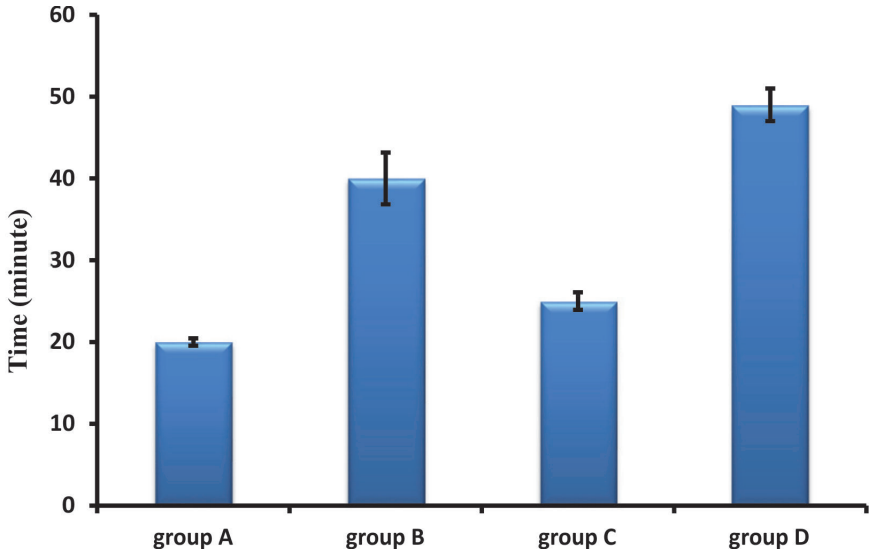
Anesthetic maintenance

The number of times anesthesia was maintained and the quantity of drugs used were significantly higher in the control group A when compared to other groups. However, the number of maintenance and the quantity of drug used were lower in group D followed by group B when compared to the control group A, and the other groups (Table 1).



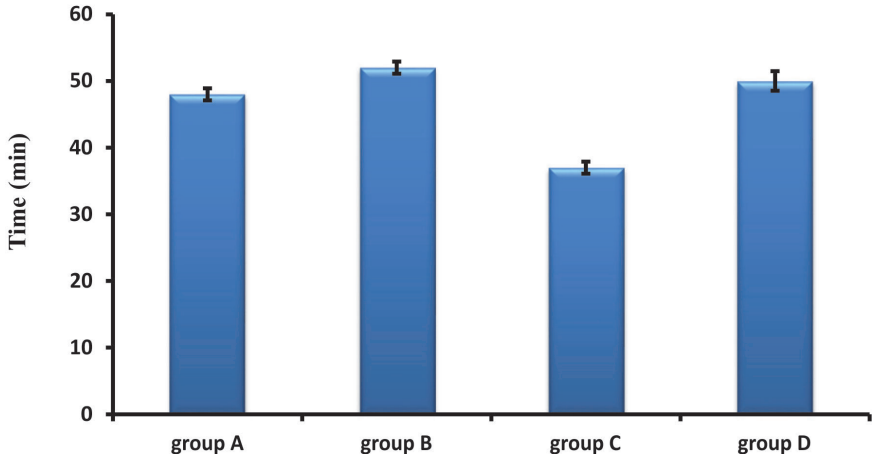
Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 1 Mean (±SEM) Onset of anesthesia in goat undergone laparotomy following iIntravenous administration of propofol combinations



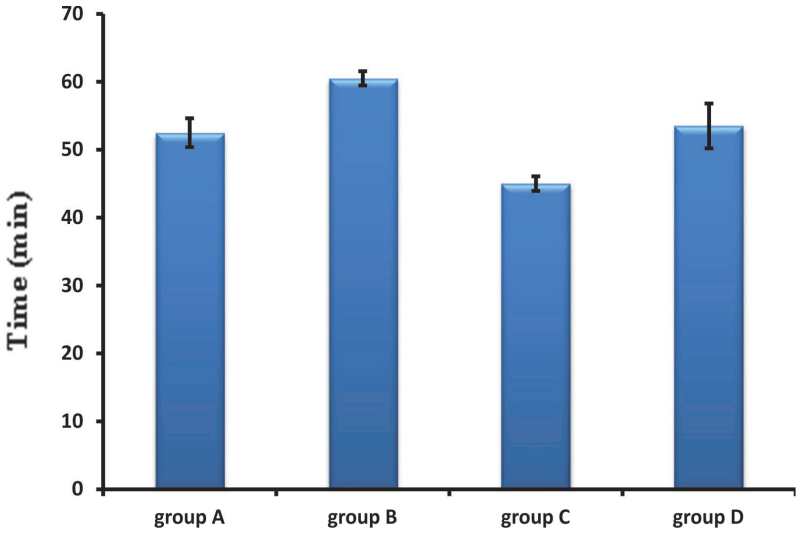
Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol . **Group D: lignocaine/ propofol.**

Figure 2 Mean (±SEM) Analgesic period in goat undergone laporatomy following intravenous administration of propofol combinations



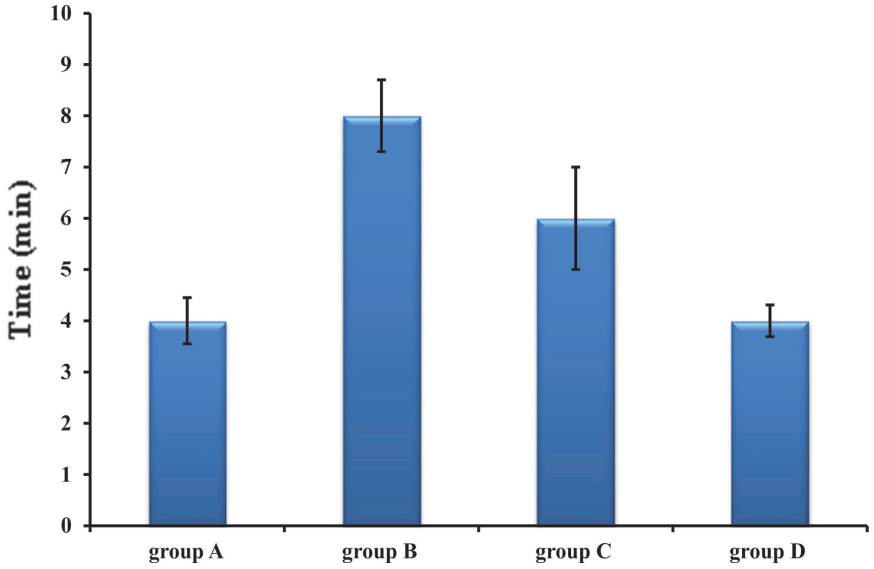
Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 3 Mean (±SEM) Sleeping time in goat undergone laporatomy following intravenous administration of propofol combinations



Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 4 Mean (±SEM) recumbent time in goat undergone laporatomy following intravenous administration of propofol combinations



Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

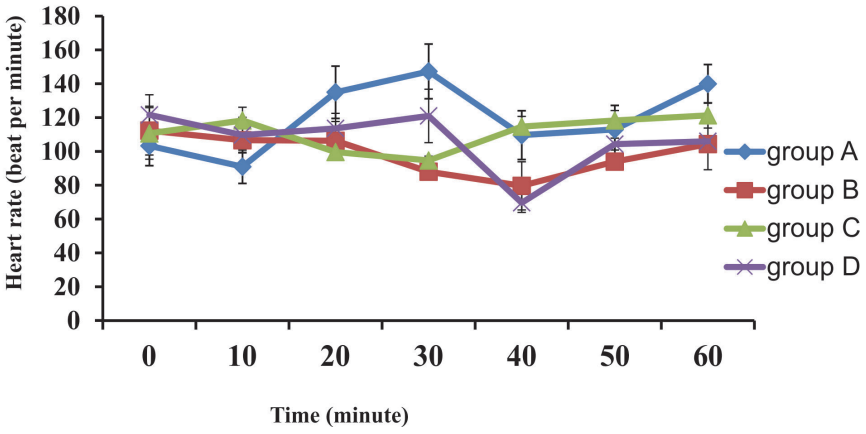
Figure 4 Mean (±SEM) Standing time in goat undergone laporatomy following intravenous administration of propofol combinations

Physiological parameters

The mean heart rate increased significantly ($p < 0.05$) in group C at 40 min post induction when compared to the control and the other groups. However, the mean heart rate was significantly ($p < 0.05$) low in groups B and D at 60 min post induction when compared to the control. Within the groups, the mean heart rate decreases significantly ($p < 0.05$) in groups B and D at 40 min post induction when compared to the baseline value of the groups (Figure 5).

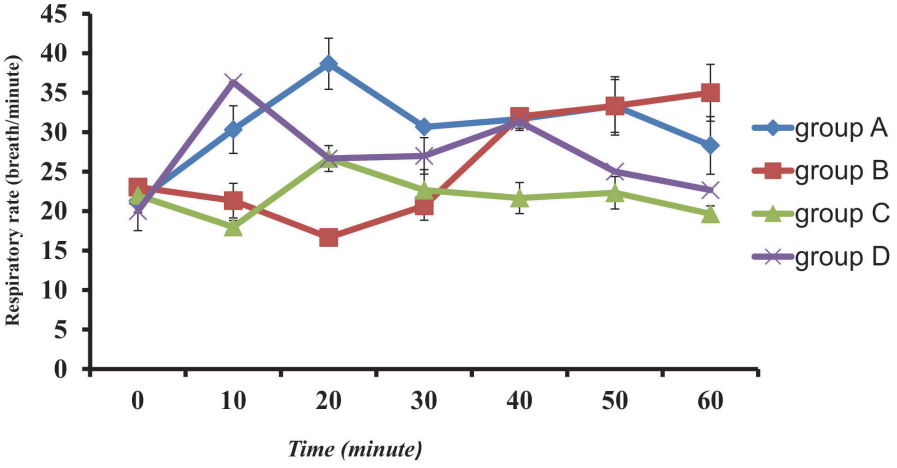
The mean respiratory rate increased significantly ($p < 0.05$) in control groups (propofol alone) at 20 min post induction when compared to other groups. However, the respiratory rate was significantly ($p < 0.05$) low in groups B and C at 30 min post induction and in group C at 40 min post induction when compared to control and other groups. Within the group, the respiratory rate was significantly ($p < 0.05$) high in control group at 20 and 50 min, and in group D from 10 min to 40 min post induction, respectively, when compared to the baseline value of the groups (Figure 6).

The mean rectal temperature of the goats increased significantly ($p < 0.05$) in groups B and D at 20 min, 50 min and 60 min post induction, respectively, when compared to the control group. However, the rectal temperature of goats in group C decreased significantly ($p < 0.05$) at 40 min post induction when compared to the control and other groups. The rectal temperature of goats in group C has no significant ($p > 0.05$) variation except at 40 min post induction when compared to the control group. However, it was significantly low from 10 min to 60 min post induction when compared to groups B and D (Figure 7).



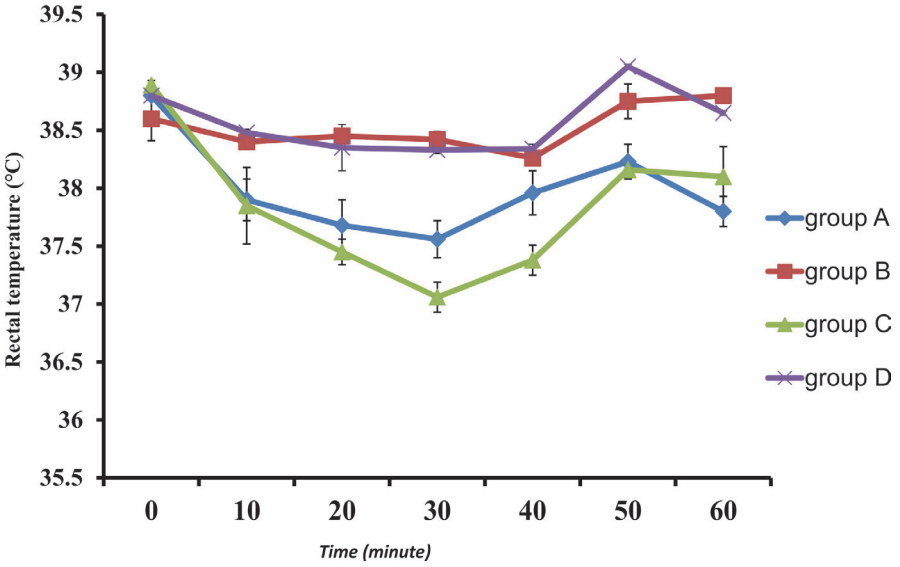
Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 5 Mean (±SEM) heart rate of goat undergone laparotomy following intravenous administration of propofol combinations



Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 6 Mean (±SEM) respiratory rate of goat undergone laparotomy following intravenous administration of propofol combination



Group A: propofol alone. **Group B: xylazine/propofol.**
Group C: tramadol/ propofol. **Group D: lignocaine/ propofol.**

Figure 7 Mean (±SEM) rectal temperature of goat undergone laparotomy following intravenous administration of propofol combinations

Table 1 Mean (SEM±) anesthetic maintenance following intravenous administration propofol combinations

Group	No. of maintenance	Quantity of drug (mls)
A	4.00 ± 0.12^{d}	17.00 ± 0.85^{d}
B	2.00 ± 0.06^{b}	8.82 ± 0.30^{b}
C	3.00 ± 0.11^{c}	13.42 ± 0.53^{c}
D	1.00 ± 0.08^{a}	4.35 ± 0.10^{a}

^{abc} figures with different superscripts in the same column are significantly different ($p < 0.05$)

DISCUSSION AND CONCLUSION

Propofol administration is known to provide short onset and duration of anesthesia, no analgesia, and a smooth and rapid recovery (Sha et al., 2013; Ukwueze et al., 2014, and Potliya et al., 2015).

The onset of propofol anesthesia in this study was short (> 1 min). Rapid onset of action is caused by rapid uptake of propofol into the central nervous system (CNS) and induction of depression that occur by enhancing the effect of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and decreasing the brains metabolic activity (Maravi et al., 2018). However, the onset of action was shorter in the group premedicated with xylazine (group B) than in other groups. This may be attributed to sedative and depressive effect of xylazine on CNS (Potliya et al., 2015; Carvalho et al., 2016, and Bayramoglu et al., 2016) before the administration of propofol. In addition, the synergistic and additive interaction between propofol and xylazine at the level of sedation and induction, respectively, may lead to a very short onset of action time observed (Naser and Mohammad, 2014).

Propofol has little or no analgesic properties (Potliya et al., 2015; Desai et al., 2016, and Karacaer et al., 2018). This was confirmed in this study where the use of propofol alone in group A provides short duration of analgesia despite high maintenance doses and quantity of propofol used in the group. However, propofol combination with xylazine and lignocaine provides analgesic duration enough to perform laparotomy. The duration of analgesia was quite longer with xylazine and lignocaine combinations than that of tramadol, while propofol combination with lignocaine provided the longest duration of action. Xylazine provides analgesia through its action on the autonomic and central nervous system (Biobaku et al., 2016). Its inhibition of locus coeruleus neurons in the pons of the lower brain stem, which attenuate the sympathoadrenal responses to noxious stimuli encountered during anesthesia and surgery, and provide hemodynamic, metabolic and hormonal stability (Potliya et al., 2015, and Bayramoglu et al., 2016). Xylazine reduces the release of noradrenalin and dopamine in the central nervous system; thus, it leads to sedation, muscular relaxation and reduction of perception of painful stimuli (Bayramoglu et al., 2016). Longer duration of analgesia, along with prolonged recovery time, was reported in dogs after premedication of propofol with xylazine (Sharma and Bhardwaj, 2010; Potliya et al., 2015). Conversely, lignocaine, amid a group of local anesthetics, provides analgesia by blocking a nerve fiber impulse through binding and inhibition of voltage-gated sodium channels (Raphael et al., 2002) and thereby provides analgesia that lasted up to 54.3 ± 5.2 minutes. Furthermore, there is no receptors competition or interaction between lignocaine and propofol (Tafur-Betancourt, 2017), and the rate of absorption and elimination of lignocaine is slow due to adrenaline incorporation (Weinberg et al., 2015). These factors might be responsible and may explain the findings in this study.

Sleeping and recumbent time were longer in the xylazine group (group B) and shorter in the tramadol group (group C). The longer sleeping and recumbent duration in group B may be a result of sedative and muscle relaxant effect of the xylazine (Shah et al. 2013, and Potliya et al., 2015). Tramadol has been reported to cause central nervous depression in humans, cat, dogs, and horse (Jong-pil Seo et al., 2011). Contrarily, in this study, no obvious sign of CNS depression was observed after administration of tramadol prior to administration of propofol; rather, excitement was observed. Short duration of sleeping and recumbent time was also observed following propofol administration. Though, it is a known fact that opioids decrease sleep time and efficiency (Barber, 2011, and Dimsdale et al., 2007).

Standing time, the period it takes an animal to stand up following anesthesia is an indicator that the animal is approaching almost full recovery stage of anesthesia. The use of propofol alone as in group A was associated with a short period of standing time due to its known fast recovery property (Ukwueze et al., 2014; Potliya et al., 2015), while xylazine combination prolonged the standing time when compared to other combinations used in this study. The prolonged standing time may be attributed to a hypnotic and powerful central muscular relaxant

effect of xylazine, leading to muscle weakness and fatigue (Ahmad et al., 2018). Tramadol also causes sensory and motor nerve conduction blockade, mediated through voltage-gated sodium channels leading to the axonal blockade (Gupta and Bhalotra, 2019). Lignocaine may have more effect on sensory nerve than locomotors nerve hence the short standing time compared to other groups.

The significant increase in the heart rate of groups A (propofol alone) and C (tramadol) during laparotomy procedure may be a result of increased pain stimuli emanating from the laparotomy incision. This showed that propofol alone and in the combination with tramadol did not provide enough analgesia during laparotomy procedure. An increased heart rate is one of the signs of nociception in both human and animal (Sacco et al., 2013), especially when decrease in the heart rate is expected. This also shows that pain response may alter the depressive effect of drugs on cardiac function.

The administration of propofol alone and propofol combinations used in this study depressed respiratory function of the goats leading to apnea. Propofol has a respiratory depressant effect (Ukwueze et al., 2014; Potliya et al., 2015, and Shanovic et al., 2018). However, propofol in combination with xylazine caused more obvious significant respiratory depression due to the combined depressive effect of both drugs on the respiratory center of the brain (Ukwueze et al., 2014). Alpha-2 agonists induce prolonged depression of thermoregulation and depress hypothalamic noradrenergic alpha-2 adrenergic receptors to cause hypothermia (Kumar et al., 2018). It is very important to take note of this serious complication in the use of propofol and xylazine combination. The cause of increase in respiratory rate in the control group (propofol alone) during laparotomy procedure despite the high quantity of anesthetic maintenance used, may also be attributed to pain stimuli because of the laparotomy procedure.

The decrease in the rectal temperature in the xylazine / propofol combination was in accordance with previous findings (Brohi et al., 2019; Ukwueze et al., 2014; Amarpal et al., 2002, and Carroll et al., 1998). The reduction in rectal temperature is considered secondary to CNS depression and reduction in the muscular activity (Kammar et al., 2014). Both drugs may have a depressive effect on the thermoregulatory center of the brain (Maravi et al., 2018). Tramadol and lignocaine have less depressive effect on temperature (Habiban et al., 2011; Ayman et al., 2015, and Ajadi et al., 2017). They seem to have a stabilizing effect on the propofol thermoregulatory depressive effect, as observed in this study. However, the significant drop in temperature at 40 min post induction in tramadol/propofol combination might be a result of the overriding effect of propofol against tramadol probably due to the large volume of the propofol used in anesthetic maintenance in that group.

The duration of analgesia was quite long with xylazine combination and short in tramadol combination, while propofol combination with lignocaine provided the longest duration of action. Sleeping, recumbent and standing time were longest in the xylazine group (group B), followed by lignocaine group and shortest in the tramadol group (group C). Both decrease and increase in physiologic parameters were observed more in xylazine and tramadol combinations than in the lignocaine combination which had more stable hemodynamic effects. Therefore, lignocaine combination significantly ($p < 0.05$) provided better analgesic, anesthetic and hemodynamic effects with moderate sleeping, recumbent and standing time when combined with propofol for induction of anesthesia during laparotomy in West Africa Dwarf goat.

CONFLICT OF INTEREST

The authors declare no competing interests.

CONTRIBUTION

Concept – OCU, CAE, AAO; Design – OCU, CAE, AAO; Supervision - CAE, AAO; Materials – OCU; Data Collection and Processing – OCU; Interpretation – CAE, AAO; Literature Search – OCU; Writing Manuscripts – OCU; Critical Review - CAE, AAO

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EVALUACIJA ANALGEZIČKOG EFEKTA KSILAZINA, TRAMADOLA I LIGNOKAINA NA ANESTETIK PROPOFOL KOD ZAPADNOAFRIČKE PATULJASTE KOZE (WAD)

SAŽETAK

Dvadeset klinički zdravih zrelih mužjaka zapadnoafričke patuljaste koze (WAD) starih između 10 i 12 mjeseci sa srednjom težinom (±SEM) od 17.5 kg je randomizirano u četiri grupe, A, B, C i D sa po pet koza u svakoj. Grupe A, B, C i D su primile fiziološku otopinu (2 ml IV), ksilazin (0.05 mg/kg tjelesne težine (TT) intravenski [IV]), tramadol (2 mg/kg TT IV) i lignokain (2.5 mg/kg TT, subkutana infiltracija u operativnom području [SC]). Sve koze iz svih grupa su anestezirane sa propofolom u dozi od 9 mg/kg TT, dok je doza održavanja iznosila 6 mg/kg TT u bolusu. Na svim kozama iz svih grupa je izvedena laparotomija. Mjereni su anesteziološki indeksi i vitalni parametri. Sam propofol (grupa A) i kombinacija propofol/tramadol (grupa C) su izazvali kratkotrajnu analegeziju uprkos visokim dozama održavanja i količni propofola koja je korištena u grupama. Međutim, kombinacija propofol/ksilazine (grupa B) je izazvala analgeziju koja je trajala dovoljno za laparotomiju, dok je kombinacija propofol/lignokain (grupa C) imala najduže djelovanje. U našem istraživanju, kombinacije i lignokaina i ksilazina su izazvale dostatnu analgeziju za izvođenje operativne procudur. Kombinacija ksilazina je, međutim, povezana sa prolongiranim snom, dužinom ležanja i oporavka, i kardijalnom i respiratornom depresijom koje su kod preživača nepoželjne jer izazivaju kardiopulmonalne i gastrointestinalne komplikacije. Osim što izaziva umjereno spavanje i vrijeme ležanja i oporavka, lignokain ima stabilizirajući efekt na kardijalne funkcije. Stoga se lignokain koji izaziva dugotrajniju analgeziju, hemodinamske efekte i umjerene anesteziološke indekse smatra anestetikom izbora u odnosu na druge lijekove kao kombinirani agens sa propofolom za indukciju anestezije prije izvođenja bolnih operativnih procedura kod zapadnoafričke patuljaste koze (WAD).

Ključne riječi: Analgezija, ksilazin lignokain, propofol, tramadol, WAD koza

CASE REPORT

COMPLICATIONS AFTER INCOMPLETE DENTAL EXTRACTIONS IN A CAT

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ABSTRACT

Exodontics is one of the most commonly performed procedures in veterinary dentistry. One of the essential steps in every dental procedure is taking preoperative and postoperative dental radiographs in order to determine the severity of the disease, root abnormalities and retained root fragments. Present case report describes a cat with signs of oral inflammation and discomfort after previous dental treatment due to signs of gingivostomatitis one month earlier. Thorough dental examination and dental radiographs revealed retained root fragments of teeth 208, 209, 308 and 309 on the left side of the maxilla and the mandible. Extraction of retained root fragments and alveolectomy were performed, followed by postoperative antimicrobial treatment. Three weeks after the procedure, the extraction sites were healing and the patient started showing usual behaviour.

Keywords: Cat, exodontics, retained roots

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INTRODUCTION

Exodontics, also known as tooth extractions, is one of the most commonly performed procedures in veterinary dentistry. There is a series of indications for tooth extraction, some of them being fractured teeth which cannot be restored, supernumerary teeth, persistent deciduous teeth, teeth affected by periodontal disease, severe chronic gingivo-stomatitis and teeth affected by odontoclastic resorption (Tutt, 2008; Dučić et al., 2023). Surgical approach is required in most cases, especially when multirooted teeth are affected (Moore and Niemiec, 2014). One of the essential steps in every dental procedure is taking preoperative and postoperative dental radiographs, in order to determine the severity of the disease, root abnormalities and retained root fragments (Niemiec, 2009; Moore and Niemiec, 2014; Shannon, 2017).

Even though dental diseases have a high prevalence in veterinary medicine, research suggests that doctors of veterinary medicine receive inadequate education in small animal dentistry during their studies (Fitzpatrick and Mellor, 2003; Gorrel, 2013; Anderson et al., 2017). When taking into consideration lack of necessary equipment and insufficiently developed skills, several complications associated with extractions can occur. Some of them include: tooth fractures, mandible/maxilla fractures, damage of adjacent teeth, iatrogenic oro-nasal communication, and repulsion of tooth root fragments into mandibular or infraorbital canal (Reiter et al., 2004). Remaining root fragments are a common complication when extracting cat's teeth. Tooth roots are often curved or hooked which makes their extraction very difficult (Woodward, 2006). Retained root fragments can cause periapical pathology, and their extraction is obligatory in most cases (Moore and Niemiec, 2014). This case report describes a cat presented with signs related to oral inflammation, oral discomfort and worsening of the general health condition after previous dental treatment.

CASE DESCRIPTION

A 5-year-old 3.2-kg female domestic shorthair cat was referred to the Clinic for Veterinary Dentistry at Veterinary Faculty in Sarajevo for a dental examination. The patient had received dental treatment involving multiple tooth extractions at another clinic one month prior to this visit due to signs of gingivostomatitis. The owner mentioned that the patient hadn't been eating properly since the treatment, and only used its right side of the mouth. The patient was an indoor/outdoor cat living in a multi-cat household. On physical examination, the patient was apathic and showed signs of oral pain and discomfort with notable hypersalivation and halitosis. Oral evaluation revealed severe inflammation of periodontal tissues with spontaneous bleeding of the gingiva when being touched, along with several root remnants located in the left side of the maxilla and the mandible. No root remnants were observed on the right side of the maxilla and the mandible.

Complete blood count (CBC), biochemical profile and SNAP FIV/FeLV Combo test were performed. The blood tests revealed neutrophilia (20.38×109/L), monocytosis (1.61×109/L), eosinophilia (1.64×109/L), hyperglycemia (16.56 mmol/L), hyperglobulinemia (67 g/L) and increased TP level (94 g/L). The patient tested positive for FIV.

In order to perform a more detailed oral examination and take dental radiographs, anesthetic induction was performed with intramuscular medetomidine-hydrochloride (80 µg/kg), butorphanol (0.4 mg/kg) and ketamine (5 mg/kg). Endotracheal intubation was performed using Magill type 3.5 mm cuffed endotracheal tube. One perioperative injection of meloxicam (0.2 mg/kg SC) was administered. Full-mouth intraoral radiographs were obtained and revealed the presence of fractured roots of the fourth premolar (tooth 208) and first molar (tooth 209) in the left side of the maxilla (Figure 1), and the third premolar (tooth 308) and first molar (tooth 309) on the left side of the mandible (Figure 2). The teeth were fractured at the point where the crown meets the dental cervix. The

fourth maxillary premolar (tooth 208) has a large distal root and two thinner mesial roots (buccal and palatal), while the first maxillary molar (tooth 209) is a small, two-rooted tooth. The mandibular third premolar (tooth 308) has two roots, mesial and distal, whereas the mandibular first molar (tooth 309) has a large mesial root and a very thin distal root.

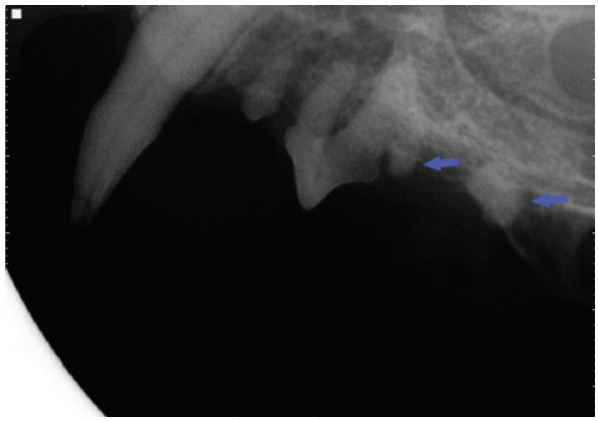

Figure 1 Left side of the maxilla; arrows are showing remnants of roots of teeth 208 and 209

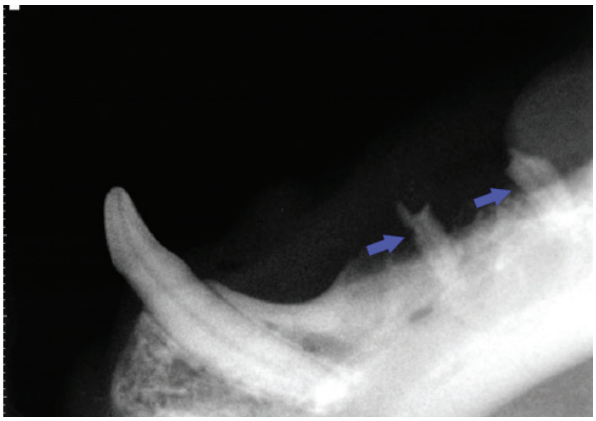

Figure 2 Left side of the mandible; arrows are showing remnants of roots of teeth 308 and 309

Left maxillary and mandibular nerve blocks were performed using 2% lidocaine-hydrochloride (2 mg/kg). Extraction was performed using 190/2 Bein root elevator with a 3 mm tip, followed by alveolectomy using a round bur on Alegra TE-95 high-speed turbine. The extraction sites were evaluated by dental radiography before being closed using 5-0 polydioxanone suture (Figures 3 and 4). Postoperative instructions included antimicrobial treatment (clindamycin, 11 mg/kg) and semi-liquid food for 10 days after the conducted method, along with liquid supplements.

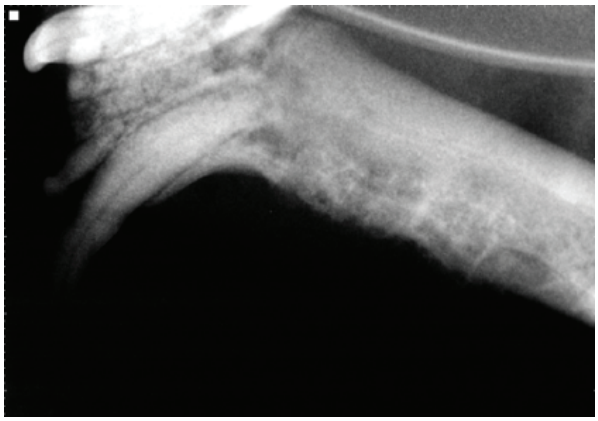

Figure 3 Postoperative radiograph of a successful extraction of teeth 208 and 209 root fragments

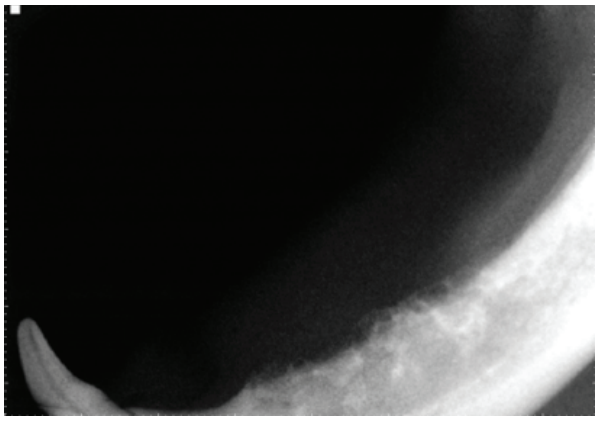

Figure 4 Postoperative radiograph of a successful extraction of teeth 308 and 309 root fragments

Reevaluation was carried out 3 weeks postoperatively. The extraction sites were healing and the owner mentioned that the patient started eating well and showing usual behaviour.

DISCUSSION AND CONCLUSION

Preoperative and postoperative dental radiographs were not performed during the initial dental treatment, nor was the owner aware of the retained root fragments.

In another case report, an author describes a cat with local and systemic complications after incomplete dental extractions, where 22 root remnants were retained (Reiter et al., 2004). A study conducted by Moore and Niemiec (2014) revealed that the rate of complete extractions was very low, and retained root fragments caused periapical pathology in 39 of 74 dogs and 27 of 42 cats evaluated in the study. A retained mesial root of the first mandibular molar associated with periapical periodontitis was detected in a male Pomeranian dog after previous dental treatment where extractions were performed (Shannon, 2017). In a research done by Ng et al. (2020), it was observed that 85 of 383 dogs that underwent a full-mouth radiographic evaluation had at least one retained tooth root fragment. A report by Galante and Beard (2004) described a chronic draining tract of the left mandible related to a retained root of the left mandibular canine tooth.

Since December 2022, there have been 7 other cases with the retained root fragments recorded at the Clinic for Veterinary Dentistry at University of Sarajevo. These patients have been treated at different clinics. However, the present case report describes a patient whose overall condition was worsening.

Dental radiography is an essential part of every dental procedure, especially when planning surgical extractions. Preextraction radiographs enable determination of the severity of a disorder, whereas postextraction radiographs serve as a proof that the extraction was performed successfully, and no root fragments were left (Niemiec, 2009; Lemmons, 2013). A very important segment of pain management in dental procedures such as the one described in this case report, is dental nerve blocks. Knowledge of the location and dimensions of the foramina in a cat's head, as well as the position and morphology of the teeth, is crucial for successful administration of dental nerve blocks prior to dental procedures (Dučić et al., 2024; Tandir et al., 2024).

Present-day veterinary curricula offer little or no training in techniques of surgical exodontics and dental radiography (Greenfield et al., 2004; Moore and Niemiec, 2014). Data from a survey done by Anderson et al. (2017), showed lack of curricular time dedicated to veterinary dentistry in veterinary schools in the USA, Canada and the Caribbean. A cross-sectional study of all UK final year veterinary students done by Perry (2014) showed that less than 40% of students felt that the teaching had prepared them for entering practice, and over 50% reported that they didn't feel confident in discussing dental problems with clients or performing oral examinations.

At Veterinary Faculty – University of Sarajevo, dental morphology of domestic animals is studied only as an elective subject in the third semester. However, this subject has been integrated in the curriculum 10 years ago, which means that doctors of veterinary medicine who graduated before that received little to no knowledge about the teeth morphology. Knowledge of teeth morphology is crucial in almost all dental procedures, along with dental radiography and adequate instruments. The lack or deficiency of any of these components will lead to professional errors that can cause both local and systemic complications in patients. Considering the fact that this is not the first case we have encountered, it is evident that there is a need for better education in the field of dental morphology and veterinary dentistry at University of Sarajevo. This would include more hours of the subject Dental morphology of domestic animals, as well as obligatory clinical practice at the Clinic for Veterinary Dentistry. Organization of educational courses for veterinarians who work in small practice in order to draw their attention to the importance of the teeth morphology and the correct use of dental equipment is also considered as an option. Additionally, a variety of educational videos are available on the Internet that can serve as a source of useful information in the field of veterinary dentistry.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – FT, ND; Supervision – FT, RA; Data Collection and/or Processing – ND, AV; Analysis and/or Interpretation – FT, AV; Literature Search – ND, RA; WritingManuscript – ND, FT; Critical Review – FT, RA, RT.

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KOMPLIKACIJE NEPOTPUNIH DENTALNIH EKSTRAKCIJA KOD MAČKE

SAŽETAK

Egzodoncija predstavlja jednu od najčešće izvođenih procedura u veterinarskoj stomatologiji. Jedan od osnovnih koraka u svakoj dentalnoj proceduri jeste preoperativno i postoperativno rendgensko snimanje koje se izvodi sa ciljem određivanja težine bolesti i dijagnosticiranja abnormalnosti korijena i fragmenata zaostalih korijena. Naš prikaz slučaja opisuje mačku sa znacima oralne inflamacije i nelagode nakon prethodnog dentalnog tretmana izvedenog zbog znakova gingivostomatitisa mjesec dana prije. Kompletan dentalni pregled i dentalni rendgenogrami su pokazali postojanje fragmenata zaostalih korijenova zuba 208, 209, 308 i 309 na lijevoj strani maksile i mandibule. Izvedeni su ekstrakcija fragmenata zaostalih korijenova i alveleoktomija uz postoperativni antimikrobni tretman. Tri sedmice nakon procedure područja ekstrakcije su zarastala, a pacijent je počeo pokazivati znakove uobičajenog ponašanja.

Ključne riječi: Egzodoncija, mačka, zaostali korijeni

PROFESSIONAL PAPER

HIGIJENSKA ISPRAVNOST, KVALITET I ODRŽIVOST VAKUUM UPAKIRANOG „BRČANSKOG PRŠUTA"

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SAŽETAK

Sušenje mesa datira još iz doba Rimljana koji su u svojim sušnicama sušili razne vrste mesa koje su konzumirali moreplovci trgovci s juga, a vremenom je suho meso stiglo i u sjeverne evropske zemlje. Ipak, bitno je naglasiti da tu nije riječ o suhom govođem mesu koje je tradicionalno za naše područje.

Tradicija pravljenja suhog mesa u našoj zemlji je stara vijekovima, a neki proizvođači su i danas zadržali stari način pravljenja ovog proizvoda. Miris i okus suhog mesa se razlikuje od područja do područja pa tako zavisi od klime, nadmorske visine, načina ishrane, vrste goveda, ali veliku ulogu ima sušnica, način soljenja, priprema robe u smislu da li se meso ohladilo nakon klanja, itd.

Na kvalitet, higijensku ispravnost, kao i održivost utječu brojni faktori od pravilnog tehnološkog procesa proizvodnje do manipulacije finalnim proizvodom. Jedna od mogućnosti produženja roka upotrebe, održavanje bolje kvalite, kao i higijenske i mikrobiološke ispravnosti je i vakuumiranje gotovih proizvoda.

Keywords: Meso, pršut, vakuumiranje

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UVOD

Higijenska ispravnost i održivost suhomesnatih proizvoda na tržištu uvjetovana je zastupljenošću i kvantitetom vrste mikroorganizama. Posebno značenje u tom smislu imaju nespecifične bakterije koje su gotovo uvijek prisutne u suhomesnatim proizvodima.

Gubitak kvaliteta namirnica djelovanjem mikroorganizama može biti u vidu infekcija koje se prenose namirnicama, zatim kao intoksikacije i najzad kao kvar namirnica. Ni jedan od ovih vidova mikrobne aktivnosti u namirnicama ne može se spriječiti sistemom represivnih mjera, koji podrazumijeva uzimanje uzoraka namirnica, vršenje njihovog ispitivanja i procjenjivanje dobivenih rezultata. Sprečavanje bolesti koje se prenose namirnicama i sprečavanja kvara namirnica mnogo bolje se postiže dobrom higijenskom praksom tokom proizvodnje i distribucije. Sa stanovišta higijene i tehnologije mesa mikroorganizme smo podijelili u četiri grupe:

1. mikroorganizmi koji se koriste u preradi mesa (jer izazivaju korisne promjene u mesu te se sve više, ova grupa mikroorganizama koristi u tehnologiji mesa),
2. mikroorganizmi indikatori (jer služe kao indikatori, odnosno pokazuju da li je obrada mesa ili nekog proizvoda od mesa rađena u odgovarajućim higijenskim i tehnološkim uslovima),
3. patogeni mikroorganizmi (mogu biti štetni po zdravlje ljudi ili životinja pa se zato i zovu patogeni i njihovo prisustvo se ne dozvoljava u finalnim proizvodima),
4. mikroorganizmi koji izazivaju kvarenje mesa i proizvoda od mesa (najveća je grupa mikroorganizama i upravo primarni zadatak tehnologije i higijene mesa je da se ne dozvoli njihovo razmnožavanje i uopće da se njihovo prisustvo svede na razumnu mjeru).

Sama mikrobiologija mesa obuhvata, između ostalog dva velika područja: prvo je zaštita potrošača od bolesti čiji su uzročnici mikroorganizmi koji se mesom prenose na čovjeka i drugo sprečavanje kvara mesa pod dejstvom mikroorganizama. Ovakva podjela je teško opravdana, ali se često radi iz praktičnih razloga. U prilog ovakvoj podjeli govori i činjenica da su mikroorganizmi koji imaju zdravstveni značaj po pravilu različitog porijekla od mikroorganizama koji kvare meso (Hadžibeganović et al. 1975).

Mikrobiološke norme u namirnicama i literature koja ih tretira je skoro nepregledna ,ali se u njoj najčešće pominju:

- mikrobiološke specifikacije – tj. maksimalno prihvaćeni brojevi mikroorganizama ili određenih grupa mikroorganizama za internu upotrebu u preduzeću (specifikacije često postavljaju i kupci na veliko)
- maksimalni broj, tj. maksimalne vrijednosti mikroorganizama za određene proizvode (uputstva) i -standardi, tj. propisima utvrđene norme.

Ovo pitanje mikrobioloških standarda za namirnice je top tema u većini zemalja dugi niz godina, a posebno danas s ekspanzijom mnogih bolesti (koje se prenose i namirnicama animalnog porijekla). U našoj zemlji mikrobiološka ispravnost suhomesnatih proizvoda načelno je regulisana Pravilnikom o mikrobiološkim kriterijumima za hranu ("Službeni glasnik BiH", 2013).

Za proizvodnju mikrobiološki ispravnih suhomesnatih proizvoda važno je da su sirovo meso i druge ingredijencije koje se koriste u tehnološkoj proceduri ispravni, ali isto tako i zadovoljavajući mikroklimatski uslovi koji vladaju u procesu proizvodnje i prometa. Svi navedeni uslovi su se sprovodili i u toku tehnološkog procesa proizvodnje vakuumiranog Brčanskog pršuta.

Nakon završenog tehnološkog postupka proizvodnje u suhomesnatim proizvodima, mogu se, zbog neadekvatnog uskladištenja ili neodgovarajuće manipulacije javiti određene organoleptičke promjene, koje su najčešće tiču mesa koje potječe od bolesnih ili nedovoljno odmorenih životinja, mesa koje je poslije klanja ostalo predugo neprerađeno, nedovoljno

iskrvareno, rashlađeno itd. Razlog kvarenju može biti i pokvarena salamura, odnosno previsoka temperature salamurenja. Najčešći vid kvarenja je gnjilenje koje obično počinje već za vrijeme prerade. Trulenje ili raspadanje se većinom javlja, odnosno razvija u toku proizvodnje, ali može dosta rjeđe da se javi i zbog dugog i neadekvatnog uskladištenja. Uzrok je višestruk, počev od mesa koje može da potiče od bolesnih, uznemirenih ili umornih životinja pa do pokvarene salamure (mutna, neprijatnog i teškog mirisa, pjenušava itd.) ili salamurenja na visokim temperaturama (Jensen et al., 2004).

Promjene nastaju adekvatno uzrocima i većinom počinju u dubini proizvoda što se može posumnjati po mekšoj konzistenciji proizvoda, a posebice pri zarezivanju (boja blijeda, siva, miris ogavno sladunjav na trulež, a nekad sladunjav ili oštro nakiseo kao i ukus, itd.). Od ostalih promjena koje se javljaju kao greška na suhomesnatim proizvodima najčešće su promjene koje nastaju u boji, mirisu, ukusu, kao i konzistenciji. Siva boja nastaje kod nedovoljnog salamurenja pri niskim temperaturama (ispod 6OC) i lošeg sastava salamure, itd. Blijeda boja nastaje pri upotrebi blijedog, vodnjikavog mesa, itd (Toldrá, F. 2010).

Kod suhomesnatih proizvoda može se pojaviti i oksidacija masnog tkiva, odnosno užeženost koja se obično javlja kod neadekvatnog skladištenja (svijetla prostorija, viša temperatura itd.), pri čemu je ovo tkivo žućkaste boje sa specifičnim mirisom i oštrim ukusom (Smajić A. 2014.).

Suhomesnate proizvode napadaju i insekti i to najčešće larve muhe sirare (Phiophilacasei). Osim ove muhe i drugi insekti mogu napasti suhomesnate proizvode kao što su sjajna – svijetla buba (Nitidulidae), crvenonoga buba šunke (Necrobiarufioes), slaninska buba (Dermesteslardarius), zatim gusjenice leptira (Aglossa pinquinalis - masna bubašvaba), larve nekih moljaca (Dysmassiaparietarella), grinje brašna (Tyroglophusfarinae) i druge. Navedene larve kod konzumenata izazivaju gađenje, a neke (muha sirara) mogu prouzrokovati i enteritise (Matković, 2018).

Nadalje, kao nedostatak suhomesnatih proizvoda je pojava tirozinskih kristala, koji nastaju u procesu salamurenja, odnosno dimljenja. Na prerezu proizvoda manifestuju se u vidu bjeličastih zrnaca, jasno su uočljiviji, a obično pobuđuju sumnju na inkalcinirane parazite (trihinela, sarkosporidije itd.) (Virgili et al.1995).

Ponekad se pri pregledu suhomesnatih proizvoda može utvrditi i miris po karbolu. Javlja se najčešće kao posljedica korištenja vlažnog drveta i strugotina za dimljenje jer tada dolazi do stvaranja para fenola koje daju navedeni miris, ali zna poprimiti i mirise okoline te ih meso lako apsorbira. Taj fenomen se naročito dešava kad se meso skladišti u prostorijama ili prevozi u transportnim sredstvima u kojima su prethodno držane neke materije jakog mirisa (eter, hlor, benzin, karbol deterdženti, dezodoransi, itd.), odnosno koje nisu dovoljno provjetrene poslije krečenja zidova, DDD, itd. (Živković, J. i Hađiosmanović 1996).

Meso, kako sirovo tako i prerađeno, u samom toku proizvodnje, kao i skladištenja i prometa, može se kontaminirati mehaničkom nečistoćom, hemijskim materijama (dezinficijensi, pesticide, i dr.), koji mogu izmijeniti organoleptička svojstva mesa i tako predstavljati potencijalnu opasnost po zdravlje ljudi.

Upravo da bi se pokušalo sve navedeno prevenirati, odnosno izbjeći u procesu proizvodnje Brčanskog pršuta, korišten je postupak pakovanja, odnosno vakuumiranje proizvoda. Pakovanje je veoma značajno za očuvanje svježeg ali i proizvoda od mesa. Danas je nemoguće zamisliti razvoj savremenih tehnologija bez odgovarajućeg pakovanja.

Vakuumiranje je vid dugotrajnog skladištenja, odnosno čuvanja namirnica izvlačenjem kiseonika iz vrećica za pohranu (čuvanje) namirnica. Naime, zrak je uz vlagu i temperaturu glavni krivac kvarenja hrane. Kvalitetno pakovanje kao što je vakuum, ne služi samo da zaštiti meso od deformacija i oštećenja, nego i od kontaminacije kao i svih drugih štetnih utjecaja. Vakuum pakovanje ima i sljedeće prednosti: smanjuje se gubitak težine, jer sprečava dehidraciju, održava

se boja mesa, osiguravaju se bolji higijenski uslovi, vrši se zaštita mesa od spoljnih agenasa, osiguravaju se bolja organoleptička svojstva mesa (mekoća, nježnost), bolje i racionalnije se koristi prostor za skladištenje, smanjuje se cijena transporta itd.

Materijal za vakuum pakovanje treba da je čvrst, nepropustan i otporan na gasove. Za pakovanje Brčanskog pršuta korištena je dupleks folija (PVC). Ovaj rad prati kvalitet, higijensku ispravnost i održivost vakuumiranog Brčanskog pršuta suho, odnosno vlažno soljenog kroz period od 185 dana na dva temperaturna režima +7 C i +15 C.

MATERIJAL I METODE RADA

U toku tehnološkog procesa proizvodnje provjerena je opravdanost različitih postupaka konzerviranja i utjecaj vakuumiranja na higijensku ispravnost, kvalitet i odživost finalnog proizvoda.

Obavljene su sljedeće pretrage:

1. organoleptička pretraga: ocjena svakog pojedinačnog uzorka je vršena opisno i bod sistemom od strane sedmočlane komisije, različitog spola i dobne starosti (Pravilnik o organizaciji službenih kontrola proizvoda životinjskog porijekla namijenjenih ishrani ljudi Službeni glasnik BiH, 2012; Listrat AB, 2016)
2. mikrobiološke pretrage: vršena su ispitivanja tokom skladištenja od 185 dana na mikroorganizme (aerobne mezofilne, psihrofilne i halogilne bakterije i dr.), kvalitativno-kvantitativnom metodom (Pravilnik o mikrobiološkim kriterijumima za hranu ("Službeni glasnik BiH", 2013).
3. fizikalno-hemijska ispitivanja: sadržaj vode, masti, ukupnih mineralnih materija, NaCl, ukupnih šećera, pH (Pravilnik o ustinjenom mesu, poluproizvodima i proizvodima od mesa "Službeni glasnik BiH", 2013)

REZULTATI

Vakuumirani *Brčanski pršut* proizveden od suho, odnosno vlažno soljenog mesa u toku skladištenjana 7^0 C odnosno 15^0 C do 185 dana nije kalirao, odnosno gubio na težini (Tabela 1,2).

Table 1 Kaliranje suho, odnosno vlažno soljenog vakuumiranog Brčanskog pršuta tokom skladištenja na 7^0 C do 185 dana

Table 1 Nominal losses of dry-salted and wet-salted vacuum-packed Brčko prosciutto during storage at 7^0 C for up to 185 days

		Masa u g (srednjavrijednost)			
Dužina skladištenja – dani	Broj uzoraka	Suho Soljeno	Kalo %	Vlažno soljeno	Kalo %
1	10	695,00	-	610,00	-
25	10	695,00	-	610,00	-
40	10	695,00	-	610,00	-
60	10	695,00	-	610,00	-
75	10	695,00	-	610,00	-
90	10	695,00	-	610,00	-
110	10	695,00	-	610,00	-
125	10	695,00	-	610,00	-
185	10	695,00	-	610,00	-

Table 2 Kaliranje suho, odnosno vlažno soljenog vakuumiranog Brčanskog pršuta tokom skladištenja na 15^0 C do 185 dana

Table 2 Nominal losses of dry-salted and wet-salted vacuum-packed Brčko prosciutto during storage at 15^0 C for up to 185 days

		Masa u g (srednjavrijednost)			
Dužina skladištenja – dani	Broj uzoraka	Suho Soljeno	Kalo %	Vlažno soljeno	Kalo %
1	10	745,00	-	710,00	-
25	10	745,00	-	710,00	-
40	10	745,00	-	710,00	-
60	10	745,00	-	710,00	-
75	10	745,00	-	710,00	-
90	10	745,00	-	710,00	-
110	10	745,00	-	710,00	-
125	10	745,00	-	710,00	-
185	10	745,00	-	710,00	-

Vakuumirani *Brčanski pršut* proizveden od suho, odnosno vlažno soljenog mesa skladišten na 7 i 15^0 C do 185 dana nije pokazivao odstupanja u fizikalno-hemijskim parametrima, odnosno sadržaj vode, NaCl-a i ukupnih šećera zadržan je do kraja skladištenja u približno inicijalnim vrijednostima.

Table 3 Fizikalno-hemijski parametri suho, odnosno vlažno soljenog vakuumiranog Brčanskog pršuta skladištenog na 7^0 C do 185 dana

Table 3 Physico-chemical parameters of dry-salted and wet-salted vacuum-packed Brčko prosciutto stored at 7^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Fizikalno – hemijskiparametri			
			Voda %	NaCl %	Ukupni šećer %	pH
1	5	suho	50,19	5,52	0,20	5,36
	5	vlažno	56,84	4,43	0,20	5,31
90	5	suho	50,04	5,53	0,20	5,39
	5	vlažno	56,17	4,47	0,20	5,39
185	5	suho	49,86	5,56	0,20	5,41
	5	vlažno	55,42	4,50	0,20	5,43

Table 4 Fizikalno-hemijski parametri suho, odnosno vlažno soljenog vakuumiranog Brčanskog pršuta skladištenog na 15^0 C do 185 dana

Table 4 Physico-chemical parameters of dry-salted and wet-salted vacuum-packed Brčko prosciutto stored at 15^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Fizikalno – hemijskiparametri			
			Voda %	NaCl %	Ukupni šećer %	pH
1	5	suho	50,19	5,52	0,20	5,36
	5	vlažno	56,84	4,43	0,20	5,31
90	5	suho	49,94	5,55	0,20	5,68
	5	vlažno	55,93	4,47	0,20	5,60
185	5	suho	49,75	5,59	0,20	5,50
	5	vlažno	55,02	4,54	0,20	5,51

Inicijalne i završne vrijednosti mikroorganizama po danima skladištenja.

Table 5 Zastupljenost mikroorganizama u suho, odnosno vlažno soljenomu vakuumiranom uzorku Brčanskog pršuta skladištenog na 7^0 C do 185 dana

Table 5 Distribution of microorganisms in dry-salted and wet-salted vacuum-packed sample of Brčko prosciutto stored at 7^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Aerobne mezofilne bakterije u 1g	Psihrofilne bakterije u 1g	Halofilne bakterije u 1g
1	5	suho	$5,3x10^3$	$1,8\ x10^2$	$5,9\ x10^2$
	5	vlažno	$5,5\ x10^3$	$2,0\ x10^2$	$6,1\ x10^2$
90	5	suho	$5,6\ x10^3$	$3,2\ x10^2$	$6,4\ x10^2$
	5	vlažno	$6,0\ x10^3$	$3,5\ x10^2$	$6,6\ x10^2$
185	5	suho	$6,2\ x10^3$	$4,2\ x10^2$	$7,0\ x10^2$
	5	vlažno	$6,3\ x10^3$	$4,4\ x10^2$	$6,8\ x10^2$

Table 6 Zastupljenost mikroorganizama u suho, odnosno vlažno soljenom vakuumiranom uzorku Brčanskog pršuta skladištenog na 15^0 C do 185 dana

Table 6 Distribution of microorganisms in dry-salted and wet-salted vacuum-packed sample of Brčko prosciutto stored at 15^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Aerobne Mezofilne bakterije u 1g	Psihrofilne bakterije u 1g	Halofilne bakterije u 1g
1	5	suho	$5,3 \times 10^3$	$1,8 \times 10^2$	$5,9 \times 10^2$
	5	vlažno	$5,5 \times 10^3$	$2,0 \times 10^2$	$6,1 \times 10^2$
90	5	suho	$5,8 \times 10^3$	$2,7 \times 10^2$	$6,2 \times 10^2$
	5	vlažno	$6,1 \times 10^3$	$3,4 \times 10^2$	$6,7 \times 10^2$
185	5	suho	$6,4 \times 10^3$	$4,6 \times 10^2$	$6,6 \times 10^2$
	5	vlažno	$6,6 \times 10^3$	$4,7 \times 10^2$	$6,9 \times 10^2$

Napomena: salmonele, koagulaza pozitivne stafilokoke, sulfit reducirajuće klostridije, E. coli te Proteus vrste nisu izolirane u ispitivanim uzorcima.

Vakuumirani *Brčanski pršut* proizveden od suho, odnosno vlažno soljenog mesa u toku skladištenja na 7^0 C odnosno 15^0 C do 185 dana nije pokazivao znatna odstupanja u organoleptičkim svojstvima te je cijelo vrijeme skladištenja zadržao svojstva I klase.

Table 7 Organoleptička ocjena suho odnosno vlažno soljenog vakuumiranog Brčanskog pršuta skladištenog na 7^0 C do 185 dana

Table 7 Organoleptic assessment of dry-salted and wet-salted vacuum-packed Brčko prosciutto stored at 7^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Organoleptička ocjena						Poeni	Klasa
			I	II	III	IV	V	VI		
1	10	suho	2,99	5,99	1,99	3,00	3,97	2,00	19,94	I
		vlažno	2,75	5,71	1,94	2,93	3,75	1,84	18,92	I
90	10	suho	2,99	5,87	1,99	2,98	3,99	1,98	19,80	I
		vlažno	2,82	5,83	1,84	2,82	3,84	1,83	18,98	I
185	10	suho	2,98	5,99	1,98	2,98	3,99	1,99	19,91	I
		vlažno	2,61	5,68	1,91	2,91	3,69	1,69	18,49	I

Legenda:

I - Spoljni izgled - bodovanje od 0-3
II - Sastav i izgled presjeka - bodovanje od 0-6
III - Konzistencija - bodovanje od 0-2
IV - Boja - bodovanje od 0-3
V - Ukus i miris - bodovanje od 0-4
VI - Prihvatljivost proizvoda - bodovanje od 0-2

Ekstraklasa 20 bodova
I klasa od 18,00 – 19,99
II klasa od 16,00 – 17,99
III klasa od 14,00-15,99
Van klase manje od 14,00 bodova

Table 8 Organoleptička ocjena suho odnosno vlažno soljenog vakuumiranog Brčanskog pršuta skladištenog na 15^0 C do 185 dana

Table 8 Organoleptic assessment of dry-salted and wet-salted vacuum-packed Brčko prosciutto stored at 15^0 C for up to 185 days

Dužina skladištenja – dani	Broj uzoraka	Vrsta soljenja	Organoleptička ocjena						Poeni	Klasa
			I	II	III	IV	V	VI		
1	10	suho	2,99	5,98	1,97	2,98	3,98	1,99	19,89	I
		vlažno	2,62	5,65	1,90	2,91	3,69	1,86	18,63	I
90	10	suho	2,98	5,89	1,99	2,88	3,98	1,94	19,66	I
		vlažno	2,81	5,72	1,80	2,85	3,80	1,80	18,87	I
185	10	suho	2,97	5,93	1,97	2,91	3,87	1,98	19,63	I
		vlažno	2,78	5,74	1,98	2,89	3,76	1,75	18,80	I

Legenda:

I - Spoljni izgled - bodovanje od 0-3
II - Sastav i izgled presjeka - bodovanje od 0-6
III - Konzistencija - bodovanje od 0-2
IV - Boja - bodovanje od 0-3
V - Ukus i miris - bodovanje od 0-4
VI - Prihvatljivost proizvoda - bodovanje od 0-2

Ekstraklasa 20 bodova
I klasa od 18,00 – 19,99
II klasa od 16,00 – 17,99
III klasa od 14,00-15,99
Van klase manje od 14,00 bodova

DISKUSIJA I ZAKLJUČAK

U suho, odnosno vlažno soljenim vakuumiranim uzorcima *Brčanskog pršuta* tokom skladištenja na 7 i 15^0 C do 185 dana nije bilo promjena u masi, odnosno kaliranju. Inicijalni sadržaj vode u suho, odnosno vlažno soljenim uzorcima iznosio je 50,19 odnosno 56,84%, a 185. dan 49,86, odnosno 55,42% tokom skladištenjana 7^0 C, a skladištenjem na 15^0 C 185. dan 49,75, odnosno 55,02%. Voda je bila neprimjetna u vidu par manjih kapi u vakuum pakovanju, što ukazuje na stabilnost proizvoda, ali i uzoraka vakuum pakovanja, odnosno PVC folije.

Do značajnijih promjena fizikalno-hemijskih parametara u suho, odnosno vlažno soljenim vakuumiranim uzorcima *Brčanskog pršuta* skladištenih na 7^0 C tokom 185 dana nije bilo. Voda je od inicijalnih vrijednosti za suho, odnosno vlažno soljene uzorke 185. dan imala neznatno promijenjene srednje vrijednosti i to 49,86%, odnosno 55,42%. Neznatne promjene su bile i u sadržaju NaCl-a od inicijalnih vrijednosti 5,56% do vrijednosti 185. dan 4,50%, dok ukupni šećer nije pokazivao odstupanja od inicijalnih vrijednosti. pH je od početne vrijednosti za suho, odnosno vlažno soljene proizvode, također, pokazao neznatna pomjeranja 185. dana do vrijednosti od 5,41, odnosno 5,43.

Značajnije promjene fizikalno-hemijskih parametara u suho, odnosno vlažno soljenim vakuumiranim uzorcima *Brčanskog pršuta* skladištenih na 150 C do 185 dana, također, nisu zabilježeni. Voda se od inicijalnih srednjih vrijednosti neznatno smanjila te je 185. dan iznosila 49,75%, odnosno 55,02%. Količina NaCl-a se, također, od inicijalnih vrijednosti neprimjetno povećala do 185. dana na vrijednosti od 5,59, odnosno 4,54%. Ukupni šećer nije pokazivao promjene u vrijednostima svo vrijeme skladištenja, a pH se neznatno povećao do 185. dana na vrijednosti od 5,50, odnosno 5,51. Znači,

sadržaj vode se kretao u granicama vrijednosti istraživanja drugih autora (Ganić i sar., 2012, Vidal 2021).

Ph vrijednosti u ispitivanim uzorcima su zadržale stabilnu vrijednost i kretale su se u rasponu od 5,41 do 5,51, jer je poželjno da se pH kreće u granicama od 5,5 do 6,0. Rezultati kod nekih drugih autora su pokazali nešto niže vrijednosti 5,20 (Krvavica, i sar., 2013). NaCl se kretao u donjim odnosno srednjim granicama za razliku od rezultata koje su navodili drugi autori 4,58-7,74 (Mediani i sar., 2022).

Inicijalna srednja vrijednost aerobnih mezofilnih bakterija kod vakuumiranog *Brčanskog pršuta* proizvedenog od suho, odnosno vlažno soljenog mesa skladištenog na 7^0 C iznosila je 5,3, odnosno 5,5x103/g, a 185. dan 6,2 odnosno 6,3 x103/g, što je znak da je pravilno i dobro rađena higijena mesa, opreme i ruku, odnosno čišćenje, pranje i dezinfekcija istih. Inicijalni broj psihrofilnih bakterija kod vakuumiranog *Brčanskog pršuta* proizvedenog od suho, odnosno vlažno soljenog mesa bio je 1,8, odnosno 2,0 x102/g, a 185. dan 4,2, odnosno 4,4 x102/g, dok je inicijalni broj halofilnih bakterija bio 5,9, odnosno 6,1 x102/g, a 185. dan 7,0, odnosno 6,8 x102/g. Salmonele, koagulaza pozitivne stafilokoke, sulfit reducirajuće klostridije, E. coli te proteus vrste nisu izolirane u ispitivanim uzorcima.

Inicijalni broj aerobnih mezofilnih bakterija vakuumiranog *Brčanskog pršuta* proizvedenog od suho, odnosno vlažno soljenog mesa skladištenog na 15^0 C iznosio je u srednjoj vrijednosti 5,3, odnosno 5,5x103/g, a 185. dan 6,4, odnosno 6,6 x103/g. Kod psihrofilnih bakterija za vakuumirani Brčanski pršut proizvedenog od suho, odnosno vlažno soljenog mesa inicijalna srednja vrijednost je iznosila 1,8, odnosno 2,0 x102/g, a 185. dan 4,6, odnosno 4,7 x102/g, dok je inicijalni broj halofilnih bakterija bio 5,9, odnosno 6,1 x102/g, a 185. dan 6,6, odnosno 6,9x102/g (Duraković, 1996).

Mikrobiološki status suho, odnosno vlažno soljenih vakuumiranih uzoraka *Brčanskog pršuta* skladištenih na 7 i 15^0 C do 185 dana bio je zadovoljavajući tj. postojeća inicijalna aerobna mikroflora (aerobne mezofilne bakterije, psihrofilne i halofilne bakterije) nisu zabilježile znatan porast, a odsutne su bile i salmonele, koagulaza pozitivne stafilokoke, sulfit reducirajuće klostridije i E. coli. Na osnovu provedenih analiza, proizvodi su proglašeni mikrobiološki ispravni tj. odgovarali su odredbama Pravilnika o mikrobiološkim kriterijumima za hranu ("Službeni glasnik BiH", 2013).

Uzorci vakuumiranog *Brčanskog pršuta* proizvedeni od suho, odnosno vlažno soljenog mesa tokom skladištenjana 7 i 15^0 C do 185 dana zadržali su odgovarajuća organoleptička svojstva bez značajnijih promjena. Organoleptičkom ocjenom poentirani su sa 19,91, odnosno 18,49 bodova za proizvod skladišten na 7^0 C i 19,63, odnosno 18,80 bodova za proizvod skladišten na 15^0 C i svrstani su u I klasu.

Na osnovu specifičnih i poželjnih organoleptičkih svojstava koje je zadržao vakuumirani Brčanski pršut skladišten na 7 i 15^0 C do 185 dana, kao i zadovoljavajućih mikrobioloških i fizikalno-hemijskih nalaza, prednost se daje pršutu pakovanom u vakuum PVC pakovanje u odnosu na stavljanje u promet suhomesnatih proizvoda na klasičan način. Bolja senzorna svojstva zadržavaju vakuumirane forme *Brčanskog pršuta* u odnosu na klasične i zadržavaju specifična organoleptička svojstva duži vremenski period, što pored navedenog opravdava ovakav način plasmana na tržištu, a posebno sa higijenskog aspekta. Uz sve navedeno, vakuumiranim proizvodima osim što miris i ukus ostaju netaknuti, zauzimaju manje prostora pri skladištenju, kompaktni su i uredni, imaju i reprezentativniji izgled (jer je svaki proizvod dostupan na uvid, a ne gube na atraktivnosti).

Postupak vakuumiranja ne iziskuje velike finansijske izdatke, kao ni posebnu tehničku ni stručnu osposobljenost, a rezultati su višestruko ekonomski isplativi (bolja senzorna svojstva, mikrobiološka održivost i higijenska ispravnost kroz duži vremenski period).

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VETERHYGIENIC SAFETY, QUALITY AND SUSTAINABILITY OF VACUUM-PACKED “BRČKO PROSCIUTTO”

ABSTRACT

The process of drying meat dates back to the time of the Romans, who used to dry various types of meat in their drying rooms, which were consumed by sailors and traders from the south. Over time, dried meat also made its way to the Northern European countries. However, it is important to emphasize that such dried meat differs from the dried beef traditional in our region. The tradition of drying meat in our country dates back centuries, and some producers still use the old methods to make this product today. The aroma and taste of dried meat vary from region to region, depending on the climate, altitude, diet, cattle breed, while the drying room, salting method, and meat preparation after slaughter also play a significant role.

Many factors influence quality, hygienic safety, and sustainability, from the proper technological production process to handling the final product. One possibility to extend the shelf life is vacuum packaging of the final products.

Key words: Meat, prosciutto, vacuum packaging

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