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ZOONOTIC DISEASES AND ANTIMICROBIAL RESISTANCE: A DUAL THREAT AT THE HUMAN-ANIMAL INTERFACE

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Abstract

With over 200 zoonotic diseases and the emergence of antimicrobial-resistant (AMR) microbes, a substantial threat looms over public health. If we are unable to cope with AMR, it suggests a potential of 10 million global fatalities by 2050. The evolving landscape of AMR transcends geographic boundaries and species, necessitating collaborative and multidisciplinary efforts. The interplay between humans, animals, and the environment highlights the profound significance of One Health in addressing these interconnected challenges. The imperative of multidisciplinary efforts to safeguard the health and well-being of our shared world, comprised of humans, animals, and the environment, can pave the way toward success. The role of surveillance, education, and public awareness in combating AMR and fostering responsible antimicrobial use is inevitable in addressing this global concern.

Key words: Antimicrobial resistance, zoonoses, One Health

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ZOONOTSKA OBOLJENJA I ANTIMIKROBNA REZISTENCIJA: DVOSTRUKA PRETNJA U RELACIJI LJUDI-ŽIVOTINJE

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Kratak sadržaj

Preko 200 zoonotskih oboljenja i sve učestalija pojava mikroorganizama rezistentnih na antimikrobne lekove (AMR) predstavljaju ozbiljnu pretnju za javno zdravlje. Ako ne uspemo da se izborimo sa antimikrobnom rezistencijom, to može da znači potencijalnih 10 miliona smrtnih slučajeva na globalnom nivou do 2050. Slika širenja AMR prevazilazi sve geografske granice i vrste, što zahteva kolaborativne i multidisciplinarne pristupe. Povezanost izmedju ljudi, životinja i životne sredine ističe značaj koncepta „Jednog zdravlja“ (One Health) u pristupu ovakvim višestruko povezanim izazovima. Ovakve multidisciplinarne aktivnosti za očuvanje zdravlja i dobrobiti našeg zajedničkog sveta sastavljenog od ljudi, životinja i okoline, su svakako imperativ koji može otvoriti put za uspeh. Uloga nadzora, edukacije i razvoja javne svesti je ključna u borbi protiv AMR i afirmisanju odgovorne primene antimikrobnih supstanci u okviru ovog globalnog problema.

Ključne reči: antimikrobna rezistencija, zoonoze, Jedno zdravlje

INTRODUCTION

Antimicrobial resistance (AMR) stands as a formidable challenge in the realm of veterinary medicine and public health, presenting a critical threat to the health and well-being of animals and humans. At its core, AMR refers to the ability of microorganisms to withstand the effects of antimicrobial agents, such as antibiotics, rendering these treatments less effective (Founou et al., 2017). In the context of One Health, this phenomenon strikes at the heart of efforts to maintain animal health, ensure food security, and safeguard public health through the prevention and treatment of infectious diseases (Saleem et al., 2022). With over 200 zoonotic diseases, the development of antibiotic-resistant bacteria and their dissemination environment and living organisms is causing a huge threat to human beings (Dafale et al., 2020).

The data, showing that approximately 4.95 million deaths were linked to bacterial antimicrobial resistance on a global scale in 2019, underscores the significant benefits of proactive infection prevention (Laxminarayan, 2022). If the current pattern of improper and excessive antibiotic use persists, projections suggest that it could lead to approximately 10 million fatalities globally by the year 2050 (Pulingam et al., 2022). The global landscape is now characterized by an escalating concern over AMR, transcending geographical boundaries and species barriers (Christaki et al., 2020). The localized issue has transformed into a pervasive challenge that spans continents, ecosystems, and sectors. The effective management of AMR is vital because it is overshadowing the remarkable development of medicine in the field of antibiotics (Marston et al., 2016). It has become the need of the hour to reinforce the impeccable mark of antibiotics, which helped save millions of lives for humans and animals.

A comprehensive strategy is imperative to confront the multifaceted challenge posed by AMR. The One Health approach emerges as a powerful paradigm, advocating for collaboration across the realms of human health, animal health, and the environment. Recognizing the intricate connections between these domains, One Health emphasizes the inextricable linkages between the health of animals, humans, and ecosystems (Olaru et al., 2023). By recognizing the interplay between veterinary medicine, medical practice, and environmental factors, the One Health approach deepens our understanding of AMR transmission and paves the way for integrated solutions that transcend disciplinary boundaries (Dafale et al., 2020).

The rise of novel, opportunistic pathogens and the increasing prevalence of drug resistance among existing pathogens represent a global concern of paramount significance (Dafale et al., 2020). Many of these emerging diseases have transcended geographical boundaries, infiltrating diverse environments worldwide. This evolving disease landscape exploits the breeding grounds for pathogenic determinants within ecosystems, utilizing any available biological host. Among these hosts, animals have emerged as a prominent breeding ground for drug-resistant pathogens (Dafale et al., 2020).

Within the intricate ecosystem of the animal gut, a myriad of microbial communities coexists, acting in synergy to benefit their host (Purohit et al., 2018). Introducing drug-resistant pathogens into this microbial landscape disrupts the delicate balance and structure of the community, facilitating the transfer of resistance genes to other pathogens within the gut (Abushaheen et al., 2020). Bacteria have developed various means to ease their transmission between animals and humans, leading to zoonotic infections with significant implications for public health. One key route for bacterial transmission is direct contact between animals and humans, allowing pathogens to transfer from one species to another. Additionally, some bacteria can spread through insects or contaminated food and water sources to reduce the gap between animal reservoirs and human populations. Once bacteria successfully cross the species barrier, their characteristics play a crucial role in shaping their virulence and pathogenicity. These pathogens may possess virulence factors, such as toxins and adhesion molecules, that enable them to adhere to host tissues, evade immune responses, and cause disease in both animal and human hosts. Furthermore, the ability of bacteria to adapt to different host environments enhances their survival and persistence, further contributing to their pathogenic potential.

The intricate interplay between humans and animals within ecosystems has given rise to a compelling paradigm known as the "One Health" approach. This approach recognizes the interconnections between human, animal, and environmental health, particularly in the context of antimicrobial resistance. It underscores the need for a holistic understanding of how the health of animals and humans are intertwined, emphasizing the importance of collaborative efforts to address the complex challenges posed by emerging pathogens and drug resistance.

ONE HEALTH AND AMR: UNDERSTANDING THE CONNECTION

The intricate web of life intertwines across species and ecosystems, forming a complex tapestry where the health of animals, humans, and the environment is interdependent (Olaru et al., 2023). In the face of global challenges, such as antimicrobial resistance (AMR), the One Health approach emerges as a compelling strategy to address the multifaceted complexities at the human-animal-environment interface (Dafale et al., 2020).

The One Health concept signifies a departure from traditional, compartmentalized approaches to health management. It acknowledges that the health of humans is deeply entwined with the well-being of animals and the environment and that disruptions in one domain can reverberate throughout the interconnected network. This holistic perspective underscores that disease processes do not respect disciplinary boundaries; they transcend species and ecosystems, necessitating collaborative efforts to unravel their complexities. Microorganisms are ubiquitous as they are present in the gut of animals, manure, soil, water bodies, and human beings. As they are interconnected and components of a cycle, drug-resistant pathogens pose a matchless threat. These pathogens reproduce at an instant rate and also produce resistance in the native gut microbiome of living organisms (Dafale et al., 2020). This is due to the indiscriminate use of antibiotics and the lack of effective policies to restrain its dissemination.

Consequently, it becomes inevitable to entail multidisciplinary teams involving medical specialists, veterinarians, ecologists, and stakeholders to manage this aggravating concern of AMR (Dafale et al., 2020). By embracing the principles of One Health, practitioners, researchers, and policymakers are better equipped to grapple with the challenges posed by emerging diseases, including those fueled by antimicrobial resistance.

The global rise of antimicrobial resistance exemplifies the urgent need for a One Health perspective (Majumder et al., 2020). As pathogens evolve and adapt in response to antimicrobial agents, they threaten both human and animal populations, transcending geographic borders and species barriers. Within this context, the interplay between animal health, human health, and the environment becomes glaringly evident. Zoonotic diseases, which are infections that can be transmitted between animals and humans, serve as poignant examples of how the interconnectedness of these realms can magnify the challenge of AMR. The transmission of resistant pathogens from animals to humans and vice versa not only heightens the potential for treatment failures but also underscores the shared vulnerabilities that traverse species lines.

The One Health approach is a call to recognize the indivisible nature of the health of animals, humans, and the environment. It reminds us that the boundaries between these domains are porous, and the solutions to complex challenges like AMR must be collaborative, multidisciplinary, and holistic 14. As we navigate the intricate web of health interactions, fostering synergies between veterinary medicine, human medicine, and environmental science is imperative. This review article seeks to delve deeper into the nexus between AMR and the One Health framework, shedding light on the ways in which these concepts intersect and offering insights into the transformative potential of collaborative approaches in safeguarding the health of our shared world.

ZOONOTIC DISEASES AND AMR: A DUAL THREAT

In the intricate tapestry of global health, zoonotic diseases emerge as a crucial juncture where the boundaries between animal and human health blur, posing a dual threat that resonates across species lines. Zoonoses, entailing 60% of the infectious ailments that a human being suffers from, are transmissible between animals and humans and have garnered increasing attention due to their potential to incite not only immediate health crises but also to contribute to a more insidious menace: antimicrobial resistance (AMR) (Rahman et al., 2020). As we navigate this complex landscape, it becomes evident that the convergence of zoonotic diseases and AMR presents a critical nexus that demands comprehensive exploration (Olaru et al., 2023).

Antibiotics are used to cure bacterial infections, increase growth to cover up decreased body weight, and enhance milk production. Their mode of action varies based on what structure of bacteria they attack. Although their use is of matchless importance, the unmetabolized antibiotics are disseminated to the surroundings, such as water bodies, soil, other animals, and human beings. It leads to modification in the sensitivity of microorganisms and they start developing resistance to antibiotics (Dafale et al., 2020). This change in sensitivity has posed a massive threat to the public and animal health (Jin et al., 2022).

Zoonotic diseases, at their core, exemplify the interconnectedness of ecosystems and species. These diseases traverse the interface between animals and humans, often sparking outbreaks that lay bare the vulnerabilities within our intertwined health systems. As agents of transmission leap from animals to humans and vice versa, they amplify the potential for the dissemination of resistant pathogens. Indeed, the movement of zoonotic infections carries with it the lurking specter of AMR—an intricate interplay that renders the treatment of such diseases increasingly challenging.

The misuse of antimicrobials in animal populations, often intended to combat zoonotic infections, has inadvertently fueled the development of resistance (Kasimanickam et al., 2021). These scenarios magnify the delicate balance that must be struck between safeguarding animal health, controlling zoonotic outbreaks, and mitigating the risk of AMR amplification. Amidst the tapestry of zoonotic diseases, specific pathogens stand out for their potential to fan the flames of AMR. These bacteria, like Campylobacter and Salmonella, cause sickness in people, and are found in animals like poultry. Because of this, farmers use medicines to kill bacteria in poultry. However, some bacteria become resistant to these medicines. This shows that we need to work on stopping diseases from spreading and making sure medicines keep working well. Campylobacter is one of the leading causes of bacterial gastroenteritis worldwide. It is commonly transmitted through the consumption of contaminated food, particularly undercooked poultry, unpasteurized milk, and contaminated water. Campylobacteriosis typically manifests as diarrhea (often bloody), abdominal pain, fever, and sometimes vomiting. While most cases of Campylobacter infection are self-limiting, severe cases can occur, especially in vulnerable populations such as the elderly, infants, and immunocompromised individuals. Campylobacteriosis is a significant public health concern, and strategies to prevent contamination of food and water sources are essential for its control. The shared susceptibility of animals and humans to these pathogens necessitates a coordinated, multidisciplinary approach that recognizes the vulnerabilities and potential consequences in both realms.

SURVEILLANCE AND MONITORING EFFORTS

In the relentless pursuit of combating antimicrobial resistance (AMR), surveillance and monitoring stand as sentinel guardians, poised to decipher resistant pathogens' intricate patterns and dynamics. Rooted in the realms of both veterinary and medical practice, these vigilant endeavors play a pivotal role in assessing the extent of AMR, identifying emerging threats, and guiding prudent interventions. Within this pivotal context, the surveillance and monitoring of AMR become linchpins that demand thorough exploration and evaluation.

Genomic surveillance offers the chance to detect genetic markers that signal resistance to antimicrobials or host adaptation, enabling timely intervention and reducing the risk of broader spread (Argimón et al., 2021). Therefore, genomic data is of huge importance to cope with the evolving sensitivity of pathogens to antimicrobials. The cornerstone of effective AMR management lies in comprehensive surveillance programs that cast a discerning eye on the intricate interplay between pathogens, antimicrobials, and the diverse environments they traverse. Conducting regular national surveys to assess the prevalence of antimicrobial resistance (AMR) provides a dependable, firsthand evaluation of AMR rates in countries that lack robust and extensive surveillance networks. This entails the use of a carefully chosen subset of surveillance locations, determined by statistically sound probability sampling techniques, to ensure accurate data that reflects the entire nation (Bertagnolio et al., 2023). These programs serve as a critical conduit for collecting and analyzing data on the prevalence, distribution, and genetic traits of resistant microorganisms. In both veterinary and medical domains, surveillance not only informs clinical decision-making but also guides policy development, enabling evidence-based strategies to tackle the looming specter of AMR. The insights garnered from surveillance initiatives empower healthcare practitioners, policymakers, and researchers with a potent arsenal to counteract the inexorable rise of resistance.

However, the path to establishing and maintaining robust surveillance systems in veterinary practice is beset with challenges. Unlike the controlled environments of clinical laboratories, the multifaceted landscapes of veterinary settings encompass diverse animal species, production systems, and geographical variations. Coordinating data collection, standardizing methodologies, and ensuring representativeness at the national level and across boundaries poses a formidable task (Anjum et al., 2021). Furthermore, the varying capacities and resources of different regions often hinder the implementation of uniform surveillance strategies. Overcoming these challenges necessitates a harmonized approach that considers the unique contexts of each setting while fostering collaborative efforts. The global stage paints a diverse tableau of AMR surveillance strategies, reflecting the nuanced dynamics of different regions and countries. While some areas have harnessed robust surveillance networks encompassing veterinary clinics, hospitals, and even environmental reservoirs, others struggle to establish the foundational infrastructure for systematic monitoring. Disparities in surveillance capacity, data sharing, and analytical capabilities further complicate the evaluation of global progress. The global stage paints a diverse tableau of AMR surveillance strategies, reflecting the nuanced dynamics of different regions and countries. While some areas have harnessed robust surveillance networks that encompass veterinary clinics, hospitals, and even environmental reservoirs, others struggle to establish the foundational infrastructure needed for systematic monitoring. Disparities in surveillance capacity, data sharing, and analytical capabilities further complicate the evaluation of global progress. The World Health Organization (WHO) has been actively funding and supporting AMR investigations in developing countries, including several African countries. One notable example is the Global Antimicrobial Resistance Surveillance System (GLASS), a collaborative effort led by WHO to collect and analyze data on AMR from around the world, including many developing countries (WHO, 2020). In Africa, the WHO has been working closely with national governments, local health authorities, and research institutions to strengthen surveillance systems for AMR, build laboratory capacity, and develop national action plans to combat AMR effectively. For instance, the WHO Regional Office for Africa has been supporting AMR surveillance initiatives in Nigeria, Kenya, Uganda, and South Africa, among others. These efforts reflect a global recognition of the importance of addressing AMR as a public health priority. By investing in surveillance, research, and capacity-building initiatives in developing countries, the WHO and other international organizations aim to create a more comprehensive understanding of the global AMR threat and develop effective strategies to combat it. Additionally, by supporting these efforts in developing regions, the global community can work towards ensuring equitable access to effective antimicrobial treatments and mitigating the spread of drug-resistant infections worldwide.

EDUCATIONAL INITIATIVES AND PUBLIC AWARENESS

In the ongoing battle against antimicrobial resistance (AMR), knowledge is unequivocally a powerful weapon that holds the potential to reshape behaviors, alter practices, and ultimately drive a transformative shift in how we interact with antimicrobial agents. For instance, vaccines are considered a better option to dodge the spread of AMR and infectious diseases (Micoli et al., 2021). As the challenge of AMR continues to cast a shadow on global health, the imperative to educate and raise awareness among key stakeholders becomes ever more pressing (Ahmed et al., 2020). From veterinarians and medical professionals to farmers and the general public, fostering a nuanced understanding of responsible antimicrobial use resonates as a linchpin in the effort to mitigate AMR's impact.

Central to the educational endeavor is the understanding that antimicrobial stewardship transcends professional boundaries and extends to every facet of society. The study reveals that insufficient training on the optimum use of antibiotics and irrational use of essential antibiotics is escalating this global issue (Saman et al., 2023). For veterinarians and medical practitioners alike, the judicious use of antimicrobials forms an integral component of their practice—forging a bridge between the realms of clinical care and public health. Equipping these professionals with comprehensive knowledge not only enhances the quality of patient care but also aligns with the larger goal of curbing the emergence and spread of resistant pathogens.

The agricultural sector, in particular, occupies a critical position in the AMR narrative. Farmers, who play a pivotal role in food production, are uniquely positioned to drive change through their choices in animal husbandry and antimicrobial use. Educating farmers about the consequences of overreliance on antimicrobials, the emergence of resistance, and the ripple effects on both animal and human health can catalyze the adoption of more sustainable and responsible practices. By forging a connection between knowledge and action, the agricultural landscape can be transformed into a bastion of AMR mitigation (Calvo-Villamañán et al., 2023).

A key conduit for driving awareness and behavioral change lies in the realm of public awareness campaigns and educational programs. These initiatives wield the power to ignite collective consciousness, shaping public perceptions and attitudes toward antimicrobial use. Whether through digital platforms, community outreach, or school curricula, these campaigns have the potential to resonate with diverse audiences, bridging the gap between the complexities of AMR and the everyday lives of individuals. By fostering an understanding of how individual actions contribute to the larger tapestry of AMR dynamics, these programs can spur a groundswell of support for responsible antimicrobial practices.

CONCLUSION

The interplay between AMR, zoonotic diseases, surveillance efforts, policy interventions, educational initiatives, and emerging research trends underscores the multifaceted nature of this critical concern. As we draw this review article to a close, a tapestry of insights and imperatives emerges-a testament to the collaborative spirit that must underpin our efforts to safeguard the well-being of our shared world. The lines between animal, human, and environmental health are not mere abstractions-they are tangible connections that shape the landscape of infectious disease dynamics, the propagation of resistance, and the efficacy of our interventions. The One Health ethos serves as a beacon, illuminating the path toward a holistic understanding of the intricate interactions that define the AMR landscape. It reminds us that no facet of this challenge can be addressed in isolation; the collective wisdom of veterinary, medical, environmental, and policy domains must converge to enact sustainable change.

As we look toward the horizon, the battle against AMR invites us to recognize that our actions today will echo far beyond the present moment. The strides we make in responsible antimicrobial use, the partnerships we cultivate, the policies we enact, and the knowledge we impart will reverberate through the intricate web of health and well-being that spans across species and ecosystems. The tapestry woven through these pages carries with it a profound call-a call to engage, to learn, to adapt, and to unite in a shared mission to safeguard both animal and human health. The journey to curb antimicrobial resistance is not a solitary pursuit; it is a symphony of collective effort, a tapestry of collaboration, and a testament to the power of our shared commitment to a healthier future.

Author's Contributions

Q.S. conceptualization, first draft, and preparation of the final draft. *K.A.U. supervision, editing*, and preparation of the final draft. *R.A.* providing resources.

Competing interest

The authors declare that there is no conflict of interest associated with the research reported in this article.

REFERENCES

1. Abushaheen M.A., Muzaheed., Fatani A.J., Alosaimi M., Mansy W., George M., Acharya S., Rathod S., Divakar D.D., Jhugroo C., Vellappally S., Khan A.A., Shaik J., Jhugroo P. 2020. Antimicrobial resistance, mechanisms and its clinical significance. Disease-a-Month, 66, 6, p.100971. doi: 10.1016/j.disamonth.2020.100971.
2. Ahmed R., Bashir A., Brown J.E., Cox J.A., Hilton A.C., Hilton C.E., Lambert P.A., Theodosiou E., Tritter J.Q., Watkin S.J., Worthington T. 2020. The drugs don't work: evaluation of educational theatre to gauge and influence public opinion on antimicrobial resistance. Journal of Hospital Infection, 104, 2, 193-197. doi: 10.1016/j.jhin.2019.10.011.
3. Anjum M.F., Schmitt H., Börjesson S., Berendonk T.U., Donner E., Stehling E.G., Boerlin P., Topp E., Jardine C., Li X., Li B. 2021. The potential of using E. coli as an indicator for the surveillance of antimicrobial resistance (AMR) in the environment. Current Opinion in Microbiology, 64, 152-158. doi:10.1016/j.mib.2021.09.011.
4. Argimón S.; Yeats C.A., Goater R.J., Abudahab K., Taylor, B., Underwood A., Sánchez-Busó L., Wong V.K., Dyson Z.A., Nair S., Park S.E., Marks F., Page A.J., Keane J.A., Baker S., Holt K.E., Dougan G., Aanensen D.M. 2021. A global resource for genomic predictions of antimicrobial resistance and surveillance of Salmonella Typhi at Pathogenwatch. Nature Communications, 2021, 12, 2879. doi:10.1038/s41467-021-23091-2.
5. Bertagnolio S., Suthar A.B., Tosas O., Van-Weezenbeek K. 2023. Antimicrobial resistance: Strengthening surveillance for public health action. PLoS Medicine, 20, 7, e1004265. doi: 10.1371/journal.pmed.1004265.
6. Calvo-Villamañán A., San Millán Á., Carrilero L. 2023. Tackling AMR from a multidisciplinary perspective: a primer from education and psychology. International Microbiology, 26, 1-9. doi: 10.1007/s10123-022-00278-1.
7. Christaki E., Marcou M., Tofarides A. 2020. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. Journal of molecular evolution, 88, 26-40. doi: 10.1007/s00239-019-09914-3.
8. Dafale N.A., Srivastava S., Purohit H.J. 2020. Zoonosis: An Emerging Link to Antibiotic Resistance Under "One Health Approach". Indian Journal of Microbiology, 60, 139-152. doi:10.1007/s12088-020-00860-z.
9. Founou R.C., Founou L.L., Essack S.Y. 2017. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS ONE, 12, e0189621. doi: 10.1371/journal.pone.0189621.

10. Jin L., Pruden A., Boehm A.B., Alvarez P.J., Raskin L., Kohn T., Li X. 2022. Integrating environmental dimensions of "One Health" to combat antimicrobial resistance: Essential research needs. Environmental Science and Technology, 56, 21, 14871-14874. doi:10.1021/acs.est.2c01651.
11. Kasimanickam V., Kasimanickam M., Kasimanickam R. 2021. Antibiotics Use in Food Animal Production: Escalation of Antimicrobial Resistance: Where Are We Now in Combating AMR? Medical Sciences, 9, 1, 14. doi:10.3390/medsci9010014.
12. Laxminarayan R. 2022. The overlooked pandemic of antimicrobial resistance. The Lancet, 399, 10325, 606-607. doi:10.1016/S0140-6736(22)00087-3.
13. Majumder M.A.A., Rahman S., Cohall D., Bharatha A., Singh K., Haque M., Gittens-St Hilaire M. 2020. Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. Infection and drug resistance, 13, 4713-4738. doi: 10.2147/IDR.S290835.
14. Marston H.D., Dixon D.M., Knisely J.M., Palmore T.N., Fauci A.S. 2016. Antimicrobial resistance. JAMA, 316, 11, 1193-1204. doi:10.1001/jama.2016.11764.
15. Micoli F., Bagnoli F., Rappuoli R., Serruto D. 2021. The role of vaccines in combatting antimicrobial resistance. Nature Reviews Microbiology, 19, 287-302. doi:10.1038/s41579-020-00506-3.
16. Olaru I.D., Walther B., Schaumburg F. 2023. Zoonotic sources and the spread of antimicrobial resistance from the perspective of low and middle-income countries. Infectious Diseases of Poverty, 12, 59. doi: 10.1186/s40249-023-01113-z.
17. Pulingam T., Parumasivam T., Gazzali A.M., Sulaiman A.M., Chee J.Y., Lakshmanan M., Chin C.F., Sudesh K. 2022. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. European Journal of Pharmaceutical Sciences, 170, 106103. doi: 10.1016/j.ejps.2021.106103.
18. Purohit H.J. 2018. Gut-Bioreactor and Human Health in Future. Indian Journal of Microbiology, 58, 3-7. doi:10.1007/s12088-017-0697-6.
19. Rahman M.T., Sobur M.A., Islam M.S., Ievy S., Hossain M.J., El Zowalaty M.E., Rahman A.T., Ashour H.M. 2020. Zoonotic diseases: etiology, impact, and control. Microorganisms, 8, 9, 1405. doi: 10.3390/microorganisms8091405.
20. Saleem Z., Godman B., Azhar F., Kalungia A.C., Fadare J., Opanga S., Marković-Peković V., Hoxha I., Saeed A., Al-Gethamy M., Haseeb A. Salman M., Khan A.A., Nadeem M.U., Rehman I.U., Qamar M.U., Amir A., Ikram A., Hassali M.A. 2022. Progress on the national action plan of Pakistan on antimicrobial resistance (AMR): A narrative review and the

implications. Expert review of anti-infective therapy, 20, 1, 71-93. doi: 10.1080/14787210.2021.1935238.

21. Saman, A., Chaudhry, M., Ijaz, M., Shaukat, W., Zaheer, M. U., Mateus, A., & Rehman, A. (2023). Assessment of knowledge, perception, practices and drivers of antimicrobial resistance and antimicrobial usage among veterinarians in Pakistan. Preventive Veterinary Medicine, 212, 105836. doi: 10.1016/j.prevetmed.2022.105836.
22. (WHO) World Health Organization. 2020. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020. Geneva, World Health Organization; 2020. Available at: https://iris.who.int/bitstream/handle/10665/332081/9789240005587-eng.pdf?sequence=1, Accessed: May, 2024.

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REVIEW OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SMOKED ANIMAL ORIGIN FOOD: A SERBIAN PERSPECTIVE

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Abstract

This review article presents various aspects of polycyclic aromatic hydrocarbons (PAH) found in smoked animal-derived foods. The article covers PAHs and smoking methods, their levels and origins, regulatory aspects, existing knowledge gaps, as well as their impact on human health and future prospects concerning smoked animal-derived foods from the Republic of Serbia. Given our tradition and the widespread appreciation for smoked meat among consumers, producers are increasingly incorporating smoking into the production process of various animal and non-animal food products to encourage consumption diversification. Over the past ten years, our research has included smoking in traditional and industrial conditions. So far, we have examined the presence of PAHs in meat products, fish products and honey. Research on PAHs in grilled and smoked cheese is underway, after which we the results of the presence of PAH compounds in all types of smoked animal origin food will be available. Our findings revealed the presence of PAHs in the majority of studies conducted in the Republic of Serbia. Therefore, it is crucial to maintain continuous monitoring of their levels. Additionally, there is a need to develop models and implement solu-

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tions aimed at reducing PAHs in animal-derived foods intended for human consumption.

Key words: smoking, food production, smoked meat, smoked fish

PREGLED SADRŽAJA POLICIKLIČNIH AROMATIČNIH UGLJOVODONIKA U DIMLJENIM PROIZVODIMA ŽIVOTINJSKOG POREKLA: PERSPEKTIVA U SRBIJI

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Kratak sadržaj

U ovom preglednom radu predstavljeni su mnogi aspekti prisustva policikličnih aromatičnih ugljovodonika u dimljenoj hrani životinjskog porekla. Obrađeni su najznačajniji PAH-ovi, vrste dimljenja, njihov sadržaj i izvori, regulativa, nedostaci u sadašnjim saznanjima, uticaj PAH-ova na zdravlje ljudi i buduća stanovišta vezana za dimljenu hranu životinjskog porekla iz Republike Srbije. Poznavajući našu tradiciju i prihvaćenost dimljenog mesa među potrošačima, naši proizvođači sve više koriste dimljenje u cilju povećanja potrošnje ostalih vrsta hrane životinjskog i ne životinjskog porekla. Tokom prethodnih deset godina, naša istraživanja su obuhvatala dimljenje u tradicionalnim i industrijskim uslovima. Do sada smo ispitivali prisustvo PAH-ova u mesnim proizvodima, ribljim proizvodima i medu. U toku su istraživanja PAH-ova u pečenom i dimljenom siru, nakon čega bismo imali rezultate prisustva PAH jedinjenja u svim vrstama dimljene hrane životinjskog porekla. Rezultati istraživanja su pokazali da su PAH-ovi prisutni u većini studija sprovedenih u Republici Srbiji, zbog čega ih treba kontinuirano pratiti i raditi na modelima i rešenjima koji dovode do njihove redukcije u namirnicama životinjskog porekla koje su namenjene za ishranu ljudi.

Ključne reči: dimljenje, proizvodnja hrane, dimljeno meso, dimljena riba

INTRODUCTION

Smoked products, and smoked meat in particular, hold a prominent place in the diets of people in the Republic of Serbia and the wider Balkan region due to their popularity. Smoked food, such as smoked meat and smoked fish products are very popular and consumed quite often in our region (Babić et al., 2017; Novakov et al., 2022).

With a deep-rooted tradition and high consumer acceptance of smoked meat, producers are leveraging smoking techniques more frequently in the food production process. Smoked meat and meat products, smoked fish and fish products, as well as smoked cheese are available on the Serbian market.

Although fish consumption in the Republic of Serbia is comparatively lower than in EU countries (FAO, 2022; FAO, 2024), fish and fish products are significant in the Serbian diet, particularly during the Christmas and Easter fasting periods. The taste of smoked carp, as one the mostly common fish species in Serbian market, resembles the taste of other traditional smoked products and therefore, affects the increase of the fish consumption (Babić et al., 2018a). Consumption of smoked fish has significantly increased over the past few decades in many European countries including the Republic of Serbia (Tešić et al., 2013; FAO, 2022; FAO, 2024), due to their receptive sensory properties, high nutritional value which includes polyunsaturated fatty acids, minerals, vitamins and proteins favorable content (Bansal and Kim, 2015; Bongiorno et al., 2015). Lately, the growing consumer dissatisfaction with industrially produced food and the growing interest in nonconventional products urges producers to look for other alternatives - traditional and authentic food, such as smoked food.

Smoking is considered as one of the oldest procedures for preserving food (Djinovic et al., 2008), but nowadays, smoking primarily serves to impart the characteristic color, aroma, flavor, and appearance to smoked food products. (Fasano et al., 2016). During the pyrolysis of organic compounds, many chemical contaminants including polycyclic aromatic hydrocarbons (PAHs) are formed (Sun et al., 2019). PAHs are a large group of ubiquitous, persistent organic pollutants consisting of two or more fused aromatic rings in their chemical structural (Babić et al., 2018b). PAHs originate from two main sources: natural (biogenic and geochemical) and anthropogenic (Yebra-Pimentel et al., 2015). Naturally, PAHs occur in fossil fuels, but are also formed during the incomplete combustion of organic materials such as sawdust, charcoal, diesel, wood and vegetation. Most of the PAHs have been proved to have teratogenicity, carcinogenicity and mutagenicity, posing a great threat to human health. Benzo(a)pyrene, as the most common compound in smoke, is metabolized in the human body into benzopyrene diol epoxide, which is then covalently bound to cellular macromolecules, including DNA. In addition, PAHs interferes with normal cell function, disrupting the cell membrane and enzyme system (ATSDR, 2012; Bogdanović et al., 2019). Many studies have shown that the major source of exposure to PAHs is through food consumption (Bansal and Kim 2015; Domingo and Nadal 2015; Yebra-Pimentel et al., 2015). EFSA (2008) estimated the dietary exposure for the sum of PAH8 (benz(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene (DhA) and benzo(ghi)perylene (BgP)) as 1.73 mg/day per person.

The main goal of this study is to highlight PAHs as very important chemical contaminants in smoked products of animal origin, to present the national and European legislation related to the present issue and give an overview of the level of contamination of smoked meat and fish products found on the Serbian market.

PAHs IN SMOKED PRODUCT OF ANIMAL ORIGIN ON THE SERBIAN MARKET

The content of PAHs in smoked meat and meat products on the Serbian market has been examined in several studies. Djinovic et al. (2008) investigated the levels of PAHs in beef ham, pork ham, bacon without skin, bacon with skin, "*čajna*" sausage and "*Sremska*" sausage originating from Serbia, produced by meat industry Zlatiborac. The tested products did not exceed the maximum permitted limits. Škrbić et al. (2014) investigated PAH levels in smoked dry fermented sausage "*Petrovská klobása*" from Serbia. The highest total concentration of 16 PAHs was found in the samples smoked in traditional smokehouse, being almost 15 times higher than the total 16 PAHs content in sausages smoked in industrial smokehouse. The most abundant PAH was phenanthrene. PAH4 was below the maximum allowed legislation levels. In a study conducted by Kartalović et al. (2015) the content of PAH4 in household-produced smoked meat were analyzed. Ham produced in traditional drying facilities and smoking cabinets in Serbia contained 11.51 µg/kg of total PAHs. Kartalović et al. (2022) analyzed the content of PAHs in traditionally smoked and dry-cured bacon "*Slavonska slanina*" and determined that it was safe for consumption. PAH4 were determined as follows: 14.84 µg/kg in the middle, 10.60 µg/kg on the surface and 17.37 µg/kg in the skin in traditional smoked meat samples, while the PAH4 content in the samples smoked in industrial conditions were below the level of quantification. Škaljac et al. (2022) found benzo[a]pyrene and PAH4 were below the limits of detection in all analyzed samples of "*Petrovská klobása*" smoked in industrial conditions. Vranešević et al. (2022) examined the content of PAHs and the possibilities of their reduction in traditional (homemade) smoked dry-cured pork loin produced in Serbia. Đinović-Stojanović et al. (2023) investigated benzo(a) pyrene and sum of PAH4 compounds levels in our types of dry fermented sausages collected from market in Belgrade, Serbia. They found that all analyzed samples PAH's levels were under limits regulated by Serbian and EU legislation. Škaljac et al. (2023) analyzed the total content of 16 PAHs in traditional dry fermented beef sausage from Serbia and found the content of BaP to be lower than the limit of detection, while contents of PAH4 were from ND to 7.42 µg/kg. Škaljac et al. (2024) examined the content of PAHs in dry cured meat products, bacon and dry fermented sausages smoked in six different chambers from the territory of Vojvodina. Benzo[a]pyrene and PAH4 were lower than the set maximum legislation values.

Smoked carp, trout and salmon are the most commonly consumed types of smoked fish in Serbia. Smoked mackerel, herring and sprats, usually canned, can also be found. Also, non-smoked fish, especially shellfish, originating from polluted areas or areas where oil has been spilled, should be checked for the PAHs presence. There are several studies regarding PAHs levels in fish produced or marketed in Serbia. Babić et al. (2018a, 2018b) analyzed PAH levels in smoked common carp meat in industrial and traditional conditions with and without filters and found that the meat was safe for consumers in accordance with European and national legislation. Novakov et al. (2017) determined the total PAHs levels of 17.67 µg/kg in canned tuna, 15.12 µg/kg in canned sardines and 57.19 µg/kg in smoked sprouts. In canned smoked sprouts benzo(a) pyrene concentration reached 8.29 µg/kg, which was above the legislation limit. Novakov et al. (2021) analyzed mussels from the Serbian market and found PAHs levels to be under the legal limits.

In addition to meat and fish, PAHs can also be found in other products of animal origin. Thus, Petrović et al. (2021) analyzed pesticides, antimicrobial drugs and PAH concentrations in honey from Serbia and found that 6.6% of polyfloral honey samples contained high PAH8 (58.9 -656.2 ng/g) concentrations.

INFLUENCE OF DIFFERENT FACTORS AFFECTING PAH LEVELS IN FOOD

Numerous factors influence the PAH content in food, making it a focal point of a majority of research studies in this field. Some investigations are focused on the differences in PAH content in traditional and industrial smoking, some on the distance from the fire to the product, some on the filters that can absorb harmful substances from the smoke (Babić et al., 2018b). There are also differences in PAHs content when different types of casings are used (Mastanjević et al., 2020). There are also studies that have shown that plastic packaging can absorb a certain amount of PAHs during product storage (Semanova et al., 2016).

Traditional and especially homemade traditional manufacturing is not standardized and is uncontrolled. PAH4 level in samples of "*Hercegovačka pečenica*" subjected to traditional smoking highly exceeded maximum limits set by the EU Regulation (12 μg/kg) by up to 10 times (Puljić et al., 2019). Pancetta, dry pork neck ("*buđola*"), pork tenderloin and "*Slavonska kobasica*" were smoked in traditional smokehouse and in an industrial chamber to see variations and influence of different smoking type on chemical contaminants content (Kartalović et al., 2020; Mastanjević et al., 2020). "*Slavonska kobasica*" contained BaP above 5 μg/kg in 4 samples and PAH4 level above 30 μg/kg in 3 samples, meaning they were not in accordance with EU regulation (Mastanjević et al., 2020). There are ways to apply traditional smoking but with the use of certain filters. In the investigations conducted by Babić et al. (2018a, 2018b) samples of common carp meat were smoked in traditional conditions using different filters. The zeolite filter appeared to be the best one solution through traditional production of smoked common carp meat. The casing type, especially for sausages is also an important way of the PAHs reduction. Mastanjević et al. (2020) found that higher total content of the 16 PAHs in sausages stuffed in natural casings than those in collagen casings. Semanova et al. (2016) proved the migration of PAHs from smoked sausages to LDPE, where the total PAH4 content decreased from 30.1 to 5.7 μg/kg, during 180 min of interaction with packaging material, proving that packaging type and length of storage also may have an important impact on PAH reduction. Direct smoking when a kiln is placed directly below food during the drying process is still the most commonly used technique. Homemade smokehouses are mostly handmade according to the needs and capabilities of the producer. Traditional smoked meat products are not under monitoring and there is no strict regulation of product variables (type of raw materials, additives, smoking conditions, relative humidity etc.). The production processes (smoking, drying and ripening) of the homemade traditional smoked products takes from one to three months. One of the most important problems in traditional smoking in households is the deposition of char particles and more volatile compounds on the surface of meat products, which results in penetration from the surface area toward inner part of smoked meat products during the storage period (Kartalović et al., 2022). The amount of PAH4 in smoked samples subjected to traditional smoking highly exceeded maximum limits set by the EU by up to 10 times (Puljić et al., 2019). The consumption of this kind of smoked products can be potentially harmful to consumers' health and that is the reason why the "as low as reasonably achievable" (ALARA) principle is in force in all EU countries. On the other hand, the amounts of the PAH4 in all smoked samples subjected to industrial smoking processes were below the limit of quantification (Puljić et al., 2019). PAH reduction in smoked products can also be affected by the distance between the fire and the product. Dry fermented beef sausages smoked in traditional condition at distance of 2 m from the smoke sours (kiln) had significantly higher contents of 16 PAHs and PAH4 than the sausages smoked at distance of 4–5 m (Škaljac et al., 2023). When errors in the smoking process occur, the rinsing method should be considered in homemade manufacturing, in order to obtain safe products (Vranešević et al., 2022).

REGULATIONS

The monitoring of PAHs has been strongly recommended by the European Union, as well as regulations by the Republic of Serbia. BaP and PAH4 (BaP, Chr, BaA and BbF) were chosen as a marker for the occurrence and effects of carcinogenic PAHs in food. Maximum level for BaP is 2 μg/kg and for the sum of PAH4 is 12 μg/kg (Commission Regulation (EU) No 915/2023; Official Gazette of the Republic of Serbia, 81/2019, 126/2020, 90/2021, 118/2021 and 127/2022).

Despite the application of GHP and GMP and HACCP to the possible extent, the current maximum levels for PAHs are not achievable in several EU Member States in certain traditionally smoked meats and meat products and traditionally smoked fish and fish products, where smoking practices cannot be altered without changing significantly the organoleptic characteristics of the food. In order not to close many small and medium size enterprises in Croatia, Ireland, Spain, Poland, Latvia, Cyprus, Sweden, Finland, Portugal and Slovakia, a derogation for local production and consumption is maintained without a time limit for certain traditionally smoked products (meat, meat products, fish and fish products) only in those Member States, namely 5 µg/kg for BaP and 30 µg/kg for PAH4 (Commission Regulation (EU) No 915/2023).

Allowed levels of BaP and PAH4 for muscle meat of most smoked fish and smoked fish products including smoked crustaceans, crabs and crab-like crustaceans are 2 and 12.0 µg/kg, respectively. Similar to European Union legislation for meat products, the EU (Commission Regulation (EU) No1255/2020) allowed Latvia, Finland and Sweden to place some traditional fish and fishery products, smoked and market in their territory, with the BaP content level lower than 5.0 µg/kg and PAH4 level lower than 30 µg/kg.

CONCLUSION

There are many techniques for quantification of PAHs. Gas chromatography-mass spectrometry (GC-MS), which has limited level of LOQ, is most commonly used, including studies that we have conducted. Our activities today are directed towards the improvement of knowledge of the sensitive detection methods for PAHs in each particular smoked food type which would considerably help consumers to avoid the risk associated with PAHs consumption. In order to decrease the level of PAHs and reduce the risk of PAHs occurrence in smoked meat products, traditional producers should be educated how to use the improved/novel smoking techniques and adjust the smoking parameters. This should result in safer smoked meat products. The results of conducted studies call for a need to monitor PAHs in traditional smoked products. In addition, likely revision of the existing rulebook for traditional smoked products might also be necessary.

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Author’s contribution

J.V., N.N. and S.V.K. made contributions to the idea of the publication, organisation of work and writing the manuscript. J.V., N.N., N.P. and S.V.K. were involved in the writing of the manuscript. K.M. reviewed the manuscript. S.Š. and B.K. gave the final approval of the manuscript to be published.

Competing interest

The authors declare that they have no competing interests.

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). 2012. Case studies in environmental medicine. Toxicity of polycyclic aromatic hydrocarbons (PAHs). Atlanta: U.S. Department of Health and Human Services, Centers for diseases control and prevention.1-68. Available at: https://www.atsdr.cdc.gov/csem/pah/docs/pah.pdf Accessed 10.04.2024.
2. Babić J., Kartalović B., Škaljac S., Vidaković S., Ljubojević D., Petrović J., Ćirković M., Teodorović V. 2018a. Reduction of polycyclic aromatic hydrocarbons in common carp meat smoked in traditional conditions. Food Additives & Contaminants: Part B, 11, 3, 208-213. doi: 10.1080/19393210.2018.1484821.
3. Babić J., Vidaković S., Bošković M., Glišić M., Kartalović B., Škaljac S., Nikolić A., Ćirković M., Teodorović V. 2018b. Content of polycyclic aromatic hydrocarbons in smoked common carp (*Cyprinus carpio*) in direct conditions using different filters vs indirect conditions. Polycyclic Aromatic Compounds, 40, 3, 889–897. doi: 10.1080/10406638.2018.1506991.
4. Babić J., Vidaković S., Škaljac S., Kartalović B., Ljubojević D., Ćirković M., Teodorović V. 2017. Factors affecting elimination of polycyclic aromatic hydrocarbons from traditional smoked common carp meat. IOP Conference Series: Earth and Environmental Science 85, 1, 012086. doi :10.1088/1755-1315/85/1/012086.
5. Bansal V., Kim K.H. 2015. Review of PAH contamination in food products and their health hazards. Environment International, 84, 26–38. doi: 10.1016/j.envint.2015.06.016.
6. Bogdanović T., Petričević S., Listeš E., Pladin J. 2019. Polycyclic aromatic hydrocarbons in selected food items coming from the Croatian market. Italian Journal of Food Science, 31, 661–680. doi: 10.14674/IJFS-1444.
7. Bongiorno T., Iacumin L., Tubaro F., Marcuzzo E., Sensidoni A., Tulli F. 2015. Seasonal changes in technological and nutritional quality of *Mytilus galloprovincialis* from suspended culture in the Gulf of Trieste (North Adriatic Sea). Food Chemistry, 173, 355-362. doi: 10.1016/j.foodchem.2014.10.029.

8. Djinovic J., Popovic A., Jira W. 2008. Polycyclic Aromatic hydrocarbons (PAHs) in different types of smoked meat products from Serbia. Meat Science, 80, 2, 449–456. doi: 10.1016/j.meatsci.2008.01.008.
9. Đinović-Stojanović J., Vranić D., Begić M., Babić Milijašević J, Milijašević M., Stefanović S, Janković S. 2023. Polycyclic aromatic hydrocarbons in dry fermented sausages from the Serbian market. Meat Technology-Special Issue, 64, 2, 312–316. doi: 10.18485/meattech.2023.64.2.59.
10. Domingo J., Nadal M. 2015. Human dietary exposure to polycyclic aromatic hydrocarbons: a review of the scientific literature. Food and Chemical Toxicolgy, 86, 144–153. doi: 10.1016/j.fct.2015.10.002.
11. European Food Safety Authority (EFSA). 2008. Scientific Opinion of the Panel on Contaminants in the Food Chain on a Request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal, 724, 1e114. doi: 10.2903/j.efsa.2008.724.
12. European Union. 2020. Commission Regulation (EU) No 1255/2020 of 7 September 2020 amending regulation (ec) no 1881/2006 as regards maximum levels of polycyclic aromatic hydrocarbons (pahs) in traditionally smoked meat and smoked meat products and traditionally smoked fish and smoked fishery products and establishing a maximum level of PAHs in powders of food of plant origin used for the preparation of beverages. Official Journal of European Union, 293, 1-4.
13. European Union. 2023. Commission Regulation (EU) No 915/2023 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Official Journal of European Union, L119/103.
14. FAO 2024. Serbia. Text by Markovic, Z., Poleksic, V. *In: Fisheries and Aquaculture*. Rome. Available at : https://www.fao.org/fishery/en/countrysector/naso_serbia Accessed: 03.06.2024.
15. FAO. 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, FAO. Available at: https://doi.org/10.4060/cc0461en. Accessed 03.06.2024.
16. Fasano E., Yebra-Pimentel I., Martínez-Carballo E., Simal-Gándara J. 2016. Profiling, distribution and levels of carcinogenic polycyclic aromatic hydrocarbons in traditional smoked plant and animal foods. Food Control, 59, 581-590. doi: 10.1016/j.foodcont.2015.06.036.
17. Kartalović B., Mastanjević K., Novakov N., Vranešević J., Ljubojević Pelić D., Puljić L. Habschied K., 2020. Organochlorine pesticides and PCBs in traditionally and industrially smoked pork meat products from Bosnia and Herzegovina. Foods, 9, 1, 97. doi: 10.3390/foods9010097.

18. Kartalović B., Okanović Đ., Babić J., Đordjević V., Janković S., Ćirković M. 2015. Polycyclic aromatic hydrocarbons in smoked ham. Procedia Food Science, 5, 144-147. doi: 10.1016/j.profoo.2015.09.042.
19. Kartalović B., Puljić L., Kovačević D., Markovčić M., Vranešević J., Habschied K. Mastanjević K. 2022. The content of polycyclic aromatic hydrocarbons in Slavonska slanina-Traditionally smoked and dry-cured bacon. Processes, 10, 2, 268. doi: 10.3390/pr10020268.
20. Mastanjević K., Kartalović B., Vranešević J., Novakov N., Habschied K. 2020. Polycyclic aromatic hydrocarbons in traditionally smoked Slavonska kobasica. Food Additives & Contaminants: Part B, 13, 2, 82-87. doi: 10.1080/19393210.2020.1712481.
21. Novakov N., Kartalović B., Mihaljev Ž., Đurić B., Vranešević J., Pelić M., Lazić D., Ljubojević Pelić D. 2022. Chemical contaminants in fish, shellfish and fish products on the Serbian market. Veterinarski Glasnik, 76, 2, 13-24. doi: 10.2298/VETGL220522012N.
22. Novakov N., Kartalović B., Mihaljev Ž., Matanjević K., Stojanac N., Habshied K. 2021. Heavy metals and PAHs in mussels on the Serbian market and consumer exposure. Food Additives and Contaminants: *Part B Surveillance,* 14, 3, 219-226. doi: 10.1080/19393210.2021.1931475.
23. Novakov N., Mihaljev Ž., Kartalović B., Blagojević B., Petrović J., Ćirković M., Rogan D. 2017. Heavy metals and PAHs in canned fish supplies on the Serbian market. Food Additives and Contaminants: *Part B Surveillance,* 10, 3, 208-215. doi: 10.1080/19393210.2017.1322150.
24. Petrović J., Ratajac R., Vranešević J., Stojanov I., Prodanov Radulović J. Kartalović B. 2021. Antimicrobial drugs, pesticides and PAHs in honey. Archives of Veterinary Medicine, 14, 2, 35-47. doi: 10.46784/eavm.v14i2.286.
25. Puljić L., Mastanjević K., Kartalović B., Kovačević D., Vranešević J., Mastanjević K. 2019. The influence of different smoking procedures on the content of 16 PAHs in traditional dry cured smoked meat "Hercegovačka pečenica". Foods, 81, 2, 690. doi: 10.3390/foods8120690.
26. Regulation on maximum concentrations of certain contaminants in food. Official Gazette of the Republic of Serbia, 81/2019, 126/2020, 90/2021, 118/2021 and 127/2022.
27. Semanova J., Sklarsova B., Simon P., Simko P. 2016. Elimination of polycyclic aromatic hydrocarbons from smoked sausages by migration into polyethylene packaging. Food Chemistry, 201, 1-6.doi: 10.1016/j.foodchem.2016.01.057.
28. Škaljac S., Jokanović M., Peulić T., Vranešević J., Kartalović B., Tomović V., Ikonić P., Šojić B. 2024. Content of polycyclic aromatic hydrocarbons in

traditionally smoked meat products from North Serbia (Vojvodina). *Fermentation,* 10, 2, 104. doi: 10.3390/fermentation10020104.

29. Škaljac S., Jokanović M., Tomović V., Kartalović B., Ikonić P., Ćućević N., Vranešević J., Ivić., Šojić B., Peulić T. 2023. Influence of traditional smoking on the content of polycyclic aromatic hydrocarbons in dry fermented beef sausage from Serbia. Food Control, 150, 109766. doi: 10.1016/j.foodcont.2023.109766.
30. Škaljac S., Jokanović M., Tomović V., Šojić B., Ikonić P., Peulić T., Ivić M., Vranešević J. Kartalović B. 2022. Color characteristics and content of polycyclic aromatic hydrocarbons of traditional dry fermented sausages throughout processing in controlled conditions. Polycyclic Aromatic Compounds, 42, 6, 3124-3134. doi: 10.1080/10406638.2020.1853183.
31. Škrbić B., Đurišić-Mladenović N., Mačvanin N., Tjapkin A., Škaljac S. 2014. Polycyclic aromatic hydrocarbons in smoked dry fermented sausages with protected designation of origin “Petrovská klobása” from Serbia. Macedonian Journal of Chemistry and Chemical Engineering, 33, 2, 227–236. doi: 10.20450/mjcce.2014.358.
32. Sun Y., Wu S., Gong G. 2019. Trends of research on polycyclic aromatic hydrocarbons in food: A 20-year perspective from 1997 to 2017. Trends in Food Science and Technology, 83, 86-98. doi: 10.1016/j.tifs.2018.11.015.
33. Tešić M., Baltić M., Teodorović V., Mirilović., Nedić D., Marković T, Marković R., Aleksić Agelidis A. 2013. Tendencija razvoja ribarstva i potrošnja ribe u Srbiji. Veterinarski Glasnik, 67, 5-6, 412-427. doi: 10.2298/VETGL1306417T.
34. Vranešević J., Kartalović B., Vidaković Knežević S., Škaljac S., Jokanović M. 2022. Reduction of polycyclic aromatic hydrocarbons to improve safety of traditional (homemade) smoked dry-cured pork loin. Polycyclic Aromatic Compounds, 43, 10, 8800-8805. doi: 10.1080/10406638.2022.2153883.
35. Yebra-Pimentel I., Fernández-González R., Martínez-Carballo E. Simal-Gándara J. 2015. A critical review about the health risk assessment of PAHs and their metabolites in foods. Critical Reviews in Food Science and Nutrition, 55, 10, 1383-1405. doi: 10.1080/10408398.2012.697497.

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PREVALENCE OF *TOXOCARA CANIS* AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES OF THE COMMUNITY IN BISHOFTU TOWN, CENTRAL ETHIOPIA

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Abstract

A cross-sectional study was conducted in order to determine the prevalence of *Toxocara canis*, potential risk factors, and knowledge, attitude, and practices in Bishoftu, central Ethiopia. Fecal samples were taken from 300 randomly selected dogs of different sex, age group, breeds, and diarrheal status. The samples were processed using a flotation technique to detect the eggs of *Toxocara canis.* The current result revealed an overall prevalence of 32.33% (95% CI: 27.07 - 37.95) of *Toxocara canis*. The prevalence of the disease was 30.30% (95% CI: 22.61 - 38.90), 31.06% (95% CI: 23.30 - 39.70), and 44.44% (95% CI: 27.94 - 61.90) in young, adult and older dogs, respectively. The prevalence of *Toxocara canis* was 32.43% (95% CI: 26.77 - 38.50) and 31.71% (95% CI: 18.08 - 48.09) in female and male dogs, respectively. The present findings indicated that there were statistically significant links between the prevalence of *Toxocara canis* regarding dog breeds and diarrheal status ($p < 0.05$). The prevalence was higher in German Shepherd breed dogs (56.52%; 95% CI: 34.49 - 76.81) than in local dog breeds (30.32%; 95% CI: 24.97 - 36.11). The prevalence of the disease was higher in diarrheic dogs (46.48%; 95% CI: 34.55 - 58.71) than in non-diarrheic dogs (27.95%; 95% CI: 22.24 - 34.24). The knowledge, attitude, and practice assessment in the present study indicated poor dog management practices and insufficient public awareness. Most dog owners (56.7%; 95% CI: 50.85 - 62.35) did not deworm their dogs regularly, while 90.67% (95% CI: 86.79 - 93.71) fed dogs raw animal products. The present finding indicated that most respondents were not aware of zoonotic canine parasites (83.7%;

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95% CI: 81.18 - 89.43) and toxocariasis (80.7%; 95% CI: 75.74 - 84.98). This study demonstrates a higher prevalence of *Toxocara canis* and lower public awareness about the disease. Therefore, this study recommends strategic deworming of dogs, better dog management, and increased public awareness in order to control the impact of the disease on animal and human health.

Key words: Attitude, Bishoftu, Dog, KAP, Prevalence, *Toxocara canis,* Zoonosis

PREVALENCIJA *TOKSOCARA CANIS* I PROCENA ZNANJA, STAVA I PRAKSE ZAJEDNICE U GRADU BISHOFTU, CENTRALNA ETIOPIJA

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Kratak sadržaj

U Bišoftu, centralna Etiopija, izvršena je studija preseka sa ciljem da se odredi rasprostranjenost *Toxocara canis*, potencijalni faktori rizika, kao i da se utvrdi kakav je stav javnosti, praksa držanja pasa i stepen informisanosti kada je u pitanju ovaj parazit. Uzeti su uzorci fecesa od 300 nasumično izabranih pasa različitog pola, starosti, rase i učestalosti pojave dijareje. Ovi uzorci su obrađeni putem tehnike flotacije kako bi se detektovala jajašca *Toxocara canis.* Dobijeni rezultat je pokazao prevalenciju od 32.33% (95% CI: 27.07 - 37.95) *Toxocara canis*. Prevalencija je bila: 30.30% (95% CI: 22.61 - 38.90) kod mladih, 31.06% (95% CI: 23.30 - 39.70) kod odraslih i 44.44% (95% CI: 27.94 - 61.90) kod starijih pasa. Prevalencija *Toxocara canis* je bila 32.43% (95% CI: 26.77 - 38.50) kod pasa ženskog pola i 31.71% (95% CI: 18.08 - 48.09) kod pasa muškog pola. Dobijeni rezultati pokazuju da je bilo značajnih statističkih razlika između prevalencije *Toxocara canis* kada je u pitanju rasa pasa i učestalost pojave dijareje ($p < 0.05$). Prevalencija je bila veća kod nemačkog ovčara (56.52%; 95% CI: 34.49 - 76.81) nego kod lokalnih rasa pasa (30.32%; 95% CI: 24.97 - 36.11). Rasprostranjenost ove bolesti bila je veća i kod pasa sa dijarejom (46.48%; 95% CI: 34.55 - 58.71) nego kod pasa koji nisu imali dijareju (27.95%; 95% CI: 22.24 - 34.24). Kada su u pitanju informisanost, stav i praksa držanja pasa, ova studija je pokazala da je način držanja pasa neadekvatan i da javnost nije dovoljno informisana o ovom parazitu. Većina vlasnika (56.7%; 95% CI: 50.85 - 62.35) ne vrši redovno čišćenje pasa od parazita, dok 90.67% (95% CI: 86.79 - 93.71) hrani pse sirovim namirnicama životinjskog porekla. Studija pokazuje i da većina ispitanika nije čula za zoonotske parazite kod pasa (83.7%; 95% CI: 81.18 - 89.43) i toksokariozu (80.7%; 95% CI: 75.74 - 84.98). Studija pokazuje visoku prevalenciju *Toxocara canis* kao i nedovoljnu informisanost javnosti o ovoj bolesti. Stoga se preporučuje čišćenje pasa od parazita, bolji uslovi držanja pasa kao i podizanje svesti javnosti o ovom problemu kako bi se kontrolisao uticaj bolesti na zdravlje životinja i ljudi.

Ključne reči: stav, Bišoftu, pas, informisanost, stav i praksa, prevalencija, *Toxocara canis*, zoonoze

INTRODUCTION

Pet animals play a significant role in society worldwide. They are important companions, contributing to the physical, social, and emotional well-being of their owners, especially children. It has been reported that pet owners visit their doctor less often, use fewer medications, and have lower blood pressure and cholesterol levels than non-pet owners (Martins et al., 2023). Dogs are the most sociable canids, adapted to human habitation worldwide. Although they offer significant benefits to many people, dogs can be a potential public health risk, since natural transmission of parasitic infections from dogs to humans may occur, either directly or indirectly through environmental factors (Robertson et al., 2000). Dogs are definitive hosts for several enteric parasites with zoonotic importance, among which *Toxocara canis* is the one that is regarded as the most common (Khante et al., 2009; Soriano et al., 2010).

Toxocariasis is a zoonotic disease caused by the larval stage of *Toxocara canis* (*T. canis*) which is the common roundworm living in the intestines of almost all animal species and humans. It is also found in a larval form in the tissues of all these animals (Despommier, 2003). It is a cosmopolitan parasite of canines and the major agent of human toxocariasis causing serious health problems in many countries (Fan et al., 2003). Epidemiological studies indicated that the prevalence of *T. canis* infection was 9.5% in Brazil (Mundim et al., 2001), 3.1% in North Central Colorado, USA (Hackett and Lappin, 2003), 8.5% in the Netherlands (Nobel et al., 2004), and 21% in central Ethiopia (Yacob et al., 2007). Mature *T. canis* nematodes live in dogs' intestines (the definitive hosts) and produce large numbers of unembryonated eggs that are excreted in the feces. It is a potential source of environmental contamination posing a high risk of infection for healthy dogs and people (Fahrion et al., 2008). These eggs are not immediately infectious; they develop to the infective stage (third-stage larvae) in the environment. Survival and development are affected by both temperature and exposure to moisture. Although many eggs may no longer be viable after 6 months, some can survive in the soil for a year or more (Himsworth et al., 2010).

Dogs can become infected by ingesting embryonated eggs from the environment, through the transplacental route in the uterus, larvae in their dam's milk, or the tissue of paratenic hosts. Humans get infected by ingestion of embryonated eggs either from the soil, dirty hands, raw fruits and vegetables, or larvae from undercooked meat of paratenic hosts. *T. canis* infection in humans can cause symptoms related to the internal organs (visceral larva migrans), eye (ocular larva migrans), or brain (cerebral larva migrans) due to larva migration (Despommier, 2003). Currently, there is an increase in the dog population in both urban and rural settlements in developing countries due to the use of dogs for security, hunting, and other recreational activities (Ceballos et al., 2014). Even though some dogs are looked after adequately, many communities still have large populations of stray domestic dogs without control and with little or no access to veterinary care. The increase in the freely roaming dog population leads to environmental contamination with dog faeces, which is a potential risk for dogs and public health due to the possibility of transmission of zoonotic parasites (Chomel and Ben, 2011).

T. canis is a highly prevalent disease in Ethiopia, with both animal and public health implications (Dubie et al., 2023; Yacob et al., 2007). However, epidemiology of the disease and the awareness of the public are inadequately comprehended in Bishoftu, central Ethiopia. Studies that generate basic information on the level of disease, environmental contamination by parasitic eggs, and human awareness are prerequisites and have significant roles in the effective prevention and control of *T. canis*. Moreover, understanding communities' perceptions towards dog husbandry, contact with pets, and knowledge about zoonotic disease are important steps towards the development and implementation of appropriate disease prevention and control strategies. Therefore, the objectives of this study were to determine the prevalence of *T. canis* infection in dogs, identify putative risk factors, and to assess the communities' knowledge, attitude, and practices regarding dog management practices, zoonotic canine parasitic diseases, and their public health implications in Bishoftu, central Ethiopia.

MATERIAL AND METHODS

Description of the study area

The study was conducted in Bishoftu, a town in central Ethiopia located in Oromia National Regional State, East Shewa Zone, located 47 kilometers southeast of Addis Ababa (Figure 1) at latitude and longitude of 8˚35'N and 40˚E with an elevation 1860 meters above sea level. It is the administrative center of Ade'a woreda. It experiences bimodal patterns of Rainfall with the main rainy season extending from June to September with an average rainfall of about 880 mm. The mean annual minimum and maximum temperatures are 12.3 °C and 27.7 *°C*, respectively with an average of 20 *°C*. The mean relative humidity is 61.3% (Yemenu and Chemeda, 2010).

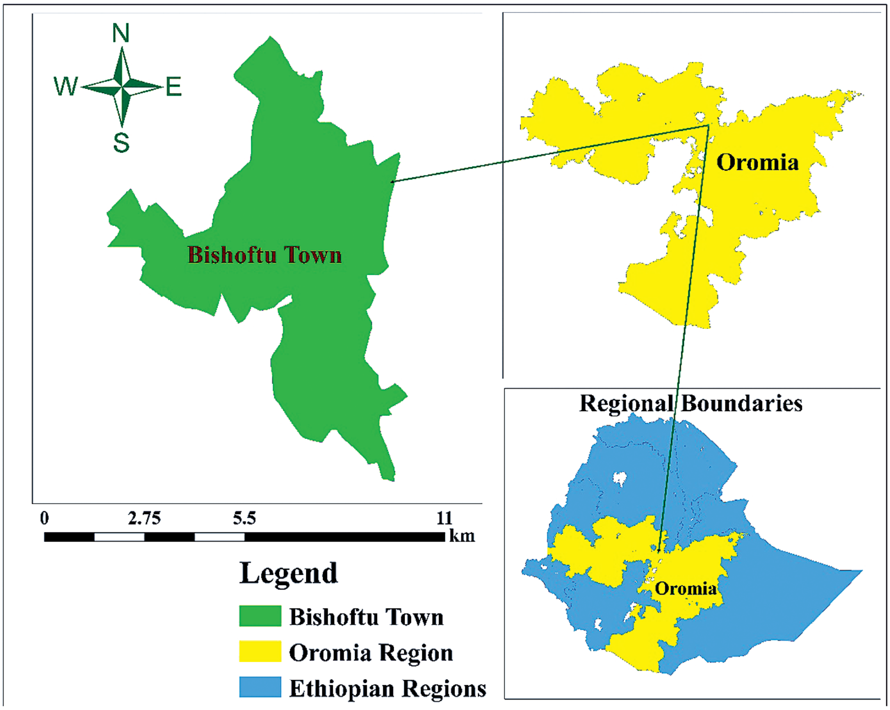

Figure 1: Map of Bishoftu town in central Ethiopia

Study design

A cross-sectional study was conducted in order to determine the prevalence of *T. canis* infection in dogs and assessment of knowledge, attitude, and practice of the community in the study area through the processing of fecal samples that were collected from randomly selected houses and a questionnaire survey.

Study Animals

The dog populations in the selected households of Bishoftu town were used as the study animals. Furthermore, interviews were conducted with dog owners to gauge their knowledge, attitudes, and perceptions regarding disease prevention and control strategies, as well as the zoonotic significance within the community. During the sampling period, health care, feeding and watering practice, dog handling, hygienic conditions, contact between people and dogs and other related information was recorded using a semi-structured questionnaire.

Sampling method and sample size determination

Simple random sampling method was the sampling technique used to select dogs for sampling of feces and the sample size included all dogs in randomly selected houses. The sample size was determined using the formula given by Thrusfield (2005), with a level of confidence of 95% and absolute precision of 5%. For the sample size determination, prevalence of 21% was used according to the study conducted by (Yacob et al., 2007) in the study area. Therefore, the required sample number was 255. However, the study was conducted on 300 dogs, with the aim to increase the accuracy of the result.

Sample collection

Fresh fecal samples weighing about 20 g were collected from each dog's rectum using disposable examination gloves. The samples were put in a separate plastic container and the sampling containers were labeled with the necessary information (breed, sex and age, fecal consistency). The samples were then transferred to an ice box, and immediately taken to Addis Ababa University College of Veterinary Medicine Parasitology Laboratory for processing.

Laboratory Diagnosis

Coprological examination for the detection of *T. canis* eggs was performed using simple floatation techniques. The flotation fluid was prepared by taking 400 g of sodium chloride (NaCl) into to1000 mL of tap water and stirring to dissolve the salt crystals (Awoke et al., 2011). About 3 gm of the fecal sample was placed into a mortar and crushed, then diluted with 45 mL flotation solution. The sample was then filtered into a beaker using a sieve, then the filtrate was poured into a clean test tube and after that a cover slip was placed on the filled test tube and left to stand for 20 minutes. The cover slip was later transferred onto a glass slide and examined under a microscope for separation of *T. canis* eggs in the sample based on their morphological characteristics according to Hendrix and Sirois (2007) and Kim et al. (2020).

Questionnaire survey for KAP assessment

Dog owners were interviewed about the knowledge, attitude, and practices of the community when it comes to disease prevention and control strategies and their zoonotic importance. During the sampling period, health care, feeding and watering practices, dog handling, hygienic conditions, contact between people and dogs and other related information was recorded using a semi-structured questionnaire.

Data management and analysis

The data collected from the field and parasitological investigations were entered into a Microsoft Excel spreadsheet. The imported data included the results of the questionnaire survey, putative risk factors, and the laboratory results of the disease status. The data was then checked and coded properly. The data was analyzed using STATA statistical software (Stata-SE version 14), and the results were presented in tabulated and frequency distribution tables. Descriptive statistics were used to summarize the KAP survey. The chi-square test was used to determine the association between presumed risk factors and the prevalence of *T. canis* infection. The potential factors were considered statistically significant factors of *T. canis* when the *p*-value was lower than 0.05 at a 95% CI.

RESULTS

Prevalence of Toxocara canis and associated risk factors

A total of 300 dogs (259 males and 41 females) were examined. Out of these, 97 dogs were found positive for *Toxocara canis*. The overall prevalence of *T. canis* in those dogs was found to be 32.3% (95% CI: 27.07 - 37.95). The current finding revealed that old dogs were more affected with *T. canis* than other age groups (Table 1). Out of the 36 examined old dogs, 16 dogs (44.4%) were infected with *T. canis,* whereas from the 132 young and 132 adult dogs 40 (30.3%) and 41 (31.1%) dogs were infected with *T. canis,* respectively.

Table 1. The prevalence of *T. canis* among dogs with different age categories

Age	Total examined	Positives	Prevalence (95% CI)	*p*-value	X^2
≤ 1year	132	40	30.30 (22.61 - 38.90)	0.252	2.760
1 -7 years	132	41	31.06 (23.30 - 39.70)		
Above 7 years	36	16	44.44 (27.94 - 61.90)		
Total	300	97	32.33 (27.07 - 37.95)		

The prevalence of *T. canis* in male and female dogs was 84 (32.4%) and 13 (31.7%), respectively, as shown in Table 2. However, there was no significant difference in the prevalence of the disease between male and female dogs ($p > 0.05$). There was a significant link between the prevalence of *T. canis* and dog breed ($p < 0.05$). The prevalence of the disease was 30.3% (95% CI: 24.97 - 36.11) in local and 56.5% (95% CI: 34.49 - 76.81) in German Shepherd breed dogs (Table 3).

Table 2: Prevalence of *T. canis* between male and female dogs

Sex	Total examined	Positives	Prevalence (95% CI)	p-value	X^2
Male	259	84	32.43 (26.77 - 38.50)	0.927	0.009
Female	41	13	31.71 (18.08 - 48.09)		
Total	300	97	32.33 (27.07 - 37.95)		

Table 3: Prevalence of *T. canis* on local and German Shepherd dog breeds

Breed	Total examined	Positives	Prevalence (95% CI)	p-value	X^2
Local	277	84	30.32 (24.97 - 36.11)	0.010	6.661
German shepherd	23	13	56.52 (34.49 - 76.81)		
Total	300	97	32.33 (27.07 - 37.95)		

The present study indicated that the presence of diarrhea was significantly associated with *T. canis* infection of dogs ($p < 0.05$). The dogs with diarrhea were more affected with the disease than dogs without clinical diarrhea. The present finding showed that the prevalence of the parasite was 46.5% (95% CI: 34.55 - 58.71) in diarrheic dogs and 27.9% (95% CI: 22.24 - 34.24) in non-diarrheic dogs as stated in Table 4.

Table 4: Prevalence of *T. canis* by fecal consistency among diarrheic and non-diarrheic dogs

Fecal consistency	Total examined	Positives	Prevalence (95% CI)	p-value	X^2
Diarrheic	71	33	46.48 (34.55 - 58.71)	0.004	8.507
Non-diarrheic	229	64	27.95 (22.24 - 34.24)		
Total	300	97	32.33 (27.07 - 37.95)		

Knowledge, attitude, and practice assessment

Regarding knowledge, attitude, and practice of the public, the study showed that of 300 dog owners, 184 (61.3%) kept dogs for security purposes, 62 (20.7%) as a pet and 54 (18%) for both security purposes and as a pet. Of 300 dog owners, 74% (95% CI: 68.65 - 78.87) provided a house for their dogs and 78 (26%) did not provide any shelter for their dogs. The majority of owners 170 (56.7%) did not take their dogs to the veterinary clinic for deworming on a regular basis and only 130 (43.3%) owners dewormed their dogs regularly. Most of the owners 60.7% (95% CI: 54.89 - 66.23) clean their dogs' houses (Table 5).

Table 5: Knowledge, attitude, and practice of the respondents on pet husbandry

Variables	No. of respondents	Proportion (95% CI)
Purpose of dog keeping		
Security	184	61.3 (55.57-66.87)
Hobby	62	20.7 (16.23-25.70)
Both	54	18.0 (13.82-22.82)
Does your dog have a house?		
Yes	222	74.0 (68.65-78.87)
No	78	26.0 (21.13-31.35)
Is your dog dewormed regularly?		
Yes	130	43.3 (37.65-49.15)
No	170	56.7 (50.85-62.35)
Do you keep the dog and its house clean?		
Dog is washed and the house is cleaned	102	34.0 (28.65-39.67)
The house is washed and cleaned	16	5.3 (3.08-8.52)
Only house cleaned only	182	60.70 (54.89-66.23)
Where does your dog defecate?		
Inside the house	41	13.7 (9.99-18.08)
Outside the compound	205	68.3 (62.74-73.56)
Anywhere within and outside the compound	54	18.0 (13.82-22.82)

Variables	No. of respondents	Proportion (95% CI)
What is the dog's waste disposal site?		
Municipal garbage site	154	51.3 (45.52-57.12)
Along the roadsides	146	48.7 (42.88-54.48)
Is your dog tied permanently?		
Yes	46	15.3 (11.45-19.92)
No	254	84.7 (80.08-88.55)
What do you feed your dog?		
Raw meat	110	36.7 (31.20-42.40)
Cooked meat	18	6.0 (3.59-9.32)
Household leftovers	10	3.3 (1.61-6.04)
Raw meat and household leftovers	162	54.0 (48.18-59.74)
How do you feed your dog?		
On the floor	60	20 (15.62-24.98)
In a bowl	240	80.0 (75.02-84.38)
Does your dog have access to the outdoors?		
Yes	265	88.3 (84.15-91.74)
No	35	11.7 (8.26-15.85)

Most dog owners (54%; 95% CI: 48.18 - 59.74) fed raw meat and household leftovers to their dogs -110 (36.7) of them fed only raw meat, 18 (36%) of dog owners fed their dogs cooked meat, while 10 (3.3%) of them fed only household leftovers. The majority of dogs (68.3%) defecated outside the compound; 41 (13.7%) dogs defecated in their house while 54 (18%) dogs defecated everywhere. In total 154 (51.3%) dog owners disposed of waste from the doghouse to the municipal landfill and 146 (48.7%) disposed of waste along roadsides. The majority of dog owners (80%) provided feed to their dogs in a bowl and 88.3% (95% CI: 84.15 - 91.74) eat outdoors (Table 5).

The present finding indicated that 93.3% (95% CI: 89.89 - 95.88) of the respondents have children, whereas 35 (11.7%) do not have children in their homes. The finding indicated that 53.7% (95% CI: 47.84 - 59.42) of the respondents and their families had experienced close contact with their dogs and 27% of them stated that there was a contact between their dogs and only their children, whereas 19.3% of them had no contact with their dogs and family at all. Similarly, 247 (82.3%) respondents reported that their children play in their compound and 40 of them stated that their children play on the roadsides, while 13 (4.3%) of children play everywhere (Table 6).

Table 6: Zoonotic disease, sanitation, and pet contact related attitude and knowledge of the respondents

Variables	No. of respondents	Proportion (95% CI)
Are children presence?		
Yes	280	93.3 (89.89-95.88)
No	20	6.7 (4.12-10.11)
Dog Contact History		
No contact with children	58	19.3 (15.02-24.26)
Very close contact with all of us	161	53.7 (47.84-59.42)
The dog only plays with children	81	27 (22.06-32.40)
Children's playing areas		
In the compound	247	82.3 (77.54-86.48)
On the **roadsides**	40	13.3 (9.70-17.71)
Anywhere	13	4.3 (2.33-7.30)
Do the children bite their nails?		
Yes	242	80.7 (75.74-84.98)
No	58	19.3 (15.02-24.26)
Do children wash their hands before a meal?		
Yes	281	93.7 (90.29-96.14)
No	19	6.3 (3.86-9.71)
What type of food do you feed your children?		
Raw vegetables	11	3.7 (1.84-6.47)
Only cooked foods	289	96.3 (93.53-98.16)
Do you know that parasitic diseases are transmitted from dog to human?		
Yes	49	16.3 (12.33-21.01)
No	257	83.7 (81.18-89.43)
Have you heard of toxocariasis?		
Yes	58	19.3 (15.02-24.26)
No	242	80.7 (75.74-84.98)

Regarding the hand-washing practice of their children before meals, the finding indicated that handwashing was practiced by 93.7% (95% CI: 90.29 - 96.14) of the respondents, and 6.3% of the respondents never wash their children's hands. The respondents indicated that 3.7% fed their children raw vegetables, whereas 96.3% of respondents fed their children only cooked foods. The level of awareness of the community on parasitic diseases indicated that 83.7% of them didn't know that parasitic diseases can be transmitted from dogs to humans, whereas 19.3% of respondents were aware of the fact that parasitic diseases can be transmitted from dogs to humans, and 80.7% (95% CI: 75.74 - 84.98) of them do not have information about the disease toxocariasis (Table 6).

DISCUSSION

The present study found a higher prevalence of *T. canis* in Bishoftu, central Ethiopia. Coprological examination of 300 samples revealed that the overall prevalence of *T. canis* was 32.3% (95% CI: 27.07 - 37.95). The finding regarding the prevalence of *T. canis* in the current study was in line with the studies that reported 39.79% in Bahir Dar (Abere et al., 2013), 38.8% in Hawassa (Dejene et al., 2013), and 36.6% in Ilam province (Alimohammad et al., 2011). However, this finding was higher than in the studies that reported 21% in Central Ethiopia (Yacob et al., 2007), 17.1% in Ambo (Endrias et al., 2010), 4.2% in Canada (Joffe et al., 2011), 7.9% in South Africa (Mukaratirwa and Singh, 2010), 5.54% in Brazil (Oliveira-Sequeira et al., 2002), 3.8% in India (Keshaw et al., 2016), 13.7% in Tanzania (Swai et al., 2010) and 26.6% in Bahir Dar (Zelalem and Mekonnen, 2012). A higher prevalence of *T. canis* in the present study might be due to the widespread parasites, poor management and feeding systems of the dogs, ecological factors required for the biology of the parasites, season of study, size of taken samples, and public awareness regarding dog health care.

The present study revealed that there was no statistically significant difference ($p > 0.05$) in the prevalence of the disease among age groups, but old dogs were more affected than other age groups. Similarly, Endrias et al. (2010) and Dubie et al. (2023) reported insignificant association of *T. canis* with age categories. The higher prevalence of the disease in older dogs might be due to the higher chance of exposure to contaminated feed or environment, the geriatric body defense system, and poor management systems. Accordingly, there was no significant difference ($p > 0.05$) observed in the prevalence of *T. canis* between male and female dogs. Likewise, several studies also reported that sex was not significantly associated with *T. canis* infection (Alimohammad et al., 2011; Dubie et al., 2023). The similarity in the prevalence of *T. canis* in female and male dogs might be due to similar management practices for both sexes.

The present study revealed a statistically significant difference ($p < 0.05$) in the prevalence of *T. canis* between local and German Shepherd dog breeds. In line with this finding, Abere et al. (2013) reported significant difference in the prevalence of the disease among dog breeds. This difference might be due to the resistance of local breeds or adaptation to the endemic parasites because of frequent exposure. The finding of the present study indicated a significant difference in the prevalence of *T. canis* between diarrheic and non-diarrheic dogs. Similarly, studies revealed that the presence of diarrhea was significantly associated with *T. canis* infection (Zelalem and Mekonnen, 2012). The findings suggested that *T. canis* should receive appropriate attention and consideration in the diagnosis of canine diarrhea.

The questionnaire survey revealed poor dog management practices and lower awareness of the public on zoonotic canine parasitic diseases. The present study showed that most dog owners clean only the dog's house and do not keep the hygiene of their dogs. Accordingly, most owners do not take their dogs to veterinary clinics for deworming on a regular basis. Similarly, several studies (Holland, 2017; Schwartz et al., 2021; Abadilla and Paller, 2022) reported poor dog management, absence of or infrequent dog deworming, and poor hygiene. The majority of dogs were fed household leftovers and raw animal products that can certainly lead to exposure to helminth infections. In line with this, Finley et al. (2008) stated the increasing provision of raw feed to dogs is a major potential source of zoonotic pathogens. The present study revealed that most dogs defecate outside the compound anywhere. The present findings and several studies indicated poor pet management practices and lower public awareness in most developing countries.

The present study revealed that over 50% of the children and their families had close contact with their dogs. The present finding was similar to a previous study in the Netherlands (Overgaauw et al., 2009). The findings imply a high risk of contracting zoonotic parasitic diseases from dogs. Hygienic practices such as hand washing after contact with dogs were practiced in most homes with dogs. In line with this finding, Stull et al. (2013) stated that owners who are not concerned about the hygienic status of their dog, should be washing their hands regularly. However, Overgaauw et al. (2009) reported that most dog owners wash their hands less frequently after a contact with their dogs. Hand hygiene plays a significant role in preventing and reducing the risk of zoonotic infections, such as toxocariasis, especially in developing countries.

The present study indicated that most dog owners (83.7%; 95% CI: 81.18 - 89.43) had poor knowledge about the zoonotic transmission of canine parasitic diseases. In line with this finding, a study conducted in Hawassa showed that

97% of the respondents were not aware of zoonotic canine parasitic diseases (Dejene et al., 2013). In another study in Ambo, 55.7% of the owners had no knowledge about the role of dogs in transmitting diseases to humans (Endrias et al., 2010). Accordingly, most dog owners (80.7%; 95% CI: 75.74 - 84.98) had no awareness about the disease toxocariasis. Similarly, several studies (Kantarakia et al., 2020; Tamiru et al., 2022; Ntampaka et al., 2022) reported lower public awareness on toxocariasis and its zoonotic transmission. The present study indicated lower public awareness of zoonotic canine parasitic diseases. Awareness about the risk of zoonotic diseases such as toxocariasis is a prerequisite for effective disease prevention, hygienic practices, and better dog management practices.

CONCLUSION

The present study revealed a widespread distribution of *T. canis* infection in dogs in Bishoftu, central Ethiopia. The study identified that dog breeds and the presence of diarrhea were significantly associated with the prevalence of *T. canis*. The prevalence of the disease was higher in German Shepherd dog breed and in dogs with the clinical diarrhea. The knowledge, attitude, and practices assessment indicated poor dog management and lower hygienic practices. Most dogs were fed raw animal products, they defecated everywhere, and were not dewormed regularly. The awareness of the public about zoonotic canine parasitic diseases and toxocariasis was lower. The current higher prevalence of *T. canis* in dogs and the lower public awareness of the disease in Bishoftu in central Ethiopia call for the need for implementation of appropriate control and prevention measures. Therefore, this study recommends strategic deworming of dogs against helminths, better dog management and hygienic practices, and awareness of the community about zoonotic canine diseases and sanitary measures to control the impact of the disease in animals and human health.

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Author's Contribution:

H.A. conception and designing, sample collection and laboratory investigation; H.A. & A.E. data analysis, results and interpretation, drafting the manuscript; A.E. revising the manuscript and approving the final version.

Competing interest

The authors declare that there is no conflict of interest associated with the research reported in this article.

REFERENCES

1. Abadilla M.G. and Paller V.G. 2022. *Toxocara canis* prevalence in soil, dog stool, and human serum samples from a rural village in Los Baños, Laguna, Philippines. Journal of parasitic diseases: official organ of the Indian Society for Parasitology, 46, 3, 889–895. doi: 10.1007/s12639-022-01507-0.
2. Abere T., Bogale B., Melaku A. 2013. Gastrointestinal helminth parasites of pet and stray dogs as a potential risk for human health in Bahir Dar town, north-western Ethiopia. Veterinary World, 6, 7, 388–392. doi:10.5455/vetworld.2013.
3. Alimohammad B., Alizaman D., Hoosain N., Abdolmahdi N., Salman A.A. 2011. Epidemiological Survey of Gastro-Intestinal Parasites in Stray Dogs and Cats in Ilam. Australian Journal of Basic and Applied Sciences, 5, 9, 1944-1948.
4. Awoke E., Bogale B., Chanie M. 2011. Intestinal nematode parasites of dogs: prevalence and associated risk factors. International Journal of Animal and Veterinary Advances, 3, 5, 374–378.
5. Ceballos N.A., Karunaratna D., Setién A.A. 2014. Control of canine rabies in developing countries key features and animal welfare implications. Revised science and technology of international Epizootics, 33, 1, 311-321. doi: 10.20506/rst.33.1.2278.
6. Chomel B.B. and Ben B. 2011. Zoonoses in the bedroom: Emerging Infectious Disease. International Journal of Animal and Veterinary Advances, 17, 167-172. doi: 10.3201/eid1702.101070.
7. Dejene G., Mesula G., Efriem D., Kassahun A., Solomon M. 2013. Gastrointestinal helminthes in dogs and community perception on parasite zoonosis at Hawassa city, Ethiopia. Global Veterinary, 11, 4, 432-440. doi: 10.5829/idosi.gv.2013.11.4.75192.

8. Despommier D. 2003. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clinical microbiology reviews, 16, 2, 265–272. doi: 10.1128/CMR.16.2.265-272.2003.
9. Dubie T., Sire S., Fentahun G., Bizuayehu F. 2023. Prevalence of Gastrointestinal Helminths of Dogs and Associated Factors in Hawassa City of Sidama Region, Ethiopia. Journal of parasitology research, 2023, 6155741. doi: 10.1155/2023/6155741.
10. Endrias Z., Yohannes S., Berhanu M. 2010. Prevalence of helminthes parasites of dogs and owners awareness about zoonotic parasites in Ambo town, Central Ethiopia. Veterinary Journal, 14, 2, 17-30. doi: 10.4314/evj.v14i2.63881.
11. Fahrion A.S., Staebler S., Deplazes P. 2008. Patent *Toxocara canis* infections in previously exposed and in helminth-free dogs after infection with low numbers of embryonated eggs. Veterinary Parasitology, 152, 1-2, 108-15. doi: 10.1016/j.vetpar.2007.11.022.
12. Fan P.C., Chung W.C., Lin C.Y., Wu. C.C. 2003. Vaccination trials against *Taenia solium* eggs in pigs injected with frozen oncospheres of *T. solium* or *T. saginata* asiatica. Journal of Microbiology, Immunology and Infection, 36, 2, 96-100.
13. Finley R., Reid-Smith R., Ribble C., Popa M., Vandermeer M., Aramini J. 2008. The occurrence and antimicrobial susceptibility of salmonellae isolated from commercially available canine raw food diets in three Canadian cities. Zoonoses Public Health, 55, 462–469. doi: 10.1111/j.1863-2378.2008.01147.x.
14. Hackett T. and Lappin M.R. 2003. Prevalence of enteric pathogens in dogs of North Central Colorado. Journal of American Animal Hospitality Association, 39, 1, 52-56. doi: 10.5326/0390052.
15. Hendrix, C.M. and Sirois, M. 2007. Laboratory Procedures for Veterinary Technicians, Mosby Elsevier, Inc., USA, ISBN 9780323045728.
16. Himsworth C.G., Skinner S., Chaban B., Jenkins E., Wagner B.A., Harms N.J., Leighton F.A., Thompson R.C., Hill J.E. 2010. Multiple zoonotic pathogens identified in canine feces collected from a remote Canadian indigenous community. American Journal of Tropical Medicine, 83, 2, 338–341. doi: 10.4269/ajtmh.2010.10-0137.
17. Holland C.V. 2017. Knowledge gaps in the epidemiology of Toxocara: the enigma remains. Parasitology, 144, 1, 81–94. doi: 10.1017/S0031182015001407.
18. Joffe D., Van Niekerk D., Gagné F., Gilleard J., Kutz S., Lobingier R. 2011. The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. The Canadian veterinary journal, 52, 12, 1323-1328.

19. Kantarakia C., Tsoumani M.E., Galanos A., Mathioudakis A.G., Giannoulaki E., Beloukas A., Voyiatzaki C. 2020. Comparison of the Level of Awareness about the Transmission of Echinococcosis and Toxocariasis between Pet Owners and Non-Pet Owners in Greece. International Journal of Environmental Research and Public Health, 17, 15, 5292. doi: 10.3390/ijerph17155292.
20. Keshaw T., Jason C., Derek T., Claude D.A., Alfre C., Cecilia H.Y., Ravindra N.S. 2016. Prevalence of Gastrointestinal Parasites in stray dogs (*Canis familiaris*) from Gre nada, West Indies. Journal of Animal Research, 6, 1, 1-5.doi: 10.5958/2277-940X.2016.00001.2.
21. Khante G.S., Khan L.A., Bodkhe A.M., Suryawanshi P.R., Majed M.A., Suradkar U.S., Gaikwad S.S. 2009. Epidemiological survey of gastrointestinal parasites of non-descript dogs in Nagpur City. Veterinary World, 2, 1, 22-23.
22. Kim H.C., Hong E.J., Ryu S.Y., Park J., Cho J.G., Yu D.H., Chae J.S., Choi K.S., Park B.K. 2020. Morphological and Molecular Characterization of *Toxocara apodemi* (Nematoda: *Ascarididae*) from Striped Field Mice, *Apodemus agrarius*, in Korea. The Korean journal of parasitology, 58, 4, 403–411. doi: 10.3347/kjp.2020.58.4.403.
23. Martins C.F., Soares J.P., Cortinhas A., Silva L., Cardoso L., Pires M.A., Mota M.P. 2023. Pet's influence on humans' daily physical activity and mental health: a meta-analysis. Frontiers in public health, 11, 1196199. doi: 10.3389/fpubh.2023.1196199.
24. Mukaratirwa S. and Singh V.P. 2010. Prevalence of gastrointestinal parasites of stray dogs impounded by the Society for the Prevention of Cruelty to Animals (SPCA), Durban and Coast, South Africa. Journal of South Africa Veterinary Association, 81, 2, 123-125. doi: 10.4102/jsava.v81i2.124.
25. Mundim M.J., Cabral D.D., Faria E.S. 2001. Endoparasites of zoonotic importance found in faecal samples from dogs in Uberlandia, Minas Gerais. Veterinary Note, 7, 73-77.
26. Nobel W.E., Robben S.R., Dopfer D., Hendrikx W.M., Boersema J.H., Fransen F., Eysker M. 2004. Infections with endoparasites in dogs in Dutch animal shelters. World parasitology, 129, 2, 40-44.
27. Ntampaka P., Niragire F., Nkurunziza V., Uwizeyimana G., Shyaka A. 2022. Perceptions, attitudes and practices regarding canine zoonotic helminthiases among dog owners in Nyagatare district, Rwanda. Veterinary Medicine and Science, 8, 4, 1378-1389. doi: 10.1002/vms3.787.
28. Oliveira-Sequeira T.C., Amarante A.F., Ferrari T.B., Nunes L.C. 2002. Prevalence of intestinal parasites in dogs from Sao Paulo state Brazil. Veterinary Parasitology, 103, 1-2, 19-27. doi:10.1016/s0304-4017(01)00575-1.
29. Overgaauw P.A., van Zutphen L., Hoek D., Yaya F.O., Roelfsema J., Pinelli E., van Knapen F., Kortbeek L.M. 2009. Zoonotic parasites in fecal samples

and fur from dogs and cats in The Netherlands. Veterinary parasitology, 163, 1-2, 115–122. doi: 10.1016/j.vetpar.2009.03.044.

30. Robertson I.D., Irwin P.J., Lymbery A.J., Thompson R.A. 2000. The role of companion animals in the emergence of parasitic zoonoses. International Journal for Parasitology, 30, 1369-1377.doi:10.1016/s0020-7519(00)00134-x.
31. Schwartz R., Bidaisee S., Fields P.J., Macpherson M.A., Macpherson C.L. 2021. The epidemiology and control of *Toxocara canis* in puppies. Parasite epidemiology and control, 16, e00232. doi: 10.1016/j.parepi.2021.e00232.
32. Soriano S.V., Pierangeli N.B., Roccia I., Bergagna H.F., Lazzarini L.E., Celescinco A., Saiz M.S., Kossman A., Contreras P.A., Ariasand C., Basualdo J.A. 2010. A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquén, Patagonia, Argentina. Veterinary Parasitology, 167, 1, 81-85. doi: 10.1016/j.vetpar.2009.09.048.
33. Stull J., Peregrine A., Sargeant J., Weese J. 2013. Pet husbandry and infection control practices related to zoonotic disease risks in Ontario, Canada. Public Health, 13, 520. doi: 10.1186/1471-2458-13-520.
34. Swai E.S., Kaaya E.J., Mshanga D.A., Mbise E.W. 2010. A Survey on Gastro- Intestinal Parasites of Non-Descript Dogs in and Around Arusha Municipality, Tanzania. International Journal of Animal and Veterinary Advance, 3, 2, 63-67.
35. Tamiru Y., Efa D., Amante M. 2022. Knowledge, Attitude, and Practice Toward Pet Contact Associated Zoonosis in Western Ethiopia. Veterinary Medicine: Research and Reports, 13, 47-58. doi: 10.2147/VMRR.S346806.
36. Thrusfield, M. 2005. Veterinary Epidemiology, Blackwell Publishing, UK, Third edition, ISBN: 978-1-405-15627-1.
37. Yacob H.T., Ayele T., Fikru R., Basu A.K. 2007. Gastro-intestinal nematodes in dog from Debre Zeit, Ethiopia. Veterinary parasitology, 148, 2, 144-148. doi: 10.1016/j.vetpar.2007.06.007.
38. Yemenu F. and Chemeda D. 2010. Climate resources analysis for use of planning in crop production and rainfall water management in the central highlands of Ethiopia, the case of Bishoftu district, Oromia region. Hydrology and Earth System Sciences, 7, 3733–3763.doi: 10.5194/hessd-7-3733-2010.
39. Zelalem G. and Mekonnin A. 2012. Prevalence of Gastrointestinal Helminthes among Dogs in Bahir Dar Town, Ethiopia. World Applied Sciences Journal, 19, 5, 595-601.

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SALMONELLA IN WILD BOARS (*SUS SCROFA*): INFLUENCE OF HUNTING AND DRESSING PROCEDURES ON MEAT SAFETY

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Abstract

Salmonella spp. is considered as a high-priority foodborne hazard for control in wild boar meat. This comprehensive study about *Salmonella* spp. in population of wild boars in Serbia was conducted with an aim to assess the influence of hunting and dressing procedures on the spread of Salmonella on wild boar carcasses and to examine the molecular similarities of strains isolated from wild boars. Samples from wild boars from twelve hunting estates in South-West Vojvodina, Serbia, were taken from 425 hunted animals, which was 25.3% of the total wild boar population in all hunting estates. Three samples were taken from each animal's skin swabs, feces, and carcass meat swabs. A total of 1,275 samples were examined using standard ISO protocols. Subtyping of the isolates was performed and compared using Pulsed-field gel electrophoresis (PFGE). Salmonella prevalence was 4.2%, and *Salmonella Enteritidis* was the dominant serotype (74.5%). Carcass meat contamination originated from the feces of the same animal. Two or more entrance wounds, damage of the abdominal cavity caused by non-expert shooting were factors found to increase Salmonella contamination on the skin and/or on carcass meat. Rain during the hunting and practice of diaphragm and peritoneum removal and the evisceration being performed on the ground/floor or in hanging position did not lead to increase in Salmonella contamination on the meat. Although the determined prevalence was not high in wild boars compared to farm pigs, it is possible that *Salmonella* entered the food chain through contaminated meat. Therefore, the

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importance of good hunting and hygiene practice in handling and dressing wild boar carcasses should not be underestimated.

Keywords: wild boar, *Salmonella*, process hygiene, game meat, hunting procedures

SALMONELLA KOD DIVLJIH SVINJA (*SUS SCROFA*): UTICAJ PROCEDURA LOVA I OBRADE NA BEZBEDNOST MESA

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Kratak sadržaj

Smatra se da je *Salmonella* spp. visokoprioritetni patogen u kontroli mesa divljih svinja. U ovom radu su prikazani rezultati obimnog istraživanje Salmonella spp. u populaciji divljih svinja u Srbiji koja su sprovedena sa ciljem da se proceni uticaj procedura lova i obrade na širenje *Salmonella* na trupovima divljih svinja kao i da se utvrde molekularne sličnosti izolovanih sojeva. Uzorci su uzeti iz 12 lovišta sa područja Jugoistočne Vojvodine u Srbiji, ukupno je uzorkovano 425 ulovljenih životinja koje su u tom momentu činile 25.3% ukupne populacije divljih svinja u lovištima. Sa svake životinje su uzorkovana tri uzorka, bris kože, feces i bris trupa. Ukupno je ispitano 1,275 uzoraka standardnim ISO protokolom. Subtipizacije je izvršena primenom elektroforeze u pulsnom polju (PFGE). Utvrđena je prevalenca *Salmonella* od 4.2%, dok je *Salmonella Enteritidis* je bila dominantni serotip (74.5%). Takođe, potvrđeno je da kontaminacija trupa potiče iz fecesa iste životinje. Dve ili više ulaznih rana i oštećenje trbušne duplje uzrokovano ne-ekspertskim pucanjem su faktori koji povećavaju kontaminaciju kože i trupa sa *Salmonella* spp. Kiša tokom lova, praksa odstranjivanja dijafragme i periotneuma i evisceracija na terenu/podu ili u visećem položaju nisu doveli do rasta kontaminacije trupova sa *Salmonella* spp. Iako utvrđena prevalenca kod divljih svinja nije velika u odnosu na farmski uzgajane svinje i dalje postoji mogućnost ulaska Salmonella u lanac hrane preko kontaminiranog mesa. Stoga ne treba potceniti značaj dobre lovne i dobre higijenske prakse prilikom rukovanja i obrade trupova divljih svinja.

Ključne reči: divlje svinje, *Salmonella*, higijena procesa, meso divljači, lovne procedure

INTRODUCTION

The European wild boar (*Sus scrofa*) is widely distributed in Europe, with a consistently increasing population since the second half of the last century (Massei et al., 2015). The biggest amount of game meat is consumed by hunters and their families, up to 1-4 kg/year per capita (Ramanzin et al., 2010), while its consumption is limited in the general population, 0.6-1.0 kg/year per capita in Austria, France, Germany and Switzerland (Atanassova et al., 2008; Membré et al., 2011). Consumers tend to follow trends of eating healthily (lower percentage of fat) and game meat is considered as a completely "natural" product because animals are raised without intensive farming.

Game meat harvesting and processing differ significantly from classical livestock meat production and represents a challenge itself. Domestic animals raised for food production in farm conditions are subject to regular veterinary health control and official *ante* and *post mortem* inspection at slaughterhouses, while in game species only *post mortem* examination is performed (Mirčeta et al., 2017). The game meat safety assurance and implementation of the concept "from forest to fork" encompasses the following: the influence of hunting estate environment, hunting and carcass dressing methods (including evisceration technique used), meat inspection after shooting, transport to the dressing/chilling facilities, etc (Petrović et al., 2014; Rodas et al., 2014; Mirčeta et al., 2017).

Salmonella frequently occurs in various types of meat used for human consumption and it is the most important pork meat pathogen in industrialized countries (EFSA, 2010). Wildlife could be transmission and accumulation vector of *Salmonella* in contact with domestic animals, direct contact with humans and through meat of water birds and wild boars (Hilbert et al, 2012). To the best of our knowledge, no outbreak of salmonellosis has ever been traced to the consumption of wild boar meat. This might be due to the general low consumption of game meat and/or the low incidence of *Salmonella* in wild boar meat (Hilbert et al., 2012). It is considered that *Salmonella* is a relevant biological hazard for hunted wild game animals, despite the fact that it is not considered a priority (Gortázar et al., 2007). It is well known that some of the hunting procedures for wild animals (such as skinning and carcass washing) ultimately lead to an increase in contamination of the carcasses (Mirčeta et al., 2017; Orsoni et al., 2020). Information on the influence of the hunting and dressing processes on *Salmonella* presence on wild boar carcass meat from Serbia is lacking.

A large study was conducted on the *Salmonella* in wild boars, with the first part investigating its epidemiology, presence and distribution (Petrović et al., 2022). The aim of this second study was to assess the influence of the hunting and dressing process on the presence of *Salmonella* in wild boars. Molecular technique (PFGE) was used to determine the source of *Salmonella* carcass contamination by examination of molecular similarities of strains isolated from different sampling sites of wild boars.

MATERIAL AND METHODS

Study area and animals

Wild boars that originated from twelve different hunting estates in the region of South-West Vojvodina were examined in this study. Ten hunting estates investigated were large, fenced hunting estates, while two were open areas. Intensive management of wild boars for hunting purposes was used in all twelve hunting estates. Intensive management in Vojvodina encompasses habitat management to preserve the natural ecosystem, continuous monitoring of health status, sampling of hunted and dead wild boars supplementary feeding, and control of predators. The samples were collected during two hunting seasons (2013-2014) as part of a large project on pathogens occurring in wild boars. Research on the prevalence of *Salmonella* was supposed to continue but had to be stopped due to the outbreak of African swine fever (Polaček et al. 2021). The number of wild boars per hunting estate varied throughout the season, but it was estimated to be 1,677, out of which 425 were sampled. This was a representative sample, with 25.3% of the total wild boar population that was present at the moment of hunting when sampling was conducted.

Hunting and sampling procedures

The hunts were performed by using “still hunting method” (fixed positions for shooting) and using rifle bullets. At the end of the hunt, animals were usually collected and eviscerated either in the field at the collection point or transported to a respective game handling establishment for evisceration and dressing (not more than 2 hours). The sampling was conducted in winter hunting seasons. The fecal samples were taken directly from the rectum, approximately 50 g. Before sampling, sponge –swabs were moisturized with

Maximum Recovery Diluent (10 ml). Swab samples were taken by swabbing skin or carcass meat surface using the sponge-swab technique (Nasco Whirl-Pak™ Speci-Sponge). Skin swab samples were collected immediately before evisceration from approximately 1000 cm^2 of skin (lateral rump-perianal-medial rump-flank-brisket-neck). Carcass meat surface samples were taken shortly after the evisceration, and all other procedures were completed in no more than 10 minutes. Four carcass meat sites corresponding to the previously sampled skin (i.e. inner side of the rump and flank, thorax and brisket) were sponge-swabbed using sterile square plastic templates, which delineated a 100 cm^2 area (in total, 400 cm^2). In this study, samples from a total of 425 freshly shot wild boars were collected, with three samples from each animal tested for the presence of *Salmonella* spp.: skin swabs, feces, and carcass meat swabs (1,275 samples in total). All samples were transported in a chill-bin with external cooling system at 4°C to the laboratory within 3 h.

Microbiological procedure

The samples were stored at 4 ^{0}C and analyses were performed within 24 hours. Isolation of *Salmonella* was performed according to the ISO 6579:2002 sponge-swab samples and fecal samples procedures (Annex D) (ISO, 2002). Producers of all bacteriological culture media were Biokar Diagnostics, France and Oxoid, Ireland, except for Salmonella differential agar (Hi media, India). Suspected colonies of *Salmonella* spp. were further confirmed using API 20 *Enterobacteriaceae* (API 20E) strips (bioMérieux, Marcy l'Etoile, France). Serotyping was performed according to the CEN ISO/TR 6579-3:2014 (ISO, 2014) with commercial antisera (Institute of Public Health of Serbia "Dr Milan Jovanović Batut", Belgrade).

Characterization of isolated Salmonella strains

In order to investigate the possible source of *Salmonella* contamination isolates from different sampling sites of wild boars (n = 20; feces = 13, carcass = 5, skin = 2 – Table 1), subtyping of the isolates was performed and compared by pulsed-field gel electrophoresis (PFGE). *Salmonella* isolates were genotyped by applying the Standardized Laboratory Protocol for Molecular Subtyping of *Salmonella* by PFGE method (CDC, 2013). The macro-restriction of the genomic DNA was done with the S*peI* and X*baI* restriction enzymes. The macro-restriction of the *Salmonella* Braenderup H9812 strain was used as a molecular size standard. The obtained profiles were statistically analyzed by using Ward's linkage of correlation coefficients between PFGE patterns of different genotypes using the SPSS cluster analysis software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Hierarchical cluster analysis was performed in order to group serotypes with similar PFGE patterns resulting in a dendrogram (Zou et al., 2010). Length of the lines between serotypes shows similarities between serotypes. The shorter the length, the more similar the serotypes are. Distances between serotypes (from 0 to 25) reflect differences between clusters.

Table 1: List of isolates examined by PFGE

Isolate Sign	Sampling site	Hunting estate	Group	*Salmonella*
312	Feces	C	B	S. Typhimurium
313	Skin	E	B	S. Typhimurium
320	Feces	I	B	S. Typhimurium
321	Carcass	I	B	S. Typhimurium
344	Feces	D	B	S. Typhimurium
346	Feces	D	B	S. Typhimurium
317	Feces	G	D	S. Enteritidis
319	Carcass	I	D	S. Enteritidis
333	Feces	D	D	S. Enteritidis
336	Feces	D	D	S. Enteritidis
354	Feces	D	D	S. Enteritidis
355	Feces	D	D	S. Enteritidis
358	Feces	D	D	S. Enteritidis
375	Feces	E	D	S. Enteritidis
376	Feces	E	D	S. Enteritidis
246	Carcass	K	D	S. Enteritidis
248	Carcass	K	D	S. Enteritidis
249	Carcass	K	D	S. Enteritidis
254	Skin	K	D	S. Enteritidis
318	Feces	E	C	S. Infantis

Data analyses

The prevalence of *Salmonella*, $\chi2$ square test and p value were calculated using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA). The results of the statistical tests were considered significant at $p<0.05$. Different hunting and dressing procedures that may have an impact on *Salmonella* prevalence were investigated. These included the following: 1. rainfall during the hunting, 2. the number of entrance wounds, 3. damage to the abdominal cavity, 4. the practice of removal of diaphragm and peritoneum, 5. the evisceration on the ground or in hanging position, 6. the order in which an animal was killed during the hunting day; and 7. the location where *Salmonella* spp. was found - skin, carcass meat and/or feces.

RESULTS

Prevalence of Salmonella spp. in wild boars

Table 2 presents overall data, including the epidemiological part of the study as well as *Salmonella* findings in feces (Petrović et al., 2022).

Table 2: Prevalence of *Salmonella* in each hunting estate (HE): prevalence in animals and positive samples

Hunting estates	No of animals in HE per year	Examined animals (% of population)	*Salmonella* positive animals (prevalence %)	*Salmonella* isolates (% of examined animals)			
				skin	carcass meat	lymph node	feces[2]
A	180	63 (35.0)	2 (3.2)	0	0	0	2 (3.2)
B	160	26 (16.3)	0	0	0	0	0
C	210	59 (28.1)	1 (1.7)	0	0	0	1 (1.7)
D	82	12 (14.6)	4 (33.3)	0	0	0	4 (33.3)
E	340	66 (19.4)	2 (3.0)	1 (1.5)	0	0	1 (1.5)
F	210	57 (27.1)	1 (1.8)	0	0	0	1 (1.8)
G	150	48 (32.0)	1 (2.1)	0	0	0	1 (2.1)
H	220	26 (11.8)	0	0	0	0	0
I	55	32 (58.2)	2 (6.3)	0	2 (6.3)	0	1 (3.1)
J	20	10 (50.0)	0	0	0	0	0
K[1]	35	15 (42.9)	5 (33.3)	2 (13.3)	2 (13.3)	1 (6.7)	2 (13.3)
L[1]	15	11 (73.3)	0	0	0	0	0
TOTAL	1677	425 (25.3)	18 (4.2)	3 (0.7)	4 (0.9)	1 (0.2)	13 (3.1)

[1] "K" and "L" are open hunting estates, all other estates have fence

[2] The results for feces were already published (Petrović et al, 2022)

The presence of *Salmonella* spp. was confirmed in 18 wild boars, with an overall prevalence of 4.2%. The highest prevalence was found in two estates, open estate “K” (33.3%) and fenced estate “D” (33.3%). Three animals had more than one *Salmonella* serotype detected. Serotyping of the 21 *Salmonella* isolates resulted in fifteen *Salmonella* Enteritidis isolates (71.4% of total number of isolates), five isolates of *Salmonella* Typhimurium (23.8%) and only one *Salmonella* Infantis isolate (4.8%).

Salmonella Typhimurium strain was detected both in feces and on the carcass meat of the same animal (hunting estate “I), so the total number of positive animals in this estate is two. In the hunting estate “K” the *Salmonella* Enteritidis strain was isolated from the skin and carcass, but not from the feces of the same animal. In all other examined animals, *Salmonella* was isolated from only one of the sampling sites. Prevalence of *Salmonella enterica* serotypes as percent of all examined samples was as follows: on carcass Enteritidis 0.7%, Typhimurium 0.2%; on skin Enteritidis 0.4%, Typhimurium 0.2 % and in feces Enteritidis 1.6%, Typhimurium 0.7%, Infantis 0.2%.

Influence of different factors on Salmonella findings

Several factors that influenced *Salmonella* presence were investigated. Rainfall during hunting increased findings of *Salmonella* on the skin ($\chi2 = 9.18$, $p = 0.01$), but not on the carcass meat ($\chi2 = 1.38$, $p = 0.5$). The possibility of detecting *Salmonella* on the skin significantly increased if boars had two or more entrance wounds ($\chi2 = 6.30$, $p = 0.04$). The $\chi2$ test indicated that the damage of the abdominal cavity caused by non-expert shooting, significantly increases the likelihood of finding *Salmonella* on the skin ($\chi2 = 7.03$, $p = 0.03$), but it was not correlated with the findings on carcasses ($\chi2 = 0.76$, $P = 0.73$). The practice of diaphragm and peritoneum removal did not significantly affect the finding of *Salmonella.* Furthermore, there was no significant difference between the evisceration being done on the ground/floor or in hanging position ($\chi2 < 3.84$, $p > 0.05$), *Salmonella* was more commonly detected in feces (3.1%), than on the carcass meat (0.9%), skin (0.7%) or mesenteric lymph node (0.2%), but this difference is not statistically significant ($\chi2 = 1.22$, $p = 0.54$).

Characterization of isolated Salmonella strains

The dendrogram carried out based on the Ward’s linkage correlation coefficient obtained between the S*peI* and *Xba*I macrorestriction PFGE profiles (Figures 1 and 2), demonstrates the existence of 8 groups of isolates. The following groups of identical isolates according to both PFGE profiles were found (isolate numbers and origin are shown in table 1): group one (320, 321), group two (344, 346), group three (246, 248, 249), and big group four (254, 317, 319, 333, 336, 354, 355, 358, 376). Identical profiles of isolates 320 and 321 as well as isolate 319, which is identical to the whole group of isolates from feces (group four) were significant in proving the hypothesis that there was a cross contamination between feces and carcass/skin.

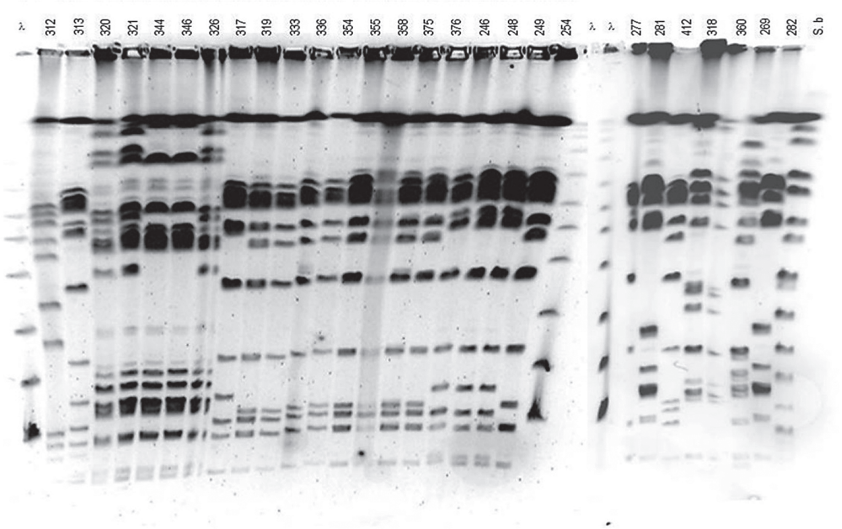

Figure 1: Pulsed-field gel electrophoresis (PFGE) macro-restriction fragment patterns of *Salmonella* sp. digested with *Xba*I enzyme.

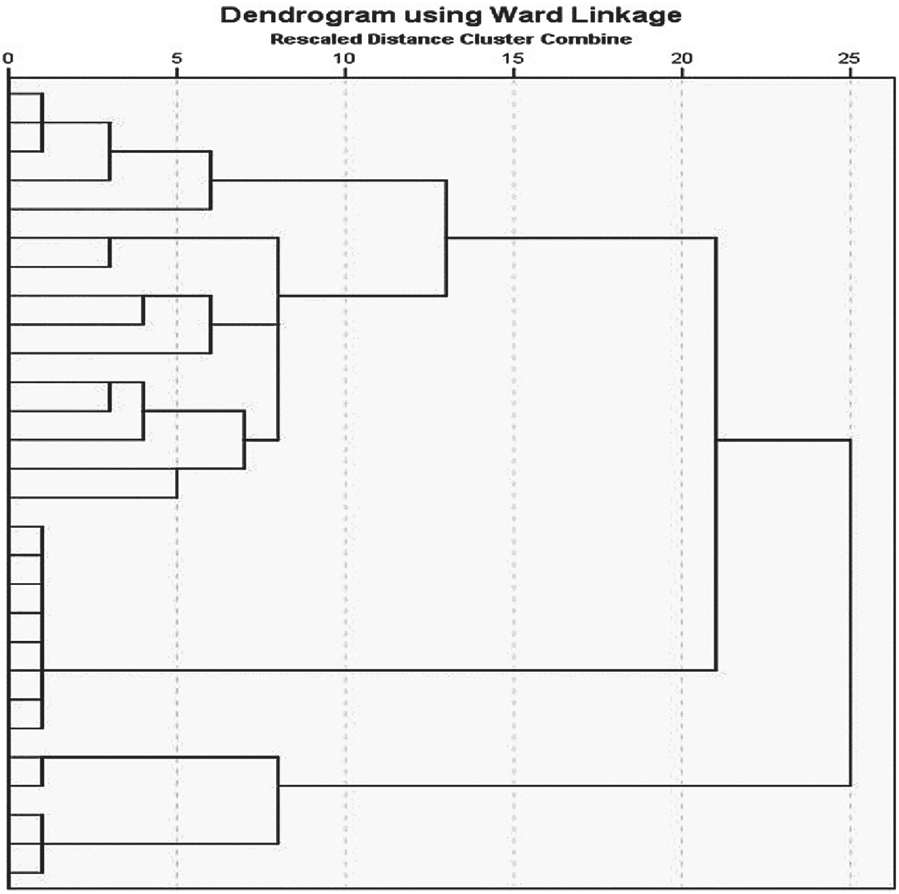

Figure 2: Dendrogram derived from the Ward linkage coefficient of correlation between the obtained PFGE *Xba*I macro-restriction profiles; Hunting estates are in quotation marks

DISCUSSION

A quarter of the wild boar population present at the tested location (25.3%) was examined for *Salmonella,* with an overall prevalence of 4.2%. The prevalence in wild boars is a little bit higher than the prevalence (2.0%) in farmed pigs from the same Vojvodina region (Stojanac et al., 2013). However, it is much lower than in finishing pigs from Hungary (up to 21.5%, Biksi et al., 2007) or free-range pigs from Spain (32.6%, Garrido et al, 2021). A relatively low prevalence (below 4%) was also found in other published studies. In one study in Italy, Orsoni et al. (2020), did not find *Salmonella* presence on carcasses of hunted wild boars. On the contrary, one earlier study by Rodas et al. (2014) determined its presence in 3.6% of investigated meat samples. *Salmonella* was more commonly detected in animals from open hunting estates compared to fenced estates, because the presence of wild boars from open hunting estates in Vojvodina was noticed around waste where a large amount of improperly removed carcasses of domestic animals were dumped (estate "K") (Petrović et al. 2022).

The dominant serotype in this study was *Salmonella* Enteritidis (71.4%), while other serotypes are less prevalent. *Salmonella enterica* serotypes Enteritidis, Typhimurium and Infantis were also found in Switzerland, Portugal and Italy (Magnino et al., 2011; Vieira-Pinto et al., 2011; Wacheck et al., 2010), but other serotypes, like *S.* Diarizonae, *S.* Manhattan have been detected in some other studies (Rodas et al., 2014). Our present findings from wild boars prove that similar *Salmonella* serotypes are present in wildlife and in domestic pigs and poultry in the same geographical area, with *S.* Enteritidis, *S.* Infantis and *S.* Typhimurium frequently found in domestic animals (47.7%, 27.9% and 13.9%, respectively) (Petrović et al., 2015). These serotypes are also the most common isolates from human infections in Serbia according to the national data (Institute of Public Health of Serbia, 2014).

As for the sample site, feces yielded most of the isolated *Salmonella* (3.1%), and skin and carcass meat were significantly less contaminated (0.7% and 0.9%, respectively). *Salmonella* presence in feces is expected and indicates possible shedding of the pathogen, which can contaminate animal skin and subsequently carcass meat surface during handling and dressing procedures (Antić et al., 2011).

In all the tested animals, a *post mortem* inspection was performed and no signs of post-mortal lesions in *Salmonella* positive animals were found. *Salmonella* in wild boars is rarely manifested through clinical signs of the disease, yet an outbreak of wild boar salmonellosis with septicemia, caused by *Salmonella* Choleraesuis has been reported in Italy (Conedera et al., 2014).

The microbiological conditions of meat from hunted animals can be compromised by poor placement of shots (in the abdomen), the evisceration and dressing in the field without access to clean water, and ageing of carcasses at ambient temperatures (Gill, 2007; Paulsen, 2011; Mirčeta et al., 2017).

This study found that damage of the abdominal cavity caused by improperly placed shot significantly increases the possibility of finding *Salmonella* on the skin, but surprisingly not on the carcass meat. These findings are to some extent consistent with Atanassova et al. (2008) and Avagnina et al. (2012), who found that animals shot in any location posterior to the diaphragm can be reasonably considered to be at higher risk of microbiological contamination of carcass meat (with general microbial load, but undoubtedly with pathogens as well) than those shot elsewhere (heart, head and neck, spine). However, these studies did not look into wild boar skin as an important source of carcass microbial contamination. Another interesting result from our study was that if an animal had been shot two or more times, there was a statistically significant increase in *Salmonella* findings on the skin but not on the carcass.

Rain during hunting increases the likelihood of finding *Salmonella* on the skin of the shot animals, probably due to a better transferability of bacteria to the swabs from the wet than from the dry skin, as demonstrated on cattle hides (Blagojević et al., 2012). On the other hand, rain was not found to increase *Salmonella* presence on carcasses (with the skin left on) immediately after evisceration - one of the reasons might be a different sampling method for skin and carcass meat (2.5 times smaller surface of carcass meat was sampled).

Interestingly, the removal of diaphragm and peritoneum, as a hygiene measure usually performed during the dressing of carcasses, had no statistical significance on the prevalence of *Salmonella* on the carcass meat. In addition, there was no difference between evisceration on the floor or while hanging. It has to be pointed out, however, that our investigation of risk factors for *Salmonella* presence is based on a very small number of contaminated skins and carcasses (0.7% and 0.9%, respectively), which pose a major limitation to the interpretation of the results.

The PFGE characterization of the *S.* Typhimurium isolates found in feces (isolate 320) and carcass meat surface of the same animal (isolate 321) was identical to indistinguishable PFGE profiles, according to S*pel* and X*bal*. This finding confirms that carcass meat contamination originated from the feces of the same animal. It was also observed that this animal had been shot expertly (i.e. one shot in the head) but eviscerated in a lying position in the field and without maintaining good hygiene practice (evisceration). Furthermore, *Salmonella* Typhimurium isolates 344 and 346 from the feces of the same animal were compared and no difference was found in the PFGE profiles. The same was the case between *Salmonella* Enteritidis isolates 246 and 248 from the same carcass. The comparison of the isolates from the same animal was done because animals may be infected with different *Salmonella* genotypes due to the various accesses to the food sources and contaminated environment (Piras et al. 2021).

Low *Salmonella* detection rate on wild boar carcass meat compared to the findings in feces as a primary source of carcass microbial contamination is to some extent expected. Despite the frequently poor carcass dressing practices observed during our study, contamination of carcass meat with enteric pathogens may be often infrequent (Gill, 2007).

CONCLUSION

This study provides valuable data on the presence of *Salmonella* spp. in wild boar population from the flat regions of Vojvodina and also clarifies some gaps in knowledge related to the epidemiology of this important foodborne pathogen. The overall *Salmonella* prevalence in wild boars from hunting estates in Vojvodina region was 4.2%, with a dominating serotype being *S.* Enteritidis. This study also confirmed that wild boar carcass meat surface contamination originates from the feces of the same animal. Although the prevalence of *Salmonella* was not found to be high in wild boars, there is still likelihood of exposure of meat consumers to this pathogen. Significant factors that influenced *Salmonella* presence on wild boar skin and carcass meat were rainfall during the hunt, two or more shots, and non-expert shooting. Therefore, good hunting practices and education of hunters in hygiene practices are essential in reducing the risk of *Salmonella* exposure to consumers of wild boar meat.

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Authors’ contributions

J.P. carried out literature research, designed a study, did the microbiology, analyzed the results and drafted the manuscript. J.M. carried out the sampling, data collection and analysis and statistics. M.V. and I.S. were involved in microbiology., R.A. was involved in data analysis. All authors read and approved the final manuscript.

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Conflict of interest

All authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence our work.

REFERENCES

1. Institute of Public Health of Serbia. 2014. Health statistical yearbook of Republic of Serbia 2013, Available at: http://www.batut.org.rs/download/publikacije/pub2013.pdf, [accessed on 15.07.2023].
2. Antić D., Blagojevic B., Buncic S. 2011. Treatment of cattle hides with Shellac solution to reduce hide-to-beef microbial transfer. Meat Science 88, 3, 498-502. doi: 10.1016/j.meatsci.2011.01.034.
3. Atanassova V., Apelt J., Reich F., Klein G. 2008. Microbiological quality of freshly shot game in Germany. Meat Science, 78, 4, 414-419. doi:10.1016/j.meatsci.2007.07.004.
4. Avagnina A., Nucera D, Grassi M, Ferroglio E, Dalmasso A, Civera T. 2012. The microbiological conditions of carcasses from large game animals in Italy. Meat Science, 91, 3, 266-271. doi: 10.1016/j.meatsci.2012.01.025.
5. Biksi I., Lorincz M., Molnar B., Kecskes T., Takacs N., Mirt D., Cizek A., Pejsak Z., Martinequ G., Sevin J., Szenci O. 2007. Prevalence of selected enteropathogenica bacteria in Hungarian finishing pigs. Acta Veterinaria Hungarica 55, 2. doi:10.1556/avet.55.2007.2.8.
6. Blagojević B., Antić D., Dučić M., Bunčić S. 2012. Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and the carcases. Veterinary Records, 170, 22, 563. doi: 10.1136/vr.100477.
7. CDC. 2013. Standard operating procedure for PulseNet PFGE of Escherichia coli O157: H7, Escherichia coli non-O157 (STEC), Salmonella serotypes, Shigella sonnei and Shigella flexneri. Centers for Disease Control and Prevention, Atlanta. Now Available at: https://pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf [accesed at 01.06.2024].
8. Conedera G., Ustulin M., Barco L., Bregoli M., Re E., Vio D. 2014. Outbreak of atypical *Salmonella* Choleraesuis in wild boars in North Eastern Italy, in: Paulsen P. Bauer A, Vodnansky M, Winkelmayer R, Smulders FJM (Ed.). Trends in game meat hygiene. Wageningen Academic Publishers, Wageningen. doi/10.3920/978-90-8686-238-2_10.

9. EFSA. 2010. Scientific opinion on a quantitative microbiological risk assessment of *Salmonella* in slaughter and breeder pigs. European Agency For Food Safety. EFSA Journal, 1547, 90.
10. Garrido V., Migura-García L., Gaitán I., Arrieta-Gisasola A., Martínez-Ballesteros I., Fraile L., Grilló MJ. 2021. Prevalence of *Salmonella* in Free-Range Pigs: Risk Factors and Intestinal Microbiota Composition. Foods. 18; 10, 6, 1410. doi: 10.3390/foods10061410.
11. Gill C. 2007. Microbiological conditions of meats from large game animals and birds. Meat Science, 77, 2, 149-160. doi: 10.1016/j.meatsci.2007.03.007.
12. Gortázar C., Ferroglio E., Höfle U., Frölich K., Vicente J. 2007. Diseases shared between wildlife and livestock: a European perspective. European Journal of Wildlife Research, 53, 241-256. doi:10.1007/s10344-007-0098-y.
13. Hilbert F., Smulders F., Chopra-Dewasthaly R., Paulsen P. 2012. *Salmonella* in the wildlife-human interface. Food Research International, 45, 2, 603-608. doi:10.1016/j.foodres.2011.08.015.
14. ISO 6579:2002, Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp. International Organization for Standardization.
15. ISO 6579-3:2014, Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.- Part 3: Guidelines for serotyping of Salmonella spp. International Organization for Standardization.
16. Magnino S., Frasnelli M., Fabbi M., Bianchi A., Zanoni M.G., Merialdi G., Pacciarini M.L., Gaffuri A. 2011. The monitoring of selected zoonotic diseases of wildlife in Lombardy and Emilia-Romagna, northern Italy, in: Paulsen P, Bauer A, Vodnansky M, Winkelmayer R, Smulders FJM (Ed.), Game meat hygiene in focus: Microbiology, Epidemiology, Risk Analysis and Quality Assurance. Wageningen Academic Publishers, Wageningen, 223-244.
17. Massei G., Kindberg J., Licoppe A., Gačić D., Šprem N., Kamler J., Baubet E., Hohmann U., Monaco A., Ozoliņš J. 2015. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Management Science, 71, 4, 492-500. doi: 10.1002/ps.3965.
18. Membré J.M., Laroche M., Magras C. 2011. Assessment of levels of bacterial contamination of large wild game meat in Europe. Food Microbiology, 28, 5, 1072-1079. doi: 10.1016/j.fm.2011.02.015.
19. Mirčeta J., Petrović J., Malesević M., Blagojevic B., Antić D. 2017. Assessment of microbial carcass contamination of hunted wild boars. Europ Journa of Wildlife Research, 63, 37. doi:10.1007/s10344-017-1096-3.

20. Orsoni F., Romeo C., Ferrari N., Bardasi L., Merialdi G., Barbani R. 2020. Factors affecting the microbiological load of Italian hunted wild boar meat (*Sus scrofa*). Meat Science, 160, 107967. doi:10.1016/j.meatsci.2019.107967.
21. Paulsen P. 2011. Hygiene and microbiology of meat from wild game: an Austrian view, in: Paulsen, P, Bauer A, Vodnansky M, Winkelmayer R, Smulders FJM (Ed.), Game meat hygiene in focus: Microbiology, Epidemiology, Risk Analysis and Quality Assurance. Wageningen Academic Publishers, Wageningen, 19–37.
22. Piras F., Spanu V., Siddi G., Gymoese P., Spanu C., Cibin V., Schjørring S., De Santis E.P.L., Scarano C. 2021. Whole-genome sequencing analysis of highly prevalent Salmonella serovars in wild boars from a national park in Sardinia, Food Control, 130, 108247, doi:10.1016/j.foodcont.2021.108247.
23. Petrović J., Babić J., Stojanov I., Velhner M. 2015. The change in antimicrobial resistance profile of meat chain-associated *Salmonella* in Serbia. Plos Food Sci, 2015, 5, 231-234. doi: 10.1016/j.profoo.2015.09.059.
24. Petrović J., Mirčeta J., Antić D., Pušić I., Urošević M. 2014. Suggested model for monitoring and control of foodborne pathogens in wild boar's meat. Archives of Veterinary Medicine, 7, 2, 57-69. doi:10.46784/e-avm.v7i2.131.
25. Petrović J., Mirčeta J., Babić J., Malešević M., Blagojević B., Prodanov-Radulović J., Antić D. 2022. *Salmonella* in wild boars (*Sus scrofa*): Characterization and epidemiology. Acta Veterinaria Belgrade, 72, 2, 184-194. doi: 10.2478/acve-2022-0015.
26. Polaček V, Mirčeta J, Prodanov-Radulović J. 2021. Key risk factors and impact of African Swine Fever spreading on pig production in Serbia. Acta Veterinaria Belgrade, 71, 4, 371-391. doi:10.2478/acve-2021-0032.
27. Ramanzin M., Amici A., Casoli C., Esposito L., Lupi P., Marsico G., Mattiello S., Olivieri O., Ponzetta M.P., Russo C. 2010. Meat from wild ungulates: ensuring quality and hygiene of an increasing resource. Italian Journal of Animal Science, 9, 318-331. doi:10.4081/ijas.2010.e61.
28. Rodas E., Bogdanova T., Bossu T., Pecchi S., Tomasseti F., De Santis P., Tolli R., Condoleo R., Greco S., Brozzi A., Bilei S., Micarelli G., Martini E., Palazzetti M. 2014. Microbiological Assessment of Freshly-Shot Wild Boars Meat in Lazio Region, Viterbo Territory: a Preliminary Study. Italian Journal of Food Safety, 21, 1, 171. doi: 10.4081/ijfs.2014.1711.
29. Stojanac N., Stevančević O., Stančić I. 2013. Estimation of the *Salmonella* spp. prevalence in pig farms with dry and wet feeding. African Journal of Microbilogica Research, 7, 25, 3272-3274. doi: 10.5897/AJMR2013.5630.

30. Vieira-Pinto M., Morais L., Caleja C., Themudo P., Torres C., Igrejas G., Poeta P., Martins C. 2011. *Salmonella* sp. in game (*Sus scrofa* and *Oryctolagus cuniculus*). Foodborne Pathogen Disease, 8, 6, 739-740. doi: 10.1089/fpd.2010.0742.
31. Wacheck S., Fredriksson-Ahomaa M., König M., Stolle A., Stephan R. 2010. Wild boars as an important reservoir for foodborne pathogens. Foodborne Pathogen Disease, 7, 3, 307-312. doi: 10.1089/fpd.2009.0367.
32. Zou W., Lin W.J., Foley S.L., Chen C.H., Nayak R., Chen J.J. 2010. Evaluation of pulsed-field gel electrophoresis profiles for identification of Salmonella serotypes. Journal of Clinical Microbiology, 48, 9, 3122-6. doi: 10.1128/JCM.00645-10.

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KNOWLEDGE AND ATTITUDES OF VETERINARY STUDENTS IN SERBIA TOWARD FARM ANIMAL WELFARE

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Abstract

In this study, veterinary students from the Faculty of Veterinary Medicine, University of Belgrade and Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad were surveyed to evaluate their knowledge and attitudes toward farm animal welfare. Data were collected from 431 students by survey consisting of 39 closed-ended questions divided into two parts (demographic characteristics and a five-point Likert scale). Results showed that female students, students aged 18 to 21 years, from veterinary high schools, from urban areas, with mixed diets, who own pets, were predominated. Younger students and students from the Faculty of Veterinary Medicine, University in Belgrade agree significantly higher ($p<0.001$) that animal welfare is necessary for sustainable agriculture, food safety, biological functioning, emotional state, and natural behavior, as well as zootechnical procedures and rearing systems impairing the welfare of farm animals compared with students of the final year of studies, and from Department of Veterinary Medicine, Faculty of Agriculture in Novi Sad. Female students, and younger students, from urban areas, who own pets, have more concerned attitudes regarding farm animal welfare ($p<0.001$). The findings of this study confirm that attitudes toward farm animal welfare are not homogeneous and are associated with students' demographic char-

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acteristics. Also, results suggest that more attention should be paid to the curriculum and program to indirectly improve the welfare of farm animals.

Keywords: attitudes, farm animals, students, welfare

ZNANJE I STAVOVI STUDENATA VETERINE U SRBIJI PREMA DOBROBITI FARMSKIH ŽIVOTINJA

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Kratak sadržaj

U ovom istraživanju, student veterine sa Fakulteta veterinarske medicine, Univerziteta u Beogradu i Departmana za veterinarsku medicine, Poljoprivrednog fakulteta Univerziteta u Novom Sadu su anketirani kako bi se ispitalo znanje i stavovi o dobrobiti farmskih životinja. Podaci su prikupljeni od 431 studenata anketom koja se sastojala od 39 pitanja zatvorenog tipa podeljenih u dva dela (demografski podaci i Likertova skala). U istraživanju su dominirali student ženskog pola, studenti od 18 do 21 godine, poreklom iz srednjih veterinarskih škola, iz urbane sredine, sa mešovitom ishranom i koji poseduju kućnog ljubimca. Mlađi studenti i studenti Fakulteta veterinarske medicine Univerziteta u Beogradu se značajno više ($p<0.001$) slažu da je dobrobit životinja bitna za održivu poljoprivredu, bezbednost hrane, emotivno stanje životinja, prirodno ponašanje, kao i da zootehničke procedure i sistem gajenja životinja narušavaju dobrobit farmskih životinja u poređenju sa studentima starijih godina i studentima Departmana za veterinarsku medicine, Poljoprivrednog fakulteta Univerziteta u Novom Sadu. Studenti ženskog pola, mlađi studenti, studenti iz urbanih sredina, koji poseduju kućnog ljubimca imaju zabrinutije stavove o dobrobiti farmskih životinja u odnosu na ostale studente ($p<0.001$). Rezultati ovog istraživanja potvrđuju da stavovi prema dobrobiti farmskih životinja nisu homogeni i da su povezani sa demografskim karakteristikama. Takođe, rezultati sugerišu da bi više pažnje trebalo posvetiti nastavnom planu i programu kako bi se indirektno poboljšala dobrobit domaćih životinja.

Ključne reči: stavovi, farmske životinje, studenti, dobrobit

INTRODUCTION

Public opinion is the strongest driving force for improving animal welfare, but veterinarians are expected to adequately present the problem of breeding farm animals based on scientific facts, taking into account animal welfare. In addition to the multitude of scientific studies that have been conducted in recent decades, increasing public concern has led to stricter legislation on the conditions of farm animal breeding.

Public concern about animals used by humans is not a new phenomenon and has increased significantly over the last century. This began with the British Animal Welfare Acts of the nineteenth century (Buller et al., 2018), continued with the publication of Ruth Harrison's book "Animal Machines" in 1964, and was followed by the development of philosophical arguments in defense of animal welfare (Singer, 1975) and animal rights (Regan, 1985). As the veterinary profession has increasingly focused on animal welfare, scientific interest in the attitudes of veterinarians and veterinary students towards animals has increased, as they can influence public opinion on animal welfare. For example, entire systems of animal production are now considered unacceptable if they are adverse to animal welfare (Ryan, 1997).

Considering the physiological and behavioral needs of animals at the state level, changes in the educational and legislative system can positively impact the improvement of welfare. Students, especially those in veterinary medicine, agriculture, and natural sciences represent future generations of professionals who will closely collaborate with stakeholders in the animal industry, thus influencing how animals will be bred and treated (Phillips et al., 2012). Understanding the attitudes of students toward animals and their knowledge of their welfare is crucial. Analyzing these variables in samples of veterinary medicine students could lead to a better understanding of how future veterinarians perceive the welfare of different species, which should be a prerequisite for successfully enhancing animal welfare (Pirrone et al., 2019)

Veterinary medicine students have an ethical and professional obligation to respect, preserve, and improve animal welfare. In Serbia, veterinarians take a professional oath of allegiance, pledging to use their skills and knowledge for the benefit of animal health and welfare. The attitudes of veterinarians toward animal welfare stem, at least partially, from their training (Crook, 2000), and veterinary students are expected to demonstrate a high level of professional interest in animal welfare. Therefore, understanding the perspectives, attitudes, and perceptions of students on these issues is fundamental, as they can serve as an indirect measure of educational adequacy and effectiveness (Heleski et al., 2005). Resolving animal welfare issues on farms is not simple, as it involves many aspects of a complex nature. The purpose of this study was to determine the knowledge and attitudes of veterinary students in Serbia toward farm animal welfare.

MATERIAL AND METHODS

The study protocol was approved by the Animal Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade, Serbia (Approval number, 03-12/2022).

During the period from December 2022 to January 2023 a total of 431 students from the Faculty of Veterinary Medicine, University of Belgrade (FVM) and the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad (DNS), were surveyed online using the Google Forms platform. The research covered students from all years of the integrated academic program. Participants were informed that participation in the survey was voluntary and anonymous and that the survey results would be used for scientific research and animal welfare improvement.

The survey consisted of 39 closed-ended questions divided into two parts. The first group of questions related to the demographic characteristics of the respondents (gender, age, education level, area of residence, dietary habits, pet ownership, farm animal ownership, year of study and study location). The second part of the survey contained a series of fifteen statements presented using a five-point Likert scale (1 - strongly disagree to 5 - strongly agree), where higher numbers indicated a higher level of empathy between students towards farm animals (Ostović et al., 2016). The survey focused on the welfare of farm animals in Serbia.

Statistical analysis

For statistical data analysis SPSS v17.0 software was used. To determine the frequency of students' responses and their attitudes, the mean value ($\bar{x}$) and standard error (SE) were calculated from the Likert scale. The differences in attitudes between different demographic characteristics of students as well as different study years were analyzed using the nonparametric Mann-Whitney U-test and Kruskal-Wallis test on the equality of the medians, adjusted for ties. When significant differences were found, the Dunn-Bonferroni post hoc test was performed. The level of significance for which results were considered statistically significant in these studies was set at $p<0.05$.

RESULTS

The demographic characteristics of the respondents (gender, age, education level, area of residence, dietary habits, pet ownership, farm animal ownership, year of study and study location) are present in Table 1. As shown in Table 1, predominated the female students (73.09% - 315/431), aged 18 to 21 years (48.26% - 208/431), from veterinary high schools (46.40% - 200/431), and urban areas (78.42% - 338/431). Students who consume mixed diet were the most represented (89.56% - 386/431), those who have pets (86.77% - 374/431), and those who do not own farm animals.

Table 1. Demographic characteristics of students from the Faculty of Veterinary Medicine, University of Belgrade (FVM), and the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad (DNS)

Demographic characteristic			Faculty				
	FVM (n=357)		DNS (n=74)		Total (n=431)		
	N	%	N	%	N	%	
Gender	Male	90	25.21	17	22.97	107	24.83
	Female	262	73.39	53	71.62	315	73.09
	Other	5	1.40	4	5.41	9	2.09
Age	18-21	175	49.02	33	44.59	208	48.26
	22-24	110	30.81	30	40.54	140	32.48
	Over 24	72	20.17	11	14.86	83	19.26
Education	Gymnasium	121	33.89	35	47.30	156	36,19
	Veterinary school	177	49.58	23	31.08	200	46.40
	Other	59	16.53	16	21.62	75	17.40
Living area	Urban	286	80.11	52	70.27	338	78.42
	Rural	71	19.89	22	29.73	93	21.58

Demographic characteristic		Faculty					
		FVM (n=357)		DNS (n=74)		Total (n=431)	
Diet	Mixed	317	88.80	69	93.24	386	89.56
	Vegetarian	32	8.96	4	5.41	36	8.35
	Vegan	8	2.24	1	1.35	9	2.09
Pet ownership	Yes	312	87.39	62	83.78	374	86.77
	No	45	12.61	12	16.22	57	13.23
Farm animal ownership	Yes	89	24.93	24	32.43	113	26.22
	No	268	75.07	50	67.57	319	74.01
Year of study	I	86	24.09	23	31.08	109	25.29
	II	66	18.49	6	8.11	72	16.71
	III	50	14.01	7	9.46	57	13.23
	IV	45	12.61	10	13.51	55	12.76
	V	47	13.17	8	10.81	55	12.76
	VI	63	17.65	20	27.03	83	19.26

Female students from FVM and DNS were the most present (73.39% - 262/357; 71.26% - 53/74), as well as students aged 18 to 21 years (49.58% - 177/357; 43.24% - 32/74), with mixed diet (88.80% - 317/357; 93.24% - 69/74), from gymnasium (49.58% - 177/357) and veterinary high school (47.30% - 35/74), students who own pets (87.39% - 312/357; 83,78% - 62/74), and those who do not own farm animals (75.07% - 268/357; 67.57% - 50/74) (Tables 2 and 3).

Table 2. Demographic characteristics of students from the Faculty of Veterinary Medicine, University of Belgrade (FVM)

FVM / year of study		I (n=86) N (%)	II (n=66) N (%)	III (n=50) N (%)	IV (n=45) N (%)	V (n=47) N (%)	VI (n=63) N (%)	Total (n=357) N (%)
Gender	Male	14 (16.28)	19 (28.79)	10 (20)	10 (22.22)	15 (31.91)	22 (34.92)	90 (25.21)
	Female	72 (83.72)	46 (69.70)	39 (78)	34 (75.56)	32 (68.09)	39 (61.90)	262 (73.39)
	Other	0	1 (1.52)	1 (2)	1 (2.22)	0	2 (3.17)	5 (1.40)
Age	18-21	85 (98.84)	61 (92.42)	28 (56)	3 (6.67)	0	0	177 (49.58)
	22-24	0	5 (7.58)	18 (36)	34 (75.56)	37 (78.72)	16 (25.40)	110 (30.81)
	over 24	3 (3.49)	0	4 (8)	8 (17.78)	10 (21.28)	47 (74.60)	72 (20.17)
Education	Veterinary school	30 (34.88)	27 (40.91)	15 (30)	13 (28.89)	15 (31.91)	21 (33.33)	121 (33.89)
	Gymnasium	41 (47.67)	29 (43.94)	27 (54)	22 (48.89)	23 (48.94)	35 (55.56)	177 (49.58)
	Other	15 (17.44)	10 (15.15)	8 (16)	10 (22.22)	9 (19.15)	7 (11.11)	59 (16.53)
Diet	Mixed	80 (93.02)	52 (78.79)	45 (90)	37 (82.22)	43 (91.49)	60 (95.24)	317 (88.80)
	Vegetarian	4 (4.65)	12 (18.18)	4 (8)	6 (13.33)	3 (6.38)	3 (4.76)	32 (8.96)
	Vegan	2 (2.33)	2 (3.03)	1 (2)	2 (4.44)	1 (2.13)		8 (2.24)
Pet ownership	Yes	80 (93.02)	62 (93.94)	38 (76)	42 (93.33)	38 (80.85)	52 (82.54)	312 (87.39)
	No	6 (6.98)	4 (6.06)	12 (24)	3 (6.67)	9 (19.15)	11 (17.46)	45 (12.61)
Farm animal ownership	Yes	20 (23.26)	20 (30.30)	10 (20)	11 (24.44)	8 (17.02)	20 (31.75)	89 (24.93)
	No	66 (76.74)	46 (69.70)	40 (80)	34 (75.56)	39 (82.98)	43 (68.25)	268 (75.07)

Based on the overall mean values (Table 4), veterinary students in Serbia agree that animal welfare is necessary for sustainable agriculture, food safety, biological functioning, the emotional state of animals, and natural behavior. However, older students as well as DNS students agreed significantly less ($p<0.001$, $p<0.05$) with the above statements compared to younger students and FVM students (Table 4).

Table 3. Demographic data of students from the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad (DNS)

DNS / year of study		I (n=23) N (%)	II (n=6) N (%)	III (n=7) N (%)	IV (n=10) N (%)	V (n=8) N (%)	VI (n=20) N (%)	Total (n=74) N (%)
Gender	Female	15 (65.22)	4 (66.67)	4 (57.14)	8 (80)	7 (87.50)	15 (75)	53 (71.26)
	Male	4 (17.39)	2 (33.33)	3 (42.86)	2 (20)	1 (12.50)	5 (25)	17 (22.97)
	Other	4 (17.39)	0	0	0	0	0	4 (5.41)
Age	18-21	22 (95.65)	6 (100)	4 (57.14)	0	0	0	32 (43.24)
	22-24	1 (4.35)	0	3 (42.86)	10 (100)	7 (87.50)	10 (50)	31 (41.89)
	over 24	0	0	0	0	1 (12.50)	10 (50)	11 (14.86)
Education	Veterinary school	8 (34.78)	4 (66.67)	5 (71.43)	4 (40)	7 (87.50)	7 (35)	35 (47.30)
	Gymnasium	9 (39.13)	1 (16.67)	1 (14.29)	2 (20)	1 (12.50)	9 (45)	23 (31.08)
	Other	6 (26.09)	1 (16.67)	1 (14.29)	4 (40)	0	4 (20)	16 (21.62)
Diet	Omnivores	22 (95.65)	6 (100)	7 (100)	10 (100)	7 (87.50)	17 (85)	69 (93.24)
	Vegetarian	1 (4.35)	0	0	0	1 (12.50)	3 (15)	5 (6.76)
	Vegan	0	0	0	0	0	0	0
Pet ownership	Yes	21 (91.30)	5 (83.33)	6 (85.71)	6 (60)	8 (100)	16 (80)	62 (83.78)
	No	2 (8.70)	1 (16.67)	1 (14.29)	4 (40)	0	4 (20)	12 (16.22)
Farm animal ownership	Yes	6 (26.09)	3 (50)	4 (57.14)	2 (20)	3 (37.50)	6 (30)	24 (32.43)
	No	17 (73.91)	3 (50)	3 (42.86)	8 (80)	5 (62.50)	14 (70)	50 (67.57)

Table 4. Mean values (±SE) of student attitudes regarding the role of animal welfare in agricultural sustainability, food safety, biological functioning, emotional state, and expression of natural behaviors

Year of study / Questions		I	II	III	IV	V	VI	In total
I	FVM	4.61±0.0^{9**}*	4.44±0.10	4.44±0.11	4.37±0.13	4.38±0.13	4.32±0.14***	4.43±0.04
	DNS	3.98±0.15	4.50±0.22	4.43±0.30	4.30±0.21	4.63±0.18	3.83±0.17	4.28±0.13
	Total	4.63±0.06$^{ab\ CdE}$	4.44±0.10^{a}	4.44±0.10^{b}	4.36±0.10^{C}	4.42±0.11^{d}	4.33±0.11^{E}	4.44±0.04
II	FVM	4.78±0.06	4.76±0.06	4.62±0.09	4.64±0.12	4.51±0.14	4.60±0.11	4.65±0.04
	DNS	4.87±0.10	4.33±0.33	5.00	4.60±0.22	5.00	4.75±0.16	4.76±0.11
	Total	4.79±0.05	4.72±0.06	4.67±0.08	4.64±0.10	4.58±0.12	4.64±0.09	4.67±0.03
III	FVM	4.72±0.07	4.61±0.08	4.76±0.08	4.69±0.09	4.57±0.12	4.71±0.09	4.68±0.03
	DNS	4.35±0.22	4.00±0.63	4.86±0.14	4.50±0.34	4.88±0.13	4.65±0.18	4.53±0.11
	Total	4.64±0.07	4.56±0.09	4.77±0.07	4.66±0.09	4.62±0.11	4.70±0.08	4.65±0.03
IV	FVM	4.77±0.06	4.77±0.09	4.98±0.02	4.82±0.07	4.77±0.10	4.73±0.11	4.83±0.03
	DNS	4.52±0.20	5.00	5.00	4.80±0.20	5.00	4.70±0.22	4.74±0.09
	Total	4.73±0.06	4.79±0.09	4.98±0.02	4.82±0.06	4.80±0.09	4.72±0.10	4.81±0.03
V	FVM	4.68±0-06*	4.64±0-10	4.88±0-07^{A}	4.89±0-05	4.43±0-14^{b}	4.67±0.10^{Ab}	4.72±0.04
	DNS	4.39±0.19	4.50±0.34	4.86±0.14	4.60±0.22	4.88±0.13	4.80±0.09	4.64±0.08
	Total	4.73±0.06	4.63±0.10	4.88±0-06^{A}	4.84±0-06^{b}	4.49±0.12^{Ab}	4.70±0.08	4.71±0.03

*** and capital letters - p<0.001; * and lowercase letters - p<0.05; an asterisk denotes significance between different faculties while letters denote significance within one faculty; I – Farm animal welfare role in sustainable agriculture, II – Farm animal welfare role in food safety and quality, III – Farm animal welfare role in biological functioning, IV – Farm animal welfare role in emotional state, V – Farm animal welfare role in expression of natural behaviors.

In Table 5 the results of the attitudes of students toward cognitive abilities in animals. Among first-year FVM students, the average values of attitudes regarding the awareness of farm animals were significantly higher (p<0.05; p<0.001) compared to the attitudes of first-year DNS students (Table 5). FVM, DNS, and first-year students believe that poultry is significantly less capable of thinking (p<0.05; p<0.001) compared to other farm animals (Table 5).

Table 5. Mean (±SE) of student attitudes regarding the cognitive abilities of farm animals

Year of study / Questions			I	II	III	IV	V	VI	In total
VI	Cattle	FVM	4.65±0.07***	4.38±0.13	4.66±0.09*	4.49±0.14*	4.53±0.10	4.44±0.14	4.53±0.05***
		DNS	4.13±0.20	4.67±0.21	4.14±0.26	3.70±0.37a	4.88±0.13a	4.40±0.28	4.27±0.12
		In total	4.54±0.07	4.40±0.12	4.60±0.09	4.35±0.14	4.58±0.08	4.43±0.12	4.49±0.04
	Pigs	FVM	4.56±0.07***	4.47±0.12	4.64±0.08	4.47±0.15	4.53±0.12	4.41±0.14	4.51±0.05*
		DNS	4.04±0.20	5.00	4.29±0.29	3.90±0.38	4.75±0.16	4.45±0.28	4.31±0.12
		In total	4.56±0.07	4.51±0.12	4.59±0.08	4.36±0.15	4.56±0.10	4.42±0.12	4.50±0.04
	Poultry	FVM	4.33±0.11**	4.06±0.14	4.30±0.13	4.20±0.18*	4.30±0.13	3.92±0.16	4.18±0.06*
		DNS	3.52±0.27a	4.17±0.40	3.86±0.40	3.40±0.34a	4.63±0.18a	4.10±0.30	3.86±0.14
		In total	4.16±0.11	4.07±0.13	4.25±0.12	4.06±0.16	4.35±0.12	3.96±0.14	4.13±0.05
	Sheep	FVM	4.57±0.09***	4.29±0.14	4.62±0.09*	4.47±0.14***	4.40±0.12	4.30±0.14	4.44±0.05***
		DNS	3.91±0.23a	4.50±0.34	4.00±0.38	3.60±0.34a	4.75±0.16e	4.30±0.28	4.12±0.13
		In total	4.43±0.03	4.31±0.10	4.54±0.05	4.31±0.07	4.46±0.03	4.30±0.02	4.39±0.05
	Goats	FVM	4.62±0.08*	4.35±0.13	4.62±0.09**	4.53±0.14*	4.47±0.12	4.32±0.14	4.48±0.05*
		DNS	4.00±0.23a	4.67±0.21b	4.00±0.37a	3.60±0.34a.b	4.88±0.13E	4.35±0.27	4.19±0.12
		In total	4.49±0.08	4.38±0.12	4.54±0.10	4.36±0.14	4.53±0.11	4.33±0.13	4.43±0.04
VII	Cattle	FVM	4.71±0.07	4.64±0.10	4.60±0.09	4.36±0.17	4.50±0.12	4.37±0.13	4.55±0.04
		DNS	4.48±0.13	4.67±0.21	4.14±0.44	4.20±0.47	4.88±0.13	4.20±0.30	4.39±0.12
		In total	4.66±0.06	4.64±0.09	4.54±0.10	4.33±0.16	4.55±.0.11	4.33±0.13	4.52±0.04
	Pigs	FVM	4.65±0.07	4.58±0.11	4.46±0.13	4.33±0.17	4.47±0.12	4.32±0.14	4.48±0.05
		DNS	4.30±0.18	4.83±0.17	4.14±0.45	4.00±0.47	4.88±0.13	4.20±0.30	4.32±0.13
		In total	4.59±0.07	4.60±0.10	4.42±0.12	4.27±0.16	4.53±0.10	4.29±0.13	4.46±0.05
	Poultry	FVM	4.38±0.11	4.24±0.12	4.37±0.15	4.04±0.18	4.26±0.16	3.97±0.16	4.22±0.06
		DNS	3.96±0.22	3.50±0.56	4.00±0.49	3.30±0.54	4.63±0.26	3.95±0.34	3.91±0.15
		In total	4.29±0.12	4.18±0.12	4.32±0.14	3.91±0.18	4.31±0.14	3.96±0.14	4.17±0.06
	Sheep	FVM	4.63±0.07	4.53±0.10	4.48±0.13	4.31±0.16	4.38±0.13	4.35±0.13	4.46±0.05
		DNS	4.44±0.16	4.50±0.34	4.14±0.46	4.00±0.47	4.88±0.13	4.10±0.30	4.31±0.13
		In total	4.59±0.07	4.53±0.10	4.44±0.12	4.26±0.16	4.46±0.11	4.29±0.13	4.41±0.05
	Goats	FVM	4.63±0.07	4.53±0.11	4.50±0.12	4.38±0.16	4.36±0.13	4.33±0.14	4.47±0.05
		DNS	4.48±0.15	4.50±0.34	4.14±0.45	4.00±0.47	4.88±0.13	4.10±0.30	4.32±0.13
		In total	4.59±0.06	4.53±0.11	4.46±0.12	4.31±0.16	4.44±0.12	4.28±0.13	4.45±0.05

*** and capital letters - p<0.001; * and lowercase letters - p<0.05; an asterisk denotes significance between different faculties while letters denote significance within one faculty; VI - Are animals sentient beings. VII - Do animals have feelings.

As shown in Table 6, first-year students and students of FVM significantly more ($p<0.05$; $p<0.001$) considered that zootechnical procedures and rearing systems impaired the welfare of farm animals compared to students of older years and students of DNS. FVM students in Serbia believed that dehorning cattle without anesthesia (4.49±0.05) and unenriched cage housing systems (4.37±0.05) most negatively influenced the welfare of farm animals.

Table 6. Mean (±SE) of student attitudes regarding zootechnical procedures on farms

Year of study / Questions		I	II	III	IV	V	VI	In total
VIII	FVM	4.60±0.08^{A**}	4.12±0.16A	4.38±0.15**	4.16±0.15A	3.72±0.18A	3.95±0.17A	4.11±0.06**
	DNS	3.87±0.26	4.00±0.26	3.43±0.30	3.80±0.39	3.88±0.23	3.60±0.30	3.78±0.13
	Total	4.46±0.09^{Aa}	4.11±0.14^{a}	4.26±0.14	4.09±0.14^{A}	3.74±0.16^{A}	3.87±0.15^{A}	4.05±0.06
IX	FVM	4.55±0.11$^{A\ a}$	4.36±0.14	4.56±0.13^{C}	4.38±0.13	4.13±0.16^{AC}	4.21±0.15$^{a\ c}$	4.38±0.06
	DNS	4.34±0.23	4.51±0.50	4.00±0.38	4.20±0.33	4.75±0.16	4.20±0.24	4.31±0.12
	Total	4.50±0.11^{A}	4.37±0.13	4.49±0.12	4.34±0.13	4.21±0.14^{A}	4.20±0.13^{A}	4.37±0.05
X	FVM	4.68±0.08^{A**}	4.41±0.14	4.32±0.16	4.33±0.16	4.04±0.17^{A}	3.95±0.18^{A}	4.32±0.06**
	DNS	4.00±0.28	3.83±0.65	4.42±0.30	3.80±0.44	4.00±0.46	3.90±0.32	3.96±0.15
	Total	4.53±0.09^{A}	4.36±0.14	4.33±0.15	4.24±0.15	4.04±0.16^{A}	3.94±0.15^{A}	4.26±0.06
XI	FVM	4.50±0.10^{A**}	4.09±0.14^{b**}	3.60±0.18^{Ab}	3.84±0.18^{A}	3.23±0.18^{Ab}	3.49±0.17^{Ab}	3.87±0.07*
	DNS	3.69±0.30	2.50±0.56^{c}	4.14±0.40^{c}	3.20±0.42	3.37±0.55	3.45±0.32	3.47±0.16
	Total	4.33±0.1.070^{A}	3.96±0.15^{ab}	3.67±0.17^{A}	3.73±0.17^{A}	3.26±0.18^{Ab}	3.48±0.15^{A}	3.80±0.06
XII	FVM	4.62±0.07^{A**}	4.00±0.16^{A}	3.58±0.20^{A}	3.82±0.18^{A}	3.74±0.19^{A}	3.89±0.16^{A}	4.01±0.06*
	DNS	3.74±0.27	3.67±0.61	3.43±0.48	3.40±0.42	3.63±0.53	3.70±0.30	3.64±0.15
	Total	4.44±0.09^{A}	3.97±0.15	3.56±0.18^{A}	3.74±0.17^{A}	3.73±0.17^{A}	3.84±0.14^{A}	3.95±0.06
XIV	FVM	4.81±0.06^{Aa**}	4.54±0.12	4.40±0.11	4.76±0.10^{d**}	4.17±0.16^{Ad}	4.32±0.15^{a}	4.53±0.05*
	DNS	4.08±0.25	4.33±0.66	4.85±0.14	4.00±0.37	4.63±0.26	4.30±0.23	4.28±0.13
	Total	4.66±0.08^{A}	4.53±0.12^{b}	4.45±0.11	4.62±0.11^{D}	4.23±0.14^{ADb}	4.31±0.13^{AD}	4.49±0.05
XV	FVM	4.65±0.07^{Aa***}	4.32±0.12	4.06±0.16^{A}	4.22±0.17^{a}	4.04±0.16^{A}	4.11±0.16^{A}	4.28±0.06***
	DNS	3.78±0.25	3.50±0.56	4.43±0.30	3.60±0.50	4.37±0.18	4.15±0.25	3.96±0.14
	Total	4.46±0.09^{A}	4.25±0.13	4.11±0.15	4.11±0.16	4.09±0.14^{A}	4.12±0.14	4.22±0.05

*** and capital letters - $p<0.001$; * and lowercase letters - $p<0.05$; an asterisk denotes significance between different faculties while letters denote significance within one faculty; VIII - tie-stall housing system without access to exercise is cruel, IX - conventional cage housing system of hens affect welfare, X – piglet castration without anesthesia affect welfare, XI - Teeth-clipping in piglets affect welfare. XII - tail docking in piglet affect welfare. XIV - dehorning cattle without anesthesia affects welfare. XV - tail docking in lambs affects welfare.

A statistically significant difference in attitudes was found between different demographic groups of students (Table 7). Female, and younger students significantly more ($p<0.001$) agree that animal welfare is essential for sustainable agriculture, food safety, biological functioning, emotional state, and natural behavior, as well as that zootechnical procedures and housing systems compromise the welfare of farm animals, compared to other students. Students aged 18 to 21, from veterinary and other high schools, from urban areas, who own pets, significantly more ($p<0.05$; $p<0.001$) believed that zootechnical procedures and housing methods compromise the welfare of farm animals compared to other students. A significant difference ($p<0.05$) in attitudes about zootechnical procedures and housing systems was found between FVM and DNS students. No significant difference was found between different demographic groups of students regarding the cognitive abilities of farm animals ($p>0.05$) (Table 7).

Table 7. Influence of student demographic characteristics on the role of animal welfare, cognitive abilities of animals, and zootechnical procedures on farms

Demographic characteristic		Role of animal welfare	Cognitive abilities of animals	Zootechnical procedures on farms
Gender	Female	4.77±0.02^{A}	3.630.03	4.34±0.04^{A}
	Male	4.49±0.06	3.51±0.07	3.57±0.10
Age	18-21	4.72±0.03	3.63±0.04	4.31±0.06^{a}
	22-24	4.67±0.05	3.56±0.04	4.08±0.0733^{a}
	over 24	4.68±0.06	3.57±0.062	3.99±0.10^{a}
Education	Gymnasium	4.62±0.05	3.57±0.05	3.91±0.08^{AB}
	Veterinary school	4.73±0.05	3.59±0.03	4.30±0.06^{B}
	Other	4.76±0.05	3.68±0.06	4.40±0.08^{A}
Living area	Urban	4.73±0.03^{A}	3.60±0.03	4.29±0.04^{A}
	Rural	4.57±0.06	3.57±0.06	3.74±0.11
Diet	Mixed	4.68±0.03^{ab}	3.58±0.03	4.12±0.05^{AB}
	Vegetarian	4.83±0.06^{a}	3.67±0.10	4.59±0.10^{B}
	Vegan	4.95±0.04^{b}	3.60±0.05	4.89±0.07^{A}
Pet ownership	Yes	4.72±0.03^{a}	3.62±0.03	4.21±0.05^{a}
	No	4.53±0.08	3.50±0.09	3.90±0.13
Farm animal ownership	Yes	4.60±0.06	3.63±0.06	3.78±0.10
	No	4.73±0.03^{a}	3.58±0.04	4.31±0.05^{A}
Year of study	I	4.74±0.04^{a}	3.62±0.04	4.49±0.07^{A}
	II	4.66±0.06	3.68±0.05	4.25±0.12^{b}
	III	4.79±0.04^{b}	3.62±0.08	4.13±.09^{A}
	IV	4.70±0.05	3.49±0.09	4.10±0.10^{A}
	V	4.59±0.08^{ab}	3.67±0.07	3.90±0.11^{Ab}
	VI	4.66±0.07	3.50±0.07	3.98±0.11^{A}
Place of study	FVM	4.70±0.03	3.60±0.03	4.23±0.05^{a}
	DNS	4.68±0.05	3.56±0.07	3.91±0.11

Capital letters - $p<0.001$; lowercase letters - $p<0.05$.

DISCUSSION

Teaching veterinary students does not only imply professional education but also plays a significant role in shaping and educating these professionals on how to deal with emotionally challenging aspects of veterinary work, as well as in developing their attitudes and opinions on animal welfare as the primary task of every veterinarian (Main et al., 2009). Veterinarians are expected to promote positive attitudes towards animals and advocate for their welfare (Hernandez et al., 2018). However, previous studies have shown that veterinary students' attitudes differ concerning animal species (Magnani et al., 2017; Mariti et al., 2018; Pirrone et al., 2019) and demographic data (Izmirli and Phillips, 2012; Pirrone et al., 2019). The results of this research are the first to address the attitudes of Serbian veterinary students towards the welfare of farm animals. Animal welfare is essential for sustainable agriculture (Keeling, 2005; Broom, 2021), food safety and quality (Viegas et al., 2011), biological functioning, emotional states, and expression of natural behaviors (Fraser, 2008; Mellor, 2016). In this study, veterinary students in Serbia agree that animal welfare is important for farm animals. However, first-year students consider animal welfare more critical for sustainable agriculture and food safety compared to older students, which is the line with the results of other researchers (Ostović et al., 2016). Animal welfare is a complex concept that includes three elements: the animal's normal biological functioning (health, productivity), its emotional state (absence or presence of pain, fear, boredom), and its ability to express certain normal behaviours (Fraser et al., 1997). Each of these elements has its own merits but none of them fully depict the animal's welfare independently; it cannot fully present the state of the animal welfare. Therefore, it is accepted that animal welfare encompasses all three areas: physical health, behavior and emotions (Duncan and Fraser, 1997; Mendl, 2001). In line with this, students in this study highly rated that animal welfare affects emotional states, biological functioning, and expression of natural behaviors. Scientific studies on veterinary students' attitudes towards animal welfare have been conducted at several British universities (Paul and Podberscek, 2000) and have shown a correlation between the year of study and students' feelings towards social and farm animals. Specifically, final-year students showed lower levels of empathy than those in the early years of study (Pollard-Williams et al., 2014). The results of this research are in line with the previous research that older students, as well as students from the Department of Veterinary Medicine, Faculty of Agriculture in Novi Sad, significantly less agreed with the statements about farm animal welfare and showed less empathy compared to younger students and students from the Faculty of Veterinary Medicine, University of Belgrade. The lower empathy for higher years of study seems counterintuitive regarding veterinary education. Physiology, anesthesia, pharmacology and many other subjects within the veterinary program are filled with information about physiological pain and stress. Lower empathy in higher years of veterinary students could be a mechanism to cope with disturbing events manifested in veterinary practice (Paul and Podberscek, 2000; Batchelor and McKeegan, 2012). Animal welfare is based on ethical principles that animals are conscious and sentient beings, capable of experiencing pain, stress, fear, frustration and other unpleasant and positive emotions (Duncan, 2006). Regarding the cognitive abilities of animals, this study found that veterinary students (from DNS compared with FVM) believe that poultry has fewer cognitive abilities and feelings compared to other farm animals, while cattle and pigs are ranked with the highest cognitive abilities. These results are in line with other research (Levine et al., 2005; Ostović et al., 2017; Shtylla Kika et al., 2023) who found that students were more likely to agree that cattle and pigs are more intelligent compared to poultry. Animal welfare protection is one of the main tasks of the veterinary profession. One of the deontological principles, the principle of equality, refers to the obligation to treat each vertebrate fairly and equally since has been established that all vertebrates can experience pain (National Research Council, 2009). According to Viñuela-Fernández et al. (2011), zootechnical procedures performed on farm animals, usually without the use of anesthesia and analgesia, are the most striking examples of inducing pain in animals. In this study, veterinary students believe that pig's castration, and cattle dehorning without anesthesia were zootechnical procedures that most disrupt animal welfare. Also, first-year students significantly inre agree that these zootechnical procedures compromise animal welfare compared to final-year students, which is following other research (Paul and Podberscek, 2000; Pollard-Williams et al., 2014; Ostović et al., 2017). In many veterinary faculties, including in Serbia, where a decline in overall empathy of students towards animals has been observed, and the curse of animal welfare is taught in the first year (Abood and Siegford, 2012). Because of that, it could be considered to include this subject in the final years of study. Additionally, students who own farm animals are less empathic towards zootechnical procedures compared to other students. Previous research has found that students who own farm animals and have farm experience have a reduced concern for animal rights and welfare own farm animals (Serpell, 2005, Herzog et al., 1991; Bjerke et al., 1998). Also, this result could be a consequence of the knowledge of zootechnical procedures by the students, and thus they are more acceptable to them compared to students who do not own farm animals. Gender distribution among students in this study revealed female predominance across all study years, consistent with the global trend of increased female presence in the veterinary profession (Lofstedt, 2003; Irvine and Vermilya, 2010). In this study, female students significantly agreed that animal welfare is essential for sustainable agriculture, food safety and quality, biological functioning, emotional state and natural living, as well as the zootechnical practices and rearing systems on farm animals compared to male students and others. These results are in agreement with those recorded by Paul and Podberscek, 2000; Serpell, 2005; Hazel et al., 2011; Van der Weijden, 2013; Ostović et al., 2017) who conducted concerning attitudes of veterinary students, veterinarians, members of veterinary faculties and consumers regarding animal welfare, indicates that female respondents tend to hold more positive attitudes compared to males. Kendall et al. (2006) reported that females' are primarily family caretakers, engaged in household tasks, and have more contact with animals; they are more likely to understand animal needs and have a more positive attitude. According to Ormandy and Schuppli (2014), the difference in attitudes towards animal welfare between genders can also be ascribed to the "moralistic" attitude of females toward the animals, while men express more "dominionistic" attitudes. In this study, the male respondents were less likely to believe that animals can experience emotions such as love, boredom, depression and anxiety. The males show more skepticism (Walker et al., 2014), since unlike females, they are also less likely to believe that animals can exhibit certain changes in behavior when experiencing suffering. As the trend of enrolling female veterinary students increases in many countries (Irvine and Vermilya, 2010; Ostović et al., 2016), it could potentially lead to improvements in animal welfare in the future. However, female veterinary graduates are more likely to focus on social rather than farm animals (Lofstedt, 2003), which means that the agriculture sector, which is covered by predominantly male veterinarians, will not benefit.

In our study, students aged 18 to 21, as well as those who graduated from veterinary secondary schools and other secondary schools, were significantly more likely to believe that zootechnical procedures and methods of keeping harm the welfare of farm animals compared to students aged 22 to 24 and over 24, and those who finished high school. These results could be ascribed that attitudes towards animal welfare become more positive from childhood to adolescence, but after that, attitudes tend to become more negative (Kendall et al., 2006; Binngiesser et al., 2013; Martens et al., 2019). These results suggest that attitudes towards animal welfare depend on age, tradition, and educational approach in a particular region. In this study, predominated students who consumed mixed diet, while significantly fewer identified as vegetarians, whose diet consists mainly of plant-based foods, while a small number declared themselves vegan, meaning they do not consume any animal products in their diet and do not use items made from fur, leather or any other animal-derived materials.

In this study, students from veterinary medicine high school have significantly higher attitudes toward zootechnical procedures compared with other secondary schools. This result can be ascribed to the fact that students who attended veterinary medicine high school had more exposure to farm animals, their breeding practices and the implementation of zootechnical procedures on farms throughout their education and practice compared to students who completed gymnasium. For these reasons, they are more informed about how poor zootechnical procedures, usually without the use of anesthesia and analgesia, and housing can disrupt the welfare of farm animals. Students who were raised in rural areas were found to be less empathic toward farm animal welfare than those with urban backgrounds. This finding could be explained by different opportunities for contact and relationship with animals offered by rural and urban environments. People from urban areas are less likely to have contact with animals they eat and are spared from watching the animal slaughter. Instead, in urban areas, animals are perceived as companions and family members, given names, and attributed human characteristics (anthropomorphism) and this could contribute to urban individuals expressing a higher level of concern for the welfare of farm animals (Pifer et al., 1994; Ormandy and Schuppli, 2014).

CONCLUSION

The attitudes of veterinary students in Serbia express a high level of empathy towards farm animals. They mostly associate animal welfare with biological functioning. The research results indicate that students do not perceive different species of animals equally in terms of cognitive abilities and zootechnical procedures. Generally, the attitudes of first-year students were more positive than those of final-year students, indicating a lower level of empathy towards animals. The results of this study raise the question of whether these future generations of veterinarians can promote animal welfare in Serbia and what their level of competitiveness will be in the international labor market. These results suggest that more attention should be paid to the curriculum and programs to indirectly improve the welfare of farm animals. Specifically, it is necessary to “convince” students that today’s animal husbandry is not only about the survival of animals, but above all about the quality of their lives. Differences among universities should be investigated further.

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Author's contributions

K.N. designed the investigation, interpreted the results, performed the statistical analysis and drafted the manuscript. D.V. performed investigation and drafting of the manuscript. M.Đ. and M.V. revised the manuscript critically and together with D.V. prepared the final draft of the manuscript. M.Đ.S. made contributions to the conception and design of the study. All authors read and approved the final manuscript.

Competing interest

The author(s) declare that they have no competing interests

REFERENCES

1. Abood S.K., Siegford J.M. 2012. Student perceptions of an animal-welfare and ethics course taught early in the veterinary curriculum. Journal of Veterinary Medical Education, 39/2, 136–41. doi:10.3138/jvme.0911.093R1.
2. Batchelor C.E.M., McKeegan D.E.F. 2012. Survey of the frequency and perceived stressfulness of ethical dilemmas encountered in UK veterinary practice. Veterinary Record, 170/1, 19–22. doi:10.1136/vr.100262.
3. Binngiesser J., Wilhelm C., Randler C. 2013. Attitudes towards animals among German children and adolescents. Anthrozoös, 26, 325–339. doi:10.2752/175303713X13697429463475.
4. Bjerke T., Ødegårdstuen T.S., Kaltenborn B.P. 1998. Attitudes toward animals among Norwegian adolescents. Anthrozoös, 11, 79–86. doi:10.2752/089279398787000742.
5. Broom D. 2021. Farm animal welfare: a key component of the sustainability of farming systems. Veterinarski Glasnik, 75/2, 145-151. doi:10.2298/VETGL210514007B.
6. Buller H., Blokhuis H., Jensen P., Keeling L. 2018. Towards farm animal welfare and sustainability. Animals, 8, 81. doi:10.3390/ani8060081.
7. Crook A. 2000. The CVMA animal abuse position–how we got here. Canadian Veterinary. Journal, 41, 8, 631–635.

8. Duncan I.J.H. 2006. The changing concept of animal sentience. Applied Animal Behavior Science, 100, 11. doi:10.1016/j.applanim.2006.04.011.
9. Duncan I.J.H., Fraser D. 1997. Understanding animal welfare, In *Animal Welfare*. Eds. M.C. Appleby and B.O. Hughes, Wallingford: CAB International, 19–31.
10. Fraser D., Weary D.M., Pajor E.A., Milligan B.N. 1997. A scientific conception of animal welfare that reflects ethical concerns. Anim. Welfare 6, 187–205.
11. Fraser D. 2008. Understanding animal welfare. Acta Veterinaria Scandinavica 50/1, S1. doi:10.1186/1751-0147-50-S1-S1.
12. Harrison R. 1964. Animal Machines: The new factory farming industry. Vincent Stuart Ltd, London.
13. Hazel S.J., Signal T.D., Taylor N. 2011. Can teaching veterinary and animal-science students about animal welfare affect their attitude toward animals and human-related empathy? Journal of Veterinary Medical Education 38: 74-83. doi: 10.3138/jvme.38.1.74.
14. Heleski C.R., Mertig A.G., Zanella A.J. 2005. Results of a national survey of US veterinary college faculty regarding attitudes toward farm animal welfare. Journal of the American Veterinary Medical Association, 226, 1538-1546. doi:10.2460/javma.2005.226.1538.
15. Hernandez E., Fawcett A., Brouwer E., Rau J., Turner P.V. 2018. Speaking up: veterinary ethical responsibilities and animal welfare issues in everyday practice. Animals, 8, 15. doi:10.3390/ani8010015.
16. Herzog H., Betchart N.S., Pittman R. 1991. Gender, sex role orientation and attitudes toward animals. Anthrozoös, 4, 184–191.
17. Irvine L., Vermilya J.R. 2010. Gender work in a feminized profession: the case of veterinary medicine. Gender & Society, 24, 56-82. doi:10.1177/0891243209355978.
18. Izmirli S., Phillips C.J. 2012. Attitudes of Australian and Turkish veterinary faculty toward animal welfare. Journal of Veterinary Medical Education, 39, 200-207. doi:10.3138/jvme.1010.130R3.
19. Keeling L.J. 2005. Healthy and happy: animal welfare as an integral part of sustainable agriculture. Ambio, 34, 316-319. doi:10.1639/0044-7447(2005)034[0316:hahawa]2.0.co;2
20. Kendall H.A., Lobao L.M., Sharp J.S. 2006. Public concern with animal well-being: Place, social structural location, and individual experience. Rural Sociological Society, 71, 399–428. 009 doi:10.1526/003601106778070617.
21. Levine E.D., Mills D.S., Houpt K.A. 2005. Attitudes of veterinary students at one US college toward factors relating to farm animal welfare. Journal of Veterinary Medical Education, 32, 481-490. doi:10.3138/jvme.32.4.481.

22. Lofstedt J. 2003. Gender and veterinary medicine. The Canadian Veterinary Journal, 44, 533-535.
23. Magnani D., Ferri N., Dalmau A., Messori S. 2017. Knowledge and opinions of veterinary students in Italy toward animal welfare science and law. Veterinary Record, 180, 225. doi: 10.1136/vr.103938.
24. Main D.C.J., Appleby M.C., Wilkins D.B., Paul E.S. 2009. Essential veterinary education in the welfare of food production animals. Revue Scientifique et Technique, 28, 611–616. doi:10.20506/rst.28.2.1900.
25. Mariti C., Pirone F., Albertini M., Gazzano A., Diverio S. 2018. Familiarity and interest in working with livestock decreases the odds of having positive attitudes towards non-human animals and their welfare among veterinary students in Italy. Animals, 8, 150. doi:10.3390/ani8090150.
26. Martens P., Hansart C., Su B. 2019. Attitudes of young adults toward animals—the case of high school students in Belgium and The Netherlands. Animals, 9, 88. doi:10.3390/ani9030088.
27. Mellor D.J. 2016. Updating animal welfare thinking: moving beyond the “Five Freedoms” towards “A Life Worth Living”. Animals, 6, 3, 21. doi:10.3390/ani6030021.
28. Mendl M. 2001. Animal husbandry: assessing the welfare state. Nature, 410, 31–32. doi:10.1038/35065194.
29. National Research Council (NRC). 2009. Recognition and alleviation of pain in laboratory animals. The National Academies Press: Washington DC, USA
30. Ormandy E.H., Schuppli C.A. 2014. Public attitudes toward animal research: a review. Animals 4, 391–408. doi:10.3390/ani4030391.
31. Ostović M., Mesić Ž., Mikuš T., Matković K., Pavičić Ž. 2016. Attitudes of veterinary students in Croatia toward farm animal welfare. Animal Welfare, 25, 21–28. doi:10.7120/09627286.25.1.021.
32. Ostović M., Mikus T., Pavicic Z., Matkovic K., Mesic Z. 2017. Influence of socio-demographic and experiential factors on the attitudes of Croatian veterinary students towards farm animal welfare. Veterinární medicína, 62/8, 417-428. doi:10.17221/172/2016-VETMED.
33. Paul E.S., Podberscek A.I. 2000. Veterinary education and students’ attitudes towards animal welfare. Veterinary Record, 146, 269-272. doi:10.1136/vr.146.10.269.
34. Phillips C.J.C., Izmirli S., Aldavood S.J., Alonso M., Choe B.I., Hanlon A., Handziska A., Illmann G., Keeling L., Kennedy M., Lee G.H., Lund V., Mejdell C., Pelagic V.R., Rehn T. 2012. Students’ attitudes to animal welfare and rights in Europe and Asia. Animal Welfare, 21/1, 87-100. doi:10.7120/096272812799129466.

35. Pifer L., Shimizu K., Pifer R. 1994. Public attitudes toward animal research: some international comparisons. Society and Animals, 2, 95–113. doi:10.1163/156853094x00126.
36. Pirrone F., Mariti C., Gazzano A., Albertini M., Sighieri C., Diverio S. 2019. Attitudes toward animals and their welfare among Italian veterinary students. Veterinary Sciences, 6, 19. doi: 10.3390/vetsci6010019.
37. Pollard-Williams S., Doyle R.E., Freire R. 2014. The influence of workplace learning on attitudes toward animal welfare in veterinary students. Journal of Veterinary Medical Education, 41, 253-257. doi.10.3138/jvme.0114-006R1.
38. Regan T. 1985. The case for animal rights. In *Defense of Animals*. Ed. P. Singer, Basil Blackwell: New York, NY, USA, pp. 13–26.
39. Ryan Y.M. 1997. Meat avoidance and body weight concerns: Nutritional implications for teenage girls. Proceedings of the Nutrition Society, 56. 519–524. doi: 10.1079/pns19970052.
40. Serpell J.A. 2005. Factors influencing veterinary students' career choices and attitudes to animals. Journal of Veterinary Medical Education, 32, 491–496. doi:10.3138/jvme.32.4.491.
41. Singer P. 1975. Animal Liberation: A new ethics for our treatment of animals; New York Review/Random House: New York, NY, USA.
42. Shtylla Kika T., Cocoli S., Ljubojević Pelić D., Puvača N., Lika E., Pelić M. 2023. Colibacillosis in modern poultry production. Journal of Agronomy, Technology and Engineering Management, 6, 6, 975-987, doi:10.55817/YZFA3391.
43. Van der Weijden J.A. 2013. Attitudes towards the use of animals of students enrolled in animal welfare and laboratory science courses in the Netherlands. MSc Thesis, Utrecht University, The Netherlands.
44. Viegas I., Vieira A., Stilwell G., Santos L.J., Fontes M.A. 2011. Is there a link between beef quality and animal welfare in traditional beef systems? New Medit: Mediterranean Journal of Economics, Agriculture and Environment, 10, 3, 17-25.
45. Viñuela-Fernández I., Weary D.M., Flecknell P. 2011. Pain. In *Animal Welfare*. Eds. M.C. Appleby, J.A. Mench, I.A.S. Olsson and B.O. Hughes, 2nd Edition, CAB International, Cambridge University Press: Cambridge, UK, 64-77.
46. Walker J.K., McGrath N., Nilsson D.L., Waran N.K., Phillips C.J.C. 2014. The role of gender in public perception of whether animals can experience grief and other emotions. Anthrozoos 27, 251–266. doi:10.2752/175303714X13903827487601.

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SEQUENCING PROTOCOL AND BIOINFORMATICS PIPELINE FOR THE AVIAN INFLUENZA VIRUS

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Abstract

The avian influenza virus (AIV), traditionally confined to avian hosts, has recently been detected in various mammal species, raising significant concerns for both animal and public health, necessitating efficient and accurate methods for virus detection and characterization. This study presents a sequencing protocol combined with a comprehensive bioinformatics pipeline designed for the sequencing and analysis of AIV genomes. The presented streamlined approach encompasses whole genome PCR-amplification of the viral genome, enabling the genome characterization and detection of viral mutations with high precision. An amplicon-based MiniSeq sequencing workflow based on a set of PCR primers targeting all genome segments was developed. Three samples from H5 high pathogenic avian influenza (HPAI) outbreaks in Serbia were sequenced using the MiniSeq platform. The protocol involves optimized sample preparation, tailored specifically for AIV, library preparation and sequencing. This is complemented by a robust bioinformatics pipeline that includes quality control, read mapping, consensus genome generation, subtyping and pathotyping, as well as statistical sequencing data. The pipeline efficiently processes raw sequencing data, ensuring high-quality genome assemblies and accurate identification of viral strains. The protocol was used on AIV samples from various avian species, demonstrating its applicability and reliability. The results highlight the protocol's capability to generate comprehensive genomic data, which is crucial for monitoring viral evolution and informing public health interventions. The described integrated approach offers a powerful

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tool for AIV surveillance and research, facilitating timely and informed decision-making in response to avian influenza outbreaks. This protocol can be readily adapted for use in various laboratory settings, contributing to global efforts in combating avian influenza and enhancing our understanding of its genomic landscape.

Key words: avian influenza, high-throughput sequencing, bioinformatics, viral genomics, molecular characterization

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PROTOKOL ZA SEKVENCIRANJE I BIOINFORMATIČKU ANALIZU VIRUSA AVIJARNE INFLUENCE

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Kratak sadržaj

Virus avijarne influence (AIV), čiji su domaćini ptice, sve češće inficira različite vrsta sisara, što izaziva značajne zabrinutosti za zdravlje ljudi i životinja, što zahteva efikasne i precizne metode za detekciju i karakterizaciju virusa. U ovoj studiji predstavljen je protokol za sekvenciranje AIV sa kompletnom bioinformatičkom analizom, dizajniranim za sekvenciranje i analizu genoma AIV. Opisani protokol obuhvata PCR-amplifikaciju celog genoma virusa, omogućavajući karakterizaciju genoma i detekciju virusnih mutacija sa visokim stepenom preciznosti. Protokol obuhvata umnožavanje svih segmenata genoma AIV primenom jednog seta prajmera u jednoj PCR reakciji. Tri uzorka dobijena tokom epidemije H5 HPAIV u Srbiji su sekvencirana korišćenjem MiniSeq platforme. Takođe, protokol obuhvata optimizovanu pripremu uzoraka, posebno prilagođenu za AIV, pripremu biblioteke i sekvenciranje. Ovaj pristup je dopunjen robusnim bioinformatičkim analizama kojim je obuhvaćena kontrola kvaliteta, mapiranje dobijenih sekvenci, generisanje kompletnog genoma, tipizacija i patotipizaciju, kao i statističku analizu podataka sekvenciranja. Bioinformatičkom analizom se efikasno obrađuju podatke sekvenciranja, osiguravajući visokokvalitetne cele genoma i preciznu identifikaciju virusnih podtipova. Protokol je primenjen i testiran na uzorcima AIV iz različitih vrsta ptica, čime je dokazana njegova primenljivost i pouzdanost. Rezultati ove studije ukazuju da razvijeni protokol generiše omogućava efikasno umnožavanje celog genoma AIV i generiše kvalitetne sekvence, što je ključno za karakterizaciju i praćenje evolucije virusa. Opisani integrisani pristup nudi moćan alat za nadzor i istraživanje AIV, olakšavajući pravovremeno i informisano donošenje odluka kao odgovor na epidemije avijarne influence. Ovaj protokol se može lako prilagoditi za upotrebu u različitim laboratorijskim okruženjima, doprinoseći globalnim naporima u borbi protiv avijarne influence i unapređenju razumevanje virusa na molekularnom nivou.

Ključne reči: avijarna influenca, sekvenciranje nove generacije, bioinformatika, genomika virsa, molekularna karakterizacija

INTRODUCTION

Avian Influenza Virus (AIV) is a highly pathogenic virus affecting both poultry and humans, with recent outbreaks causing significant economic and health impacts worldwide. The rapid evolution and genetic diversity of AIV necessitate robust and efficient methods for accurate detection, characterization, and surveillance. Advances in sequencing technologies have provided new opportunities, yet challenges remain in integrating these technologies into a streamlined workflow. Current literature highlights the need for comprehensive approaches combining sample amplification, high-throughput sequencing, and bioinformatics analysis (Imai et al., 2018; Puryear et al., 2023; Ip et al., 2023). Recent studies have demonstrated various methodologies for sequencing AIV, each contributing valuable insights into the virus's genetic makeup and evolution. However, there remains a gap in providing an all-encompassing protocol that can facilitate seamless transitions from sample collection to data analysis. This study presents a sequencing protocol with an accompanying bioinformatics pipeline specifically designed for AIV. This approach integrates the entire process, from sample amplification to sequencing and bioinformatics analysis, into a single, cohesive workflow. This protocol can be of use in various of AIV genomic studies, ultimately aiding in better understanding and management of AIV outbreaks, enabling more effective monitoring and control measures.

MATERIALS AND METHODS

Ethics statement

The animal procedures conducted in this study adhered strictly to Serbian and European regulations on animal experimentation and notifiable diseases. All samples were collected as part of the official surveillance of Avian Influenza, supervised by Serbian veterinary directorate and in accordance with Serbian veterinary legislation, as these procedures are routinely performed for official surveillance.

Sample collection and nucleic acid extraction

Carcasses of birds (both domestic and wild) with suspected AIV infection, collected during 2021-2022 were delivered to our laboratory for detection of AIV. Organs were harvested and prepared for nucleic acid extraction. Viral RNA extraction was performed with IndiMag Pathogen Kit (Indical Bioscience, Germany) as described by the manufacturer. The presence of the AIV genome in these samples was confirmed by targeting the detection of matrix gen (M gen), using RT-qPCR with TaqMan-based one-step RT-qPCR with oligonucleotide primers and probes and thermal profiles described by Spackman et al. (2002) and by using commercial kit RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen, ThermoFisher Scientific), according to the manufacturer's instructions. All samples were processed in a BSL2+ laboratory in strict compliance with biosafety procedures. Out of all the tested samples, three were selected for sequencing, based on the Ct values (below 28).

PCR amplification for whole genome sequencing

Since the beginning and the end of each genome segment are homologous, whole genome amplification was performed using only two primers (MBTuni-12 and MBTuni-13) described by Zhou et al. (2009). The PCR amplification was performed using SuperScript™ IV One-Step RT-PCR System (ThermoFisher Scientific, USA) and the PCR was performed in 25 µl reaction volumes with 1.5 µl forward and reverse primers (10 pmol/µl), 12.5 µl of SuperScript IV buffer mix, 0.5 µl of SuperScript enzyme mix, 5 µl of extracted RNA and DEPC water up to 25 µl. The PCR thermal profile was as follows: reverse transcription at 48°C for 45 minutes, DNA polymerase activation and reverse transcriptase inactivation at 98°C for 2 minutes, followed by 5 cycles of denaturation at 94°C for 30 seconds, amplification at 45°C for 30 seconds, and elongation at 72°C for 3 minutes. This was further followed by 35 cycles of denaturation at 94°C for 30 seconds, amplification at 57°C for 30 seconds, and elongation at 72°C for 3 minutes, with a final elongation at 72°C for 10 minutes. The PCR amplification was performed using a PCR instrument -Thermocycler Gradient (Eppendorf, Germany).

Whole genome sequencing

To assess the PCR amplification, the concentration of dsDNA was measured using Qubit 4 (ThermoFisher Scientific, USA), following the manufacturers' instructions, followed by purification with the GeneJET PCR Purification Kit (ThermoFisher Scientific, USA). Before library preparation, samples were diluted to a working concentration of 1 ng/μl, instead of the recommended 100 ng/μl described in the library preparation protocol, because the low viral RNA concentration in the samples makes it challenging to achieve 100 ng/μl of DNA even after PCR amplification (although it should be noted that the protocol supports DNA concentration of 1 ng/μl to 500 ng/μl). Library preparation and sequencing was performed with the DNA Prep kit (Illumina, USA), following the manufacturers' instructions, with one modification (described in the previous sentence). The libraries were pooled and sequenced (2x150 paired-end) on the MiniSeq benchtop sequencer (Illumina, USA). The datasets generated in this study are available in NCBI GenBank under the accession numbers PP853088- PP853111.

Sequencing data analysis

Raw sequencing reads were initially assessed for quality using FastQC (v0.11.9) (Andrews, 2010). Statistical data on the raw reads were generated with Prinseq (v0.20.4) (Schmieder and Edwards, 2011). To ensure high-quality data, low-quality bases, primers, and adapters were trimmed using Trim Galore (v0.6.7) (Krueger, 2012). Reference-guided genome assembly was conducted using BWA (v0.6) (Li and Durbin, 2009), Samtools (v1.16.1) (Li et al., 2009), and iVar (v1.3.1) (Grubaugh et al., 2019). Coverage statistics were obtained using weeSAM (v1.6) (Aydemir and Baykal, 2018). A custom reference genome was constructed with the Vapor tool (v1.0.2) (Southgate et al., 2020) utilizing a custom-built database containing all Avian Influenza Virus (AIV) genomes from the NCBI GenBank. Subtyping was performed with the Blast command line tool with a custom-made database comprised of all available

AIV HA and NA genome segments from GISAID. The pathogenicity of the viruses was checked by identifying the presence of multi–basic hemagglutinin cleavage sites using the EMBOSS Transeq tool (Rice et al., 2000).

RESULTS and DISUSSION

Out of all the samples that tested positive for AIV presence by used RT-qPCR, three AIV positive sample materials were chosen for further analysis, PCR amplification and whole genome sequencing. A total of 2.6 Gbp of information was obtained from a sequencing run with a density of 321±3 K/mm^2, with 78.56±0.42% of the clusters passing quality control (QC) filters. Approximately 87.16% of Read1 and 92.43% of Read2 sequences contained high-quality bases, meeting the ≥ Q30 base calling QC standard, as generated by FastQC and Prinseq. BLAST analysis of the AIV hemagglutinin and neuraminidase sequences identified two viruses as belonging to the H5N1 subtype and one to the H5N8 subtype. All AIVs were categorized within clade 2.3.4.4b. The high pathogenicity of the viruses was confirmed by the presence of multi-basic hemagglutinin cleavage sites. Consensus genome was generated for all samples. The complete genomes of all three virus samples yielded equal or greater than 3000-fold sequence coverage depth. The results of this study demonstrate the efficacy of this comprehensive sequencing and bioinformatics pipeline in accurately identifying and characterizing AIV from field samples. The high-throughput sequencing yielded 2.6 Gbp of data, with a significant proportion of high-quality reads, underscoring the robustness of the initial quality control and data preprocessing steps. The initial quality assessment using FastQC and the subsequent data trimming with Trim Galore ensured that dataset maintained high standards, with over 87% of Read1 and 92% of Read2 sequences meeting the ≥ Q30 quality threshold. This high level of quality is essential for reliable downstream analyses. Our PCR protocol successfully amplified the whole genome of the viruses, enabling the simultaneous detection of two different subtypes. The reference-guided genome assembly using BWA, Samtools, and iVar was highly effective, as evidenced by the coverage depth exceeding 3000-fold for all samples. High depth is critical for ensuring the accuracy of variant calling and genome assembly, providing a solid foundation for subsequent analyses. Constructing a custom reference genome with the Vapor tool, utilizing a comprehensive database of AIV genomes from NCBI GenBank proved advantageous. This approach enhances the accuracy and relevance of genomic analyses by tailoring the reference to include the most recent and locally relevant strains, reducing bias, and improving assembly quality. It al-lows for more precise detection of region-specific mutations and pathogenicity markers, providing detailed evolutionary insights and adaptability to rapidly evolving pathogens like AIV. BLAST analysis and phylogenetic classification identified three H5N1 and one H5N8 subtypes, all belonging to clade 2.3.4.4b. The ability to accurately subtype the viruses and place them within a specific clade demonstrates the effectiveness of this pipeline and the utility of using a custom reference genome. Most of the papers describing AIV sequencing use in-house developed protocols and pipelines (Puryear et al., 2023) or focus on the utilization of Oxford Nanopore technology (Imai et al., 2018) and that is why this paper presents an integrated protocol and bioinformatics pipeline designed to streamline the entire process of AIV. It combines sample amplification, sequencing, and data analysis into a cohesive workflow. By providing a comprehensive, all-in-one solution, the paper aims to facilitate scientists' efforts in efficiently obtaining and analyzing high-quality genomic data, enhancing the accuracy and speed of AIV research and surveillance.

CONCLUSION

In conclusion, the conducted study presents a simple yet effective sequencing and bioinformatics pipeline for the analysis of AIVs. The high quality of the sequencing data, combined with the use of a custom reference genome, allowed for accurate identification, assembly, and pathogenicity assessment of the viruses. These results contribute valuable data to the global understanding of AIV diversity and evolution and highlight the importance of continued surveillance and methodological advancements in the field. Future studies could build on this approach by incorporating additional bioinformatics tools and expanding the scope of bioinformatic analysis.

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Author's Contribution

V.G. carried out literature research, designed the study, prepared samples for sequencing, sequenced the samples, analyzed the results, and drafted the manuscript. T.P. carried out the RT-qPCR testing of samples and, together with V.G., drafted the manuscript. G.L. was involved in sample collection and data analysis. B.Đ. collected the samples and prepared them for testing. D.V. and J.N. were involved in data analysis and the final polish of the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

REFERENCES

1. Andrews S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. [Online] Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed 10 March 2024.
2. Aydemir O., Baykal A.T. 2018. weeSAM: A Web-Based Application for Classification of Single-Cell RNA-Seq Data. Bioinformatics, 34, 6, 1065-1067. doi:10.1093/bioinformatics/bty075.
3. Grubaugh N.D., Gangavarapu K., Quick J., Matteson N.L., De Jesus J.G., Main B.J., Tan A.L., Paul L.M., Brackney D.E., Grewal S., Gurfield N., Van Rompay K.K.A., Isern S., Michael S.F., Coffey L.L., Loman N.J., Andersen, K.G. 2019. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. Genome Biology, 20, 8. doi:10.1186/s13059-018-1618-7.
4. Imai K., Tamura K., Tanigaki T., Takizawa M., Nakayama E., Taniguchi T., Okamoto M., Nishiyama Y., Tarumoto N., Mitsutake K., Murakami T., Maesaki S., Maeda T. 2018. Whole genome sequencing of influenza A and B viruses with the MinION sequencer in the clinical setting: a pilot study. Frontiers in Microbiology, 9, 2748. doi: 10.3389/fmicb.2018.02748.
5. Ip H.S., Uhm S., Killian M.L., Torchetti M.K. 2023. An evaluation of Avian influenza virus Whole-Genome sequencing approaches using Nanopore technology.Microorganisms, 11, 2, 529. doi:10.3390/microorganisms11020529.
6. Krueger F. 2012. Trim Galore: A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files.

[Online] Available at: http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/ Accessed 10 March 2024.

7. Li H., Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics, 25 14, 1754-1760. doi:10.1093/bioinformatics/btp324.
8. Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25, 16, 2078-2079. doi:10.1093/bioinformatics/btp352.
9. Puryear W., Sawatzki K., Hill N., Foss A., Stone J. J., Doughty L., Runstadler J. 2023. Highly pathogenic Avian influenza A(H5N1) virus outbreak in New England Seals, United States. Emerging Infectious Diseases, 29, 4, 786–791. doi: 10.3201/eid2904.221538.
10. Rice P., Longden I., Bleasby A. 2000. EMBOSS: The European Molecular Biology Open Software Suite. Trends in Genetics, 16, 6, 276-277. doi:10.1016/s0168-9525(00)02024-2.
11. Schmieder R., Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics, 27, 6, 863-864. doi:10.1093/bioinformatics/btr026.
12. Southgate J., Bull M., Brown C., Watkins J., Corden S., Southgate B., Moore C., Connor T. 2020. Influenza classification from short reads with VAPOR facilitates robust mapping pipelines and zoonotic strain detection for routine surveillance applications. Bioinformatics, 36, 6, 1681–1688. doi: 10.1093/bioinformatics/btz814.
13. Spackman E., Senne D. A., Myers T.J., Bulaga L.L., Garber L.P., Perdue M.L., Suarez D.L. 2002. Development of a Real-Time Reverse Transcriptase PCR assay for Type A influenza virus and the Avian H5 and H7 hemagglutinin subtypes. Journal of Clinical Microbiology, 40, 9, 3256–3260. doi: 10.1128/jcm.40.9.3256-3260.2002.
14. Zhou B., Donnelly M.E., Scholes D.T., St George K., Hatta M., Kawaoka Y., Wentworth D.E. 2009. Single-Reaction genomic amplification accelerates sequencing and vaccine production for classical and swine origin human influenza A viruses. Journal of Virology, 83, 19, 10309–10313. doi: 10.1128/jvi.01109-09.

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Examples of references:

Articles in journals:

1. Stojanović D., Maličević Ž., Ašanin R. 2002. The use a new model for the investigation of sepsis. Acta Veterinaria, 52, 2/3, 125-131.
2. Chen J. and McClane B.A. 2015. Characterization of *Clostridium perfringens* TpeL toxin gene carriage, production, cytotoxic contributions, and trypsin sensitivity. Infection and Immunity, 83, 2369–2381. doi:10.1128/IAI.03136-14.
3. Williams R.B. 2015. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34, 159-180. doi: 10.1080/03079450500112195.
4. Bailey M.A., Macklin K.S., Krehling J.T. 2013. Use of a multiplex PCR for the detection of toxin-encoding genes *netB* and *tpeL* in strains of *Clostridium perfringens.* ISRN Veterinary Science, Article ID 865702, 1-4. doi:10.1155/2013/865702.

Books:

5. Ficken, M. D. and Wages, D. P. 1997. Necrotic enteritis in Diseases of Poultry, Eds. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, Iowa State University Press, Ames, Iowa, USA, 10th edition, ISBN xxx-xxx-xxxxx-xx-x.

Chapters in books:

6. Plumb J.A. and Hanson L.A. 2011. Sturgeon viruses. In *Health maintenance and principal microbial diseases of cultured fishes*. Eds. J.A. Plumb, L.A. Hanson, 3rd edition, Blackwell Publishing, 219-225.

Articles in proceedings:

7. Giangaspero A., Marangi M., Pati S., Cafiero M.A., Camarda C., Sparagano O.A.E. 2011. Investigating the presence of acaricide residues in laying hens naturally infected by the red mite *Dermanyssus gallinae*. In *Book of Abstracts*, The 12th Asian food conference 2011, BITEC Bangna, Bangkok, Thailand, 27.
8. Vidanović D., Petrović T., Šekler M., Debeljak Z., Vasković N., Matović K., Plavšić B., Dmitrić M. 2018. Avian influenza in Serbia: epidemiological situation during

2016–2017. In *Programme and Abstract book*, 11th International Congress for Veterinary Virology, 12th Annual Meeting of EPIZONE, 27-30.08.2018, University of Veterinary Medicine Vienna, Vienna, Austria, 118 (p187).

9. Lazić G., Lazić S., Bugarski D., Grubač S., Lupulović D., Samojlović M., Petrović T. 2018. Human enteroviruses in river water and sewage in Vojvodina. In *Book of Abstracts*, International Scientific Conference "Green economy and environment protection", Belgrade, 23-25. April 2018, edited by Larisa Jovanović, Belgrade, Naučno stručno društvo za zaštitu životne sredine "ECOLOGICA", 95-96. ISBN 978-86-89061-11-6.

Lows and Regulations:

10. European Union. 2003. Commission Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, Official Journal of the European Union, L 268:29. **https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2003R1831:20100901:EN:PDF**

Citations with organisations as authors:

11. European Food Safety Authority. 2016. Peer review of the pesticide risk assessment of the active substance benzoic acid. EFSA Journal, 14(12):4657-n/a. **http://dx.doi.org/10.2903/j.efsa.2016.4657.**

Software:

12. Statistica (Data Analysis Software System). 2006. v.7.1., StatSoft, Inc., USA (**www.statsoft.com**).

Web Links:

13. OIE: Animal Diseases. Available at: **http://www.oie.int/en/animal-health-in-the-world/information-on-aquatic-and-terrestrial-animal-diseases/.** Accessed 07.08.2019.
14. European Centre for Disease Prevention and Control (ECDC). Historical data by year - West Nile fever seasonal surveillance. Available at: https://ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical Accessed 31.07.2019.

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