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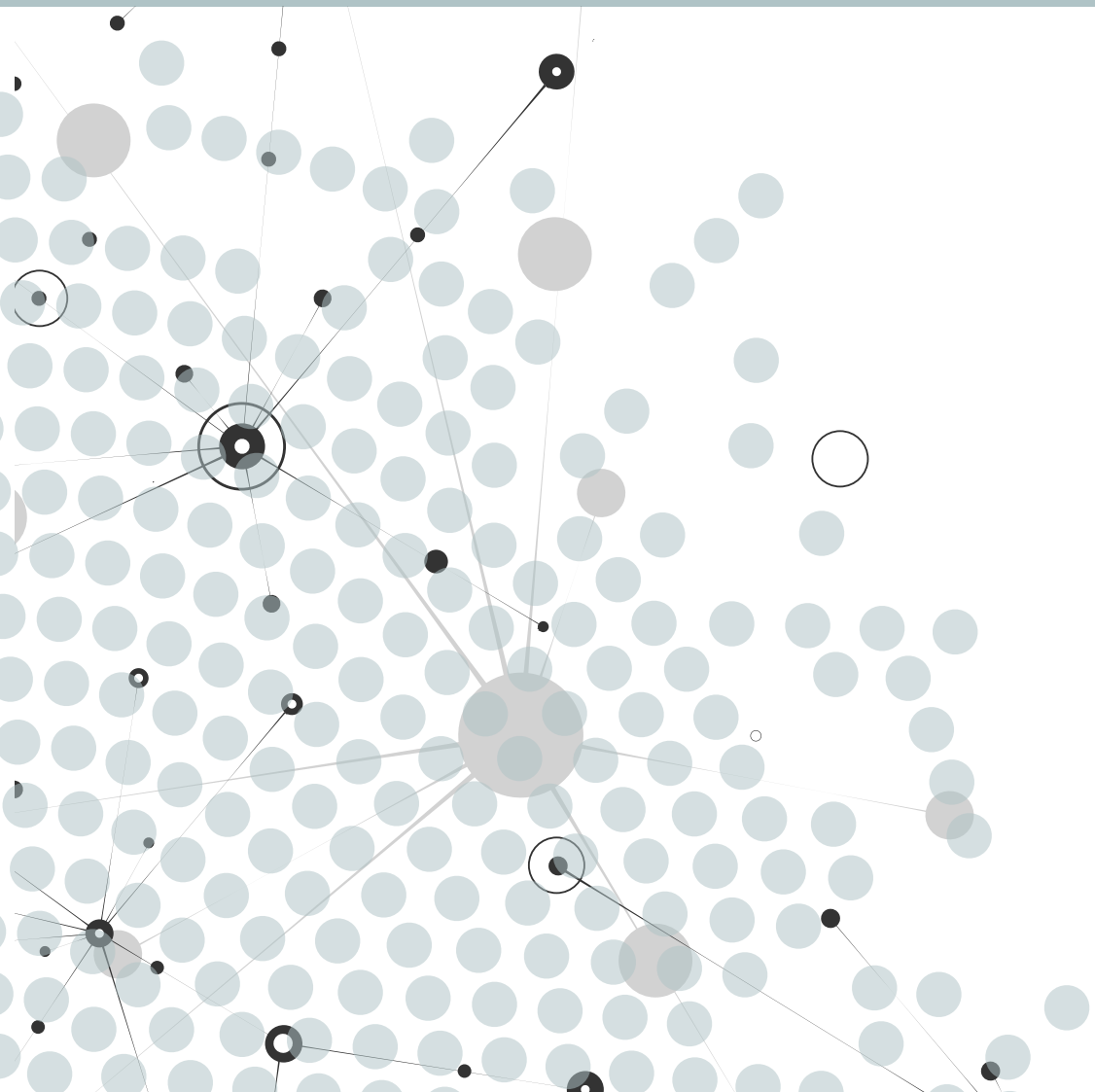

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EDITORIAL

Dear colleagues,

We are pleased to present to you this special issue of Veterinaria, Supplement 74(1), 2025. This edition features a curated selection of Conference Papers that vividly reflect the spirit and scientific contributions of the recently concluded 10th International Congress on Advances in Veterinary Sciences & Technics (ICAVST 2025), held from July 21–25, 2025, at the Faculty of Veterinary Medicine, University of Sarajevo, Bosnia and Herzegovina.

Our goal with this special issue is to enhance the visibility and longevity of the key messages delivered at the Congress, presented in the form of full papers and abstracts, and to make them accessible to a wider academic audience.

The Congress brought together dedicated researchers and professionals from Turkey, Latvia, Austria, Pakistan, Bosnia and Herzegovina, and Russia, who shared their work through various presentation formats. Due to the high number of participants—particularly those who delivered oral presentations—we have selected a portion of these contributions for full-format publication in this issue.

A broader collection of full texts and abstracts will be published in the 2025 ICAVST Proceedings Book, which will be available on the official ICAVST platform.

It is important to note that all articles in this supplement have undergone a brief internal review by the Veterinaria Editorial Team. However, unlike regular issues of the journal, these contributions were not subjected to the standard double-blind peer review process.

We invite you, our esteemed readers, to explore these texts, evaluate their content, and draw insights that may benefit your own academic and professional endeavors.

In closing, I would like to extend my sincere gratitude to the ICAVST leadership team for their constructive collaboration. We look forward to seeing future Congresses organized with the same level of professionalism, dedication, and excellence as this 10th edition in Sarajevo.

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


BOSANSKI BRDSKI KONJ

CONFERENCE PAPER

NANOPORE SEQUENCING IN VETERINARY HEALTH SCIENCES: A REVIEW

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ABSTRACT

In veterinary health, the ability to perform field-portable, real-time long-read sequencing, which is unattainable with traditional platforms, makes nanopore sequencing one of the most revolutionary tools. In recent years, the use of nanopore sequencing technology in veterinary medicine has gained significant attention focusing on pathogen detection, antimicrobial resistance (AMR) profiling, livestock genetic identification, microbiome studies, and disease monitoring in wildlife and domesticated animals. Its usefulness in field diagnostics and outbreak response is invaluable in remote resource-limited region because it is portable and can deliver results in a short time. Despite the obstacles of higher error rates and the need for bioinformatics skills, chemistry, base calling algorithms, and workflow of nanopore sequencing are improving its precision and accessibility. In this paper, we review the existing literature on nanopore sequencing technology, its veterinary applications, advantages over other sequencing methods and propose integrated One Health strategies for the future. As presented in the review, technology offers prospective opportunities that could improve animal health, food safety, and monitoring of zoonotic threats in the context of humans, animals, and environment.

Keywords: Nanopore sequencing, Oxford Nanopore Technologies, veterinary genomics, pathogen detection, antimicrobial resistance

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INTRODUCTION

Improvement in the scientific methods of DNA sequencing has revolutionized the field of biological science research (Ji et al., 2024). Introduction of long-read systems, particularly single-molecule sequencing systems has changed the long-ruled sequencing methods such as Sanger sequencing or short-read systems (Kumar et al., 2024). Oxford Nanopore Technologies (ONT) has led the development of portable devices that can sequence the DNA or RNA as they pass through the nanopores in a device (Chen et al., 2023). With no prior PCR amplification, these new types of sequencers provide real-time, in-situ data processing and have ultra-long reads (Koivunen, 2019). Nanopore sequencing has gained attention in the field of veterinary genetics due to its in-field pathogen surveillance ability (Santos et al., 2020). Present article aims to review the applications of nanopore sequencing technology in the field of veterinary health science.

Principles and Mechanisms of Nanopore Sequencing

Nanopore technology operates based on DNA or RNA molecules being threaded through nanoscale protein pores deeply set within/surrounded by and part of a membrane (Ying et al., 2022). The signal processing schemes perform more complex interpretations to form base calls from patterns of unique iconic current disruptions generated by each nucleotide entering the pore. With the ONT platform, base calls generated by Guppy and Dorado neural networks can give meaning to sequences captured by the raw signal. With improved base calling models and newer chemistries (R10.4, Q20), the accurate raw readout has been boosted to over 99 percent as reported in (El-Lagta et al., 2024). Specifically, nanopore sequencing reads native molecules: DNA alterations such as methylation are preserved and can be accessed during the sequencing process (Gouil and Keniry, 2019). With varying degrees of portability from fully portable MinION and Flongle to high-output PromethION, ONTs can also be designed to allow adaptive sampling where sequencing reads are altered dynamically based on the user's specifications for enrichment and rejection in real-time (Pugh, 2023). Thus, the core nanopore mechanism single-molecule, long-read, real-time sequencing is highly beneficial to veterinary medicine.

Applications in Veterinary Health Sciences

Pathogen Detection and Surveillance

Within a short period, nanopore sequencing has been applied for the identification and genomics characterization of animal pathogens. For example, during outbreaks of foot-and-mouth disease (FMD), the MinION portable sequencers allowed on-site sequencing and processing of the FMD virus. Brown et al. (2021) demonstrated that any MinION could produce a full-consensus FMDV genome (serotypes A, O, Asia-1) within minutes and cross-referenced via Illumina for instant serotype characterization, yielding 100% overlap for consensus FMDV genome (Brown et al., 2021). Tick-borne zoonoses surveillance experienced the same benefit, such as in a metagenomic nanopore study of Ethiopian livestock ticks where the field isolates of Rickettsia, Francisella, Spiroplasma, Ehrlichia, Borrelia, a Babesia parasite, and even a poxvirus were detected (Chadd et al., 2025). Another advance has been in detection of pathogen in ecological systems Telussa et al. (2025) using MinION for environment poultry waste pathogen surveillance revealed 98% sufficiency in known resistance genes and 96% inferable virulence in comparison to reference assemblies (Telussa et al., 2025). The portability and speed of nanopore sequencing makes it possible to track and perform real-time One Health surveillance from livestock to wildlife for tracking outbreak detection.

Antimicrobial Resistance (AMR) Profiling

Nanopore sequencing technology is capable of rapidly profiling antimicrobial resistance (AMR) genes in samples from animal associations (Slizovskiy, 2024). The long reads are likely to span entire resistance loci, plasmids, facilitating the connection through mobile genetic elements. The MinION was utilized in the Indonesian slaughterhouse study to confirm the identification of 98 % of the known AMR genes, including phenotypic resistance prediction at 91 % concordance to laboratory tests (Telussa et al., 2025). These results outperformed those generated by Illumina MiSeq in alignment with the hybrid-reference-Illumina detected 95% of ARGs. Moreover, with nanopore sequencing, entire bacterial or metagenomic genomes can be uncultured sequenced, enabling the discovery of novel resistance genes and their genomic loci. For instance, real-time nanopore sequencing was used during rapid AMR profiling during bloodstream infections, thus, enabling its use for in-field veterinary diagnosis. In conclusion, these studies prove the importance of veterinary AMR surveillance through the use of nanopore technology, enabling comprehensive resistome analysis at unprecedented speeds and from local (ONT, 2023).

Livestock Genetics and Breeding Programs

In livestock genomics, there has been a groundbreaking development in long reads with nanopore sequencing. The accumulation of ONT data has been used to generate covering animal genomes as well as to identify structural variations of specific traits. One of the most recent projects is the bovine genome project where ONT was used to provide telomere-to-telomere bovine chromosomes assemblies. ONT ultra-long reads were used to achieve very high consensus accuracies of ~Q51 (99.999%) (Li et al., 2023). This accomplishment illustrates the outstanding capability of nanopore technology to decipher highly complex immune-gene aggregate sequences and the structural variations which are impossible to achieve on short reads. Lowpass nanopore sequencing has been applied to genotype imputation to make genotyping by sequencing more efficient, especially in breeding. Lamb et al. (2023) showed that 0.1x coverage ONT reads, when imputed with densely spaced SNP panels, not only demonstrated high-correlation (>0.91) with genomic estimated breeding values (GEBVs) of the SNP-chip values, but also low-coverage reads which reflected diversity in breeding were shown to have high genomic estimate of breeding value (>0.92) compared with other highly correlated (>0.85) SNP-chip values (Lamb et al., 2023). Research has indicated that a significantly reduced coverage of 0.1x ONT reads, approximately 5 million reads, can yield accuracy in GEBV that is comparable to, or even surpass, that provided by low-density SNP chips. The inherent characteristics of nanopore technology facilitate the generation of reads that overlap multiple genetic markers, thereby contributed to enhanced phasing efficiency and improved imputation capabilities. Consequently, the application of genotype level analysis utilizing nanopore sequencing is deemed feasible in practical agriculture settings. For instance, in a controlled experiment involving the MinION workflow, an average through imputations, achieving a correlation of 0.92 with trait predictions that employed SNP chip-based methodologies. This level of accuracy was attained utilizing portable sequencing systems, demonstrating the practical applicability of this technology in the field of genomics (ONT, 2024).

Microbiome Studies (Nutrition, Immunity, and Disease)

The influence of microbiomes on animal nutrition, immunity, and health permeates even into nanopore sequencing. Its ability to

read long reads facilitated higher resolution taxonomic sequencing of entire 16S rRNA and some other marker genes which enabled the analysis of bacterial strains. With MinION, the pig gut microbiome was sequenced by producing 4.8 million long reads with an average length of 1.7 kb. Using MinION sequencing, 214 bacterial genera were identified as compared to 183 with Illumina data (Tort-Miró et al., 2025). Measures of microbiome diversity were highly correlated at the broader taxonomic levels, yet some discrepancies at the genus level still accounted for remain (Tort-Miró et al., 2025). Both nanopore and Illumina systems captured key community patterns (high numbers of Firmicutes in the microbiota of healthy sows) (Son et al., 2024). The influence of age in companion animals was illustrated in a study where dogs were Illumina/Nanopore sequenced. The study showed that the beneficial gut genera decline with age in dogs, while pathogenic genera increase. Such examples highlight why some draw attention to long read result being comparable to short read results (r0.96 on phylum) while giving full length operon/amplicon data (Tort-Miró et al., 2025). Furthermore, nanopore technology can be used to meta-transcriptomics or direct RNA sequencing of the microbiome, which ONT refers to as providing insights into the "working of microbes". The real-time and long-read metagenomics enabled by ONT promise to be powerful in examining the microbiome of animals in relation to their diet, infection tendencies, and immune condition.

Wildlife and Conservation Genetics

Conservation genomics nanopore sequencing is another area of focus. The affordability and field-portability of technology make it possible to sequence endangered species without collecting and sending samples. One of the most complete marine mammal genomes, the hourglass dolphin, was sequenced using a single flow cell, and under 10 percent of the cost that is typically used. This was done using a PromethION run on a laptop. The portable MinION enabled the first fully on-site assembly of the red-fronted brown lemur genome in Madagascar. These advances greatly reduce barriers to low-resource settings (ONT, 2024). Adaptive nanopore sequencing also performed well for environmental monitoring, as demonstrated in Aotearoa where New Zealand soil samples were sequenced for distinct, expertly endangered kakapo genotypes, promising non-invasive monitoring of wildlife (Urban et al., 2023). Rapid field-deployable sequence can leverage wildlife forensics for anti-trafficking purposes by identifying species in biological samples quickly (Alketbi, 2024). In total, conservation genomics entering a new stage because of nanopore tools that provide access-even in compliance with the Nagoya Protocol-and facilitate One-Health stewardship by the locals (Urban et al., 2023).

Previous Research in Veterinary Science Applications of Nanopore Sequencing

Table 1 summarizes the key studies that have applied nanopore sequencing in various areas of veterinary science, including pathogen detection, genomic surveillance and antimicrobial resistance profiling.

Table 1 Nanopore Sequencing research conducted in Veterinary Health Sciences (2019–2025)

Country	**Study site (setting)**	Species Studied	**Pathogens studied or microbiome focus**	Reference
Belgium	Field & diagnostic cattle (Belgian herds)	Cattle (calves)	*Mycoplasma bovis* (resp. pathogen), antimicrobial resistance markers (via Nanopore GWAS)	Bokma et al. 2021
USA	Dairy herd / proviral DNA diagnostics	Cattle (dairy herd)	*Bovine leukemia virus* proviral genome sequencing and transmission analysis	Pavliscak et al. 2021es New Roman
Spain	Canine gut faeces	Dogs	Microbiome: high-quality metagenome-assembled genomes (MAGs), ARGs, prophages & plasmid linkages	Cuscó et al. 2022
Türkiye	Tick pools from Anatolia	Ticks (field-collected)	Bacterial pathogens in infections; same-day ID via metagenomic Nanopore plus AMR prediction	Ergunay et al. 2023
Mongolia	Grazing ruminant tick surveillance	Ticks	Bacterial (Rickettsia spp., *Coxiella burnetii*, *Borrelia*, *Anaplasma*), viruses (Alongshan, Beiji nairovirus), *Theileria*, *Babesia*	Ergunay et al. 2024
UK	Canine clinical cases (urine/skin)	Dogs	Pathogen detection; AMR profiling	Ring et al. 2023~
Poland/Bulgaria	*Ixodes ricinus* & *Dermacentor reticulatus* pools	Ticks	Rickettsia spp. (e.g. *R. asiatica/raoultii*), *Neoehrlichia mikurensis*, *Anaplasma phagocytophilum*, *Coxiella burnetii*	Nelson et al. 2024
South Africa	Tick genomics and endosymbiont assembly	Ticks	Tick genome assembly & *Coxiella*-like endosymbiont characterization	Meiring et al. 2025
Bhutan	Canine-borne pathogen surveillance (One Health study)	Blood-borne vectors (ticks/fleas)	*Mycoplasma haemocanis*, *Ehrlichia canis*, *Anaplasma platys*, *Bartonella*,	Huggins et al. 2024
Spain	Swine farms – gut microbiome (sows & piglets)		Gut bacterial diversity; potential pathogens (*EscherichiaShigella*) vs. beneficial taxa (Lactobacillus etc.)	TortMiró et al. 2025
Japan	Mastitic bovine milk samples		Bovine mastitis pathogens (*E. coli*, *Strep. uberis*, *K. pneumoniae*, *S. aureus*) identified via 16S rRNA Nanopore in ~6 h	Usui et al. 2023
Saskatchewan feedlot, Canada (chronically ill cattle)	Nasopharyngeal swabs of cattle with unresponsive respiratory disease	None	*Moraxella bovoculi*, *Mannheimia haemolytica*, *Mycoplasma dispar*, *Pasteurella multocida* (BRD bacteria + ARGs)	Freeman et al. 2022
Jena, Germany (Federal Salmonella reference lab)	Isolated *Salmonella enterica* from bovine outbreaks	None	Multiple *Salmonella enterica* serovars + genetic markers for antimicrobial resistance & virulence	Thomas et al. 2023

Country	**Study site (setting)**	Species Studied	**Pathogens studied or microbiome focus**	Reference
Nebraska Veterinary Diagnostic Center, USA	Tissue / serum samples from sick pigs	None	Mixed viruses: bovine viral diarrhea virus, bovine herpesvirus1, influenza A virus, Seneca Valley virus, etc.	Neujahr et al. 2024
Le Crotoy wetlands, France (wild poultry surveillance)	Cloacal/tracheal swabs from domestic birds and wild waterfowl	None	Highly pathogenic avian influenza A(H5N1) (whole genome)	Croville et al. 2024
Kandy District, Sri Lanka (dairy farms)	Cow dung/env. samples from dairy production sites	None	*Enterobacter cloacae* complex (multidrugresistant; blaNDM, blaCTXM, etc.)	Kumari et al. 2025
Wisconsin, USA (dairy cattle surveillance)	Farms affected by the spillover of HPAI from dairy cattle	None	Highly pathogenic avian influenza A H5N1 (metagenomic & targeted)	Caserta et al. 2024
St. Lawrence Estuary, Canada (marine wildlife virology)	Pinniped necropsy samples from seals	None	HPAI H5N1 wholegenome sequences (GridION)	Lair et al. 2024

Comparative Advantages of Nanopore Sequencing

Nanopore techniques, as with other technologies, come with several advantages over the traditional techniques. Unlike short-read platforms, the ONT's long reads surpass short read platforms by capturing entire repeat sequences, structural variants, and full genes (Kaplun et al., 2023). Consequently, this provides extensive genome assemblies as well as long-read haplotypes phasing. An example of ONT's advantages includes the construction of gapless assemblies of cattle chromosomes in the part of the immune loci (Li et al., 2023). Devices like MinION and Flongle can be transported to any location with Internet access and can even be used on a smartphone with SmidgION. Unlike laboratory-based sequencers, which take days to provide results, farm and clinic samples can be analyzed on spot. Sequencing results are delivered instantly, enabling immediate data analysis. Unlike other techniques, this approach does not involve PCR amplification which reduces bias and preserves encoding (marks). Just as examples of base alterations such as 5mC or m6A, can be noted within the sequencing process without any extra steps being taken (Buytaers et al., 2021), this sorts of shifts aid in the discover of solutions to problems in the regulation of genes as well as epigenetics of viruses which could not be resolved using standard methods. The nanopore device processes a wide range of inputs, including tiny amplicons, cfDNA, long genomic DNA, double stranded DNA, and even native RNA, all in a single workflow on one device (Si et al., 2024). To determine the isoforms, all transcriptomes (cDNA or direct RNA) can be sequenced. In RNA-seq, host DNA can be depleted or enriched through adaptive sampling, so modification does not need to be made to the library. ONT offers one-sample and high-throughput devices (Flonge, GridION, PromethION) (Tytgat,

2022). This flexibility benefits either a single field trial or a complete consortial project. The pricing structure is equally advantageous. A MinION flow cell costs roughly 900$ and provides ~15-30 Gb, more than enough to sequence bacteria or small eukaryotes, and can often be washed and reused. EPI2ME cloud runs base calling and data analysis using AI/ML on custom proprietary pipelines developed by ONT, applying them through an open preference system. GPU-accelerated on-board base calling and machine-learned variant calling processes raw data into biological data for quicker than ever before. Such efficiencies are particularly beneficial for veterinary research. The capability to phase distance SNPs and perform genomic selection more efficiently is possible through drone-enabled nanopore reads. Moreover, the potential to recapture entire viral genome in a single read is equally impressive (Romagnoli et al., 2023). According to ONT resources, the benefits are described as follows: short reads from 50 bp to over 4 Mb, high quality genomes at any read length with the least contig, and resolved genomic regions beyond the reach of short-read amplicons which are free from amplification bias enabling the detection of base modifications (ONT, 2024).

Limitations and Technical Challenges

As promising as nanopore sequencing is, it certainly has its disadvantages. The raw nanopore read accuracy has been blamed for a Q < Q25, resulting in 640 bases errors per bacterial gut-strain genome (Bejaoui et al., 2025). Even with improvements in chemistry chemistry and base caller (R10.4.1, SUP models) claiming over 99% single-molecule precision, there are still challenges in single-read precision tasks that arise from higher rates (El-Lagta et al., 2024). This is why many programs add high coverage together with consensus polishing, most often short-read hybrids, to achieve a reference quality sequence. As an example, Bejaoui et al. (2025) have shown that greater Nanopore error indicated lesser accuracy in nanopore phylogenies than those generated by Illumina during outbreak tracing (Bejaoui et al., 2025). Some errors due to context have been reported, but dual reader pores (R10) rectify these context errors (Tytgat et al., 2020). When combined with flow cells, MinION output of over 10-30 Gb of data per run makes it less efficient for large genomes or high depth metagenome projects. Smaller ONT devices attempt to solve some of these problems but come at greater cost and infrastructural need. Long-read libraries might need other requirements as more-molecular-weight DNA like well-prepped libraries may pose very low-input and heavily degraded samples. Some devices for protein nanopore experiments have a very short shelf life and with use, can wear out after a few dozen hours of runtime. (ONT, 2023). Nanopore data analysis requires a strong bioinformatics background, particularly with base calling, alongside high computational power. The evolution of pipelines is coming too rapidly, which makes it hard to keep up.

CONCLUSION

In a short period of time, these technologies have become an essential asset in veterinary health sciences. This tool enables new pathogen detection and large-scale animal genomics and microbiome characterization, thanks to its long reads, real-time data processing, and field probability. The advantages of nanopore technology are already being harnessed with complete livestock genome assembly and onsite breeding stock genotyping. As with other technologies, nanopore faces limitations relating to error rates and throughput, which are being gradually resolved with enhanced hardware and software. Importantly, nanopore technologies align with the One Health paradigm as they integrate human, animal and environmental genomics. With nanopore tools, *In-Situ* Lab and similar multidisciplinary

projects could empower local professionals to perform genomic surveillance, while providing remote oversight.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Conception: RMKS, MH; Design: SA, HA; Supervision: RMKS, MH; Materials: NH, ED; Data Collectionand/orProcessing:HA; Analysis and/or Interpretation of the Data: RMKS, MH; Literature Review: SA, NH, ED; Writing: RMKS, MH, HA; Critical Review: HA

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SEKVENCIRANJE POMOĆU NANOPORA U VETERINARSKIM ZDRAVSTVENIM NAUKAMA: PREGLED

SAŽETAK

U veterinarskoj medicini, mogućnost izvođenja terenskog, realnog sekvencioniranja dugih nizova u stvarnom vremenu, nije bilo moguće sa tradicionalnim platformama, a što čini nanopore sekvencioniranja jednom od najrevolucionarnijih tehnologija. U posljednjim godinama, upotreba nanopore tehnologije u veterinarskoj medicini privukla je značajnu pažnju, s fokusom na detekciju patogena, profilisanje antimikrobne rezistencije (AMR), genetsku identifikaciju stoke, istraživanja mikrobioma i praćenje bolesti kod divljih i domaćih životinja. Njena korisnost u terenskoj dijagnostici i odgovoru na epidemije je neprocjenjiva u udaljenim i resursima ograničenim područjima, jer je prenosiva i može pružiti rezultate u kratkom vremenu. Uprkos izazovima poput veće stope grešaka i potrebe za bioinformatičkim znanjem, hemija, algoritmi za bazno očitavanje i radni tok nanopore sekvencioniranja stalno se poboljšava, čime se povećava preciznost i dostupnost.

U ovom radu pregledali smo postojeću literaturu o nanopore tehnologiji, njenoj veterinarskoj primjeni, te istakli prednosti u odnosu na druge metode sekvencioniranja i predlažemo integrisane strategije u okviru koncepta Jedno zdravlje (One Health) u budućnosti.

Kako je prikazano u radu, tehnologija nudi perspektivne mogućnosti koje mogu unaprijediti zdravlje životinja, sigurnost hrane i praćenje zoonotskih prijetnji u kontekstu ljudi, životinja i okoliša.

Ključne riječi: Nanopore sekvencioniranje, Oxford Nanopore Technologies, veterinarska genomika, detekcija patogena, antimikrobna rezistencija

CONFERENCE PAPER

CLIMATE CHANGE AND DEATH INVESTIGATION: REDEFINING EXPERIMENTAL FORENSIC SCIENCE

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ABSTRACT

One of the deadliest weather-related occurrences in the world is heat, and as climate change continues, heat-related mortality could rise sharply. According to The Lancet Countdown on Health and Climate Change's 2020 report, an additional 475 million heatwave incidents occurred worldwide in 2019, exposing vulnerable populations, which resulted in excess mortality and morbidity. The number of deaths from heat-related causes among those over 65 has increased by 53.7% during the previous 20 years, with a total of 296,000 deaths in 2018. The burden of heat-related mortality linked to recent decades of human-induced climate change was the subject of another multinational investigation. Human health has already been impacted by climate change and will continue to be so through direct, indirect, and diffuse pathways. Heat-related risks may get worse or get better as a result of interactions between climate change and other trends including urbanization, population growth and aging, and socioeconomic development. High uncertainty among heat-related mortality predictors (such as human behaviour and adaptation) are frequently the cause of such large variations in heat mortality; in contrast, smaller estimations of adverse health effects are produced by slower future population increase and greater adaptability.

Keywords: Climate change, death, experimental, forensic, postmortem

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1. Introduction / Background

In forensic practice, the diagnosis of heat stroke-related deaths is typically one of exclusion. This is due to the fact that both gross and histological postmortem findings in heat-related fatalities are non-specific and lack pathognomonic features, while biochemical analyses do not provide definitive markers. Consequently, a comprehensive assessment of circumstantial evidence, in conjunction with detailed autopsy findings, is essential to rule out alternative causes of death, including alcohol or drug intoxication. (Dervišević et al., 2023b). Heat waves, exacerbated by climate change, have already resulted in thousands of fatalities worldwide. These deaths are often due to a combination of extreme heat and high humidity, with wet-bulb temperatures exceeding 35 °C-conditions under which the human body can no longer effectively cool itself through perspiration, leading to fatal heat stress. In addition to direct effects, climate change contributes to a range of intermediate causes of death, including crop failures, droughts, flooding, severe weather events, wildfires, and sea-level rise. These phenomena may not be immediately fatal but significantly increase vulnerability to injury, disease, malnutrition, and displacement. Climate

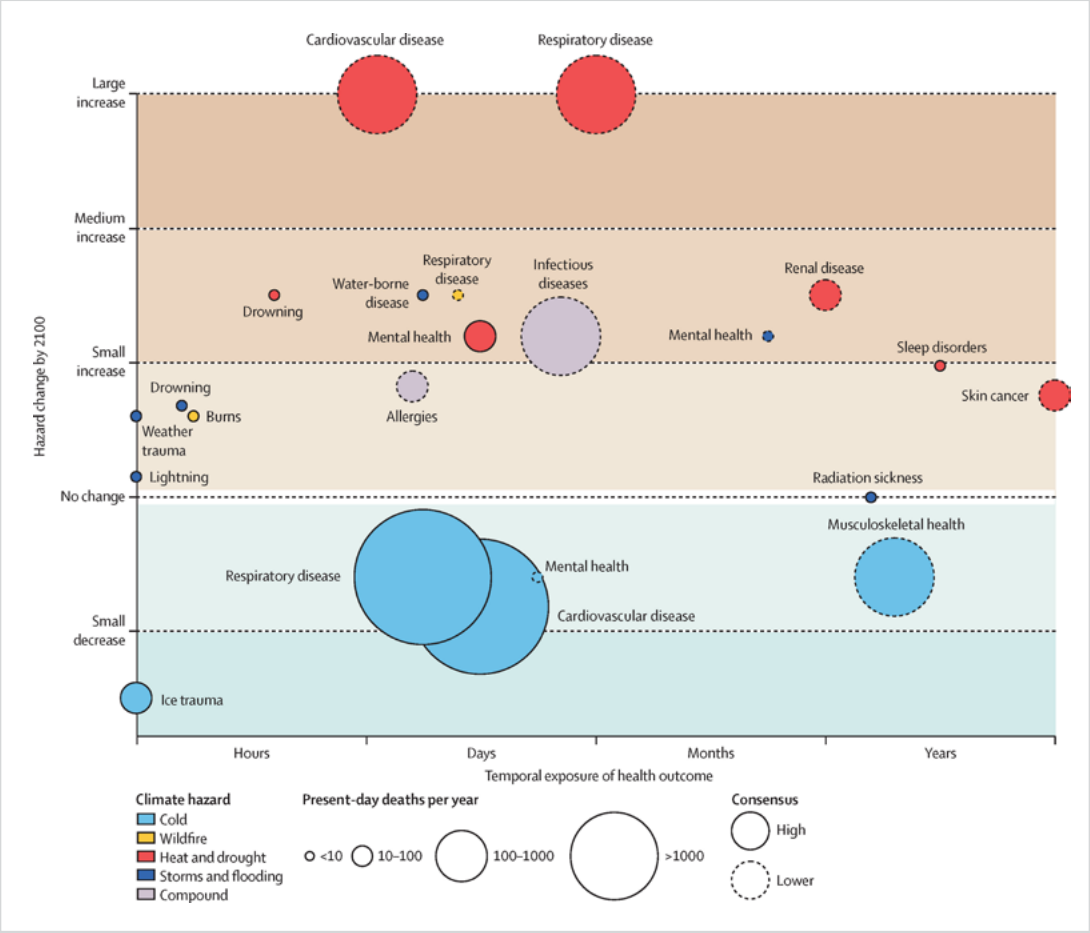

Figure 1 Synthesis of UK climatic patterns and weather-related mortality. The circle sizes show current estimates of annual human mortality from different climate hazards, categorized by the number of deaths (less than 10, between 11 and 100, between 101 and 1000, and more than 1000 annually). Certain climate hazards are represented by the colours of circles (Mitchell et al., 2024). Copyright, Elsevier 2024

change is likely the biggest threat to human life in the future, but nuclear war and biodiversity loss also present serious existential threats. The findings of this research suggest that, on average, burning about 1000 tons of fossil carbon (producing 3700 tons of CO_2) results in one premature death in the future. Measuring carbon emissions in human lives helps non-experts grasp the figures and makes energy policy goals more evident. It is obvious that it is inherently wrong to enable a policy to cause manslaughter. The large mortality tolls resulting from this research and attributable to present carbon emissions have obvious and immediate implications for energy policy. Many deaths during heat waves stem not just from body overheating, but also from heat stress, which can exacerbate pre-existing medical conditions, leading to fatal outcomes (Pearce and Parncutt, 2023). The circle's size represents the current mortality rate, which is primarily caused by heat, drought, and cold hazards, however infectious disease-related mortality is also largely caused by compound climate hazards (Mitchell et al., 2024). As they are also linked to the greatest projected increase in hazard, the largest heat and drought circles show a mortality rate of 100–1000 deaths annually, which is predicted to increase (Figure 1, y axis). Although the projected rise in the hazard is significantly smaller, there is a great deal of uncertainty in the expert replies, and the current mortality rate for several of the cold mortality causes is substantially higher (>1000 deaths annually). The risk to the elderly and heat-related cardiovascular diseases currently dominates mortality outcomes in all but the smallest category.

2. Sudden Cardiac Death (SCD)

Refers to death resulting from unexpected circulatory arrest (van den Tweel and Wittekind, 2016). Estimates of the annual occurrence of sudden cardiac death vary depending on the sources used for the case identification, applied definitions, and methods for extrapolating rates, as well as the autopsy rates conducted in each country (Illing et al., 2020). In prospective studies conducted in the USA, China, Ireland, and the Netherlands, which use standardized definitions and various surveillance sources for case identification, the rate of SCD ranges from 40 to 100 per 100,000 in the general population (Fukuda et al., 2015; P. Zhao et al., 2016). The annual occurrence of SCD grows with age, being 100 times less common in people under the age of 30 (0.001%) compared to those over 35 (Luterbacher et al., 2004). Globally, there is a rising number of deaths occurring indoors with increasing indoor temperatures, such as in bathrooms while taking a bath or in saunas. Autopsy rates are generally low and vary considerably between countries, with rates falling below 10% of all deaths in the United States, compared to 23.8% in Finland (Illing et al., 2020; Luterbacher et al., 2004). Some protocols for conducting autopsies in suspected SCD cases can differ significantly, even in the different regions of the same country (Ferron et al., 2006). These discrepancies in autopsy rates and procedures likely contribute to variations in the occurrence and the causes of SCD (Thommen, 2005). Scientific data regarding the role of hyperthermia as a cause of sudden cardiac death (SCD) remain limited highlighting the need for further research in this area (Dervišević et al., 2023a; Dervišević et al., 2023c).

The causes of sudden cardiac death (SCD) are varied, with structural heart alterations being a key contributor. Cardiac remodeling, a common feature in several cardiovascular disorders—including hypertrophic cardiomyopathy, dilated cardiomyopathy, and chronic heart failure—frequently underlies these structural changes (Iba et al., 2025a; Sacco et al., 2023) (Figure 2). SCD can also result from coronary conditions such as arterial spasms, progressive atherosclerosis, ischemia, or myocardial infarction. However, not all cases are linked to ischemic events; some occur in individuals with inherent conduction system disorders. Furthermore, external substances can act as triggers in certain instances of SCD (Sacco et al., 2023). Differentiating the underlying cause of death in such cases using standard autopsy and histopathological methods remains highly challenging. Moreover, histopathological

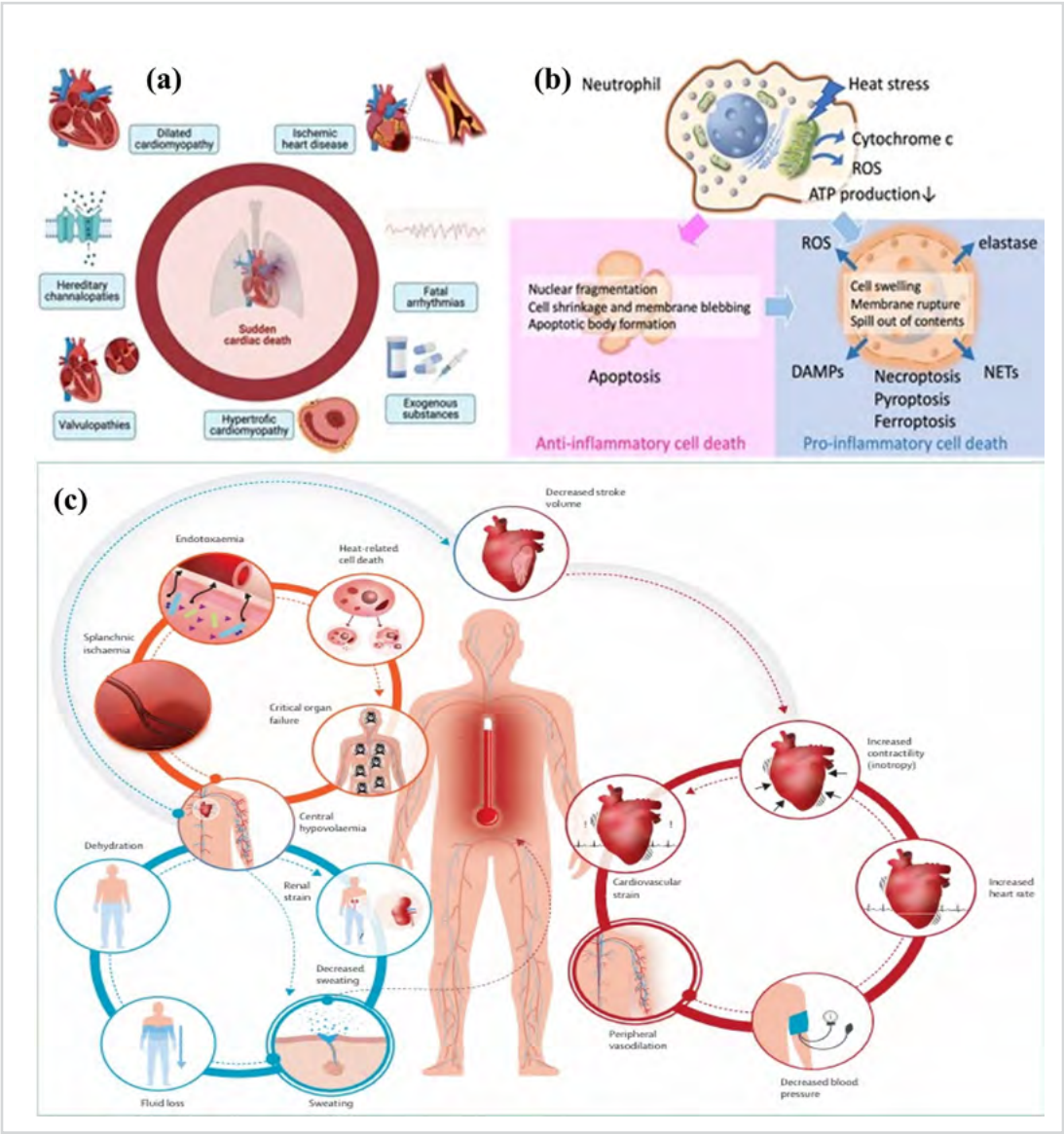

Figure 2 (a) Causes of SCD (Sacco et al., 2023). Copyright MDPI © 2023. b) Pro-inflammatory and anti-inflammatory cell death mechanisms in heatstroke (Iba et al., 2025a). Copyright BMC © 2025. (c) An example of the physiological mechanisms underlying heat stress in humans (Ebi et al., 2021). Copyright The Lancet © 2021

evaluations often suffer from interobserver variability, influenced by the pathologist's level of experience and subjective interpretation of tissue morphology. As a result, histological findings alone may not provide definitive conclusions regarding the precise cause of death. This limitation poses significant implications not only for epidemiological research and prevention strategies but also for legal contexts, where establishing the exact cause of death and any associated responsibilities is crucial (Sacco et al., 2023). The two main ways that the human body reacts to heat stress are by secreting perspiration onto the skin, which then evaporates and dissipates body heat, and by shifting blood flow towards the skin (vasodilation), which enhances heat transmission from muscles to skin and ultimately to the environment. Together with extra thermal

information from temperature-sensitive nerve cells in the skin and other parts of the body, the brain controls these physiological heat loss reactions. Non-thermal cues like cytokines, metaboreceptors (a kind of chemoreceptor that reacts to metabolic products produced by working muscles), and dehydration can also have an impact on this regulation. These physiological heat stress reactions can have varying effects on individuals and are required to restrict increases in core temperature (Ebi et al., 2021).

Elevated body temperatures can negatively affect the function of mitochondrial electron transport chain components, with complex I showing particular sensitivity. This disruption compromises mitochondrial performance, diminishes ATP synthesis, and results in an energy deficit within the cell, thereby promoting cellular dysfunction and lowering resistance to physiological stress (Iba et al., 2025b). The damage to mitochondria induces apoptosis (Iba et al., 2025a). Exposure to extreme heat (typically ≥43 °C) can trigger apoptosis through mitochondrial-mediated pathways, including the release of cytochrome c into the cytosol, ultimately resulting in programmed cell death. This biological response forms the basis of hyperthermia-based cancer treatments, which exploit elevated temperatures to selectively induce apoptosis in tumor cells (Iba et al., 2025a). However, because apoptosis is an energy-dependent process requiring adequate ATP levels, cells experiencing severe ATP depletion are more likely to undergo necrosis instead, a form of cell death that often triggers an inflammatory response. In summary, hyperthermia-induced mitochondrial dysfunction-characterized by increased membrane permeability, excessive reactive oxygen species (ROS) production, and disruption of the electron transport chain-can initiate both apoptotic and necrotic pathways, ultimately contributing to multiple organ failure. Therefore, damage to mitochondria caused by elevated temperatures represents a key pathway contributing to cell death during heatstroke (Iba et al., 2025a). Heat stress can trigger multiple forms of programmed cell death.

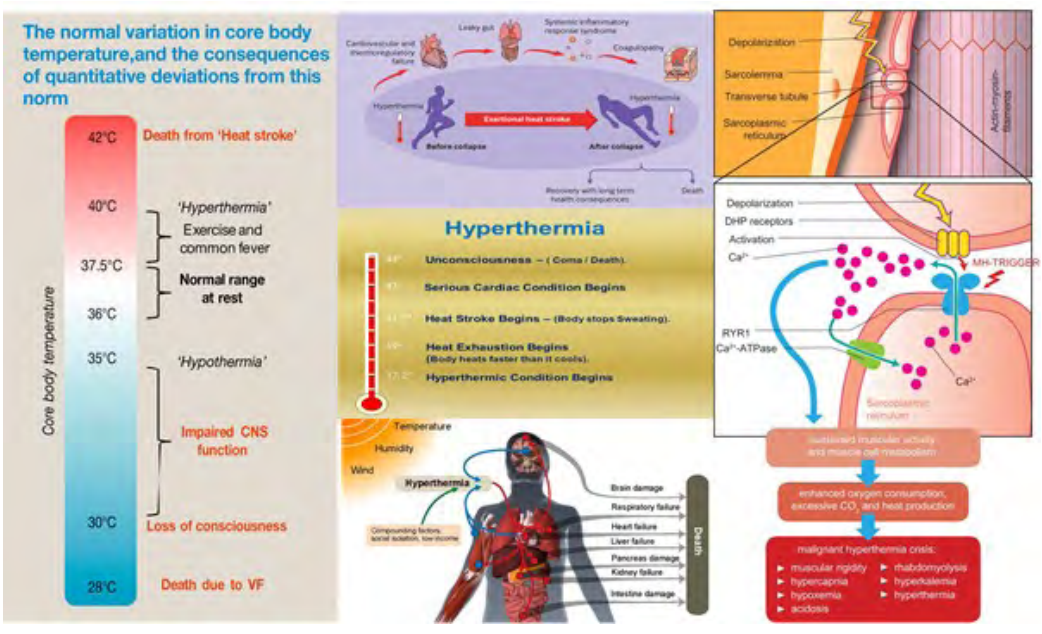

Figure 3 Summary of the main pathophysiological factors participating in exertional heat stroke, from hyperthermia to death (Iba et al., 2025a). Copyright MDPI © 2019

Among them, apoptosis is a non-inflammatory process marked by cell shrinkage and fragmentation of the nucleus. During apoptosis, cells break down into apoptotic bodies that are efficiently removed by phagocytes. In contrast, proinflammatory forms of cell death—such as necroptosis, pyroptosis, and ferroptosis—exhibit common characteristics including cellular swelling and nuclear membrane rupture. These processes result in the release of intracellular components, which in turn stimulate inflammatory responses (Figure 3).

Poor blood flow in hypoxic areas directly contributes to their ease of heating, as higher temperatures are more readily attained due to the limited heat dissipation by perfusion. Since tumour cells are far more susceptible to heat than cells in an environment with enough oxygen, it is generally believed that hyperthermia kills them specifically. Furthermore, hyperthermia at 41–43 °C enhances membrane permeability and decreases DNA damage repair, both of which improve the efficacy of medications that kill tumour cells. Lastly, perfusion and extravagation are improved even at relatively low temperatures, increasing medication delivery (van Rhoon et al., 2020a).

3. Key connections between human health and climate change

There is a correlation between elevated mortality risk and both high and low temperatures. From 2000 to 2019, it was estimated that suboptimal temperatures caused 5,083,173 fatalities annually, or 9.43% of all deaths globally. Furthermore, it is anticipated that the excess mortality linked to temperature would continue to rise until the 2050s. The temperature shift may result in a variety of illnesses in addition to an increase of fatalities, as seen in Figure 4. For instance, exposure to excessively high temperatures has been linked to an increased risk of hospitalization and ED visits for conditions affecting the respiratory, metabolic, and cardiovascular systems. Climate and environmental changes brought on by global warming may increase the likelihood of a number of health consequences (Q. Zhao et al., 2022). The vast majority of harmful chemicals affect important targets inside the body; hence the relevant "environment" is the body's interior chemical environment. This will help us reinstate human health as the main emphasis of exposure science. Lastly, biological monitoring for exposure assessment would be encouraged by concentrating on the interior chemical environment. The body's internal chemical environment is referred to as the "environment" in this context, and the amounts of chemically active substances in this internal environment are referred to as "exposures." Instead of using a bottom-up strategy that analyses food, water, air, and so forth, it makes more sense to use a top-down strategy based on biomonitoring (such as blood sampling) to investigate the exposome, current research indicates that non-genetic factors account for roughly 90% of the risks of chronic diseases (Rappaport, 2011).

Figure 5 shows the temperature ranges for the various effects. A gentle heat treatment, on the other hand, has not been linked to any toxicity and may cause a variety of alterations in cellular and molecular physiology. An increase in temperature may have an impact on a number of targets within the cell, such as the cytoskeleton, membranes, and macromolecule production. Evidence of immunological activation and the emergence of systemic immune responses is also present. Particle energy rises with temperature, making collisions more effective and accelerating chemical reactions. The temperature rises by 10 °C when the reaction rate is increased, and the temperature coefficient values differ depending on the enzyme. For the majority of enzymes found in animal cells and tissues, the ideal temperature range is 35 – 50 °C (Pereira Gomes et al., 2019).

Water makes up the majority of the human body, making up about 60% of its weight. The intracellular space contains around 40% of this aqueous proportion, followed by the extracellular space with 2%, the interstitial space with 15%, and the intravascular space with 5%. Although the human body needs a lot of water, it is unable to store much of it, so in order to keep internal equilibrium, water must be consumed. Water produces the heat

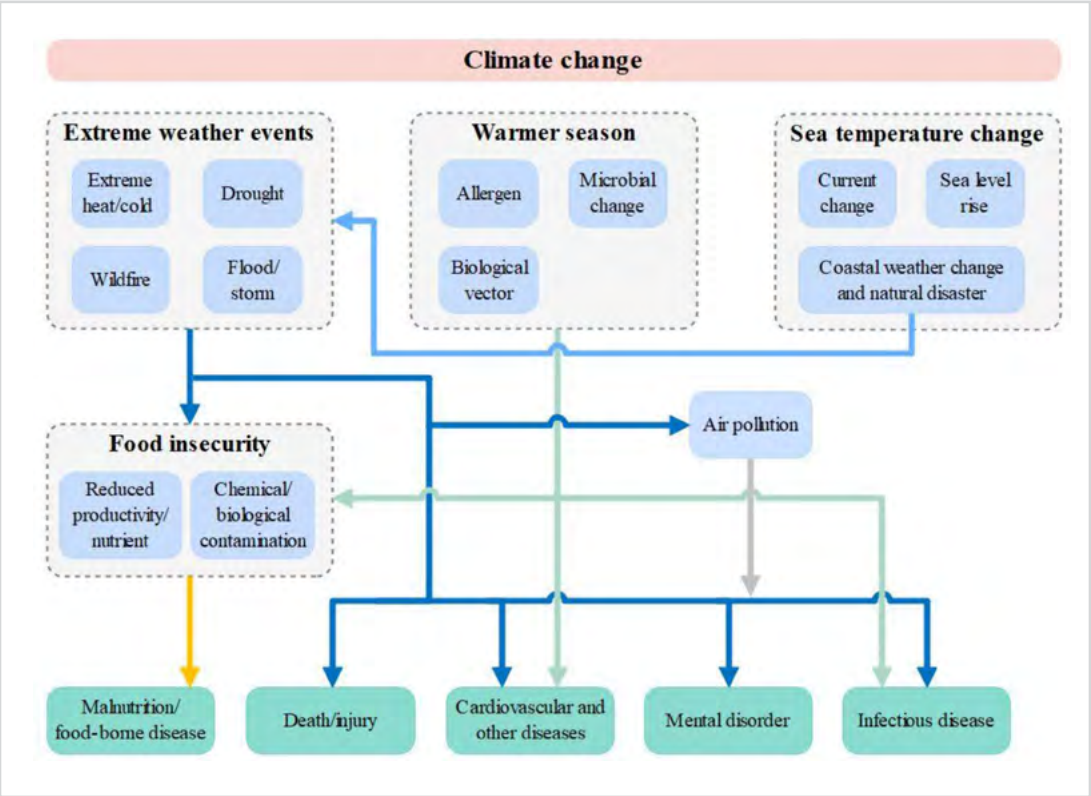

Figure 4 Key connections between health outcomes and climate change, including four groups extreme weather events, warmer season, sea temperature change and food insecurity (Q. Zhao et al., 2022). Copyright, Elsevier 2022

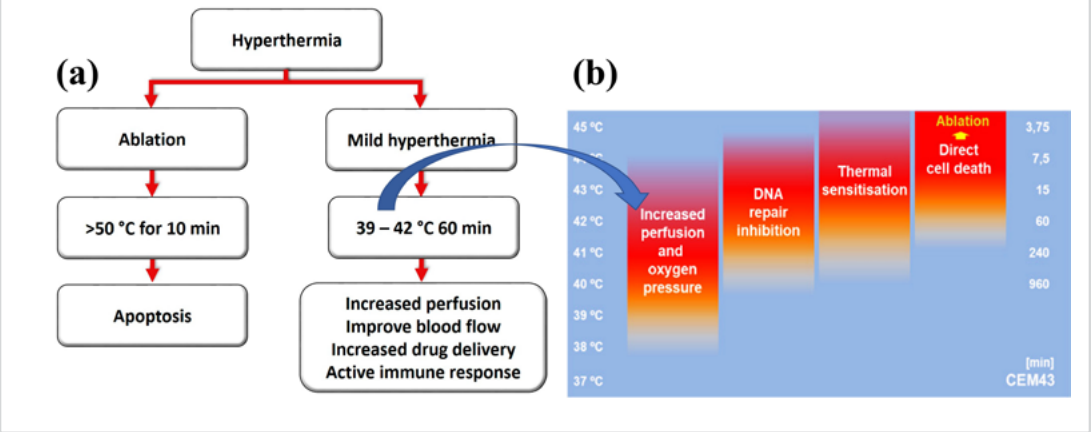

Figure 5 (a) Depending on the severity and duration, mild hyperthermia and ablation cause different types of cell damage (Gomes et al., 2019). Copyright MDPI © 2019. (b) Various biological mechanisms of hyperthermia, including the temperature at which the effect starts and, if relevant, the maximum temperature at which it stops or transitions to a higher one (van Rhoon et al., 2020a). Copyright Elsevier © 2020

energy required to break H-bonds when it is liquid at 37°C. The duration of this operation is roughly 1 to 20 picoseconds (1 ps = 10−12 s). Because a new bond is formed with a different molecule as soon as the first one breaks, molecules are in perpetual motion. This phenomenon is referred to as "flickering clusters," which are transient clusters of hydrogen bonds that form in the liquid phase of water. The patient's cardiac output is closely related to the T° variations that occur after intravenous fluid administration. An elevation of 1°C in the patient's core body temperature may occur for every 4 litres of saline solution given. Within the first 48 hours after a cardiac arrest, there is a correlation between increased mortality and each degree Celsius above 37.7°C (Robayo-Amortegui et al., 2024). As shown in Figure 6(a), lowering core body temperature in traumatic brain injury patients can reduce cerebral metabolism by as much as 6% for every degree Celsius that the temperature is lowered. This reduces cellular damage. Finally, as shown in Figure 6(b), water disease affects the integumentary system via the same mechanism of capillary leakage, venous congestion, and water extravasation into the interstitial space.

Figure 6 (c-d) summarizes the key pathophysiological mechanisms involved in exertional heat stroke. During physical exertion, hyperthermia develops when the cardiovascular system can no longer maintain effective thermoregulation. This results in altered blood flow, increasing intestinal permeability and allowing gut contents to enter the bloodstream—a process described by the leaky gut hypothesis. Both hyperthermia and translocated intestinal contents trigger a systemic inflammatory response syndrome (SIRS), which contributes to disseminated intravascular coagulation (DIC), manifesting as coagulopathy. These pathological responses often persist after collapse, continuing until the individual is cooled and consciousness is restored. The primary outcomes of exertional heat stroke are either fatality or survival accompanied by long-term health complications. The world is witnessing an increasing number of sudden deaths resulting from hyperthermia. Current scientific evidence suggests a causal link between hyperthermia and cardiac response, in line with the pathophysiological sequence of events associated with hyperthermia, although the exact mechanism of onset remains unknown (Richmond et al., 2015). From a forensic medicine perspective, there is no data indicating the exact pathophysiological mechanism that leads to sudden cardiac death. One of the significant advancements in clinical cardiology involves the identification of biochemical markers indicative of myocardial damage. In forensic medicine, researchers have been seeking a biochemical marker that would serve as the gold standard for post-mortem cardiac and non-cardiac mechanisms leading to ischemia and subsequent necrosis of cardiomyocytes (Marui et al., 2017). There is currently no data available on the exact percentage of deaths occurring during bathing, but there is an increasing amount of research focused on what precisely happens pathophysiological during such events (Mørch et al., 2017). Studies conducted thus far present unclear and unresolved pathophysiology and suggest the need for further investigation, as it is crucial to understand that death occurs during exposure to high water temperatures. Bathing in hot water has been associated with sudden death (Walter and Carraretto, 2016).

Although forensic medicine primarily addresses whether a death is violent, it is also very useful to help identify the cause of sudden deaths and the markers that can provide post-mortem evidence of the most likely cause of death. Among sudden deaths, it often turns out that cardiac aetiologies are the most common (Walter and Carraretto, 2016). Normal body temperature is approximately 37 ° (33.2–38.2 °C). This range becomes even narrower when rectal measurements are used instead of oral, tympanic, or axillary methods (Yang et al., 2017). Normal fluctuations in body temperature occur throughout the day, throughout the month, and across a lifetime.

Even slight deviations in core temperature can activate thermoregulatory mechanisms, and variations outside the physiological range can be life-

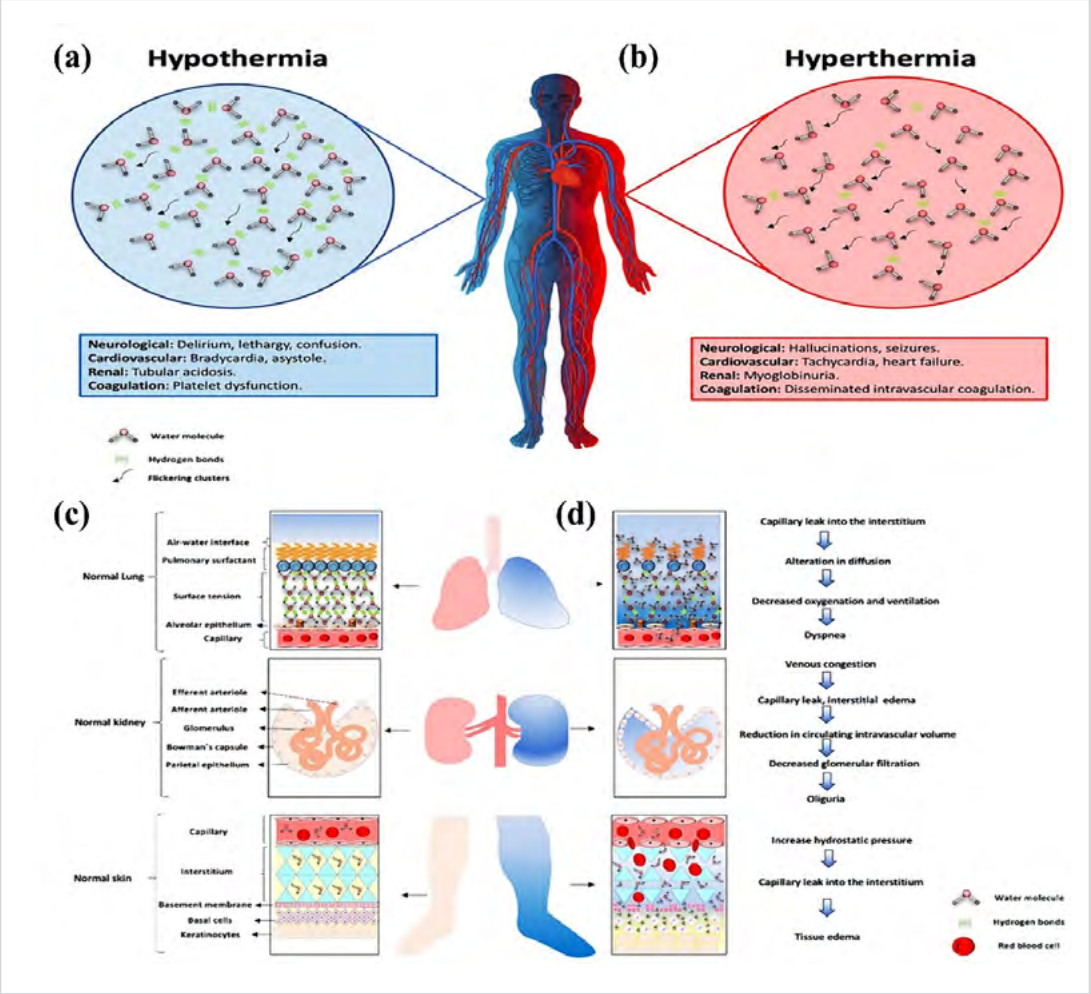

Figure 6 (a-b) The effects of water behaviour on the human body during hypothermia and hyperthermia. (c-d) The human body, which is mostly made of water, has amazing biochemical qualities and is essential for functions like thermoregulation and protein transport [22]. Copyright Frontiers in Medicine © 2024

threatening. A measured body temperature that goes above 41,9 °C can cause cytotoxicity, protein denaturation, and damaged DNA, potentially leading to organ failure. Conversely, if the body temperature falls in hypothermia, changes in cardiovascular, haematological, neuromuscular, and respiratory functions associated with this can also be fatal (Lepock, 2003).

Core body temperature is preserved within a range of ±6 °C (10-55°C) in environments, meanwhile the skin temperature fluctuates with the external environment. Oral temperature usually falls between 36.5 and 37 °C, whereas the rectal measured temperature is typically 0.5 °C higher (Natarajan et al., 2015). The normal range of body temperature is from 36 to 37.5 °C (Natarajan et al., 2015). During physical activity, body temperature can reach 38-40°C, while exposure to extreme cold can cause it to drop to 35°C.

Body temperature varies depending on the measurement site. n thermoregulation studies, the body is generally divided into two regions:

the skin, which fluctuates with environmental conditions, and the inner core (internal organs), which maintains a more stable temperature. The preoptic area of the anterior hypothalamus is essential for regulating temperature. Nerve receptors are typically more sensitive to heat than to cold, with stimulation of certain brain areas promoting sweating, while cooling can impair heat dissipation mechanisms.

Additionally, there are significantly more receptors for detecting cold than for heat, all of which signal to the hypothalamus.

Hyperthermia can be caused by heat stroke, infectious diseases, hypothalamic damage, necrosis, malignity and any stimulus that could activate immune cells to release endopyrogens (Heled et al., 2013; Lassche et al., 2019). Pathohistological findings in cardiovascular causes of sudden death vary depending on whether the event is acute, subacute, or chronic, leading to specific and nonspecific signs on the heart. In acute and subacute cases, such as those caused by hyperthermia, nonspecific unclear fibrosis or endocardial fibrosis with focal necrosis and eosinophilic infiltrates is often observed.

Previous studies of heart tissue exposed to heat have identified focal areas of necrotic fibers, which show fragmentation of microfilaments and create gaps between cardiomyocytes (Dervišević et al., 2023a).

Signs of relaxation and blood extravasation during hyperthermia have also been recorded, resulting from increased cardiac output and vasodilation as a response to high temperatures. Cardiac tissue has shown significant oedema with thinning of muscle fibres surrounding haemorrhagic areas. Classic microscopic examination of the heart following exposure to high temperatures has revealed moderate atherosclerosis, accompanied by necrotic foci and oedema (Quinn et al., 2014). A multidisciplinary approach that integrates basic medical sciences—such as medical biochemistry, pathology, and pathophysiology—through clinical cardiology can aid forensic medicine in identifying the cause of death more precisely and simply. This could be a significant study in the realm of fundamental research, contributing to clarifying the causes of death in forensic practice.

4. Discussion

Global warming is expected to have a greater impact on health due to temperature, although it is still unknown how population aging may affect these patterns. According to the findings, heat-related mortality in 800 locations across 50 countries/areas will rise by 0.5%, 1.0%, and 2.5%, respectively, at 1.5°C, 2°C, and 3°C of global warming; of these, 1 in 5 to 1 in 4 heat-related deaths can be ascribed to population aging. Even though progressive warming alone is predicted to reduce cold-related mortality, population aging will largely reverse this trend, resulting in a net increase in cold-related mortality of 0.1%–0.4% at 1.5–3 °C global warming (Chen et al., 2024). When global warming hits 1.5°C, 2°C, and 3°C, population aging alone causes an average increase in future temperature-related excess mortality of 0.8% (95% eCI: 0.6% to 0.9%), 1.7% (1.2% to 2.1%), and 2.6% (0.9% to 3.5%) for the non-optimal (cold and hot) temperature-related mortality, as seen in Figure 7. By contrast, under the maximum amount of warming (3 °C), climate alone was responsible for 3.0% (i.e., 0.08%/2.67%) of the net changes in non-optimal temperature-related mortality. Approximately two-thirds of the countries or regions analyzed (36 across all warming scenarios) showed a significant increase in mortality burden attributable to population aging. In contrast, only a few countries exhibited a significant decrease in mortality linked to aging—specifically 5, 3, and 2 countries at warming levels of 1.5 °C, 2 °C, and 3 °C, respectively.

One of the main causes of the rise in temperature-related mortality is aging.9. According to Yang et al. (2021), between 128,000 and 229,000 people died in China in the 2090s as a result of heat-related causes. The historical exposure-response relationship between temperature and the old population across various age categories is shown in Figure 8. In particular, the senior

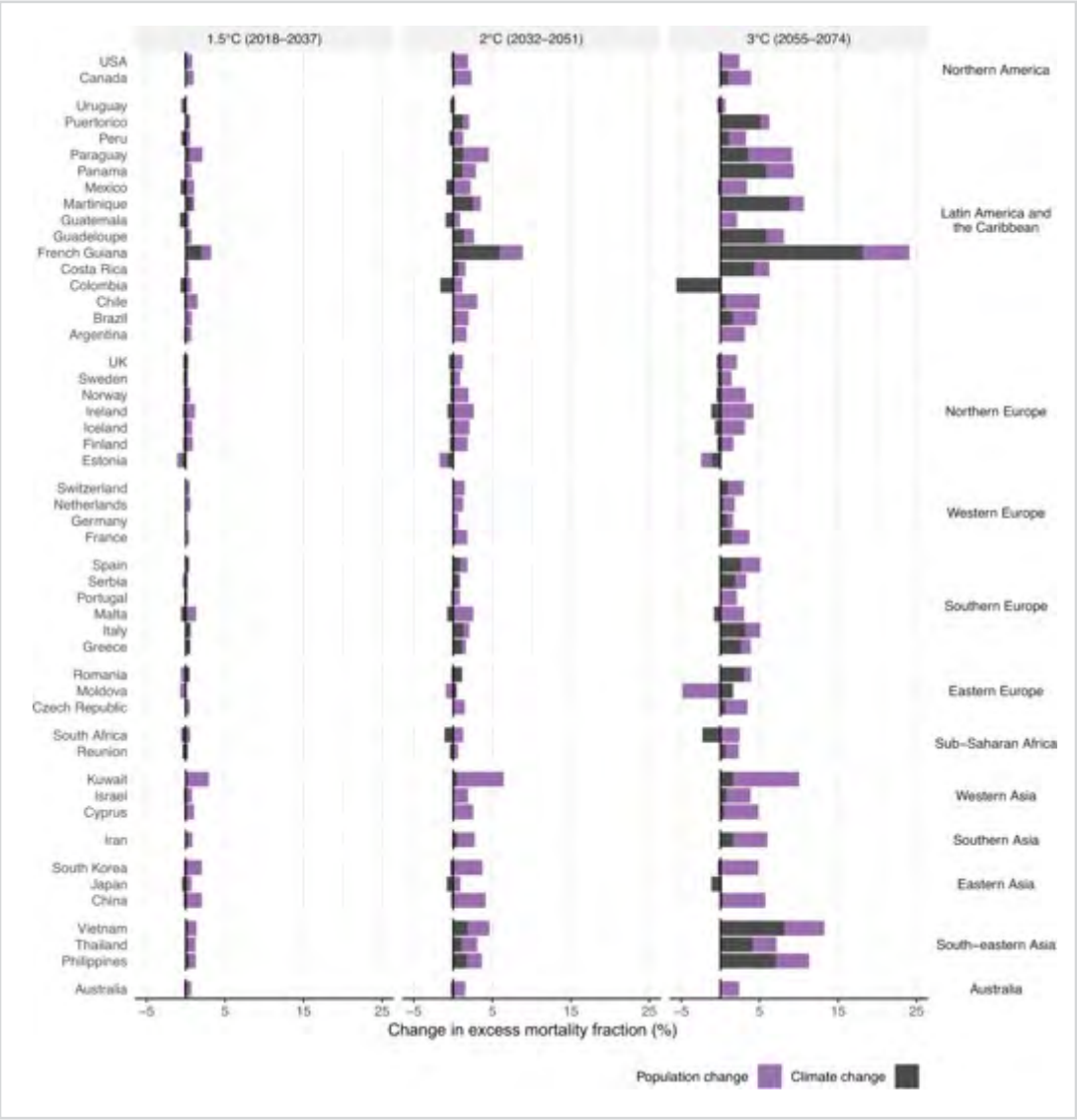

Figure 7 Changes at the country or area level related to climate change and population aging are presented for global warming scenarios of 1.5°C, 2°C, and 3°C, using 20-year periods compared to the historical baseline from 1995 to 2014. The 20-year moving average of global mean temperature is projected to first exceed 1.5°C, 2°C, and 3°C above pre-industrial levels (defined as 1850–1900) during the intervals 2018–2037, 2032–2051, and 2055–2074, respectively (Chen et al., 2024). Copyright, Nature © 2024

population in Nantong experienced an increase in the minimum mortality temperature (MMT) as they became older. The MMT was 24, 25, and 26 °C for the elderly (65–79, 80–89, and 90+, respectively). Compared to MMT, the mortality risk (RR) at the 97.5th percentile (31 °C) was 1.192 (95% CI:1.139–1.246), 1.340 (95% CI:1.283–1.399), and 1.492 (95% CI:1.402–1.588), respectively, and at the 2.5th percentile (1 °C) was 1.280 (95% CI:1.209−1.355), 1.708 (95% CI:1.583−1.844), and 2.217 (95% CI:1.981−2.480), respectively. When selected a maximum lag time of 10–21 days for temperature, 4–6 days for relative humidity, and 4–8 days for air

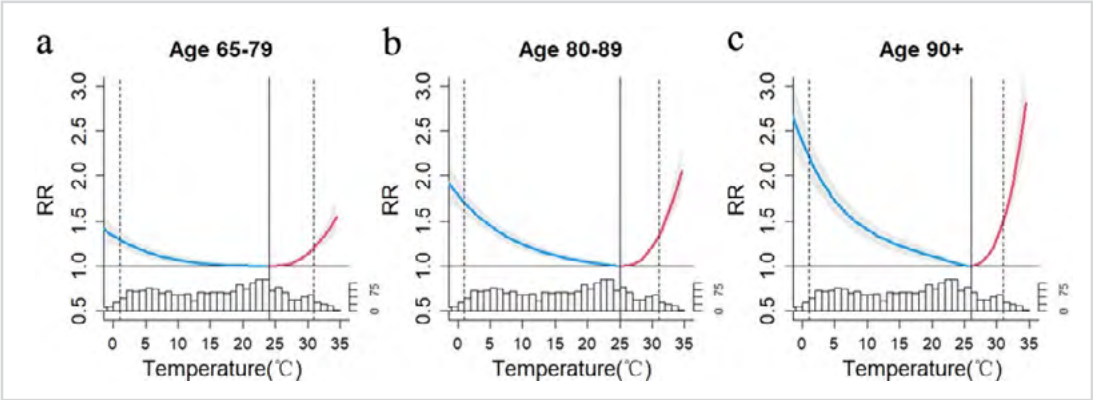

Figure 8 (a) ages 65–79; (b) ages 80–89; and (c) ages 90+. The bars show the frequency of temperatures from 2012 to 2017 at intervals of 1 °C. The associated 95% confidence intervals are indicated by the error band. The vertical dashed lines show the 2.5th and 97.5th temperature percentiles, whereas the vertical solid line shows the minimal mortality temperature (Huang et al., 2023). Copyright, Nature © 2023

pollutants, the temperature–mortality relationships were comparable (Huang et al., 2023).

Annual hot and cold degree days were used to regress the annual age-standardized mortality rates (ASMRs). Furthermore, people 75 years of age and older are predicted to see greater declines in ASMR as a result of less cold weather. When the analysis was stratified by the two most common causes of non-accidental and non-cancer deaths, it was found that, over the years under all scenarios, the increases in cardiovascular ASMR due to heat were greater than the increases in respiratory ASMR due to heat, especially during the 2090s (e.g., the increases for cardiovascular and respiratory deaths in the 2090s were 135.99% and 113.85%, respectively), while the decreases in respiratory ASMR due to less cold weather were larger over the years under all scenarios (Wang et al., 2022). In the current projection, age was anticipated to be a significant effect modifier (Figure 9). The increase in both hot and net effect under representative concentration pathways (RCP) was remarkably steep, with the net change from 0.12% in 2030s to 89.25% in 2090s (Table 1).

Table 1 Projected percentage increases in age-standardized mortality rates for Hong Kong are shown for the 2030s, 2050s, 2070s, and 2090s, relative to the baseline period of 2014–2018

RCP	2030s Hot	2030s Cold	2030s Net	2050s Hot	2050s Cold	2050s Net	2070s Hot	2070s Cold	2070s Net	2090s Hot	2090s Cold	2090s Net
2.6	3.20	−3.37	−0.29	4.69	−4.06	0.44	5.01	−4.52	0.27	5.15	−3.80	1.15
4.5	3.62	−3.78	−0.29	12.28	−5.27	6.36	17.54	−6.62	9.76	19.18	−7.12	10.69
6.0	−1.33	−1.91	−3.21	2.78	−4.30	−1.64	13.93	−6.71	6.29	23.42	−8.25	13.24
8.5	3.81	−3.55	0.12	21.30	−7.30	12.44	54.13	−9.82	38.99	116.49	−12.58	89.25

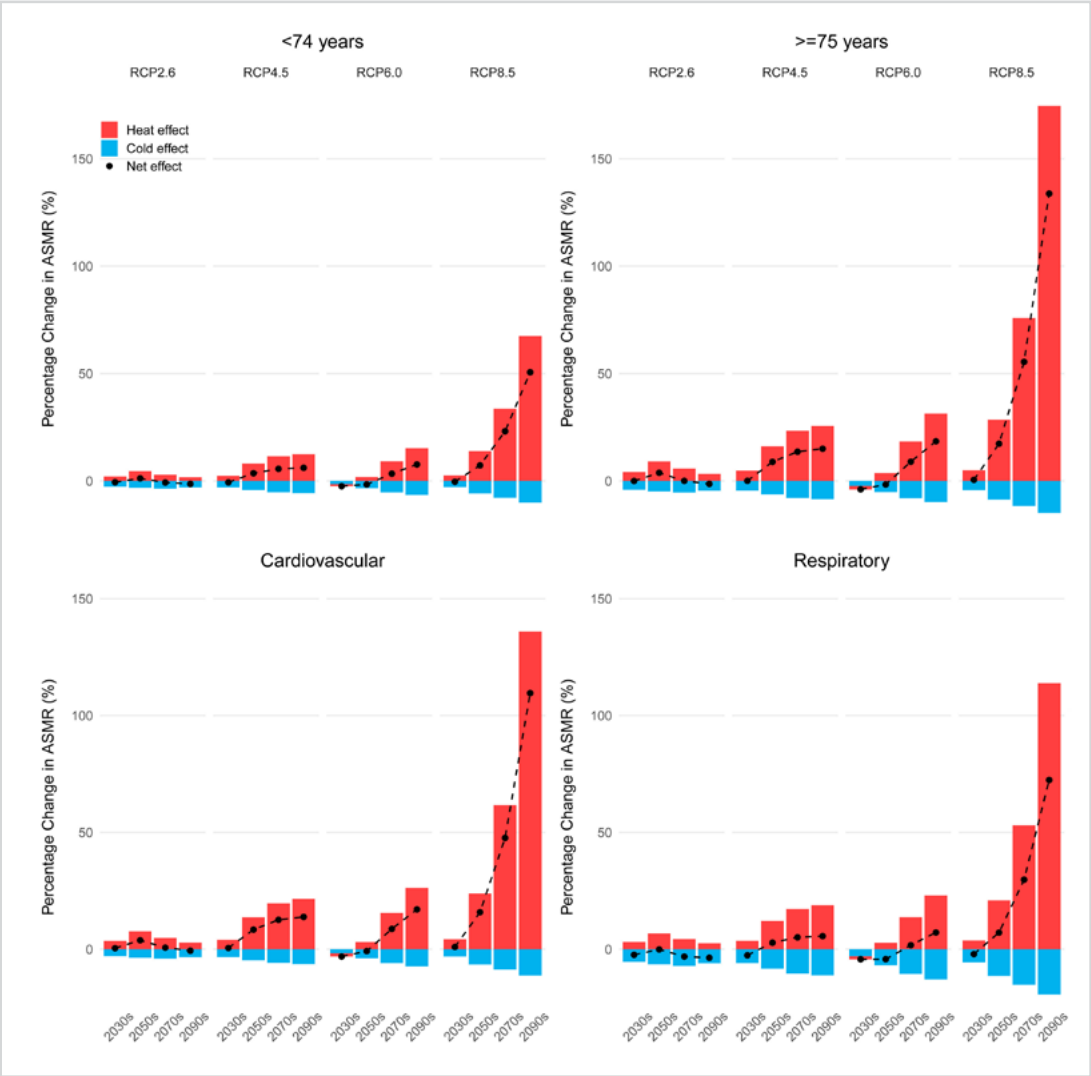

Figure 9 Using 2014–2018 as a reference baseline, calculate the average percentage changes in ASMR by age and cause of death in Hong Kong during the 2030s, 2050s, 2070s, and 2090s under various climate change scenarios. Age-standardized mortality rate, or ASMR (Wang et al., 2022). Copyright, Elsevier © 2022

5. Challenges and limitations in the implementation of technologies in forensic sciences

The forensic science specialist must confront and resolve a number of issues brought about by the exponential advancement of technologies, the creation of disruptive technologies, and the solutions that are developed on a regular basis. Although there are certain benefits to using new technologies, there are also technical drawbacks that need to be addressed. In the case of cameras, which have limited precision and resolution in images and 3D models, the technical constraints that are shown are contingent upon the features of the technological equipment utilized. Despite advancements in technology, challenges still exist in getting past technical constraints to guarantee the accuracy of forensic findings. Financial difficulties arise from the substantial investment needed to deploy cutting-edge

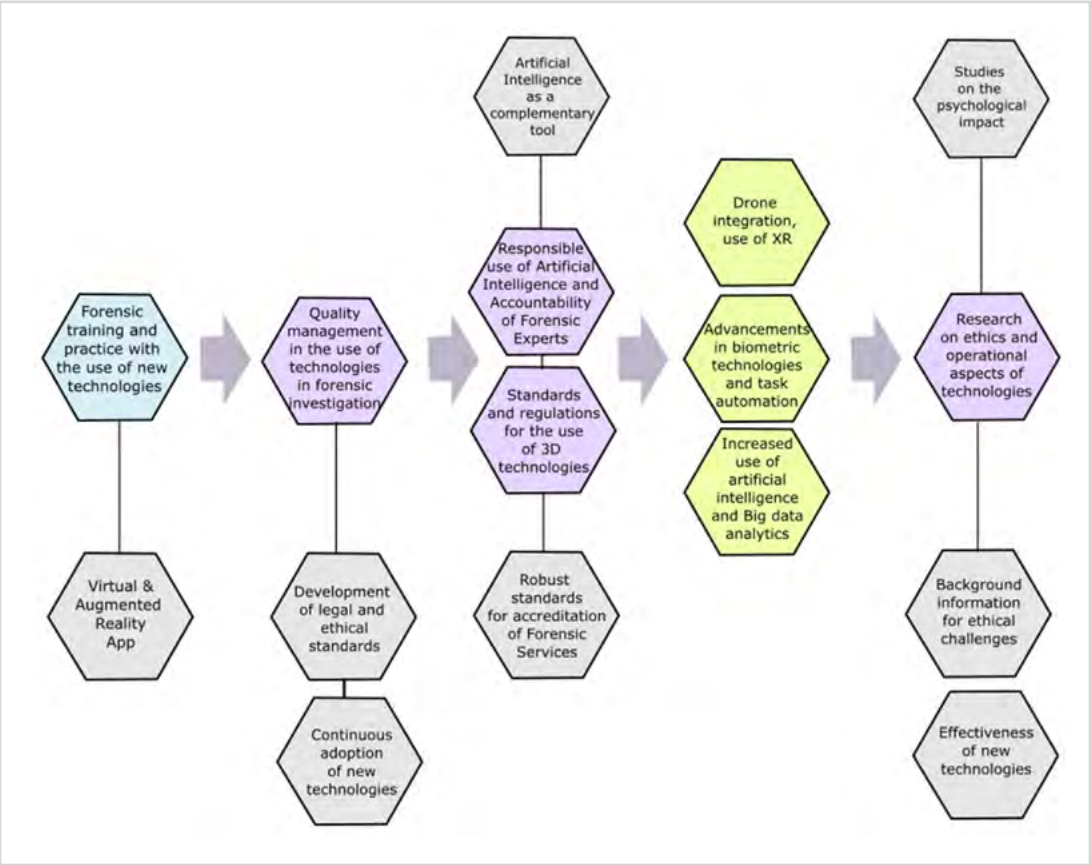

Figure 10 Upcoming advancements in forensic investigation technology use (Chango et al., 2024). Copyright, MDPI 2024

technologies. There are moral and legal issues with artificial intelligence use. Strict ethical standards have been implemented as a result of numerous inquiries. The significance of considering AI as an additional tool rather than a replacement for human judgment is highlighted by worries about the possibility of false information. As a result, taking preventative action is essential. To reduce these hazards, safety procedures must be followed and the detrimental effects must be recognized. Even with better sensors and more processing power, some forensic systems still struggle with accuracy. Before widespread adoption, thorough validation is necessary to guarantee dependable outcomes. Large-scale data-generating technologies, such 3D laser scanning, make information management difficult (Chango et al., 2024). The term "climate change" then describes how those components have changed throughout time. This includes, among other things, natural changes brought on by variations in the sun's activity or the tilt of the earth's axis. The phrase has become frequently used to describe manmade climate change in recent years. One crucial component of the job done by forensic pathologists and forensic anthropologists is estimating the post mortem delay. Although most people believe that temperature is the most significant factor influencing decomposition, some formulations also include relative humidity for a more thorough approximation. Anthropogenic climate change affects both of these factors (Strack and Smith, 2023). It is well recognized that various techniques used in forensic investigations can pose risks to both human health and the environment. This is often due to the chemical composition of the powders and liquids applied, as well as their

environmental impact. As a result, identifying safer alternatives—particularly for materials like fingerprint developers—has become a priority. Natural substances such as seaweed, certain spices, and chalcones have been suggested as more eco-friendly and cost-effective options. Digital forensics is one of the forensic and crime scene investigation fields that is expanding, as seen in Figure 10. Both the sharp rise in the amount of data being captured and the growing complexity of the digital environment are proving to be problems in this field (Thompson, 2024).

CONCLUSION

There is an urgent need for collaborative, multidisciplinary research to better understand the pathophysiological impacts of climate change on immunological disorders. To accurately measure how climate change influences immune function and disease patterns, inform mitigation and adaptation strategies, and evaluate their effectiveness, innovative data science approaches, novel biomarkers, and economic modeling are essential. Efforts to tackle disparities in climate change impacts must be grounded in the principles of justice, equity, diversity, and inclusion (JEDI). The health of people, animals, and the ecosystem as a whole are all at existential risk due to climate change. The danger of serious diseases has increased as a result of the shifting exposuresome. Linked to immunological dysregulation, which includes autoimmune disorders, cancer, allergies, and asthma. Although there has been progress in creating treatment options for these conditions, these strategies are insufficient to address the problems brought on by climate change.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: ED; Design: ED, TA; Supervision: HS, AZČ; Materials: TA, ED; Data Collection and/or Processing: TA; Analysis and/or Interpretation of the Data: TA; Literature Review: HS; Writing: ED, TA, AZČ; Critical Review: HS

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KLIMATSKE PROMJENE I ISTRAGA SMRTI: REDEFINISANJE EKSPERIMENTALNE FORENZIČKE NAUKE

SAŽETAK

Toplota je jedan od najsmrtonosnijih vremenskih fenomena u svijetu, a kako klimatske promjene napreduju, smrtnost uzrokovana toplotom bi se mogla naglo povećati. Prema izvještaju The Lancet Countdown on Health and Climate Change iz 2020. godine, tokom 2019. godine zabilježeno je dodatnih 475 miliona slučajeva toplotnih talasa širom svijeta, koji su izložili ranjive populacije i doveli do povećane smrtnosti i obolijevanja. Broj smrtnih slučajeva uzrokovanih toplotnim udarom kod osoba starijih od 65 godina povećan je za 53,7% u posljednjih 20 godina, sa ukupno 296.000 smrtnih slučajeva u 2018. godini. Teret smrtnosti povezan s visokim temperaturama, a koji je povezan s decenijama klimatskih promjena izazvanih ljudskim djelovanjem, predmetom je brojnih naučnih istraživanja. Klimatske promjene su već uticale na ljudsko zdravlje i nastaviće se njihovo djelovanje rezultirajući povećanjem broja smrtnosti od toplotnih udara. Rizici povezani s vrućinom mogu se pogoršati ili poboljšati kao rezultat interakcije između klimatskih promjena i drugih trendova, uključujući urbanizaciju, rast i starenje populacije te socioekonomski razvoj. Visok stepen nesigurnosti među prediktorima smrtnosti povezanih s vrućinom (kao što su ljudsko ponašanje i prilagodba) često je uzrok velikih varijacija u procjenama smrtnosti; nasuprot tome, sporiji rast stanovništva i veća sposobnost prilagodbe proizvode niže procjene štetnih zdravstvenih efekata.

Ključne riječi: Eksperiment, forenzika, klimatske promjene, postmortum, smrt

CONFERENCE PAPER

EVALUATION OF THE HEMATOLOGICAL RESPONSE TO VARIOUS THERAPEUTIC APPROACHES IN THE MANAGEMENT OF OPEN WOUNDS: AN EXPERIMENTAL STUDY IN RODENTS

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ABSTRACT

Hematological analyses are important in veterinary and human medicine for early detection of homeostatic changes and assessment of systemic responses to injury, stress, and therapy. In this study, the hematological response of Wistar rats with surgically induced wounds was analyzed following treatment with natural and synthetic therapeutic agents.

Twenty-four healthy adult Wistar rats were randomized into six groups (n = 4): surgical control (C), gentamicin ointment (AB), Manuka honey MGO 250+ (MH1), Manuka honey MGO 550+ (MH2), chlorine dioxide (CL), and absolute control without incision and therapy (CC). After a standardized abdominal incision (except CC), treatments were applied twice daily for 7 days. On day 7, hematological parameters and peripheral blood smears were analyzed.

Significant intergroup differences were found in WBC ($p = 0.030$), lymphocytes ($p = 0.020$), RBC ($p = 0.002$), MCV and MCH ($p < 0.001$), RDW-CV and RDW-SD ($p < 0.001$). Elevated estimated marginal means were most consistent in MH1, MH2, and CC, particularly for WBC, lymphocytes, RBC, and red cell indices, suggesting a more pronounced hematological response.

Poikilocytosis was present in all groups, with annulocytes most prevalent (up to 6.01% in MH1), followed by dacryocytes and ovalocytes. The leukogram showed lymphocyte dominance in all groups, with the highest percentage in MH1 (65.75%), and the highest neutrophil percentage in AB (47.75%).

This study provides insight into potential differences in the hematological response to natural and synthetic wound treatments in an animal model, and serves as a basis for further research into the biological safety and efficacy of the therapies used.

Keywords: Chlorine dioxide, experimental wound, gentamicin, Manuka honey, poikilocytosis

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INTRODUCTION

The skin, as the body's primary protective barrier, responds to injury by initiating a cascade of complex biological processes aimed at restoring its structural integrity and reestablishing its thermoregulatory, endocrine, and sensory functions (Al-Masawa et al., 2022). Being the largest organ in mammals, the skin functions as a multilayered interface between the organism and its environment, providing critical protection to internal organs against external insults, while simultaneously playing a central role in maintaining fluid homeostasis, regulating body temperature, and supporting immunological, neurological, and metabolic activities (Percival et al., 2015; WHO, 2009; DeBoer and O'Connor, 2004).

However, wounds represent a significant disruption of this protective barrier, often accompanied by bacterial contamination. This contamination remains one of the primary causes of delayed healing and wound complications, as wounds frequently harbor diverse microorganisms—many of which are potentially pathogenic and capable of triggering purulent infections (Kožár et al., 2018; Rijal et al., 2017). The increasing prevalence of antibiotic-resistant bacteria, virulent strains, and novel pathogens has intensified the search for effective, alternative antimicrobial agents for use in both human and veterinary medicine (Chapnik and Wilkins, 2014).

Among the most promising alternatives is chlorine dioxide (ClO_2), a water-soluble gas with potent broad-spectrum antimicrobial properties. It has been widely utilized in water purification and the food industry, and is increasingly recognized in healthcare settings for its efficacy against bacteria, viruses, and fungi, combined with low cytotoxicity (Simpson et al., 1993; Sanekata et al., 2010; Wen et al., 2017; Venkatnarayanan et al., 2017; Bridges et al., 2018; Palcsó et al., 2019).

In parallel, natural substances such as manuka honey, derived from *Leptospermum scoparium*, have gained substantial attention due to their proven antibacterial, anti-inflammatory, and immunostimulatory effects (Mandal and Mandal, 2011; Ahmed et al., 2003). Manuka honey promotes wound healing through multiple mechanisms, including modulation of the local immune response, stimulation of tissue regeneration, and inhibition of microbial colonization (Yao et al., 2003).

In both human and veterinary medicine, the evaluation of hematological parameters plays a fundamental role in the early detection of pathological conditions, monitoring of therapeutic efficacy, and overall assessment of health status (Katica and Gradaščević, 2017; Katica and Delibegović, 2019). These parameters are key indicators of physiological and pathological processes, and offer insight into systemic alterations induced by local injuries or treatments (Ihedioha, 2004).

Despite the well-documented therapeutic properties of manuka honey and chlorine dioxide, current literature remains limited regarding their systemic hematological effects when applied topically to skin wounds. Most available studies focus on antimicrobial efficacy or healing outcomes, while data on hematological responses, particularly in experimental models are scarce. Furthermore, existing research primarily investigates oral or parenteral administration routes, leaving a significant gap in understanding the systemic implications of localized wound treatments.

Therefore, the aim of this study was to evaluate the hematological response to topical application of manuka honey, chlorine dioxide, and antibiotic in rats with surgically induced skin wounds. By assessing hematological parameters as systemic biomarkers, this study seeks to provide a deeper understanding of the organism's response to various wound therapies and contribute to the identification of effective alternative treatment options.

MATERIAL AND METHODS

Ethics committee approval

This study was approved by the Ethics Committee of the Veterinary Faculty of the University of

Sarajevo, who gave a positive opinion under number: 07-03-1103-2/24, from 04.12.2024.

Animal model

In the research 24 clinically healthy adult rats of both sexes, Wistar strain, aged 2-3 months, with an average weight 180-250 g were used. The rats had free access to food and water provided during the experiment, and 12-hour rotations of light and dark. The ambient temperature was maintened between 20-23^{0}C and humidity 60%±10% (Katica and Gradaščević, 2017).

General experimental procedure and study groups

The twenty-four rats were randomly divided into six groups depending on which treatment was used. The first group C (4 rats) received no therapy and served as the surgical control. The second group A (4 rats) was treated with gentamicin ointment (gentamicin Bosnalijek 1mg/g). The third group MH1 (4 rats) received Manuka honey MGO 250+ (Manuka Health, Te Awamutu, New Zealand). Fourth group MH2 (4 rats) received Manuka honey MGO 550+ (Manuka Health, Te Awamutu, New Zealand). The fifth group CLO (4 rats) was treated with chlorine dioxide (Dioxy Activ Supra), and the sixth group CC (4 rats) consisted of rats without surgical incision and without therapy, serving as the absolute control group.

Surgical procedure

Prior to the surgical intervention, all tested animals were acclimatized to the experimental conditions. General anesthesia was induced by intramuscular injection of 5 mg/kg xylazine hydrochloride 2% (2% Xylazin, CpPharma, Bergdorf, Germany) and 60 mg/kg ketamine hydrochloride (International B.V. Netherlands). Once surgical anesthesia was confirmed, the rats abdominal hair was carefully removed to ensure clear visibility and access to the surgical site. A 4 cm longitudinal incision was made through skin and subcutaneous tissue along the median abdominal line, exposing a wound surface in constant contact with the non-sterile bedding. After 24 hours, each animals received treatment depending on the group it was in. Therapies were administered twice daily for seven (7) consecutive days.

Study design

Hematological – biochemical procedures

On the seventh day of the experiment, peripheral blood samples were taken by tail vein puncture in EDTA vacutainers with a volume of 3 ml. The puncture site was previously disinfected with standard disinfectants. Analysis of hematological parameters was performed using a Mindray BC-20S automated hematology analyzer. The following parameters were determined: red blood cell count (RBC, $\times10^{12}$/L), hemoglobin concentration (HGB, g/L), hematocrit (HCT, L/L), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width – coefficient of variation (RDW-CV) and standard deviation (RDW-SD, fL), white blood cell count (WBC, $\times10^9$/L), granulocyte count and percentage (Gran#, Gran%, $\times10^9$/L), lymphocyte count and percentage (Lym#, Lym%, $\times10^9$/L), monocyte count and percentage (Mon#, Mon%, $\times10^9$/L), and platelet count (PLT, $\times10^9$/L).

Cells of the leukocyte order were differentiated, and numerical values were expressed as percentages after 1000 such cells were analyzed. Differentiated: lymphocytes (L) (%), monocytes (M) (%), neutrophils (N) (%), basophils (B) (%) and eosinophils (E) (%).

Values are shown in percentages (Bajrić et al., 2020; Katica et al., 2019).

Quantification of poikilocytotic erythrocytes

Blood smears of peripheral blood were made, dried in air and stained by the Giemsa method. They were processed according to standard technical laboratory procedures. Poikilocytes were assessed semiquantitatively (Christopher et al., 2014).

On each original stained smear, 2000 erythrocytes were counted and characterized at a microscopic magnification of 1000 X. Poikilocytes were defined on the basis of standard morphology, and counting was limited to representative monolayer

fields in which about half of the erythrocytes touched but did not overlap. The number and type of poikilocytes was recorded and expressed as a percentage of the red blood cells. Poikilocytosis was then classified as: absent (0%), rare (0.05-0.5%), mild (>0.5-3%), moderate (>3-10%) and pronounced (>10%).

Statistical data processing

All data are presented as arithmetic means and standard deviations (SD). The normality of distribution within groups was assessed using the Shapiro-Wilk test. For variables that showed a normal distribution ($p > 0.05$), one-way analysis of variance (ANOVA) was applied to determine intergroup differences. In addition, estimated marginal means with 95% confidence intervals were calculated and visualized to better illustrate intergroup variation and deviation from the overall mean. Data analysis was done using SPSS software (version 21.0. IBM). The level of statistical significance was set at $p<0.05$.

RESULTS

Results of peripheral blood parameters

For all variables of peripheral blood parameters across all experimental groups, the results of the Shapiro-Wilk (SW) test indicated a normal distribution of data ($p > 0.05$). Therefore, the data met the assumptions for parametric statistical analysis. Table 1 presents the arithmetic means and standard deviations (Mean ± SD) for each measured parameter across all groups.

Table 1 Arithmetic means and standard deviations (Mean ± SD) of hematological parameters in all experimental groups. Data normality was assessed using the Shapiro-Wilk test (SW); p-values greater than 0.05 indicate normal distribution

		PLT (x10^9/L)	RDW-SD (fL)	RDW-CV	MCHC (g/L)	MCH (pg)	MCV (fL)	HCT	HGB (g/L)	RBC	Mon%	Lym%	Gran%	Mon# (x10^9/L)	Lym# (x10^9/L)	Gran# (x10^9/L)	WBC (x10^9/L)
C	Mean	1039.75	33.13	0.15	320.25	18.15	56.75	0.46	148.00	8.14	0.04	0.48	0.48	0.10	1.19	1.16	2.45
	SD	154.79	0.63	0.00	5.56	0.44	0.72	0.02	9.90	0.36	0.00	0.03	0.03	0.01	0.28	0.15	0.43
	SW test	0.968	0.997	0.993	0.994	0.963	0.828	0.925	0.823	0.879	0.928	0.898	0.893	0.863	0.919	0.856	0.921
	p	0.828	0.991	0.972	0.975	0.798	0.163	0.565	0.15	0.334	0.584	0.42	0.397	0.272	0.533	0.245	0.541
AB	Mean	990.50	27.63	0.13	316.75	17.00	53.70	0.47	148.50	8.71	0.05	0.47	0.48	0.14	1.50	1.52	3.16
	SD	117.69	0.94	0.00	5.50	0.43	1.24	0.02	4.44	0.34	0.00	0.04	0.04	0.02	0.37	0.19	0.57
	SW test	0.917	0.937	0.828	0.908	0.927	0.837	0.965	0.963	0.987	0.848	0.923	0.9	0.95	0.873	0.86	0.787
	p	0.519	0.634	0.163	0.473	0.577	0.186	0.809	0.798	0.942	0.22	0.555	0.432	0.714	0.31	0.262	0.08
MH1	Mean	1055.25	29.13	0.14	310.75	16.80	54.13	0.50	156.00	9.29	0.04	0.52	0.44	0.16	1.96	1.65	3.76
	SD	96.57	1.60	0.01	2.22	0.22	0.69	0.02	5.60	0.32	0.00	0.03	0.03	0.03	0.47	0.40	0.87
	SW test	0.89	0.965	0.985	0.801	0.927	0.856	0.772	0.881	0.961	0.971	0.873	0.912	0.935	0.836	0.951	0.931
	p	0.383	0.813	0.933	0.103	0.577	0.245	0.061	0.343	0.784	0.85	0.31	0.494	0.625	0.185	0.722	0.6
MH2	Mean	1179.00	30.25	0.15	310.00	16.63	53.63	0.49	152.75	9.20	0.04	0.51	0.45	0.14	1.90	1.69	3.73
	SD	165.92	2.31	0.01	5.23	0.21	1.24	0.01	5.85	0.37	0.01	0.04	0.04	0.03	0.46	0.30	0.73
	SW test	0.978	0.836	0.953	0.859	0.926	0.934	0.885	0.96	0.868	0.999	0.812	0.772	0.991	0.764	0.931	0.805
	p	0.891	0.185	0.735	0.256	0.572	0.615	0.359	0.78	0.291	0.998	0.126	0.061	0.962	0.052	0.6	0.111
CLO	Mean	1274.00	30.75	0.15	318.50	16.95	53.18	0.45	158.50	8.47	0.05	0.46	0.50	0.13	1.28	1.39	2.80
	SD	253.93	1.17	0.00	0.58	0.31	0.97	0.03	27.70	0.42	0.01	0.05	0.05	0.05	0.28	0.27	0.53
	SW test	0.953	0.91	0.971	0.729	0.972	0.97	0.935	0.885	0.882	0.955	0.952	0.941	0.853	0.886	0.969	0.761
	p	0.734	0.483	0.85	0.024	0.855	0.84	0.627	0.361	0.349	0.748	0.727	0.658	0.235	0.364	0.833	0.049
CC	Mean	901.25	32.85	0.15	315.50	17.58	55.78	0.46	145.50	8.28	0.05	0.52	0.43	0.22	2.34	1.90	4.45
	SD	50.77	1.37	0.01	5.57	0.31	0.51	0.03	7.94	0.48	0.01	0.04	0.04	0.10	0.77	0.53	1.37
	SW test	0.854	0.885	0.881	0.957	0.92	0.93	0.847	0.946	0.803	0.912	0.886	0.895	0.9	0.944	0.892	0.853
	p	0.24	0.362	0.343	0.759	0.538	0.594	0.217	0.689	0.108	0.492	0.366	0.404	0.431	0.677	0.391	0.236

The analysis of variance (ANOVA) results are summarized in Table 2. Statistically significant differences among groups were observed for several parameters, including WBC ($p = 0.030$), lymphocyte count (Lym#; $p = 0.020$), RBC ($p = 0.002$), MCV ($p < 0.001$), MCH ($p < 0.001$), RDW-CV ($p = 0.001$), RDW-SD ($p < 0.001$), and PLT ($p = 0.037$). Other parameters did not show significant differences ($p > 0.05$). Table 2 shows the arithmetic means and standard deviations for each parameter across all groups.

Table 2 Analysis of variance (ANOVA of blood parameters between six groups of rats tested)

	Sum of squares	df	Mean square	F	p
WBC (x10n9/L)	10.68*	5	2.14	3.230	**0.030**
Gran# (x10n9/L)	1.32	5	0.27	2.399	0.078
Lym# (x10n9/L)	3.93*	5	0.79	3.569	**0.020**
Mon# (x10n9/L)	0.03	5	0.01	2.540	0.066
Gran%	0.01	5	0.00	2.073	0.116
Lym%	0.02	5	0.00	1.957	0.134
Mon%	0.00	5	0.00	1.305	0.306
RBC (x10n12/L)	4.55**	5	0.91	6.191	**0.002**
HGB (g/L)	512.21	5	102.44	0.607	0.696
HCT	0.01	5	0.00	2.128	0.062
MCV (fL)	39.95**	5	7.99	9.101	**0.000**
MCH (pg)	6.54**	5	1.31	11.798	**0.000**
MCHC (g/L)	342.71	5	68.54	1.297	0.077
RDW-CV	0.00**	5	0.00	7.517	**0.001**
RDW-SD (fL)	90.43**	5	18.09	8.734	**0.000**
PLT (x10n9/L)	357445.71*	5	71489.14	3.027	**0.037**

Quantification of poikilocytotic forms of erythrocytes and leukogram results (%)

Table 3 Determined poikilocytotic forms of RBCs in groups expressed in (%) from 2,000 analyzed RBCs

Types of poikilocytotic forms RBC	Group CC	Group C	Group AB	Group MH1	Group MH2	Group ClO
Ovalocytes	0.35	0.52	0.32	0.33	0.48	0.60
Dacrocytes	0.48	0.52	1.00	1.10	1.08	0.53
Anulocytes	5.00	4.53	3.85	6.01	5.66	5.47
Echinocytes	0.05	0.11	0.06	0.06	0	0.18
Stomatocytes	1.31	0.72	0.40	1.22	0.60	0.80
Drepanocytes	0	0	0	0	0	0
Schistocytes	0.15	0.16	0.18	0.13	0.10	0.18
Codocytes	0.30	0.16	1.31	0.55	0.80	1.48
Acanthocytes	0.01	0	0	0	0	0.01
Spherocytes	0.13	0.57	0.41	0.17	0.22	0.25

Table 4 Leukogram results (%)

Groups	Neutrophil	Lymphocyte	Monocyte	Basophil	Eosinophil
CC	35.25	61.5	1.25	0	1.75
C	41.0	57.00	0.25	0	1.75
AB	47.75	51	0	0	1.0
MH1	33.5	65.75	0.25	0	0.25
MH2	40.25	50.0	0	0	2.25
ClO	42.0	55.25	0.25	0	2.5

Original microscopic photos of individual poikilocytotic forms of erythrocytes from six groups of tested rats (Figure 1)

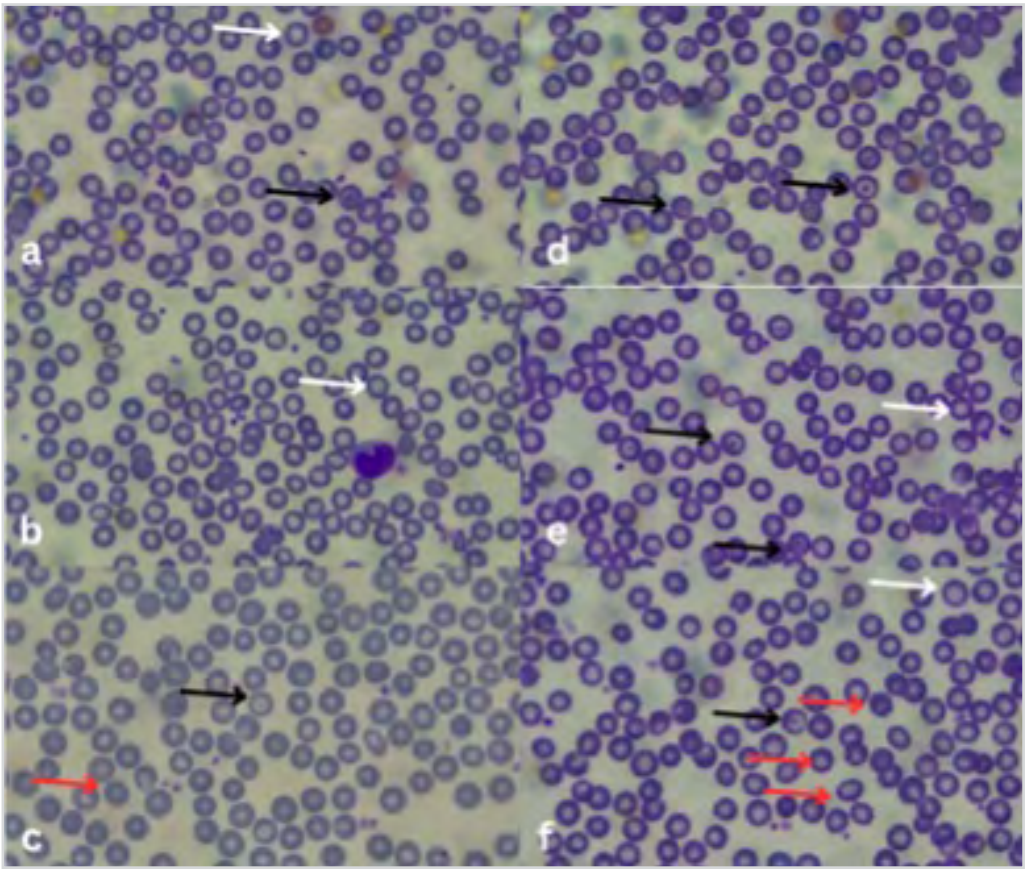

Figure 1 Poikilocytotic forms of erythrocytes a) AB group; b) CC group; c) C group; d) MH1 group; e) MH2 group and f) CLO group. Black arrows indicate target cells, white arrows anulocytes and red arrows echinocytes

DISCUSSION AND CONCLUSION

The skin, as the largest organ, protects internal organs and maintains homeostasis; however, its integrity can be compromised by wounds that often harbor diverse microorganisms. Bacterial contamination remains one of the primary causes of complications in the wound healing process, as many of these microbes can be potentially pathogenic and lead to purulent infections (Percival et al., 2015; Kožár et al., 2018; Rijal et al., 2017).

With the emergence of antibiotic-resistant bacteria, more virulent strains, and new pathogens, the need to find effective antimicrobial agents for wound treatment in veterinary medicine has become increasingly urgent (Chapnik and Wilkins, 2014).

Chlorine dioxide (ClO_2) has gained notable attention as a promising agent due to its strong antimicrobial properties against a wide range of bacteria, fungi, and viruses. Originally utilized as a gaseous, water-soluble disinfectant in water purification and the food industry, it is now increasingly considered for medical purposes. Its broad-spectrum activity combined with relatively low toxicity makes it a compelling option for healthcare applications (Sanekata et al. 2010; Wen et al. 2017; Venkatnarayanan et al. 2017; Bridges et al. 2018; Palcsó et al. 2019).

The therapeutic potential of manuka honey, particulary its role in modulating immune responses and microbial control, has been acknowledged in previous studies, which underlines its relevance in wound healing contexts (Mandal and Mandal, 2011; Ahmed et al., 2003; Yao et al., 2003).

The CC group exhibited the highest WBC (4.45×10^9/L), indicating strong systemic immune activation. In contrast, the untreated control group (C) showed the lowest WBC (2.45×10^9/L), suggesting a suppressed hematological response. The antibiotic group (AB) showed moderate elevation (3.16×10^9/L), while the manuka honey-treated groups MH1 (3.76×10^9/L) and MH2 (3.73×10^9/L) presented significantly increased values. These findings confirm the capacity of both natural and synthetic topical agents to influence systemic immune profiles.

Lymphocyte count followed a similar trend, with significantly higher values in CC (2.34×10^9/L), MH1 (1.96×10^9/L), and MH2 (1.90×10^9/L) compared to control (1.19×10^9/L), confirming immune modulation. Leukogram revealed a lymphocyte-dominant profile, especially in MH1 (65.75%) and CC (61.5%), while AB had the highest neutrophil percentage (47.75%), possibly reflecting acute antimicrobial activity induced by antibiotic treatment.

The increased RBC values in MH1 and MH2 compared to control, along with moderately altered RDW-SD values, suggest stimulated erythropoesis and red blood cell turnover. However, MCV and MCH values in these groups were lower than those in the control group, indicating microcytic and hypochromic characteristics. These findings are consistent with previous studies reporting that honey administration can modulate hematological parameters even in the absence of disease (Aliyu et al., 2012). Akinbami et al. (2013) reported that alterations in RBC, Hb, PCV, RDW, and lymphocytes can impact disease progression and prognosis. However, in their study, honey was administered orally, whereas in our model manuka honey was applied topically, possibly explaining the localized and moderate hematological changes observed.

A related study on burn injury reported significantly decreased RBC, MCV, and MCH values following thermal damage, with WBC counts varying depending on the treatment protocol. Platelet levels remained comparable to healthy controls, and neutrophils and eosinophils were largely unaffected (Kulyar et al., 2022). These results are in line with our findings, suggesting that immune modulation may be highly context-dependent and influenced by the extent of tissue injury and reparative mechanisms.

Morphological erythrocyte analysis revealed increased anulocytes in MH1 (6.01%) and MH2 (5.66%) compared to the control group (4.53%), potentially indicating regenerative erythropoiesis

or oxidative stress. Elevated frequencies of dacryocytes and codocytes in MH1, AB, and CC further support the presence of systemic stress or treatment-induced hematological adaptation. Interestingly, codocytes were most prominent in the ClO_2 group (1.48%), suggesting that chlorine dioxide may influence red cell membrane dynamics.

Chlorine dioxide (ClO_2), traditionally used as a gaseous water-soluble disinfectant in industrial and food sectors, is gaining increasing attention in healthcare due to its potent antimicrobial properties and low toxicity. It has demonstrated efficacy against bacteria, viruses, fungi, and other pathogens, and is recognized for accelerating wound healing, particularly in burn injuries (Young, 2016). In our study, ClO_2 induced moderate WBC (2.80×10^9/L) and lymphocyte (1.28×10^9/L) elevations, accompanied by an increased eosinophil count (2.5%), indicating immunological activity and systemic reactivity. In accordance with the above, our research corresponds with the study conducted by Young (2016).

Platelet count remained within the expected physiological range across all experimental groups, showing no marked thrombocytic response to treatment.

Taken together, our findings support that topical applications of manuka honey and chlorine dioxide can modulate hematological profiles and immune responses. Manuka honey demonstrated more pronounced effects on leukocyte and erythrocyte parameters, while chlorine dioxide showed relevant activity, particularly in red cell morphology and eosinophilic response. These results reinforce the therapeutic potential of both natural and synthetic topical agents in wound management. However, it is important to note that the relatively small number of animals per group and the short observation period (7 days) represent limitations of this study. These factors may have influenced the extent of the hematological changes observed and should be addressed in future research through larger sample sizes and extended monitoring.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

CONTRIBUTIONS

Conception – M.K., N.K-D.; Design – M.K., N. K-D., N.K.; Supervision – M.K., N. K-D.; Materials – M.K., N. K-D., N.K.; Data Collection and Processing – N. K-D., M.K., N.Č., N.K.; Interpretation – N. K-D, M.K., N.Č.; Literature Review – N. K-D, M.K.; Writing – N. K-D.; M.K.; Critical Review – M.K.; N. K-D

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EVALUACIJA HEMATOLOŠKOG ODGOVORA NA RAZLIČITE TERAPIJSKE PRISTUPE U LIJEČENJU OTVORENIH RANA: EKSPERIMENTALNA STUDIJA NA GLODARIMA

SAŽETAK

Hematološke analize imaju značajnu ulogu u veterinarskoj i humanoj medicini, jer omogućavaju rano otkrivanje promjena u homeostazi organizma i procjenu sistemskog odgovora na povredu, stres i terapijske intervencije.

U ovom istraživanju analiziran je hematološki odgovor Wistar štakora sa hirurški izazvanim ranama nakon tretmana prirodnim i sintetičkim terapijskim sredstvima.

Dvadeset četiri klinički zdrava odrasla Wistar štakora randomizirana su u šest grupa (n = 4): hirurška kontrola (C), gentamicin mast (AB), Manuka med MGO 250+ (MH1), Manuka med MGO 550+ (MH2), hlor dioksid (CL) i apsolutna kontrola bez incizije i terapije (CC). Nakon standardizirane abdominalne incizije (osim u grupi CC), tretmani su primjenjivani dva puta dnevno tokom 7 dana. Sedmog dana provedena je analiza hematoloških parametara i perifernih krvnih razmaza.

Značajne razlike između grupa utvrđene su za WBC ($p = 0.030$), limfocite ($p = 0.020$), RBC ($p = 0.002$), MCV i MCH ($p < 0.001$), RDW-CV i RDW-SD ($p < 0.001$). Povišene procijenjene marginalne srednje vrijednosti najčešće su zabilježene u grupama MH1, MH2 i CC, posebno za WBC, limfocite, RBC i eritrocitne indekse, što ukazuje na izraženiji hematološki odgovor.

Poikilocitoza je bila prisutna u svim grupama, s annulocitima kao najzastupljenijim oblikom (do 6,01% u MH1), zatim dakriocitima i ovalocitima. Leukogram je pokazao dominaciju limfocita u svim grupama, s najvećim procentom u MH1 (65,75%), dok je najveći procenat neutrofila zabilježen u AB (47,75%).

Ovo istraživanje pruža uvid u moguće razlike u hematološkom odgovoru na prirodne i sintetičke tretmane rana u animalnom modelu te predstavlja osnovu za dalja istraživanja biološke sigurnosti i efikasnosti primijenjenih terapija.

Ključne riječi: Gentamicin, hlor-dioksid, eksperimentalna rana, manuka med, poikilocitoza

CONFERENCE PAPER

EFFICIENCY OF STABLE LIQUIDE CHLORINE DIOXIDE (ClO_2) IN SANITATION OF WATER DISTRIBUTION SYSTEM IN POULTRY PRODUCTION-SYNTHESIS OF THEORETICAL AND EXPERIMENTAL FINDINGS

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ABSTRACT

Modern veterinary practice faces growing challenges in the field of biosecurity, especially in intensive livestock and poultry production. One of the most underestimated, yet critical, factors in maintaining the health status of animals is the quality of drinking water and the hygienic safety of the water distribution system. Although in everyday veterinary practice, the leading causes of health disorders are often attributed to diet, environmental factors, or genetic potential, numerous cases indicate that water is a key vector of microbial contamination, primarily through biofilm structures that form in internal water distribution systems. Due to the increasing incidence of resistant bacterial strains and the limited effectiveness of conventional disinfectants, such as chlorine, there is a growing need for new, safer, and more effective sanitation methods.

Stabilised liquid chlorine dioxide (ClO_2) is emerging as a potent alternative to conventional agents. This paper represents a synthesis of experimental and review findings related to the use of stabilised liquid chlorine dioxide for the rehabilitation of the drinking system in laying hens suffering from colisepticemia. Through a case study of a farm with a recorded problem of systemic infection caused by Escherichia coli, the causes of contamination, remediation measures, as well as the effects achieved in terms of biofilm reduction, mortality and microbiological load of water were analyzed. Along with the case report, the paper analyzes and expands the context of the effectiveness of stabilised liquid chlorine dioxide, based on the literature and previous studies. Stabilised ClO_2, in contrast to its gaseous form, offers safer handling, a prolonged residual protection effect, and minimal toxicity.

The paper emphasises the importance of microbiological control and improved sanitation of water supply systems in poultry production, and highlights stabilised liquid chlorine dioxide as a highly effective tool in the fight against biofilm and resistant bacterial strains.

Keywords: Animal production, disinfection, prevention, stable liquid chlorine dioxide

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INTRODUCTION

Effective control of pathogenic microorganisms is essential for maintaining animal health and ensuring food safety in modern animal production (Gagić, 1988; Gagić, 2000; Asaj, 2003;). Within biosecurity measures, disinfection plays a crucial role, focusing on reducing microbiological contamination in facilities, equipment, water, and the environment. Despite its importance, disinfection is often treated as a routine and technically simple task, leading to an underestimation of its complexity and the key factors that determine its success. As a result, commonly used disinfectants, such as chlorine and its compounds, continue to dominate practice, even though evidence suggests their limited efficacy, particularly in the presence of biofilms and organic matter. (Gagić et al, 2013; Ališah, 2020.)

Biofilm represents a sophisticated organizational form of microbial communities that adhere to both biotic and abiotic surfaces, enveloped in a self-produced polysaccharide matrix. This structure enables microorganisms to survive extreme conditions and develop resistance to antimicrobials and the host's immune response. In poultry production, biofilm formation within water distribution systems creates a persistent source of contamination in drinking water, thereby directly jeopardizing animal health, increasing mortality rates, and impairing productivity. (Ališah, 2020; Pivić, 2025.) A significant challenge is that water, a key vector for pathogen transmission, is often neglected in veterinary diagnostics and therapy. Health issues in animals are more frequently attributed to factors like inadequate nutrition, housing, or hygiene, while water quality and the sanitation of drinking systems are not analyzed until chronic health problems arise. This oversight not only delays problem resolution but can also lead to unnecessary and ineffective use of antibiotics and additives, exacerbating the risk of antimicrobial resistance and leaving residues in animal products. (Gagić et al, 2013; Hadžiabdić et al, 2013, Mujaković et al, 2022.)

Given the limitations of conventional disinfectants and the complexity of biofilm infections, modern research is increasingly focusing on stabilized liquid chlorine dioxide (ClO_2) - a broad-spectrum oxidant that, unlike its gaseous form, can be safely and effectively used in various sanitary applications. ClO_2 possesses exceptional antimicrobial properties, including the ability to eradicate biofilm and destroy spores and viruses resistant to other agents. Its selective oxidation mechanism, prolonged effectiveness in aquatic systems, and absence of toxic by-products make it an ideal candidate for contemporary disinfection practices in veterinary and livestock operations. (Gagić, 2000; Plavšić, 2011.)

This paper presents the results of a study conducted on a laying hen farm where a mass outbreak of colisepticemia occurred due to contamination in the feeding system. (Gagić et al, 2013.) After conventional measures, including water chlorination and antibiotic treatments, yielded little to no results, a remediation program utilizing stabilized liquid chlorine dioxide was implemented. The methodology includes diagnostics of the system (such as ATP luminometry and cultural isolation), application of ClO_2 at various concentrations and time intervals, and monitoring outcomes through measurement of microbial load and flock mortality.

Additionally, the paper offers a theoretical analysis of the properties of ClO_2, its advantages over traditional disinfectants, and modern disinfection principles, including the stages of mechanical cleaning, sanitary washing, and proper application techniques (Ališah, 2020.). The goal is to provide an integrated model for disinfecting water supply systems that can be applied in real production conditions without disrupting the production process. Also, this work aims to enhance biosecurity practices in the poultry industry while addressing global challenges related to food safety, antimicrobial resistance, and public health.

Disinfection as a complex veterinary and hygienic procedure

Disinfection, defined as the set of measures aimed at destroying, reducing, or inactivating pathogenic

microorganisms on non-living surfaces, is a fundamental component of biosecurity in livestock and poultry production (Asaj, 2003.) Unlike sterilization, which involves the complete destruction of all microorganisms, disinfection seeks to reduce the microbiological burden to a level that does not jeopardize animal health or the safety of animal-origin products. (Gagić, 1988).

However, disinfection procedures are often carried out routinely without sufficient professional understanding of the prerequisites, limitations, and optimal methods of application. This approach significantly diminishes the effectiveness of disinfection measures, contributes to the development of microbial resistance, and leads to the inefficient use of disinfectants. (Ališah, 2020; Ališah et al, 2023.)

The classic process of disinfection consists of three main phases: mechanical cleaning, sanitary washing, and the application of disinfectants. Mechanical cleaning removes up to 90% of microorganisms from the target surface. Sanitary washing is performed with warm water and detergents, further reducing organic matter and making pathogenic microorganisms more susceptible to chemical agents. Skipping these preparatory steps in favor of "2-in-1" or "3-in-1" commercial products often results in ineffective disinfection, particularly in the presence of biofilm. (Asaj, 2003; Gagić, 2012.)

The Problem of Biofilm in Water Supply

A major challenge in modern water supply systems is the presence of biofilm: a complex microbial community that forms on the inner surfaces of pipelines, drinkers, and reservoirs. Biofilm consists of microorganisms (commonly bacteria from the Enterobacteriaceae family) (Ališah, 2020; Pivić, 2025), embedded in a self-produced polysaccharide matrix that shields them from disinfectants, antibiotics, and the host's immune system. Additionally, biofilm facilitates the horizontal transfer of genes, including those responsible for antimicrobial resistance, complicating treatment responses.

In poultry production, biofilm in animal watering systems can continuously introduce pathogenic microflora, such as *Escherichia coli, Salmonella spp., Pseudomonas spp*., and other opportunistic bacteria. These pathogens enter the body through drinking water, leading to respiratory, digestive, or systemic infections such as colisepticemia. This results in increased mortality, reduced egg production, and higher antibiotic usage. (Hadžiabdić et al, 2013.)

Limitations of Classic Disinfectants: Chlorine and Chloramine

Chlorine and its compounds (chlorine lime, caporite, chloramines) have been utilized for decades as primary disinfectants in livestock and community hygiene. While their application is simple, cost-effective, and efficient under certain conditions, they also come with several limitations. Chlorine is highly reactive in the presence of organic matter, leading to the formation of by-products such as trihalomethanes and chloramines, which are toxic and potentially carcinogenic. Moreover, at a pH greater than 7.5, chlorine's efficiency declines significantly, and its ability to eliminate biofilm is limited due to its inability to penetrate the polysaccharide matrix. (Plavšić, 2011; Gagić et al, 2013.)

When in contact with metals, chlorine can exhibit strong corrosive effects, posing additional technical challenges in systems with metal components. Its use in confined spaces also requires caution because of its potential toxicity to both operators and animals.

Stabilized Liquid Chlorine Dioxide (ClO_2): A Modern Broad-Spectrum Oxidant

In response to the shortcomings of conventional disinfectants, stabilized liquid chlorine dioxide (ClO_2) has been developed as an innovative solution that combines high biocidal efficacy with safety in application. Unlike the gaseous form of ClO_2, which is unstable and potentially explosive, the stabilized liquid formulation allows for easy storage, transport, and application without the need for additional protective measures. (Gagić et

al, 2013.)

ClO_2 acts as a selective oxidant, transferring one electron to electron-rich centers within the organic molecules of microorganisms. This reaction destroys cell membranes, denatures proteins, and inactivates enzymes, all without generating harmful by-products. (Mujaković et al, 2022).

ClO_2 is particularly effective at dismantling biofilm structures, as it can oxidize both the matrix and the cells within it. Its stability across a wide pH range (1.5-10), along with its non-toxicity and long-lasting residual action in drinking water, make it highly suitable for use in livestock facilities without disrupting production. Furthermore, ClO_2 does not produce chlorinated by-products or leave any unpleasant odor or taste in water and food. (Ališah et al, 2023.)

Additionally, ClO_2 demonstrates virucidal and sporicidal properties that exceed those of chlorine, and its application has been documented in various industries, from wastewater and swimming pool treatment to the decontamination of spaces contaminated with *Bacillus anthracis* spores. (Gagić, 2000; Hadžiabdić et al, 2013.)

Location of the Experiment and Research Objectives

The experimental study was conducted on a commercial farm dedicated to breeding laying hens for table eggs, located in the central part of Bosnia and Herzegovina. The facility is a closed-type operation, featuring two lines of automated six-storey cages manufactured by Salmet, with a total capacity of 7,800 Lohmann Brown laying hens. The feeding system utilizes nipple drinkers, providing a continuous flow of water ad libitum, while the ventilation system is automated and designed to accommodate a maximum capacity of 3.0 m^3/h/kg of live weight.

The facility sources groundwater from its own catchment area, which includes a protective tank with a volume of 7,000 liters. Water from the reservoir is delivered to the building via a 75-meter-long supply line. A critical epidemiological concern was identified in the immediate vicinity, as there were two pig fattening facilities located above the catchment area, which posed a risk of fecal contamination to the reservoir (Gagić et al, 2013.)

Experimental Subjects and Health Status

At the time of the observed health issues, the farm housed 7,800 three-month-old laying hens. By the end of February, their health and growth rates were above the standard for this genetic line. However, beginning on February 22, there was a sudden increase in daily mortality, with as many as 50 hens dying each day, exhibiting symptoms of systemic coli-septicemia. Initial treatment included antibiotic therapy (non-resorptive antibiotics) and vitamin supplementation (AD_3E and amino acids), but there was no clinical improvement.

Pathoanatomical and microbiological examinations confirmed the presence of *Escherichia coli* in the parenchymatous organs and a positive coli titer in the drinking water.

Methodology and Phases of the Experiment

The research protocol was divided into four phases with the following goals:

1. Confirming that the water and water distribution system were the source of the infection.

2. Sanitizing the system using stabilized ClO_2.

3. Establishing a permanent disinfection program.

4. Implementing parallel anti-stress therapies.

Phase I – Diagnostic Evaluation

Water and biofilm samples were collected at three checkpoints:

- Accumulation tank

- Entrance shaft (external system)

- Ends of the pipes in each of the cages (internal network)

The following methods were employed:

- ATP luminometer (Charm Sciences Inc., USA) for bioluminescent quantification of contamination in relative light units (RLU).

- Microbiological culture on selective media for the identification of *E. coli*.

Samples were collected at the following times:

1. Before any treatment

2. 5 and 15 minutes after rinsing the system with clean water

3. After each treatment with ClO_2.

Phase II – Initial Sanitation with Stabilized Liquid ClO_2

Double disinfection of the internal system was performed:

- Shock treatments at night with 4 ml of stabilized liquid ClO_2 per liter of water (4‰) for five consecutive nights.

- Daily prophylactic treatments with 2 ml/l (2‰) via water medication for five days.

After each shock treatment, the system was emptied and filled with a new solution.

Phase III – Sanitation Maintenance Program

A monthly and continuous regimen was established, including:

- Permanent disinfection of the accumulation tank (adding ClO_2 directly to the tank)

- Monthly shock treatments of both the external and internal systems (4‰)

- Continuous disinfection of incoming water into the building (2‰).

Phase IV – Shock Treatment

After each shock treatment, a three-day course of vitamin C was administered per laying hen each day, through drinking water.

Instruments and Measurement Parameters

- ATP Luminometry: Measurements were taken at the sampling site within 5 seconds of swab contact with luciferase. Values above 20,000 RLU were considered unacceptable for sanitation.

- Microbiological Analyses: Standard methods of inoculation on selective media, with incubation at 37 °C for 24 hours.

- Mortality: The number of deaths recorded daily was analyzed in three-time intervals: before treatment, during treatment, and after the implementation of the rehabilitation program.

The effectiveness of treatment with stabilized liquid chlorine dioxide (ClO_2) was evaluated based on three key indicators: daily mortality of laying hens, the degree of microbiological contamination of water (measured by ATP luminometry), and the presence of pathogenic bacteria (E. coli) in the water supply. Analyzing data from various phases of the experiment revealed a significant difference between the initial conditions and the post-treatment period.

Mortality of Laying Hens

At the start of the study, the average daily mortality rate was 14.8 hens, which is more than ten times above the technological standard for the Lohmann Brown breed (1.4 hens per day). After completing the first two phases of sanitation, the average daily mortality decreased to 2.1 hens, and by the third month, it stabilized at 1.06 hens per day, which is below the standard.

Table 1 Overview of average mortality values of laying hens in four time periods

Remediation Phase	Mortality (individuals/day)
Before Treatment	14.8
During Shock Treatment	2.1
During Maintenance	1.06
Benchmark	1.4

Note: The reduction in mortality was noticeable within the first five days of shock treatment.

ATP Luminometry – Degree of Contamination

The readings from the ATP luminometer displayed a high degree of contamination in the internal water supply system prior to treatment, with values exceeding 450,000 RLU at the ends of the pipes in the batteries. After applying ClO_2 at a concentration of 4‰, these values dropped to below 0.5 RLU.

Table 2 ATP Values at the ends of the tubes

Sample Location	Pre-treatment (RLU)	After Shock Treatment (RLU)
Battery 1 – Floor 1	465,000	0.00
Battery 2 – Floor 3	505,000	0.00
Battery 1 – Floor 5	487,000	0.32
Battery 2 – Floor 5	538,000	0.45

These results confirm that ClO_2 at a high concentration (4‰) effectively eliminated the biofilm and reduced the microbial burden to a sanitation-acceptable level (≤ 20,000 RLU).

Microbiological Analysis

Microbiological tests on selective media for E. coli indicated that water samples from the ends of the pipes before treatment were significantly contaminated, often showing overgrown colonies (>10_2 CFU/mL). After treatment, all samples from the internal system tested negative for E. coli.

Table 3 Presence of *E. coli* at defined control points

Sample Location	Before Treatment	After Treatment
End of Pipe – Battery 1	+++ (overgrown)	Negative
End of Pipe – Battery 2	+++ (overgrown)	Negative
Accumulation Pool	Negative	Negative
Manhole at the Entrance to the Facility	Negative	Negative

*Note: The absence of E. coli in the external system further confirms that the source of infection was localised within the internal distribution system of the facility, where biofilm had formed.

Presence of Residual ClO_2

Test strips confirmed the presence of residual ClO_2 at all ends of the system during daily treatment (2‰), indicating that the disinfecting potential of the water was sustained throughout the day.

Table 4 Detection of residual ClO_2 during prophylactic treatment

Location	Residual ClO_2 present
Pipe – Floor 1, Battery 1	Yes
Pipe – Floor 5, Battery 2	Yes
Pipe – Floor 3, Battery 1	Yes
Pipe – Floor 2, Battery 2	Yes

CONCLUSION

This study provides a comprehensive evaluation of the efficacy of stabilized liquid chlorine dioxide (ClO_2) as a sanitation agent for farm water supply systems, with particular emphasis on biofilm elimination and the control of colisepticemia caused by *Escherichia coli*. The results unequivocally demonstrated that ClO_2 represents a superior alternative to conventional disinfectants such as chlorine and chloramines, especially in the context of eradicating microbiological contaminants and biofilms in drinking water systems. Application of stabilized ClO_2 at concentrations of 4‰ for nocturnal shock treatments and 2‰ for daily prophylactic disinfection resulted in a significant reduction in ATP luminometry readings, from over 450,000 RLU to below 1 RLU. This indicates effective biofilm removal from the internal surfaces of the pipes, an outcome that conventional chlorination could not achieve. Mortality in laying hens decreased from 14.8 to 2.1 birds per day in the initial days of ClO_2 treatment, with further stabilization to 1.06 after three months, which is below the technical standard for this genetic line. This substantial reduction in mortality reflects improved flock health and the elimination of infection sources. Microbiological analyses following treatment confirmed the complete elimination of *E. coli* and other pathogenic bacteria from the water supply system, confirming ClO_2's successful suppression of contamination in drinking water. Stabilized liquid ClO_2 does not produce harmful byproducts such as trihalomethanes or chloramines, making it safe for use in food production. Its long-lasting residual effect in water systems ensures continuous protection, reducing the need for frequent disinfectant application.Stabilized liquid ClO_2 offers several operational advantages: it is safe to handle, requires no special storage conditions, leaves no unpleasant odor, and does not affect the taste of water or eggs. Its proven residual activity, as demonstrated in this study, provides extended microbiological safety in closed water systems that are often difficult to access for cleaning. The use of ClO_2 does not necessitate interruptions in the production cycle, which is crucial for commercial farms where each non-productive day results in economic loss. This makes ClO_2 application cost-effective, particularly when compared to expenses associated with treatment, production losses, and potential public health implications from antibiotic residues and pathogenic microorganisms in eggs. Given its proven biofilm elimination capability, broad-spectrum antimicrobial activity, and technical advantages, ClO_2 has clear potential for wider application in the livestock and food industries-from sanitation of water and ventilation systems to treatment of working surfaces and packaging materials. Its established efficacy in dentistry, dairy processing, and foodservice disinfection further underscores its versatility and safety.The most direct indicator of sanitation success was the sharp decrease in flock mortality-from 14.8 to just 2.1 birds per day within the first five days of treatment, followed by stabilization at 1.06, below the technological norm. This change suggests not only the elimination of the infection source (*E. coli*) but also an overall improvement in flock health. Concurrently, the need for further antibiotic use was eliminated, aligning with modern principles of reducing antimicrobial usage in livestock production.Additionally, the supplementary administration of vitamin C as an anti-stress therapy following shock treatments had a positive effect, supporting the immune response of the birds during recovery. This synergy between chemical sanitation and nutritional support provides a practical and scalable model for implementation in intensive poultry production systems.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

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

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EFIKASNOST STABILIZOVANOG TEČNOG HLOR-DIOKSIDA (ClO_2) U DEZINFEKCIJI SISTEMA ZA DISTRIBUCIJU VODE U PERADARSKOJ PROIZVODNJI – SINTEZA TEORIJSKIH I EKSPERIMENTALNIH NALAZA

SAŽETAK

Savremena veterinarska praksa suočava se s rastućim izazovima u oblasti biosigurnosti, posebno u intenzivnoj proizvodnji stoke i peradi. Jedan od najpotcjenjenijih, a ipak ključnih faktora u održavanju zdravstvenog statusa životinja jeste kvalitet pitke vode i higijenska sigurnost vododistributivnih sistema. Iako se u svakodnevnoj veterinarskoj praksi glavni uzroci zdravstvenih problema često pripisuju ishrani, faktorima sredine ili genetskom potencijalu, brojni slučajevi ukazuju na to da je voda ključni vektor mikrobne kontaminacije, prvenstveno putem biofilm struktura koje se formiraju u unutrašnjim sistemima za distribuciju vode. Zbog sve češće pojave rezistentnih bakterijskih sojeva i ograničene efikasnosti konvencionalnih dezinfekcionih sredstava, poput hlora, raste potreba za novim, sigurnijim i efikasnijim metodama sanacije.

Stabilni tečni hlor dioksid (ClO_2) pojavljuje se kao snažna alternativa konvencionalnim sredstvima. Ovaj rad predstavlja sintezu eksperimentalnih i preglednih nalaza vezanih za primjenu stabilnog tečnog hlora dioksida u rehabilitaciji sistema za piće kod koka nosilja oboljelih od koliseptikemije. Kroz studiju slučaja na farmi sa zabilježenim problemom sistemske infekcije izazvane bakterijom *Escherichia coli*, analizirani su uzroci kontaminacije, mjere remedijacije, kao i postignuti efekti u pogledu smanjenja biofilma, smrtnosti i mikrobiološkog opterećenja vode. Pored izvještaja o slučaju, rad analizira i širi kontekst efikasnosti stabilnog tečnog hlora dioksida na osnovu literature i prethodnih istraživanja. Stabilni ClO_2, za razliku od svog gasovitog oblika, omogućava sigurnije rukovanje, produžen efekat rezidualne zaštite i minimalnu toksičnost.

Rad naglašava važnost mikrobiološke kontrole i unapređenje sanitacije sistema za vodosnabdijevanje u peradarskoj proizvodnji, te ističe stabilni tečni hlor dioksid kao izuzetno efikasno sredstvo u borbi protiv biofilma i rezistentnih bakterijskih sojeva.

Ključne riječi: Animalna proizvodnja, dezinfekcija, prevencija, stabilizirani tečni hlor dioksid

CONFERENCE PAPER

PRELIMINARY CHECKLIST OF ICHTHYOFAUNA AND MARINE MAMMALS OF BOSNIA AND HERZEGOVINA'S COASTAL WATERS: A BASELINE FOR CONSERVATION, MONITORING, AND ONE HEALTH APPROACHES

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ABSTRACT

Bosnia and Herzegovina's (B&H) limited coastal zone along the Adriatic Sea represents a unique and understudied marine ecosystem. This study presents the first structured preliminary inventory of marine fish and mammal species in the country's territorial waters, compiling 110 species (106 fish, 4 marine mammals) from historical records, grey literature, and local knowledge sources. Using a standardized Observation Quality Index (OQI), species were categorized into high, medium, or low confidence tiers based on data quality criteria. The checklist identified 21 bony fish and three marine mammals with high-confidence presence, while most elasmobranchs (13 species) and bony fish (69 species) fell into the medium-confidence category, reflecting regional data scarcity and underreporting. The findings offer a critical biodiversity baseline for future research, marine conservation, and environmental management in the region. Moreover, the study highlights the value of integrating data quality assessment and the One Health approach, recognizing the interconnectedness of marine ecosystem health, biodiversity conservation, and human well-being. This inventory supports national and regional efforts to meet international biodiversity monitoring commitments and underscores the need for continued marine research and cross-border collaboration in the Adriatic Sea.

Keywords: Checklist inventory, marine ichtyofauna, marine mammals, One Health

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INTRODUCTION

In the context of accelerating climate change and increasing anthropogenic pressures on the world's oceans, inventories of fish species and marine mammals have become indispensable tools for ocean monitoring and conservation. Fish and marine mammals serve as keystone and sentinel species within marine ecosystems, indicating that changes in their populations often signal broader ecological shifts. By systematically recording the presence, abundance, distribution, and health status of these species, scientists are better equipped to detect and understand ecosystem-level responses to stressors such as rising ocean temperatures, acidification, overfishing, and pollution (Bianchi et al., 2022; Plon et al., 2024).

Fish species are vital not only for ecological balance but also for global food security. Many communities, particularly in coastal and island regions, rely heavily on fish for dietary protein and economic stability. Inventories help detect declines in key commercial species, allowing for evidence-based fisheries management that can prevent overexploitation and ensure long-term sustainability (Copernicus Marine Service, n.d.; McQuatters-Gollop et al., 2019). Moreover, data on species range shifts caused by warming waters - such as poleward migrations or changes in spawning patterns enable scientists and policymakers to anticipate and adapt to emerging ecological and economic challenges.

Marine mammals, on the other hand, act as critical sentinel species-organisms whose health reflects the state of the broader marine environment. Changes in their behavior, reproductive success, or disease prevalence often serve as early warning signals of ecosystem distress. For example, increases in marine mammal strandings or disease outbreaks may indicate rising levels of pollution, harmful algal blooms, or shifts in prey availability (Frontiers in Marine Science, 2023; Plon et al., 2024). As long-lived, top-level predators, marine mammals also integrate the effects of multiple environmental stressors across time and space, offering a holistic view of ocean health.

Importantly, the collection and integration of this data are central to the One Health paradigm, which underscores the interconnectedness of human, animal, and environmental health. By informing conservation actions, public health planning, and sustainable resource management, inventories of fish and marine mammals provide a critical foundation for climate-resilient decision-making. Without accurate and regularly updated species inventories, efforts to maintain healthy marine ecosystems and safeguard human well-being in a changing climate would be severely undermined (Frontiers in Marine Science, 2023).

B&H is located in the western part of the Balkan Peninsula, in Southeastern Europe. It borders Croatia to the north, west, and southwest, Serbia to the east, and Montenegro to the southeast. Although it is predominantly a landlocked country, B&H has limited access to the sea, with a coastline of about 24 kilometers and the town of Neum on the Adriatic Sea. This narrow coastal strip represents the country's only maritime connection to open waters, which is important for tourism and trade, but also limits the development of maritime infrastructure and access to international sea routes.

A total of 207 species of fauna were found in the sea of B&H during the last century, of which 97 were species of fish, which is slightly more than a quarter of the total number of 380 species of fish for the Adriatic Sea (Šoljan, 1948). If we consider that the number of fish species in the Adriatic has increased over the past thirty years from 407 to 456 and the new six that were registered by the authors of the book Ichthyofauna of the Adriatic Sea (Šoljan, 1980; Jardas, 1996; Dulčić and Kovačić 2020; Kovačić, 2023), we can assume that the number of species in the B&H sea has also increased proportionally. More serious research that would result in a comprehensive or taxon-specific inventory of the marine flora and fauna of B&H has not been done so far.

Although comprehensive research covering all taxonomic groups has not yet been conducted in B&H's marine area, certain groups have been

explored sporadically since 2011, primarily due to the efforts of individual scientist who initiated targeted studies on sharks and rays. Between 2011 and 2022, a series of publications (Gajić and Lelo, 2011a; Gajić and Lelo, 2011b; Gajić and Lelo, 2014; Gajić, 2014; Gajić, Kahrić and Dedić, 2014; Kahrić and Gajić, 2015; Kahrić, Gajić, Dedić and Hadžić, 2015; Gajić and Kahrić, 2015; Kahrić, 2016; Gajić, Kahrić and Lelo, 2017; Kahrić, 2017; Kahrić, 2018; Kahrić, Gajić and Muhamedagić, 2018; Kahrić and Gajić, 2018; Kahrić, 2019; Kahrić, 2020; Kahrić, 2022) have contributed to the most consistent and structured documentation of cartilaginous fish in B&H territorial waters. These studies significantly enriched the knowledge of local elasmobranch populations, highlighting their occurrence, distribution, and ecological relevance. In contrast, bony fishes have received considerably less attention, with only occasional investigations conducted in the 21st century, thus leaving notable gaps in the understanding of their current status and trends (Kahrić and Gajić, 2016; Karalić, 2022).

This discrepancy underlines the importance of compiling and reviewing existing data to establish a unified biodiversity baseline, as presented in this work. The synthesis of previously scattered records enables a more accurate assessment of species richness, data reliability, and potential knowledge gaps. Moreover, it provides a reference point for future monitoring and management efforts.

Other marine taxonomic groups, particularly marine mammals, remain underexplored. Their presence in B&H's territorial waters is mostly occasional, often linked to prey availability or proximity to fish farms, with documented occurrences of individuals entering the area sporadically (Kahrić, 2016). Despite their ecological significance, no systematic surveys have been conducted so far.

Moreover, the study highlights the value of integrating data quality assessment and the One Health approach, recognizing the interconnectedness of marine ecosystem health, biodiversity conservation, and human well-being. In this context, recent histopathological research on elasmobranchs has identified liver and spleen lesions (Gajić et al., 2020), which are likely linked to bioaccumulated contaminants such as heavy metals, persistent organic pollutants, and remnants of war-related debris - indicating early signs of ecosystem degradation with potential implications for food safety and public health. Furthermore, the high prevalence of microplastics in both fish digestive tracts and benthic sediments-particularly in species like *Mullus barbatus* - underscores the pervasive nature of pollution in B&H's coastal zone (Kahrić et al., 2019). Microplastics in marine sediment primarily affect benthic or demersal fish species, such as skates, rays, and the aforementioned *Mullus barbatus* (Kahrić et al., 2016).

These findings illustrate how environmental stressors may compromise the ecological integrity of marine populations and, by extension, affect human communities reliant on seafood resources and coastal ecosystem services. Complementing this perspective, B&H participated in the regional IPA-Adriatic De Fish Gear project, which represented the first comprehensive, harmonized assessment of marine litter across the Adriatic and Ionian Seas. The project included monitoring of various environmental matrices.- such as beach litter, surface water, seafloor, and biota - within the Neum-Klek Bay, contributing valuable data on the distribution and composition of marine debris in the country's coastal zone (Vlachogianni et al., 2017). This initiative further supports the One Health framework by recognizing marine litter, especially microplastics, as a cross-cutting pressure with ecological, economic, and human health implications. The integration of such monitoring efforts with biodiversity assessments is essential for designing evidence-based management strategies and mitigating long-term impacts on both marine ecosystems and local communities.

In light of these findings, adopting a coordinated, One Health-oriented strategy - aligned with regional and international frameworks - is essential for addressing ecological risks and reinforcing the resilience of B&H's marine ecosystems.

In regions where marine biodiversity is poorly documented and financial or technical resources are limited, compiling a preliminary checklist using existing literature, historical records, and grey sources is not only a pragmatic approach-it is also a scientifically valid and strategically valuable one. This foundational step is particularly important for countries like B&H, where marine biodiversity remains underrepresented in global biodiversity databases (Convention on Biological Diversity, n.d.).

Despite limitations in completeness or timeliness, the creation of a preliminary checklist plays a critical role in establishing a baseline for biodiversity research. Even when data are derived from older publications, student theses, local monitoring programs, or technical reports, such syntheses consolidate fragmented knowledge into a structured, accessible format. This allows researchers and decision-makers to assess what is already known and to identify key knowledge gaps that future studies should address (Corsi, 2004; Bianchi et al., 2022).

One of the most important benefits of this approach is its ability to fill critical data gaps. In the absence of recent or comprehensive field surveys, preliminary checklists may represent the only available organized source of marine species data for a given region. This is particularly relevant in emerging or post-conflict countries, where biodiversity research has not kept pace with environmental or conservation challenges (Copernicus Marine Service, n.d.; Frontiers in Marine Science, 2023).

Additionally, drawing on grey literature-including student dissertations, unpublished monitoring reports, and environmental consultancy documents-helps to recognize and integrate valuable local knowledge that is often overlooked in conventional peer-reviewed sources. While these materials may not always undergo formal publication, they often contain methodologically robust and context-specific observations that enhance regional biodiversity assessments (Corsi, 2004).

The preliminary checklist approach is also notably cost-effective. It enables scientists and conservation professionals to make meaningful progress without large budgets or the logistical burdens of full-scale fieldwork. When properly documented, these compilations can meet accepted scientific standards and serve as credible references for marine conservation and management planning (McQuatters-Gollop et al., 2019). Moreover, these checklists provide a solid foundation for future research, collaboration, and funding. A well-documented checklist can support grant applications, justify detailed field-based surveys, or serve as a baseline for reassessing species distributions or conservation statuses in the future (Corsi, 2004).

Finally, even preliminary biodiversity data play a crucial role in conservation planning and environmental policy. In contexts where data are sparse, structured information—even from older or grey sources-can inform marine spatial planning, environmental impact assessments, and conservation prioritization under international frameworks such as the Convention on Biological Diversity or the EU Marine Strategy Framework Directive (Convention on Biological Diversity, n.d.; Bianchi et al., 2022).

In summary, while inherently limited, the development of preliminary marine species checklists using existing resources represents a scientifically grounded, resource-efficient, and strategically powerful approach. It allows under-resourced countries to actively participate in the global marine biodiversity dialogue and lays the groundwork for long-term monitoring and marine stewardship

MATERIAL AND METHODS

This research employed a comprehensive inventory approach to assess marine fauna biodiversity in the target region, integrating historical records with contemporary data sources. The foundational dataset originated from a fauna inventory conducted in the 1980s by Dr. Tonko Šoljan, which served as a critical baseline for

species presence and distribution (Bianchi et al., 2022). Recognizing the limitations of relying solely on historical data, this inventory was systematically updated and validated through a multi-source verification process (Corsi, 2004). All species names have been verified and standardized according to the World Register of Marine Species (WoRMS) for marine invertebrates and mammals, and FishBase and Eschmeyer's Catalog of Fishes for fish taxa (WoRMS, 2025; Froese & Pauly, 2025; Fricke et al., 2025).

To ensure the reliability and scientific value of species occurrence data collected from diverse sources, this study implemented a standardized framework for assessing the quality of observations. Given that the data originate from both historical and contemporary sources-ranging from peer-reviewed literature to interviews with local fishers-it was essential to establish a transparent method for evaluating the credibility, accuracy, and taxonomic consistency of each record. This methodology follows established practices in biodiversity monitoring and ecological data quality assessment (Yoccoz et al., 2001; Fletcher et al., 2019; OBIS, 2021). The marine species inventory compiled in this study draws from historical scientific records (e.g., Šoljan, 1980s baseline fauna checklist), peer-reviewed literature and regional biodiversity assessments, grey literature (e.g., student theses, environmental reports, unpublished surveys), online media (e.g., news portals, forums), community knowledge (e.g., fisher interviews, oral history).

The Observation Quality Index (OQI) is a quantitative tool used to measure the reliability and scientific confidence of species occurrence data-especially useful in biodiversity studies where data come from mixed sources like old records, interviews, and non-peer-reviewed reports. In the context of marine species inventories, where resources and field observations might be limited, the OQI helps researchers assess the credibility of each species record, prioritize which records to verify or monitor further, filter out unreliable data from conservation or policy analysis, and document limitations transparently in biodiversity baselines. This method draws on best practices from ecological monitoring (Fletcher et al., 2019), data quality management in biodiversity databases (Costello et al., 2013), and data validation principles from the Ocean Biodiversity Information System (OBIS, 2021). In many countries-especially those with limited marine research infrastructure (like Bosnia and Herzegovina)-available biodiversity data may be old or incomplete, collected from local communities, spread across unpublished reports, theses, or grey literature. The OQI provides a structured way to integrate and evaluate all these sources fairly and scientifically. Instead of discarding lower-quality data outright, you score it transparently and make decisions accordingly.

The OQI works by assigning numerical scores to each observation across multiple quality criteria (such as source type, date, frequency of reports, and location accuracy). Each criterion is scored individually (e.g., 1-4 points), and the final OQI is calculated as the average of these scores:

OQI = Sum of all criteria scores / Number of criteria

Each observation was assigned a score for all six criteria (Table1).

Table 1 Scoring system and interpretation of OQI score

Criterion	Scoring System
1.Source reliability	4 = Peer-reviewed article 3 = Grey literature (e.g., reports, theses) 2 = News/media 1 = Interviews/community knowledge

Criterion	Scoring System	
2. Temporal Relevance	4 = Recent (<10 years) 3 = Medium-term (10–30 years) 2 = Historical (>30 years) 1 = Unknown	
3. Taxonomic Certainty	4 = Expert-identified with clear documentation 3 = Matched in WoRMS 2 = Field ID without expert 1 = Unverified/local names	
4. Geographic Precision	3 = Exact location (e.g., GPS or named site) 2 = Approximate region 1 = Unknown or vague	
5. Observation Frequency	4= ≥5 independent sources 3 = 3–4 sources 2 = 2 sources 1 = 1 source	
6. Cross-Validation	3 = More than two different source types confirm 2 = Two types confirm 1 = One type only	
OQI Score	**Interpretation**	**Action**
3.5–4.0	High Confidence	Include in baseline, usable for policy
2.5–3.4	Moderate Confidence	Use cautiously, prioritize for re-check
<2.5	Low Confidence	Flag for verification, not used for trend analysis

This scoring helps prioritize which species records need further validation, and which can be used confidently in decision-making, such as marine spatial planning or environmental reporting under frameworks like the EU Marine Strategy Framework Directive (McQuatters-Gollop et al., 2019).

RESULTS

After we have taken into account all available data, the preliminary list contains a total of 110 species, of which 4 are mammals, 3 of which have high reliability of presence and one has low reliability. Of the ichthyofauna, all 13 species belonging to the class Elasmobranchii are with medium confidence, while in bony fishes, 21 species are present with high confidence, 69 with medium confidence and 3 species with low confidence according to Observation Quality Index (Table 2).

Table 2 Preliminary checklist of marine mammals and ictyofauna

N	Species Name	Source Reliability	Temporal Relevance	Geographic Precision	Taxonomic Certainty	Obs. Freq.	Cross-Val.	OQI	Confidence
	Klasa MAMMALIA Linnaeus, 1758								
1	*Balaenoptera physalus* (Linnaeus, 1758)	2	4	3	2	1	1	2,17	L
2	*Delphinus delphis* (Linnaeus, 1758)	4	4	3	4	4	2	3,50	H
3	*Monachus monachus* (Hermann, 1779)	4	4	3	3	3	3	3,33	H
4	*Tursiops truncatus* (Montagu, 1821)	4	4	3	4	4	2	3,50	H
	Klasa ELASMOBRANCHII Bonaparte, 1838								
1	*Dasyatis pastinaca* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
2	*Mustelus asterias* (Cloquet, 1819)	4	4	3	4	2	2	3,17	M
3	*Mustelus mustelus* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
4	*Mustelus punctulatus* (A. Risso, 1827)	4	4	3	4	2	2	3,17	M
5	*Myliobatis aquila* (Linneaus, 1758)	4	4	3	4	2	2	3,17	M
6	*Prionace glauca* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
7	*Raja clavata* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
8	*Raja miraletus* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
9	*Scyliorhinus canicula* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
10	*Scyliorhinus stellaris* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
11	*Squalus acanthias* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
12	*Squatina squatina* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
13	*Torpedo marmorata* (Risso, 1810)	4	4	3	4	1	1	2,83	M
	Klasa ACTINOPTERYGII Klein, 1885								
1	*Acipenser sturio* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
2	*Aidablennius sphynx* (Valenciennes, 1836)	4	2	3	4	1	1	2,50	M
3	*Alosa fallax* (Lacepède, 1803)	4	2	3	4	1	1	2,50	L
4	*Aphia minuta* (Risso, 1810)	4	2	3	4	1	1	2,50	M
5	*Arnoglossus kessleri* (Schmidt, 1915)	4	2	3	4	1	1	2,50	M
6	*Arnoglossus laterna* (Walbaum, 1792)	4	2	3	4	1	1	2,50	M
7	*Arnoglossus thori* (Kyle, 1913)	4	2	3	4	1	1	2,50	M

N	Species Name	Source Reliability	Temporal Relevance	Geographic Precision	Taxonomic Certainty	Obs. Freq.	Cross-Val.	OQI	Confidence
8	*Atherina (Hepsetia) boyeri* (Risso, 1810)	4	2	3	4	1	1	2,50	M
9	*Atherina hepsetus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
10	*Belone belone* (Linnaeus, 1760)	4	4	3	4	4	4	3,83	H
11	*Microlipophrys dalmatinus* (Steindachner & Kolombatovic, 1883)	4	2	3	4	1	1	2,50	M
12	*Blennius ocellaris* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
13	*Parablennius sanguinolentus* (Pallas, 1814)	4	2	3	4	1	1	2,50	M
14	*Boops boops* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
15	*Callionymus maculatus* (Rafinesque, 1810)	4	2	3	4	1	1	2,50	M
16	*Cepola macrophthalma* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
17	*Chelidonichthys lastoviza* (Bonnaterre, 1788)	4	2	3	4	1	1	2,50	M
18	*Chromis chromis* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
19	*Citharus linguatula* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
20	*Conger conger* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
21	*Coris julis* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
22	*Coryphoblennius galerita* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
23	*Dentex dentex* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
24	*Dentex gibbosus* (Rafinesque, 1810)	4	2	3	4	1	1	2,50	M
25	*Dicentratchus labrax* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
26	*Diplodus annularis* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
27	*Diplodus puntazzo* (Cetti, 1777)	4	4	3	4	4	4	3,83	H
28	*Diplodus sargus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
29	*Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817)	4	2	3	4	1	1	2,50	M
30	*Engraulis encrasicolus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	L
31	*Eutrigla gurnardus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M

N	Species Name	Source Reliability	Temporal Relevance	Geographic Precision	Taxonomic Certainty	Obs. Freq.	Cross-Val.	OQI	Confidence
32	*Gobius bucchichi* (Steindachner, 1870)	4	2	3	4	1	1	2,50	M
33	*Gobius cobitis* Pallas, 1814	4	2	3	4	1	1	2,50	M
34	*Gobius cruentatus* Gmelin, 1789	4	2	3	4	1	1	2,50	M
35	*Gobius geniporus* Valenciennes, 1837	4	2	3	4	1	1	2,50	M
36	*Gobius niger* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
37	*Hippocampus guttulatus* (Cuvier, 1829)	4	4	3	4	4	3	3,67	H
38	*Hippocampus hippocampus* (Linnaeus, 1758)	4	2	3	4	2	2	2,83	M
39	*Labrus merula* Linnaeus, 1758	4	4	3	4	4	4	3,83	H
40	*Lepidopus caudatus* Euphrasen, 1788	2	4	3	4	1	1	2,50	L
41	*Lepidotrigla cavillone* (Lacepède, 1801)	4	2	3	4	1	1	2,50	M
42	*Lesueurigobius suerii* (Risso, 1810)	4	2	3	4	1	1	2,50	M
43	*Lichia amia* Linnaeus, 1785	4	2	3	4	1	1	2,50	M
44	*Lithognathus mormyrus* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
45	*Merluccius merluccius* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
46	*Mola mola* (Linnaeus, 1758)	4	4	3	4	1	1	2,83	M
47	*Monochirus hispidus* Rafinesque, 1814	4	2	3	4	1	1	2,50	M
48	*Mugil cephalus* Linnaeus, 1758	4	4	3	4	2	2	3,17	M
49	*Mullus barbatus* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
50	*Mullus surmuletus* Linnaeus, 1758	4	4	3	4	4	4	3,83	H
51	*Oblada melanurus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
52	*Oedalechilus labeo* (Cuvier, 1829)	4	2	3	4	1	1	2,50	M
53	*Ophidion barbatum* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
54	*Pagellus acarne* (Risso, 1827)	4	2	3	4	1	1	2,50	M
55	*Pagellus bogaraveo* (Brünnich, 1768)	4	2	3	4	1	1	2,50	M
56	*Parablennius gattorugine* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
57	*Parablennius tentacularis* (Brunnich, 1768)	4	4	3	4	2	2	3,17	M

N	Species Name	Source Reliability	Temporal Relevance	Geographic Precision	Taxonomic Certainty	Obs. Freq.	Cross-Val.	OQI	Confidence
58	*Pagellus erythrinus* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
59	*Platichthys flesus* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
60	*Salaria pavo* (Risso, 1810)	4	2	3	4	1	1	2,50	M
61	*Sardina pilchardus* (Walbaum, 1792)	4	4	3	4	4	4	3,83	H
62	*Sarpa salpa* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
63	*Sciaena umbra* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
64	*Scomber scombrus* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
65	*Scorpaena notata* Rafinesque, 1810	4	2	3	4	1	1	2,50	M
66	*Scorpaena porcus* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
67	*Scorpaena scrofa* Linnaeus, 1758	4	4	3	4	4	4	3,83	H
68	*Seriola dumerili* (Risso, 1810)	2	4	3	3	2	2	2,67	M
69	*Serranus hepatus* (Linnaeus, 1758)	4	4	3	4	4	3	3,67	H
70	*Serranus scriba* Linnaeus, 1758	4	4	3	4	4	4	3,83	H
71	*Solea solea* (Linnaeus, 1758)	4	4	3	4	2	2	3,17	M
72	*Sparus aurata* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H
73	*Sphyraena sphyraena* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
74	*Spicara flexuosum* (Rafinesque, 1810)	4	2	3	4	1	1	2,50	M
75	*Spicara maena* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
76	*Spondyliosoma cantharus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
77	*Symphodus cinereus* (Bonnaterre, 1788)	4	2	3	4	1	1	2,50	M
78	*Symphodus mediterraneus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
79	*Symphodus ocellatus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
80	*Symphodus roissali* (Risso, 1810)	4	2	3	4	1	1	2,50	M
81	*Symphodus tinca* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
82	*Synapturichthys kleinii* (Risso, 1827)	4	2	3	4	1	1	2,50	M
83	*Syngnathus tenuirostris* (Rathke, 1837)	4	2	3	4	1	1	2,50	M

N	Species Name	Source Reliability	Temporal Relevance	Geographic Precision	Taxonomic Certainty	Obs. Freq.	Cross-Val.	OQI	Confidence
84	*Trachinus draco* Linnaeus, 1758	4	4	3	4	2	2	3,17	M
85	*Trachinus radiatus* Cuvier, 1829	4	2	3	4	1	1	2,50	M
86	*Trachurus mediterraneus* (Steindachner, 1868)	4	2	3	4	1	1	2,50	M
87	*Trachurus trachurus* (Linnaeus, 1758)	4	2	3	4	1	1	2,50	M
88	*Tripterygion melanurus* Guichenot, 1850	4	2	3	4	1	1	2,50	M
89	*Tripterygion tripteronotum* (Risso, 1810)	4	4	3	4	3	3	3,50	H
90	*Uranoscopus scaber* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
91	*Xiphias gladius* Linnaeus, 1758	4	2	3	4	1	1	2,50	M
92	*Zeugopterus regius* (Bonnaterre, 1788)	4	2	3	4	1	1	2,50	M
93	*Zeus faber* (Linnaeus, 1758)	4	4	3	4	4	4	3,83	H

DISCUSSION AND CONCLUSION

The preliminary inventory of marine mammals and ichthyofauna biodiversity in B&H, comprising 110 species including 4 marine mammals and 106 fish species provides a foundational assessment of species presence and data reliability through the application of the Observation Quality Index (OQI). This tool enabled us to categorize the confidence level of species occurrences into high, medium, and low, thus offering a structured method to evaluate the quality of the existing biodiversity records.

The detection of four marine mammal species, with three identified with high confidence, underscores their credible and likely recurring presence within the limited marine territory of B&H, specifically the Adriatic Sea near Neum. This is in line with regional cetacean distributions noted in the central and southern Adriatic basins (Holcer et al., 2015; Fortuna, 2006). The single low-confidence mammal record, however, signals a potential knowledge gap or rare vagrancy that requires targeted verification through future surveys or citizen science efforts. Within ichthyofauna, the classification of all 13 Elasmobranchii (sharks and rays) species under medium-confidence status reflects the general scarcity and patchiness of elasmobranch data in the Adriatic Sea, a concern previously highlighted by Ferretti et al. (2013). Elasmobranchs are known to be under-reported due to their elusive behavior, low abundance, and limited fisheries data in this region (Dulčić and Dragović, 2017), making the medium-confidence categorization consistent with regional patterns of data deficiency.

Bony fishes presented a more detailed distribution across the confidence spectrum, with 21 species confidently confirmed, 69 species marked with medium confidence, and only three species categorized with low confidence. This skew toward medium confidence highlights moderate uncertainty, possibly due to inconsistencies in species identification, underreporting, or outdated records. Nevertheless, the relatively high number of high-confidence records may

reflect better documentation of commercially relevant or commonly observed species. This result supports previous findings that coastal fish biodiversity in the Adriatic region is relatively well documented, especially in the northern and central sectors (UNEP/MAP, 2012), although data may still be sparse for certain microregions like B&H's limited coastline. Our result of 106 total species, with 13 Elasmobranchii, could suggest that the studied area harbors about 25-30% of the total known fish biodiversity of the Adriatic Sea, Elasmobranchii species also indicates a relatively healthy representation of these often vulnerable taxa, particularly given their slow growth rates, late maturity, and low reproductive rates.

The use of the Observation Quality Index proved essential in assessing not just species richness but the reliability of each record. This tiered approach enables prioritization in future research and conservation. High-confidence species can form the core of national biodiversity monitoring programs, while medium- and low-confidence species highlight areas where further survey effort is urgently needed. Additionally, these results stress the importance of integrating citizen science platforms and harmonizing data standards across borders to enhance the spatial and temporal resolution of biodiversity data (Boero et al., 2015).

Overall, this inventory, though preliminary, reveals a diverse but unevenly documented marine biota in Bosnia and Herzegovina. Continued efforts to improve data quality, particularly for elasmobranchs and low-confidence taxa, are crucial for informed conservation planning, fisheries management, and reporting obligations under international biodiversity frameworks such as the EU Marine Strategy Framework Directive and the Convention on Biological Diversity.

From a One Health perspective, maintaining accurate and high-quality marine biodiversity inventories is not only essential for ecosystem conservation but also for safeguarding human and animal health. Marine mammals and fish serve as indicators of ocean health, and their well-being is closely linked to environmental conditions, pollution levels, and anthropogenic pressures-all of which can have direct or indirect impacts on human populations. In this context, the quality and reliability of species occurrence data become critical for early detection of ecological imbalances, zoonotic risks, and food safety issues related to fisheries. Strengthening biodiversity monitoring through a One Health lens supports a more integrated and preventative approach to environmental management, public health, and sustainable development in Bosnia and Herzegovina and the wider Adriatic region.

Given the limited marine area of Bosnia and Herzegovina and its ecological connection to the greater Adriatic Sea, continued regional cooperation, capacity building, and the integration of standardized monitoring methods are essential. Strengthening biodiversity inventories with verified, high-quality data will be vital for supporting sustainable marine resource management and fulfilling international conservation obligations.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: MČ, AK; Design: MČ, AK; Supervision: AA; Materials: MČ,AK; Data Collection and/or Processing: MČ, AK; Analysis and/or Interpretation: MČ, AK; Literature Search: MČ, AK; Writing– Original Draft: MČ, AK; Critical Review: MČ, AK, AA

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PRELIMINARNA LISTA IHTIOFAUNE I MORSKIH SISARA U PRIMORSKIM VODAMA BOSNE I HERCEGOVINE: OSNOVA ZA ZAŠTITU, MONITORING I PRISTUP "ONE HEALTH"

SAŽETAK

Ograničena obalna zona Bosne i Hercegovine duž Jadranskog mora predstavlja jedinstven i nedovoljno istražen morski ekosistem. Ova studija prikazuje prvi preliminarni strukturirani popis morskih vrsta riba i sisara u teritorijalnim vodama zemlje, obuhvatajući 110 vrsta (106 riba i 4 morska sisara), prikupljenih iz historijskih izvora, sive literature i lokalnog znanja. Korištenjem standardizovanog Indeksa kvaliteta opažanja (OQI), vrste su svrstane u kategorije visoke, srednje ili niske pouzdanosti na osnovu kriterija kvaliteta podataka. Spisak je identificirao 21 vrstu košljoriba i tri vrste morskih sisara s visokim stepenom pouzdanosti, dok je većina hrskavičavih riba (13 vrsta) i košljoriba (69 vrsta) svrstana u kategoriju srednje pouzdanosti, što odražava nedostatak regionalnih podataka i slabu dokumentaciju. Nalazi predstavljaju ključnu osnovu biodiverziteta za buduća istraživanja, očuvanje mora i upravljanje okolišem u regiji. Pored toga, studija naglašava važnost integracije procjene kvaliteta podataka i pristupa „One Health", prepoznajući međusobnu povezanost zdravlja morskih ekosistema, očuvanja biodiverziteta i ljudskog blagostanja. Ovaj inventar podržava nacionalne i regionalne napore za ispunjavanje međunarodnih obaveza u praćenju biodiverziteta i ističe potrebu za kontinuiranim istraživanjima mora i prekograničnom saradnjom u Jadranskom moru.

Ključne riječi: Inventar vrsta, morska ihtiofauna, morski sisari, One Health

CONFERENCE PAPER

AN ASSESSMENT OF CARDIAC HISTOPATHOLOGICAL CHANGES IN DOXORUBICIN DOSE-DEPENDENT ANIMAL MODELS

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ABSTRACT

One effective anthracycline human chemotherapy drug that is frequently used to treat solid and haematological cancers is doxorubicin (DOX). The dose-dependent cardiotoxicity of some medications can result in irreversible heart failure, limiting their clinical utility. Understanding the pathophysiology and early detection of DOX-induced cardiac injury is made possible by animal models, especially rats, using acute models of DOX cardiotoxicity due to less time-consuming operations. The aim of this research is to determine a potential cardiotoxic DOX dose in gender-specific Wistar wild-type rats using light microscopy for evaluating morphological changes of the heart.

Adult Wistar rats (n=10), including males (n=5) and females (n=5), were treated with doxorubicin intraperitoneal injection in different doses (25 mg/kg, 30 mg/kg and 40 mg/kg) per male rat and female rat, respectively. Rats were sacrificed after 48 hours and 72 hours for the models of 25 mg/kg and 30 mg/kg, while the rats of the 40 mg/kg model group were sacrificed 24 hours after. The myocardium of the left ventricle is analysed using a light microscope under magnifications of ten and twenty times.

Male Wistar rats developed more pronounced morphological changes of the left ventricle compared to female Wistar rats, resulting in myocardial interstitial oedema and disorganisation of myocyte architecture.

Male Wistar wild-type rats develop a more aggressive form of acute cardiotoxicity caused by doxorubicin compared to female Wistar wild-type rats.

Keywords: Cardiotoxicity, doxorubicin, myocyte injury

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INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic derived from *Streptomyces peucetius*, has been widely used in oncology due to its broad-spectrum activity against various solid tumours and hematologic malignancies (Belger et al., 2023). Despite its efficacy, its clinical use is severely limited by a well-documented risk of cardiotoxicity, which can manifest acutely or chronically. Acute cardiotoxicity may present as arrhythmias or transient myocardial dysfunction within days of administration, while chronic effects may develop months to years later, potentially progressing to irreversible heart failure (Chatterjee et al, 2010). The mechanism of DOX-induced cardiotoxicity is multifactorial and includes the generation

of reactive oxygen species (ROS), disruption of mitochondrial function, interference with topoisomerase-II beta in cardiomyocytes, and apoptosis. The heart is particularly vulnerable to oxidative damage due to its relatively low levels of endogenous antioxidant enzymes compared to other organs (Rawat et al., 2021). In recent years, increasing attention has been given to sex-based differences in drug response and toxicity. Hormonal, genetic, and molecular differences between males and females can significantly influence the pharmacokinetics and pharmacodynamics of therapeutic agents. Yet, preclinical studies often neglect to analyse male and female responses separately. Understanding these differences in the context of DOX cardiotoxicity is crucial for developing targeted cardioprotective strategies and improving patient outcomes (Belger et al., 2023; Rawat et al., 2021). This study focuses on the early morphological effects of DOX-induced cardiotoxicity using an acute rat model. We hypothesise that male and female Wistar rats exhibit differing degrees of myocardial damage following DOX administration, potentially due to inherent biological differences. This work contributes to the growing body of literature emphasising the importance of sex as a biological variable in toxicological research.

MATERIALS AND METHODS

Animal Selection and Ethical Approval

Ten healthy adult Wistar rats comprising five males and five females, aged 8 to 10 weeks and weighing between 220 and 300 grams, were procured from an accredited animal facility. Animals were acclimatised for one week under controlled environmental conditions (temperature 22 ± 2°C, humidity 55 ± 10%, 12-hour light/dark cycle). All experimental protocols were approved by the Institutional Animal Care and Use Committee and conducted in compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Doxorubicin Administration

Rats were randomly divided into three experimental groups based on the dose of DOX administered: 25 mg/kg, 30 mg/kg, and 40 mg/kg. DOX was diluted in sterile saline and administered as a single intraperitoneal injection. The time points for sacrifice were set as follows: 72 hours post-injection for 25 mg/kg, 48 hours and 72 hours for 30 mg/kg, and 24 hours for 40 mg/kg. These intervals were selected based on preliminary studies and literature indicating the onset of acute cardiotoxicity within these windows.

Tissue Collection

At the respective time points, rats were anesthetized using a combination of ketamine (80 mg/kg) via intraperitoneal injection. Once deep anesthesia was confirmed, rats were euthanised by cervical dislocation. Hearts were immediately excised, rinsed in cold phosphate-buffered saline (PBS), and fixed in 10% neutral-buffered formalin for 72 hours.

Histological Processing and Staining

Fixed heart tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and mounted on glass slides. Hematoxylin and eosin (H&E) staining was performed for general histological evaluation. Sections were examined using a light microscope at magnifications of 10× and 20×.

Morphological Assessment

Histological analysis focused on identifying specific features of myocardial injury, including interstitial oedema, cytoplasmic vacuolization, myofibrillar disarray, nuclear morphology, necrosis, and inflammatory cell infiltration. Each parameter was semi-qualitatively scored with a value of 1 (yes) and 0 (no) and then summed up to a mean value by two independent pathology researchers. Average scores for each rat were calculated and compared between sexes and across doses.

RESULTS

Overview of Morphological Findings

Histological examination revealed dose-dependent myocardial damage in all experimental groups. The extent and severity of damage were more pronounced in male rats across all doses. Common alterations observed included myocardial fibre disorganisation, interstitial oedema, cytoplasmic fragmentation, and focal necrosis.

25 mg/kg Group (48 hours and 72 hours)

At the lowest dose, male rats exhibited mild interstitial oedema and occasional vacuolization of cardiomyocytes with partial change in morphology of nuclei after both periods (Figure 1). In contrast, female rats showed minimal structural disruption, with mostly preserved myofibrillar integrity. No significant inflammatory infiltration, haemorrhage or necrosis was noted in either sex (Figure 2).

30 mg/kg Group (48 hours and 72 hours)

Histological changes became more pronounced at this intermediate dose, but morphological changes remained the same in both periods, i.e. after 48 hours and 72 hours. Male rats demonstrated highly expressed severe interstitial oedema, moderate myocardial disorganisation, cytoplasmic vacuolization with hyperemia (Figure 3). Female rats exhibited mild to moderate interstitial oedema, mild myocardial disorganisation and mild cytoplasmic vacuolization with hyperemia.

40 mg/kg Group (24 hours)

Rats exposed to the highest dose showed severe cardiac pathology. In males, widespread necrosis, interstitial oedema with haemorrhage, and inflammatory cell infiltration were evident, together with changes in nuclear morphology. Myocardial fibres were disorganised and fragmented (Figure 4). Female rats also showed signs of damage, much less interstitial oedema, but with lesser severity of myocyte disorganisation and with mild focal necrosis changes (Figure 5). Nuclear morphology for both sexes in this group was changed.

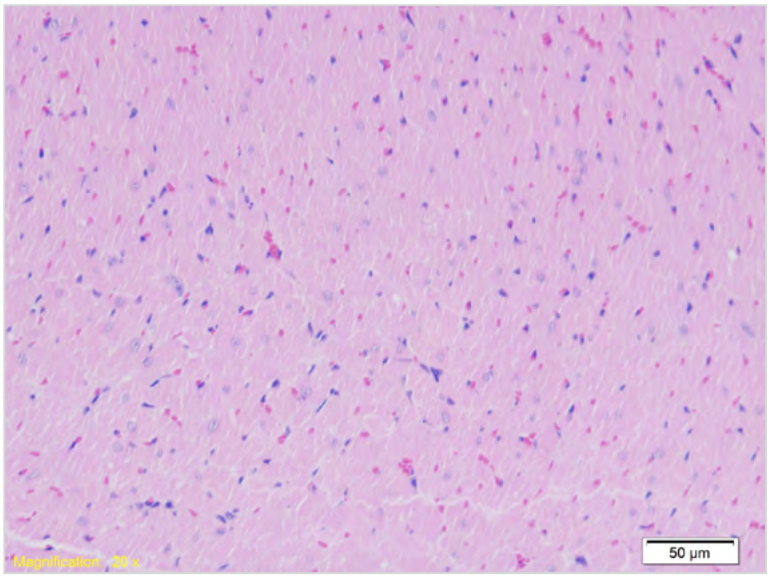

Figure 1 Representative photomicrograph of a male rat in the 25 mg/kg group

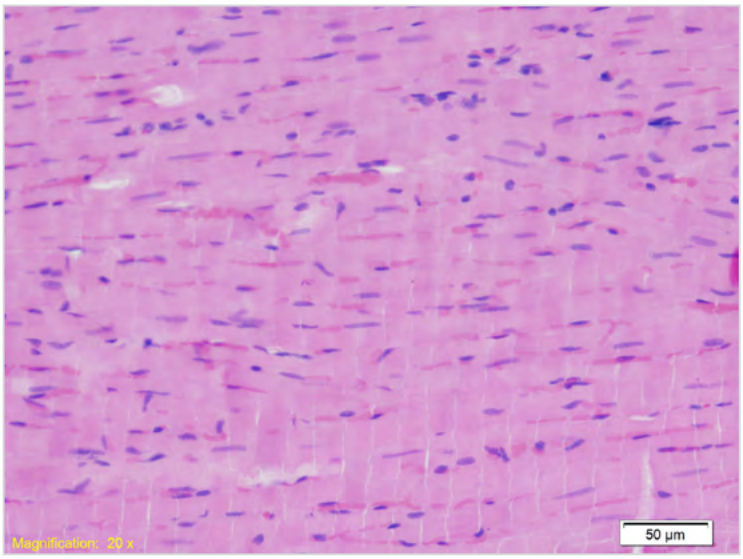

Figure 2 Representative photomicrograph of a female rat in the 25 mg/kg group

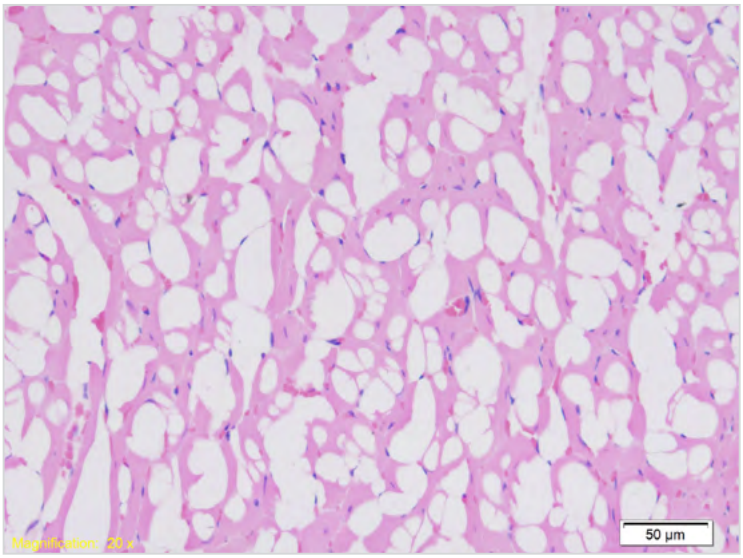

Figure 3 Representative photomicrograph of a male rat in the 30 mg/kg group

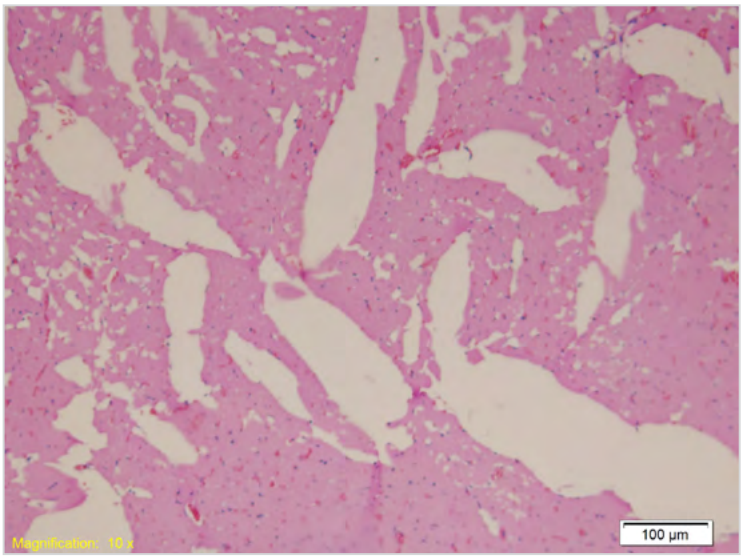

Figure 4 Representative photomicrograph of a male rat in the 40 mg/kg group

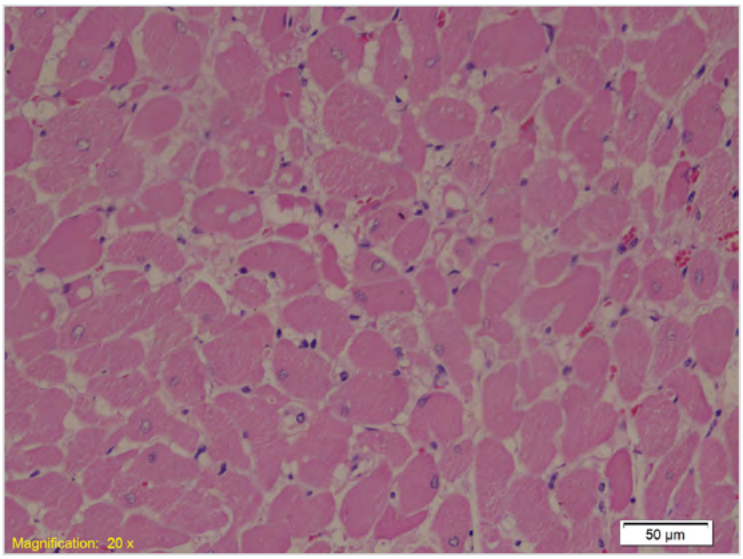

Figure 5 Representative photomicrograph of a female rat in the 40 mg/kg group

DISCUSSION AND CONCLUSION

Our study reveals clear sex-based differences in the morphological manifestations of acute DOX-induced cardiotoxicity in Wistar rats. Male rats consistently displayed more severe myocardial damage than their female counterparts, particularly at higher DOX doses. These findings suggest that biological sex may be a critical factor in modulating the heart's response to chemotherapeutic injury. One plausible explanation lies in hormonal influences. Estrogen has been reported to exert cardioprotective effects through several mechanisms, including enhancement of mitochondrial function, reduction of oxidative stress, and upregulation of anti-apoptotic pathways. Male rats, with lower circulating estrogen levels, may thus be more vulnerable to the mitochondrial and oxidative damage induced by DOX (Rattanasopa et al., 2019). Several mechanisms have been implicated in DOX-induced cardiotoxicity, with oxidative stress, ROS generation, and apoptosis being the most extensively documented. Beyond these primary pathways, additional contributing factors include mitochondrial dysfunction, dysregulation of iron homeostasis, disruption of intracellular Ca^{2+} balance, impaired autophagy, enhanced nitric oxide production, activation of inflammatory mediators, and altered expression of genes and proteins involved in apoptotic signalling (Chatterjee et al., 2010; Osataphan et al., 2020). DOX has also been shown to suppress DNA methyltransferase 1 (DNMT1) activity, resulting in reduced global DNA methylation. This epigenetic alteration is associated with dysregulation of mitochondrial regulatory genes such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (TFAM) in cardiac tissue. Furthermore, Dox exposure modulates microRNA expression profiles and perturbs histone deacetylase (HDAC) activity, further contributing to its cardiotoxic effects (Rawat et al., 2021; Rattanasopa et al., 2019 Osataphan et al., 2020). Additionally, sex-specific gene expression profiles in cardiac tissue could contribute to differential responses. Previous studies have shown that genes involved in detoxification, inflammation, and cell death pathways are expressed at different levels in male versus female myocardium. These molecular differences may affect the rate and extent of DOX-induced injury (Agostinucci et al.,2023; Dulf et al., 2023). Our findings are consistent with human studies reporting that male patients are more likely to develop anthracycline-related cardiac complications, particularly in pediatric oncology. This underlines the importance of including

both sexes in preclinical research and adopting sex-specific analyses in drug toxicity studies (Camilli et al., 2024). However, due to the fact that this is a pilot study about the determination of sex-related morphological changes in rat hearts caused by doxorubicin administration, the study is limited by its small sample size and lack of a control group, which could have provided a baseline for comparison. Future research should involve larger cohorts and explore the underlying molecular mechanisms driving sex differences in DOX cardiotoxicity. In conclusion, this study demonstrates that male Wistar rats exhibit more severe morphological damage than females in an acute model of DOX-induced cardiotoxicity. These results highlight the necessity of considering sex as a biological variable in preclinical toxicological studies. Recognising and understanding such differences may inform the development of tailored cardioprotective strategies and improve risk stratification in patients undergoing anthracycline-based chemotherapy.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Conception: RJ, ELS, MK, AF; Design: RJ, ELS, MK, AF; Supervision: RJ, ELS, MK, AF; Materials: RJ, ELS, MK, AF; Data Collection and/or Processing: RJ, ELS, MK, AF; Analysis and/or Interpretation: RJ, ELS, MK, AF; Literature Search: RJ, ELS, MK, AF; Writing – Original Draft: RJ, ELS, MK, AF; Critical Review: MK, AF

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PROCJENA HISTOPATOLOŠKIH PROMJENA NA SRCU U DOZNO-OVISNIM MODELIMA ŽIVOTINJA TRETIRANIM DOKSORUBICINOM

SAŽETAK

Jedan od djelotvornih antraciklinskih hemoterapijskih lijekova koji se često koristi za liječenje solidnih i hematoloških karcinoma kod ljudi je doksorubicin (DOX). Kardiotoksičnost nekih lijekova koja zavisi od doze može dovesti do nepovratnog zatajenja srca, što ograničava njihovu kliničku primjenu. Razumijevanje patofiziologije i rana detekcija srčanog oštećenja izazvanog DOX-om omogućeno je kroz modele na životinjama, posebno na štakorima, koristeći akutne modele DOX kardiotoksičnosti zbog manje vremenski zahtjevnih procedura. Cilj ovog istraživanja je utvrditi potencijalnu kardiotoksičnu dozu DOX-a kod spolno specifičnih Wistar štakora divljeg tipa korištenjem svjetlosne mikroskopije za procjenu morfoloških promjena na srcu.

Odrasli Wistar štakori (n=10), uključujući mužjake (n=5) i ženke (n=5), tretirani su intraperitonealnom injekcijom doksorubicina u različitim dozama (25 mg/kg, 30 mg/kg i 40 mg/kg) po mužjaku i ženki. Štakori su žrtvovani nakon 48 sati i 72 sata za modele sa 25 mg/kg i 30 mg/kg, dok su pacovi iz grupe s dozom od 40 mg/kg žrtvovani nakon 24 sata. Miokard lijeve komore analiziran je pomoću svjetlosne mikroskopije pri uvećanjima od deset i četrdeset puta.

Mužjaci Wistar štakori razvili su izraženije morfološke promjene lijeve komore u poređenju sa ženkama, što je rezultiralo intersticijskim edemom miokarda i dezorganizacijom arhitekture miocita.

Mužjaci Wistar pacova divljeg tipa razvijaju agresivniji oblik akutne kardiotoksičnosti izazvane doksorubicinom u poređenju sa ženkama Wistar pacova divljeg tipa.

Ključne riječi: Doksorubicin, kardiotoksičnost, oštećenje miocita

CONFERENCE PAPER

MONITORING OF AN ORPHANED BOSNIAN MOUNTAIN HORSE FOAL: A ONE-YEAR CASE STUDY

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ABSTRACT

This case report describes a foal of the Bosnian Mountain Horse (*Equus ferus caballus*) type, found in a cachectic and life-threatening condition in Eastern Bosnia, in April 2024. The foal, approximately two months old and orphaned, was adopted by local residents. It was fed a diet based on diluted cow's milk, forage and concentrates, supplemented with minerals and vitamins. The foal was monitored until it reached one year of age. Morphometric measurements included front cannon girth, thorax girth, body length, height at withers, and height at croup. Despite non-standard feeding and care conditions, the foal showed progressive recovery and development comparable to healthy foals of the same breed.

Keywords: Bosnian mountain horse, orphan foal, morphometry

INTRODUCTION

The Bosnian Mountain Horse (also known as the Balkan Pony or Bosnian Pony) is considered the oldest and most versatile indigenous horse breed in the Balkans. It was primarily developed in Bosnia and Herzegovina and later spread throughout the region, particularly during the era of the former Yugoslavia (Bunevski et al., 2019; Mesaric et al., 2015; Trajkovski and Bunevski, 2007). The Bosnian mountain horse is a warmblooded oriental indigenous breed, a product of the hot karst and strict selection. It belongs to the group of small horses, it is very hardy, resistant and modest in its diet. It is irreplaceable on rocky and hilly terrain, and its gait is very safe. It is mostly brown, reddish and black in color. It serves as a pack horse that, under a load of 100 to 120 kg, reaches a speed of 5-6 km per hour on a flat track, and can travel up to 40 km during the day. It is also used for towing and riding (Srebočan and Gomerčić, 1996).

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This breed has faced a drastic population decline, particularly during and after the 1992–1995 war in Bosnia and Herzegovina, when many animals were abandoned. Presently, populations survive in isolated locations such as the Kruzi plateau at the foot of Mount Cincar (Katica et al., 2010).

Orphaned foals represent a management challenge in equine rearing, as the absence of maternal imprinting and nutrition can impact both welfare and growth (Tateo et al., 2009). There is limited literature on orphan foal rearing in the Bosnian Mountain Horse population, and this case study seeks to contribute data on growth under alternative nutritional and environmental conditions.

CASE PRESENTATION

In early spring, an orphan male foal of Bosnian Mountain Horse breed was found in cachectic condition on Gosina mountain, near the village of Hrenovica (Pale municipality, Bosnia and Herzegovina). The foal was estimated to be between 1–2 months of age and was unable to stand or walk independently (Figure 1). It was taken in by local residents and initially treated with vitamin and mineral supplementation provided by a local veterinerian.

Due to the unavailability of mare's milk, a surrogate mare, or commercial milk replacer, the foal was fed with cow's milk diluted 1:1 with boiled and cooled water. In addition, it had free access to fresh water, hay, and a mash made of bran and oats soaked in water. The foal was kept in a fenced pasture with a simple shelter, providing protection from weather conditions.

Despite the challenges associated with early orphaning, the foal adapted well to the new environment. No major health issues (e.g., diarrhea, colic, respiratory infections) were observed throughout the monitoring period.

Figure 1 Initial condition of the foal at time of rescue and local rescuerer (April 2024)

Morphometric measurements were conducted monthly from the 2nd to the 12th month of age using a standard flexible measuring tape. All measurements were conducted by the same trained individual to ensure consistency. The following body dimensions were recorded: height at withers, height at croup, body length, chest girth and cannon bone circumference (front limb).

Table 1 Monthly morphometric values of the Bosnian Mountain Horse foal from 2 to 12 months of age

	Age in month										
Specification (cm)	2	3	4	5	6	7	8	9	10	11	12
Height at withers	89	95	101	106	108	115	120	123	129	129	130
Height at croup	90	91	92	110	111	120	123	126	130	131	131,5
Body length	85	86	87	98	106	115	126	128	129.5	130	131
Thorax girth	90	91	92	106	125	128	135	137	139	139	140
Cannon girth	14	15	16	18	20	22	23	23.5	24	24	25

Ethics committee approval

This study was approved by the Ethics Committee of the Veterinary Faculty of the University of Sarajevo, who gave a positive opinion under number: 07-03-583-2/25, from 19.06.2025.

DISCUSSION AND CONCLUSION

The orphan foal monitored in this study exhibited a consistent and proportional growth pattern throughout the observation period, aligning with findings reported by Bochiș and Țăpălagă (2012), who described normal growth trajectories in orphaned warmblood foals reared on artificial feeding. The morphometric data collected in this study indicate a developmental progression comparable to their findings, with notable increases in withers and croup height, chest girth, and cannon bone circumference.

Unlike the findings of Bochiș and Țăpălagă (2012) where peak growth occurred in the fourth month and gradually declined afterward, our foal exhibited more rapid gains between the fifth and eighth months. This discrepancy may be attributed to different feeding protocols, environmental adaptation, or individual variability. Notably, the foal in the present study showed no signs of developmental disturbances, such as diarrhea or respiratory complications, throughout the observation period, which likely contributed to its uninterrupted growth.

In agreement with the results of Yakan et al. (2012), the values recorded in this study—particularly withers height (130 cm) and croup height (131.5 cm) at twelve months—exceeded several previously reported norms for orphan foals and even surpassed those of some mare-raised foals, including two half-siblings from the same mother (HSM). This may be influenced by sex-related growth tendencies, as multiple studies have shown that colts are generally larger and grow faster than fillies (Hintz et al., 1979; Knight and Tyznik, 1985; Thompson, 1995).

The increase in cannon bone circumference from 14 cm to 25 cm is particularly noteworthy, confirming healthy skeletal development and supporting the observations of Yakan et al. (2012) that adequate nutrition and care can ensure normal skeletal growth in orphan foals.

Overall, the findings of this study reinforce the notion that, with proper management, a balanced diet, and absence of health complications, orphan foals can reach morphometric values comparable to-or even exceeding-those of mare-reared counterparts.

This case aligns with reports emphasizing the resilience of the Bosnian Mountain Horse in harsh conditions and minimal management (Katica et al., 2010; Srebočan and Gomerčić, 1996). The absence of health complications also underscores the importance of a hygienic and stable environment

in managing orphan foals.

Despite lacking maternal support and being fed a non-standard diet, the foal demonstrated a healthy and progressive growth trajectory. Although cow's milk does not provide the immunological benefits of mare's colostrum, early supplementation with vitamins and minerals may have contributed to initial immune support and overall development. Furthermore, the breed's genetic adaptation to karst terrain and selective breeding at the Goražde stud may explain its ability to thrive under suboptimal conditions (Bunevski et al., 2019).

In conclusion, this case highlights the potential of the Bosnian Mountain Horse to achieve satisfactory growth outcomes despite orphanhood and suboptimal feeding protocols. These findings reinforce the value of conserving and studying this genetically and culturally significant breed. Future research should explore long-term health and performance outcomes and compare growth profiles with foals raised under standard conditions.

AKNOWLEDGEMENTS

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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

CONTRIBUTIONS

Conception – MK, NKD; Design – MK, NKD; Supervision – MK, NKD; Materials – MK, NKD, A.A; Data Collection and Processing – NKD; Interpretation – NKD, MK, AA.; Literature Review – NKD, MK; Writing – NKD; MK; Critical Review – MK, NKD

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PRAĆENJE SIROČETA BOSANSKOG BRDSKOG KONJA: JEDNOGODIŠNJA STUDIJA SLUČAJA

SAŽETAK

Ovaj prikaz slučaja opisuje ždrijebe bosanskog brdskog konja (Equus ferus caballus), pronađeno u kahektičnom i po život opasnom stanju u istočnoj Bosni, u aprilu 2024. godine. Ždrijebe, staro otprilike dva mjeseca i bez majke, usvojili su lokalni stanovnici. Hranjeno je razrijeđenim kravljim mlijekom, kabastom hranom i koncentratima, uz dodatke minerala i vitamina. Praćeno je do navršene jedne godine starosti. Morfometrijska mjerenja uključivala su obim prednje cjevanice, obim grudnog koša, dužinu tijela, visinu u grebenu i visinu u sapi. Uprkos nestandardnim uslovima ishrane i njege, ždrijebe je pokazalo postepeni oporavak i razvoj uporediv sa zdravim ždrjebadi iste pasmine.

Ključne riječi: Bosanski brdski konj, morfometrija, siroče, ždrijebe

ORAL ABSTRACTS

MONITORING CHANGES IN THE EYES - THE SIGNIFICANCE OF THE POST-MORTEM INTERVAL IN ASPHYXIA

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ABSTRACT

Post-mortem ocular changes, particularly those affecting the sclera, are valuable indicators in forensic pathology for the estimation of post-mortem interval (PMI) and the evaluation of tissue decomposition. Previous studies have shown that dark scleral spots can appear within hours after death, influenced by eyelid position and environmental conditions. Our research aimed to examine how postmortem scleral changes progress over time and whether they are affected by environmental or physiological factors.

Twelve Wistar rats were divided into four groups: K – control group, autopsy performed immediately after death (n=3); A – 24-hour postmortem interval (n=3); B – 48-hour interval (n=3); C - 72-hour interval (n=3). All rats died by asphyxia due to hanging. Temperature measured during the study included: antemortem core temperature, ambient temperature, postmortem core temperature, postmortem eye temperature, and eye temperature at autopsy. Each eye sample was examined macroscopically for corneal and scleral changes relative to the postmortem interval.

Ambient temperature significantly influenced the postmortem body temperature of the rats. With increasing postmortem interval, ocular changes, such as corneal dryness and appearance of scleral spots, became more pronounced.

This pilot study demonstrated that, despite anatomical differences from human cadavers, the Wistar rat model provides a reliable experimental framework for studying postmortem scleral changes and related temperature patterns. These findings can enhance forensic investigations and support future research on ocular indicators of the postmortem interval.

Keywords: post-mortem interval, ocular temperature, sclera, forensics

ANALIZA PROMJENA NA OČIMA – ZNAČAJ POSTMORTALNOG INTERVALA KOD ASFIKSIJE

SAŽETAK

Postmortalne promjene oka, posebno one koje zahvataju bjeloočnicu (scleru), značajni su pokazatelji u forenzičkoj patologiji za procjenu postmortalnog intervala (PMI) i procjenu razgradnje tkiva. Prethodna istraživanja su pokazala da se tamne mrlje na bjeloočnici mogu pojaviti unutar nekoliko sati nakon smrti, a na njihov razvoj utiču položaj kapaka i uslovi okoline. Naše istraživanje imalo je za cilj ispitati kako postmortalne promjene na bjeloočnici napreduju tokom vremena i da li su pod uticajem faktora okoline ili fizioloških faktora.

Dvanaest Wistar pacova podijeljeno je u četiri grupe: K - kontrolna grupa, obdukcija urađena odmah nakon smrti (n=3); A – postmortalni interval od 24 sata (n=3); B – interval od 48 sati (n=3); C – interval od 72 sata (n=3). Svi pacovi su umrli od asfiksije uslijed vješanja. Tokom studije, mjerene su i sljedeće temperature: antemortalna temperatura jezgre, temperatura okoline, postmortalna temperatura jezgre, postmortalna temperatura oka i temperatura oka u trenutku obdukcije. Svaki uzorak oka makroskopski je pregledan radi promjena na rožnici i bjeloočnici u zavisnosti od postmortalnog intervala.

Temperatura okoline imala je značajan uticaj na postmortalnu tjelesnu temperaturu pacova. Sa produžavanjem postmortalnog intervala, očne promjene, poput sušenja rožnice i pojave mrlja na bjeloočnici, postale su izraženije.

Ova pilot-studija pokazala je da, uprkos anatomskim razlikama u odnosu na ljudske kadavere, Wistar model pacova predstavlja pouzdani eksperimentalni okvir za proučavanje postmortalnih promjena na bjeloočnici i povezanih temperaturnih obrazaca. Ovi nalazi mogu unaprijediti forenzičke istrage i podržati buduća istraživanja očnih pokazatelja postmortalnog intervala.

Ključne riječi: Bjeloočnica, forenzika postmortalni interval, temperatura oka

ORAL ABSTRACTS

ADVANCING MEAT CLASSIFICATION AND VETERINARY TRAINING THROUGH 3D TECHNOLOGY

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ABSTRACT

The use of different distant learning methods in veterinary medicine has become more popular since the COVID-19 pandemic. Technologies such as virtual reality (VR), 3D models, video lectures, and other digital tools have enhanced the educational experience by enabling interactive 3D visualization, particularly in teaching anatomy and simulating clinical procedures. A crucial aspect of veterinary hygiene and public health is the accurate categorization of meat, a responsibility that lies with veterinary professionals. This classification is vital not only for regulatory compliance but also for combating food fraud - a persistent issue that undermines food safety, public health, and consumer trust. In light of frequent food fraud cases both locally and globally, it is essential for professionals and consumers alike to understand the distinguishing characteristics of various meat categories. This study aimed to assess the ability of twenty-five fourth-year veterinary students at University of Sarajevo to identify meat categories and describe muscle anatomy using three different teaching methods: traditional classroom learning, computer-based learning with 3D scanned models, and immersive VR. To support this goal, an innovative virtual resource "3DMeat" was developed using 3D scanning technology. The findings of this study indicate that technology-based learning tools, especially immersive and interactive approaches like VR and 3D visualization, can substantially improve student engagement and knowledge retention when learning complex topics such as meat categorization. This research also aimed to bridge the gap between professional, legally defined meat classifications and commonly used market terms by explaining the morphological and anatomical features of muscles associated with each category.

Keywords: Food fraud, Meat categories, 3D technology

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UNAPREĐENJE KLASIFIKACIJE MESA I VETERINARSKE EDUKACIJE POMOĆU 3D TEHNOLOGIJE

SAŽETAK

Upotreba različitih metoda učenja na daljinu u veterinarskoj medicini postala je popularnija nakon pandemije COVID-19. Tehnologije poput virtuelne stvarnosti (VR), 3D modela, video predavanja i drugih digitalnih alata poboljšale su obrazovno iskustvo omogućavajući interaktivnu 3D vizualizaciju, posebno u nastavi anatomije i simulaciji kliničkih procedura. Tačna klasifikacija mesa predstavlja ključni aspekt veterinarske higijene i javnog zdravlja, a za nju su odgovorni veterinarski stručnjaci. Ova klasifikacija je od suštinskog značaja ne samo radi usklađenosti s propisima, već i u borbi protiv prevara s hranom – čestog problema koji ugrožava sigurnost hrane, javno zdravlje i povjerenje potrošača. S obzirom na učestale slučajeve prevara s hranom, kako na lokalnom tako i na globalnom nivou, od suštinske je važnosti da i stručnjaci i potrošači razumiju karakteristike različitih kategorija mesa. Cilj ove studije bio je procijeniti sposobnost dvadeset pet studenata četvrte godine Veterinarskog fakulteta Univerziteta u Sarajevu da prepoznaju kategorije mesa i opišu anatomiju mišića koristeći tri različite metode učenja: tradicionalnu nastavu u učionici, učenje putem računara uz 3D skenirane modele i imerzivnu VR. U tu svrhu razvijen je inovativni virtuelni resurs "3DMeat" korištenjem tehnologije 3D skeniranja. Rezultati istraživanja pokazuju da alati za učenje zasnovani na tehnologiji, posebno imerzivne i interaktivne metode poput VR-a i 3D vizualizacije, mogu značajno poboljšati angažman studenata i retencijue znanja prilikom učenja složenih tema kao što je klasifikacija mesa. Istraživanje je također imalo za cilj da premosti jaz između profesionalnih, zakonski definisanih klasifikacija mesa i uobičajenih tržišnih pojmova, objašnjavajući morfološke i anatomske karakteristike mišića koje su povezane sa svakom kategorijom.

Ključne riječi: Prevara s hranom, kategorije mesa, 3D tehnologija

ORAL ABSTRACTS

THE FORENSIC SIGNIFICANCE OF CORE TEMPERATURE IN IDENTIFYING PRIMARY AND SECONDARY HYPOTHERMIA AS A CAUSE OF DEATH: A PILOT STUDY ON WISTAR RATS

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ABSTRACT

Hypothermia is defined as a body core temperature below 35 °C and can be caused by internal or external stress. Primary hypothermia is caused by excessive exposure to low environmental temperature without any medical conditions prior to that. Secondary hypothermia is caused by alteration in thermoregulation by disease, trauma, surgery, drugs, or infections. The aim of the research is to investigate core temperature values in rats subjected to specific water temperatures at five different time points. It focuses on distinguishing between primary and secondary hypothermia in these rats.

The total 21 Wistar rats were divided into three experimental groups as: Control group rats exposed only to hypothermic condition (n = 7); Alcohol + hypothermia (n = 7); and Benzodiazepines + hypothermia (n = 7). The temperature spots analyzed in the study were: normal core temperature, core temperature during injection of 0,3 ml ketamine, temperature of immersion and the temperature at the onset of hypothermia and temperature at the time of death.

In our study the comparative analysis of body temperatures at various time points following submersion in water revealed significant differences among the study groups treated with either alcohol or benzodiazepines and the control group. Notable differences were observed in baseline temperature, post-anesthesia induction temperature, and immediate post-submersion temperature. Specifically, significant differences were discovered among the alcohol and benzodiazepine groups ($p < 0.001$) and ranging from the alcohol and control groups ($p < 0.001$). The analysis of survival times following induced hypothermia revealed a statistically significant difference among the three experimental groups ($p = 0.04$), though subsequent post-hoc comparisons did not demonstrate significant differences in mean survival times.

There is a difference in survival time between primary and secondary hypothermia groups, depending on consumption and intoxication with alcohol or benzodiazepines. The analysis of survival times following induced hypothermia showed a statistically significant difference among the groups.

Keywords: Forensic, Hypothermia, Primary, Secondary, Survival time

SUDSKO-MEDICINSKI ZNAČAJ TEMPERATURE TIJELA U IDENTIFIKACIJI PRIMARNE I SEKUNDARNE HIPOTERMIJE KAO UZROKA SMRTI: PILOT STUDIJA NA PACOVIMA SOJA WISTAR

SAŽETAK

Hipotermija se definiše kao pad unutrašnje tjelesne temperature ispod 35°C, a može nastati kao posljedica vanjskih uticaja ili poremećaja u organizmu. Primarna hipotermija rezultat je direktnog izlaganja hladnom okruženju bez prethodnih zdravstvenih problema, dok sekundarna hipotermija nastaje zbog oslabljenog mehanizma termoregulacije usljed bolesti, traume, farmakoloških sredstava, infekcija ili hirurških intervencija. Cilj ovog istraživanja bio je analizirati promjene centralne tjelesne temperature kod pacova izloženih određenim temperaturnim uslovima vode, u više vremenskih tačaka, kako bi se procijenile razlike između primarne i sekundarne hipotermije.

Ukupno 21 pacov soja Wistar bio je podijeljen u tri eksperimentalne grupe: kontrolnu grupu, koja je bila izložena samo hipotermijskim uslovima (n = 7), grupu koja je bila pod uticajem alkohola i hipotermije (n = 7), te grupu koja je bila pod uticajem benzodiazepina i hipotermije (n = 7). Temperature koje su analizirane su: fiziološka tjelesna temperatura, temperatura tokom injekcije 0,3 ml ketamina, temperatura prilikom uranjanja u vodu, temperatura na početku razvoja hipotermije, te temperatura u trenutku smrti.

Dobijeni rezultati pokazuju značajne razlike u dinamici tjelesne temperature između grupa koje su bile pod uticajem alkohola ili benzodiazepina u poređenju sa kontrolnom grupom. Posebno su bile izražene razlike u početnoj temperaturi, temperaturi nakon anestezije i neposredno nakon izlaganja hladnoj vodi. Statistička analiza je pokazala izrazito značajne razlike između grupe s alkoholom i one s benzodiazepinima ($p < 0,001$), kao i između alkohola i kontrolne grupe ($p < 0,001$). Također, utvrđena je statistički značajna razlika u vremenu preživljavanja među sve tri eksperimentalne grupe ($p = 0,04$), iako naknadne post-hoc analize nisu potvrdile značajne razlike u prosječnim vremenima preživljavanja.

Na osnovu provedenog istraživanja može se zaključiti da konzumacija alkohola i benzodiazepina utiče na tok i ishod hipotermije, što se ogleda u razlikama u vremenu preživljavanja između pacova sa primarno i sekundarno izazvanom hipotermijom. Ove razlike su statistički potvrđene i ukazuju na potencijalnu forenzičku vrijednost analize centralne tjelesne temperature prilikom utvrđivanja uzroka smrti.

Ključne riječi: Hipotermija, primarna, sekundarna, sudska medicina, vrijeme preživljavanja

ORAL ABSTRACTS

TISSUE PRESERVATION WITH ELNADY TECHNIQUE: IMPORTANT REMARKS

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ABSTRACT

Formalin, as a proven carcinogenic and toxic chemical, is aiming to be reduced or completely removed from use in all spheres. Among different alternatives for formalin use, the plastination technique is widely known as unique method in tissue preservation. Due to its expensiveness, more affordable method has been invented by Professor Fawzy Elnady. The Elnady technique aims to detoxify formalin specimens used in classes by using cheaper and available chemicals. The specimens obtained are non-toxic, durable, odorless, dry, soft and flexible. They can be used in the teaching of anatomy, but also in other subjects such as embryology, pathology, parasitology, clinics and forensic veterinary medicine. During preparation, formalin is used for tissue fixation, acetone for dehydration, glycerin for impregnation and corn starch for drying of specimens. In this study, we observed effectiveness of the method on more than 100 different specimens. The results showed that lower quality specimens are obtained when the organs are stored in formalin for longer time. Usually, these specimens became darker with the decrease of visibility of structures. Furthermore, we noticed that lungs become darker with tissue sensitive to damages. The liver preparation resulted in dark, non-flexible specimen. We suggest these changes could be due to the time specimens were in glycerin since before this phase specimens had adequate characteristics. Throughout the specimens we also noticed the bad impact organ membranes had if left on the organ. With the acetone, they became too dry and their remains decreased the quality of the specimens. In our study, we confirmed the results of the author but we suggest that this method is not adequate for every type of organ and that organ membranes should be removed prior to process. We emphasize the importance of fresh specimens use, since older formalin specimens after preparation were not of great quality.

Keywords: Alternatives, anatomy, Elnady, plastination

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PREZERVACIJA TKIVA ELNADY TEHNIKOM: VAŽNE NAPOMENE

SAŽETAK

Formalin, kao dokazano kancerogena i toksična hemikalija, sve se više nastoji smanjiti ili potpuno ukloniti iz upotrebe u svim područjima. Među različitim alternativama za upotrebu formalina, tehnika plastinacije poznata je kao jedinstvena metoda očuvanja tkiva. Zbog svoje visoke cijene, razvijena je pristupačnija metoda koju je izumio profesor Fawzy Elnady. Elnadyjeva tehnika ima za cilj detoksikaciju formalinskih preparata korištenih u nastavi, upotrebom jeftinijih i dostupnih hemikalija. Dobiveni preparati su netoksični, izdržljivi, bez mirisa, suhi, mekani i fleksibilni. Mogu se koristiti u nastavi anatomije, ali i u drugim predmetima poput embriologije, patologije, parazitologije, klinike i forenzičke veterinarske medicine. Tokom pripreme koristi se formalin za fiksaciju tkiva, aceton za dehidraciju, glicerol za impregnaciju te kukuruzni škrob za sušenje preparata. U ovom istraživanju promatrali smo efikasnost metode na više od 100 različitih preparata. Rezultati su pokazali da se dobivaju preparati lošije kvalitete kada su organi duže vrijeme skladišteni u formalinu. Takvi preparati obično su tamniji, s manjom vidljivošću struktura. Također smo primijetili da pluća postaju tamnija i osjetljiva na oštećenja. Priprema jetre rezultirala je tamnim, nefleksibilnim preparatom. Smatramo da su te promjene povezane s trajanjem boravka preparata u glicerinu, budući da su prije te faze pokazivali zadovoljavajuće osobine. Kod preparata također smo uočili negativan utjecaj membrana organa ako bi ostale na organima. U kontaktu s acetonom postajale bi presuhe, a njihovi ostaci smanjivali su kvalitetu preparata. U našem istraživanju potvrdili smo rezultate autora, no smatramo da metoda nije pogodna za sve vrste organa te da se membrane organa trebaju ukloniti prije procesa. Naglašavamo važnost korištenja svježih preparata, jer stariji organi čuvani u formalinu nakon pripreme nisu bili zadovoljavajuće kvalitete.

Ključne riječi: Alternative, anatomija, Elnady, plastinacija

ORAL ABSTRACTS

RADIOLOGICAL ASSESSMENT OF MARINE SPECIES IN NEUM BAY (BOSNIA AND HERZEGOVINA): IMPLICATIONS FOR ONE HEALTH AND FOOD SAFETY

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ABSTRACT

Environmental radioactivity is often an overlooked factor in the One Health framework, despite its potential to impact human health through bioaccumulation and trophic web transfer. This study presents a radiological assessment of marine species along Bosnia and Herzegovina's Adriatic coastline, using mussels (*Mytilus galloprovincialis*) and sea cucumbers (*Holothuria sp*.) as bioindicator species.

Samples from each species were collected from Neum Bay and analysed using gamma spectrometry. Activity concentrations of radionuclides K-40, Be-7, Pb-210, Cs-137 and Ra-226 were determined based on their characteristic gamma emission lines or those of their decay products. All activity concentrations are expressed on dry weight (d.w.).

In mussels, the dominant radionuclide was K-40 (340.6 Bq/kg), followed by Pb-210 (15.6 Bq/kg), Be-7 (14.5 Bq/kg), Cs-137 was present at low level (0.1 Bq/kg), and Ra-226 was below the detection limit (<0.4 Bq/kg). In contrast, sea cucumbers showed lower K-40 activity (123.4 Bq/kg), but higher levels of Pb-210 (49.8 Bq/kg), Be-7 (36.9 Bq/kg), and detectable Ra-226 (7.7 Bq/kg), while Cs-137 was not detected.

The activity concentration of Po-210 in *Mytilus galloprovincialis* was not measured directly, however it can be estimated using established relationship between Po-210 and Pb-210 observed in mussels, particularly from the MARIS program. Using a conservative ratio of 15:1, the estimated 210Po activity concentration in Neum Bay mussels is approximately 90 Bq/kg (f.w.).

From a public health perspective, it is likely that ingestion dose would be underestimated due to the absence of alpha-emitting Po-210 data in mussels. These findings highlight the importance of integrating environmental radionuclide monitoring into food safety and marine ecosystem protection strategies, particularly in under-monitored regions such as Bosnia and Herzegovina.

Keywords: Bioindicator species, environmental radioactivity, food safety, One Health, radionuclide risk

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RADIOLOŠKA PROCJENA MORSKIH ORGANIZAMA U NEUMSKOM ZALJEVU BOSNE I HERCEGOVINE: IMPLIKACIJE ZA ONE HEALTH PRISTUP I SIGURNOST HRANE

SAŽETAK

Radioaktivnost okoliša često se zanemaruje unutar One Health pristupa, iako može imati značajan uticaj na ljudsko zdravlje putem bioakumulacije i prijenosa kroz trofički lanac. Ova studija prikazuje radiološku procjenu morskih vrsta duž jadranske obale Bosne i Hercegovine, koristeći dagnje (*Mytilus galloprovincialis*) i morske krastavce (*Holothuria sp*.) kao bioindikatorske vrste.

Uzorci obje vrste sakupljeni su u Neumskom zaljevu i analizirani gama spektrometrijskom metodom. Koncentracije aktivnosti radionuklida K-40, Be-7, Pb-210, Cs-137 i Ra-226 određene su na osnovu njihovih karakterističnih gama emisijskih linija ili linija produkata raspada. Sve vrijednosti izražene su na suhe mase (s.m.) uzoraka.

Kod dagnji je dominantni radionuklid bio K-40 (340,6 Bq/kg), zatim Pb-210 (15,6 Bq/kg), Be-7 (14,5 Bq/kg), dok je Cs-137 bio prisutan u niskoj koncentraciji (0,1 Bq/kg), a Ra-226 ispod granice detekcije (<0,4 Bq/kg). Nasuprot tome, morski krastavci su pokazali nižu aktivnost K-40 (123,4 Bq/kg), ali višu aktivnost Pb-210 (49,8 Bq/kg), Be-7 (36,9 Bq/kg), te Ra-226 (7,7 Bq/kg), dok Cs-137 u ovom slučaju nije detektovan.

Iako koncentracija aktivnosti Po-210 u dagnjama nije direktno mjerena, moguća je njena procjena na osnovu poznatog odnosa između Po-210 i Pb-210, koji je utvrđen u prethodnim istraživanjima, posebno kroz MARIS program. Primjenom konzervativnog omjera 15:1, procijenjena aktivnost Po-210 u dagnjama iz Neumskog zaljeva iznosi oko 90 Bq/kg po svježoj masi uzorka.

Iz perspektive javnog zdravstva, nedostatak direktnih podataka o alfa-emiteru Po-210 u dagnjama može dovesti do potcjenjivanja doprinosa stvarnoj godišnjoj efektivnoj dozi putem ishrane. Ova studija pokazuje potrebu za sistemskim uključenjem monitoringa radioaktivnosti u programe kontrole sigurnosti hrane i zaštite morskog okoliša, posebno u nedovoljno istraženim obalnim područjima poput Bosne i Hercegovine.

Ključne riječi: Bioindikatorske vrste, ekološka radioaktivnost, One Health, rizik od radionuklida, sigurnost hrane

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- dijagnostike spongiformne encefalopatije (BSE),
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Precise titles of Tables and Figures should be embedded in the manuscript at the appropriate place. For example: „Table 1. Results of....".
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Main text sub-divided into:

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4. Introduction,
5. Materials and Methods,
6. Results,
7. Discussion and Conclusion,
8. Acknowledgements,
9. References.

Short Communications and short clinical case reports for publication as short communications should follow the same format as Research Article, but the text must not exceed 1,500 words with no more than twenty references.
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Journal names in the reference list should be written in accordance with the Journal Title Abbreviations list available at:
https://images.webofknowledge.com/images/help/WOS/A_abrvjt.html
Harvey N, Kingsley M, Terek F. 2019. Anatomical aspect of the red fox lung. Veterinaria, 64(3), 20-5.
Include the doi number if one exists
When more than a six authors:
Catanese G, Grau A, Valencia JM, Garcia-March JR, Vázquez-Luis M, Alvarez E, et al. 2018. Haplosporidium pinnae sp. nov., a haplosporidian parasite associated with mass mortalities of the fan mussel, Pinna nobilis in the Western Mediterranean Sea. J Invertebr Pathol, 157, 9-24.
Books:
Brown C, Laland K, Krause J. 2011. Fish Cognition and Behavior. 2nd ed. Oxford, UK: Wiley-Blackwell.
Chapters in books:
King AS. 1993. Apparatus urogenitalis. In Baumel JJ, King AS, Breazile JE, et al (Eds), Handbook of Avian Anatomy: Nomina Anatomica Avium

(2nd ed., pp. 329-90). Cambridge, USA: Nuttall Ornithological Club.
Conference proceedings:
Bregoli M, Dediasi K, Pasolli C. 2006. Antibiotic resistant E. Coli in free-ranging alpine wild ruminants, in: VII Conference of the European Section of the Wildlife Diseases Association. p. 78.
Web Adresses:
Papazoglou LG, Basdani E. 2015. Exploratory laparotomy in the dog and cat. https://www.cliniciansbrief.com/article/exploratory-laparotomy-dog-cat (accessed 4.18.17).
Theses:
Donker SA. 2010. Arctic ground squirrels in the Southwest Yukon Territory: evidence for habitat specific demography and source-sink dynamics. MSc, University of British Columbia, Vancouver, Canada.
Watts AJR. 2012. Nutritional status and trophic dynamics of the Norway lobster Nephrops norvegicus (L.). PhD, University of Glasgow, Glasgow, UK.

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If possible, use standard abbreviations. In general, use of abbreviations other than standard abbreviations and units of measures should be kept to a minimum. In the Abstract, a term should be abbreviated only if it is used at least 3 times in the structured abstract. The term must be expanded at first mention, with the abbreviation given in parentheses after the expanded term.
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- ✓ Results
- ✓ Discussion and conclusion;
- ✓ Acknowledgements;
- ✓ References

Tables should be created in MS Word® and embedded in the text of the article as well as a separate excell document.
Figures and images with proper resolution and size are embedded in the text of the article as well as a separate TIFF or JPEG file.
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