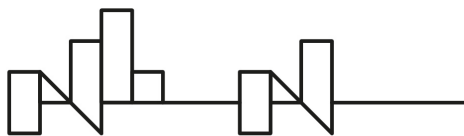


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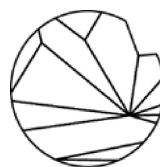
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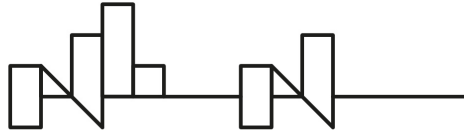
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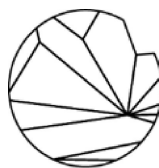
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THE IMPACT OF GLOBAL CHANGE ON THE ENVIRONMENT, HUMAN AND ANIMAL HEALTH

International Scientific Conference

PROCEEDINGS of scientific abstracts and contributions



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Košice, Slovakia**

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PLENARY LECTURES

ONE HEALTH - A WAY TO BETTER WORLD?

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The concept of *One Health* is already well-established in veterinary medicine and has strong connections with human and environmental health, forming a unique trio. In the classical vision of *One Health*, the focus was often on zoonoses, which has further developed during the recent COVID-19 pandemic. Undoubtedly, the issue of zoonotic diseases and the transmission of diseases between animals and humans cannot be marginalized. However, the question remains open as to whether it is the most significant problem of the modern world. We tend to make cognitive errors and place our visions and difficulties at the centre of the world. I am aware of this burden, and at the same time, I see the serious problems we face in the wealthier part of the world, known as the affluent global north.

Certainly, objectively measured health indicators are impressive, and we observe improvements on a global scale in many parameters: a decrease in the number of neonatal deaths, an increase in life expectancy, and the practical elimination of rabies (at least with strong syndrome). However, in evolution, there are no free lunches, and all gains come with certain costs, with the system behaving according to the principle of trade-offs and evolutionary compromises. We pay for development and rapid urbanization with exposure to stressful environmental factors such as artificial light, excessive noise levels, pollution, and frequent interpersonal contacts, at a very high price. This price is an increasing discomfort in life, a decrease in the level of happiness, and even the development of serious mental illnesses such as depression and bipolar disorder. However, this problem is rarely discussed in the concept of *One Health*, while contact with animals could serve as a remedy to improve the situation.

Certainly, the effects and mechanisms of animal-assisted therapy have been well described, but it is interesting to consider the broader context. How does the human brain react to contact with the wild nature, and how does it influence our subjective and objective indicators of happiness? There are several mechanisms explaining this phenomenon: contact with green (as a colour), the calming effect of rustling trees, and the specific choral singing of birds. In detail, it is the

observations of birds that provide a lot of evidence, and based on these studies, specific types of therapies are developed, grounded in evidence-based solutions: forest bathing, ornithological walks, mindfulness sessions.

Acquaintance with birds improves the diagnostic effectiveness for doctors interested in birding, but more importantly, it influences combating fatigue and burnout among veterinary and medical professionals. However, individual approaches are important, which brings us closer to considering individual preferences within the One Health concept, which traditionally operated at the population level.

In conclusion, we face a significant challenge in incorporating well-being parameters into the concept of One Health, but fortunately, we have an increasing amount of scientific research data that shows it is possible and worth implementing the proposed solutions. The key, however, lies not in searching for further evidence in scientific papers but in investing our own time and resources in contact with the wild nature. I highly encourage everyone to do so.

EMERGING INFECTIOUS DISEASES AND WILDLIFE: LEAST KNOWN SIDE OF THE ONE HEALTH PARADIGM

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The definition of One Health per se always includes pathogens and diseases affecting wildlife. Undoubtedly, this is a neglected part of the entire scope of One Health. Evidence accumulated over the last decades clearly shows that diseases caused by emerging pathogens are one of the greatest threats to biodiversity and one of the most pronounced aspects of biological invasion. In my presentation, I will attempt to summarize the main reasons for the emergence of infectious diseases (EIDs) in wildlife and outline how these diseases affect wildlife populations, especially in the case of endangered vertebrate species. The causes of EID in wildlife can be summarized in many ways, and indeed they often overlap. For clarity, EID causes are divided into two main areas: (i) global and ecosystem changes and alterations, and (ii) movement of pathogens and their hosts. Examples presented include helminthiasis of mountain gorillas, toxoplasmosis in marine wildlife, the giant liver fluke *F. magna*, avian malaria, TINC in testudines, mammalian morbilliviruses, diseases associated with *Angiostrongylus cantonensis*, and several others. Finally, the perspectives and limitations of the management of EID in wildlife are briefly discussed.

LARGE SCALE ENVIRONMENTAL CHANGES AND WILDLIFE HEALTH IN THE NORTH

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ABSTRACT

Climate changes, physical landscape changes and changes in human landscape use may have a major impact on the health of wildlife. Heat, changes in humidity and changed winter conditions may cause problems for some species, increased loads of parasites and vectors may cause declining health in other species. Species adapted to a cold climate with low pathogen exposure may be especially vulnerable. Current health problems in muskoxen (*Ovibos moschatus*), wild reindeer (*Rangifer tarandus*) and moose (*Alces alces*) are described and discussed in the context of anthropogenic change.

INTRODUCTION

The World have changed rapidly the last century, and it is still changing. We humans adapt more or less successfully to our new environment, by changing behaviour. The wild animals around us, adapted through long time evolution to an environment that not was dominated by anthropogenic changes may have less opportunities for adaption.

Climate change is already evident. We see warmer mean temperatures, more frequent and hotter heat waves, longer growth season, more and heavier precipitation and more unpredictable weather.

Globalization implies that travel and trade connects all parts of the world and pave the way for efficient transmission not only of goods and humans, but also invasive species, parasites and pathogens.

Economic development implies increased encroachment on wildlife habitats with building of houses, factories, roads, railroads, power lines and power dams. This infrastructure may create obstacles for natural animal movement and cause habitat loss and fragmentation.

Increased population density, increased leisure time, increased tourism travel, better outdoor equipment and increased availability of terrain vehicles promotes increased human presence and disturbance in habitats previously relatively undisturbed and in all seasons.

Arctic and boreal mammals are adapted to an environment with a long, cold and relatively dry winter and a short and intense growth period. A warmer, wetter and wilder climate may create an environment that these animals cannot cope with.

In addition, there has been relatively few parasite and pathogen species present in these environments, and as a general rule they and their hosts have lived in balance. The climate changes, however, may shift this balance in favour of the parasites and pathogens, while the animals already struggle to adapt to their new situation.

Moose

The moose is well adapted to a cold and dry winter and a short growth season. Earlier spring and later fall may cause heat stress for the moose, while parasites and pathogen vectors may experience extended swarming periods and shortened generation span. Deer ked (*Lipoptena cervi*), sheep tick (*Ixodes ricinus*) and the nasal bot fly (*Cephenemyia ulrichii*) are species that may affect moose health and fitness. Red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) may in contrast benefit from a warmer climate and tolerate a higher burden of parasites and pathogens. This may cause a situation of apparent competition, i.e. competition mediated through a common parasite.

Population performance in Norwegian moose populations has declined the last decades, especially in the southern parts of the country. Can this be ascribed to changes in health, or is this a result of poor population management, feed overexploitation and hunter selection of the wrong animals?

Reindeer

Reindeer are herd animals that migrate between different pastures to optimize feeding through the seasons. Encroachment and disturbance cause habitat loss and fragmentation, which may prevent natural migration. In addition to the loss of food resources that this may imply, hindered migration may cause parasite aggregation and thereby increased parasite burdens. Increased use of reindeer habitats for grazing by free-roaming sheep and use of salt licks visited by both livestock and wildlife, may create ideal hot spots for disease transmission and spillover between species. Reindeer warble flies (*Hypoderma tarandi*), nasal bot flies (*Cephenemyia trompe*),

sheep gastrointestinal parasites and necrobacillosis caused by *Fusobacterium necrophagum* may constitute additional challenges to a species that already is threatened by climate change and habitat fragmentation. Chronic Wasting Disease is a severe threat for the survival of several reindeer populations.

The muskox is adapted to an extreme climate with very long, cold, dry and dark winters. The little population that was introduced to Norway a century ago is surviving in an «island» of relatively cold mountain climate, but surrounded by valleys with a more temperate climate. The population has, however, experienced several population declines driven by disease. Hot and humid weather, rain on snow and spillover of domestic sheep pathogens are factors that seem to have played important roles in these events.

In conclusion, it is easy to become pessimistic about the future for animals adapted to cold climate and undisturbed habitats. Realizing the challenges we have created and continue to create for these species, we ought to behave so that we not worsen the situation. Allowing the animals to live relatively undisturbed in large enough areas of suitable habitat, is necessary to secure their future.

ONE HEALTH – STORIES OF A COVID VACCINE AND AFRICA DEVELOPMENTS

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ABSTRACT

As we emerge from the Covid-19 pandemic, one can reflect on the number of outbreaks that appear to increase in recent years. According to the OIE, over 70% of emerging infectious diseases are zoonotic. Global warming, urbanization, and increasing encroachment of wildlife habitats, have led to an increase in pathogens that were previously limited in scope. While there is considerable debate on the origins of the Covid-19 virus, its ability to infect multiple species is not in doubt. Besides humans, some species such as mink and large cats also experienced significant mortalities. Our vaccine efforts began when the first reports of the virus from dogs in Hong Kong were mentioned. We settled on a sub-unit approach for our animal vaccine with an adjuvant that was ideal for multiple species. The spike trimer of SARS-CoV 2 virus was generated ensuring the trimer was locked in the pre-fusion state. The resulting vaccine was highly efficacious in cats and dogs. However, the severity of the disease did not warrant a full development of the product for cats and dogs. The mink on the other hand were highly susceptible and also contributed to variants that spilled over into humans. Therefore, the vaccine was tested and approved for use in mink. While the vaccine was widely used in mink, vaccines were also provided to Zoos to help prevent the disease in valuable animals. Our vaccine has been provided to several zoos in the USA where more than 300 species of animals have received it. There were no reports of any adverse events due to the vaccine and continue to serve these animals as needed.

Learnings from Covid and the complexity of the World biology with future potential threats are leading us to develop projects in different geographical areas with continuous care approach, that represents prediction, prevention, detection, and treatment. One of those important geographical areas is the Sub Saharan Africa, where during the last six years we were implementing a joint initiative with Bill & Melinda Gates Foundation (BMGF) called A.L.P.H.A. (Advancing Livestock Productivity and Health in Africa). Based on the initial success in building diagnostic hubs in Africa, increase access to quality products and services supported by training stakeholders with focus on gender balance first in Nigeria, Uganda,

Ethiopia and Tanzania, we have just signed the second phase for another five years with BMGF, expanding under A.L.P.H.A. Plus name to more than 11 countries. A development of set of digital tools such as LabCard app and others, are supporting the implementation of the initiative.

IMPACTS OF GLOBALISATION AND CLIMATE CHANGE ON WATERBORNE PARASITES: THE CASE FOR *CRYPTOSPORIDIUM*

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ABSTRACT

Parasites are considered as the «neglected pathogens». Among the twenty WHO-designated neglected tropical diseases (NTD), two are viral, five bacterial/fungal, one is non-infectious (snakebite envenoming) and twelve are parasitic. Of these, 12 parasitic NTD there are 10 that are zoonotic or potentially zoonotic, and seven that can be transmitted to people by either contaminated food or water. These are truly diseases that belong to the One Health paradigm. Although the potential impacts of climate change on water supply (quantity) have received considerable attention, there has been relatively little focus on how water quality is likely to be affected by climate change. However, according to the Intergovernmental Panel on Climate Change (IPCC), climate change is not only going to result in an increased likelihood of water-related disasters (drought and flood risks, and the associated societal damages), but also is going to affect water quality. As Anna Michalak, Professor of global ecology at the Carnegie Institution for Science in Stanford, California, said in the New York Times: “*When we think about climate change, we are used to thinking about water quantity — drought, flooding, extreme rainfall and things along those lines. Climate change is just as tightly linked to issues related to water quality, and it's not enough for the water to just be there, it has to be sustainable.*” Indeed, higher water temperatures and more frequent floods and droughts are projected to exacerbate many different forms of water pollution – from sediments to pesticide and pathogens.

However, climate change is not the only global change that is affecting parasites. Although we recognise how globalisation is enabling diseases to sweep around the planet (think Covid-19), it is less usual to consider parasites, or, in particular, waterborne parasites. Nevertheless, while globalisation is bringing together unexpected combinations that can result in the emergence and reemergence of some waterborne parasites, urbanisation, with its implicit greater concentration and connectedness of people, also increases the speed at which new infections, including parasites, are spread under the pressures of overwhelmed water and sanitation infrastructure.

Thus climate change, globalisation, and urbanisation are all impacting on waterborne parasitic diseases.

In order to bring waterborne parasites into focus, the first parasite on the menu will be a schistosome; schistosomiasis is now considered to be largely a tropical disease (and also one of the WHO NTD) with the last human cases in Europe reported in Portugal in 1967. However, globalisation means that this parasite returned to Europe in 2015 – and climate change variables may enable successful spread and establishment elsewhere in Europe. Lack of expectation of this parasitoses in patients who have not travelled outside of Europe may also delay diagnosis, potentially affecting both individual patient health and also further spread.

However, the emphasis of this presentation will be on *Cryptosporidium*, a protozoan intestinal pathogen. This parasite is particularly well suited to survival and spread in damp cool environments, such as in Norway, but it is also common in tropical climates and it is recognised as being one of the most important causes of paediatric diarrhoea in developing countries. However, increasing data from molecular studies indicate that a particular concern regarding this parasite may lie not in globalisation bringing this parasite from tropical countries to Europe, but the reverse direction of travel. Some species of *Cryptosporidium* infect both people and ruminant livestock, and this parasite not only also has a considerably impact on the welfare and productivity of young ruminant livestock, but zoonotic transmission is also important. However, current evidence indicates that zoonotic species are relatively under-represented in both Asian and African countries.

Data from studies that we have conducted in different countries (including Ethiopia and Norway) will be used to demonstrate how globalisation and climate change are of relevance for this parasite, particularly considering diagnostic tools, the current geographical distribution of genotypes and species of *Cryptosporidium*, and water supply infrastructure.

CLIMA CHANGE, LIVESTOCK AND ANTIMICROBIAL RESISTANCE

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ABSTRACT

This paper is focused on the impact of climate change on farm animals and its effect on antimicrobial resistance. It also proposes possible solutions in relation to the necessary reduction in the use of antimicrobial substances within the framework of the "One health" concept.

INTRODUCTION

The climate change has numerous impacts on ecosystems, human and animal health and food production, which also affects antimicrobial resistance (AMR). WHO (2017) recognize that antibiotic resistance is one of the major threats to public health and food safety in the 21st century. High adaptive abilities of microorganisms contributed to the rapid activation of mechanisms of resistance to antimicrobial substances of various nature. The list of animals' and humans' infections, which are less and less responsive to antibiotic therapy, is growing every year.

Higher temperatures can be associated with increased frequency of horizontal gene transfer, (Kim et al., 2008; Vegge et al., 2012), as well as an increase in antimicrobial resistant infections. (MacFadden et al., 2018; McGough et al., 2020; Pepi and Focardi, 2021). MacFadden et al. (2018) found out that raise of temperature about 10°C was associated with increases in antibiotic resistance for the common pathogens *Escherichia coli* (of 4.2%), *Klebsiella pneumoniae* (of 2.2%), and *Staphylococcus aureus* (of 2.7%). The climate change also contributes to the emergence and spread of AMR in the environment due to the continuing disruption of the environment by extreme weather patterns (Burnham 2021). Temperature, oxygen and carbon dioxide concentrations in the environment can also influence the survival and proliferation of bacteria, and the rate at which they acquire resistance (Liao et al., 2019; Gupta et al., 2016).

The effects of climate change on livestock diseases depend on the geographical region, type of land, disease characteristics, and animal susceptibility (Thornton et al., 2009). According to Nardone et al. (2010) animal health can be affected directly or indirectly by climate change. Directly animal health can be affected in relation to the increase of temperature, which increases the potential for morbidity and death. The indirect effects are related with microbial communities (pathogens or parasites), spreading of vector-borne diseases, food-borne diseases, host resistance,

and feed and water insufficiency (Nardone et al., 2010; Thornton et al., 2009; Tubiello et al., 2008). Global warming and changes in precipitation affect the quantity and spread of vector-borne pests such as flies, ticks, and mosquitoes. In addition, disease transmission between hosts will be more likely to happen in warmer conditions (Thornton et al., 2009). Impact of heat stress on livestock depends on temperature, humidity, species, genetic potential, life stage, and nutritional status.

Livestock influence on climate is realised through a change of used land, feed production, animal production, manure, and processing and transport. Livestock contribute 14.5% of the total annual anthropogenic greenhouse gas emissions globally (Gerber et al., 2013). Feed production and manure emit CO₂, nitrous oxide (N₂O), and methane (CH₄), which consequently affects climate change. Animal production increases CH₄ emissions. Processing and transport of animal products and a change of used land contributes to the increase of CO₂ emissions.

According to Allen et al. (2018) while cumulative carbon dioxide (CO₂) emissions dominate anthropogenic warming over centuries, temperatures over the coming decades are also strongly affected by short-lived climate pollutants (SLCPs), complicating the estimation of cumulative emission budgets for ambitious mitigation goals. Using conventional Global Warming Potentials (GWP) to convert SLCPs to “CO₂ equivalent” emissions misrepresents their impact on global temperature. A new way of calculating GWP* that relates cumulative CO₂ emissions to date to current SLCP emission rates and accurately shows the impact of emissions of both long- and short-lived pollutants on radiative forcing and temperature over a wide range of time horizons would reduce the expected warming in 2030 by 28% in relation to the recent scenario. While previous calculations did not address the rate of degradation of greenhouse gases in the atmosphere and simply treated one greenhouse gas as another, the new GWP* methodology takes into account the difference in the rate of degradation of greenhouse gases. While CH₄ is completely degraded after 50 years, CO₂ remains in the atmosphere for more than a thousand years. If these differences are taken into account, the position of agriculture as one of the significant producers of greenhouse gases changes completely. Livestock can have a positive effect on the climate. CO₂, which is released into the atmosphere during burning of fossil fuels, will return to its fossil form in millions of years. In the case of agricultural production, however, the cycle is already "closed" within a maximum of a few years. The growth of plants, algae or bacteria absorbs part of the emitted CO₂. Although agricultural and livestock systems are a source of greenhouse gases, in a more comprehensive view they even absorb more carbon than they produce, helping to reduce the carbon footprint of other industries (Pulina, 2023).

METHODS

The aim of our work was to create a complex screening system of climate change impact on farm animals and its effect on AMR from the point of prevention, prophylaxis and biosecurity practices on reducing usage of antimicrobial substances and AMR developing. We were based on a professional literature (PubMed, Web of Science) and especially on the results of the authors' scientific research projects and their practical experiences from farms audits.

RESULTS

The first and the most important step to prevent the development of AMR on livestock farms is to maintain good animal health. This can be achieved by introducing and, above all, by consistently observing preventive measures including the principles of good husbandry practice (management), vaccination program (prophylaxis) and observing the principles of biological safety (biosecurity).

From a practical point of view, it is necessary to create comprehensive analysis with focus on determining the critical control points (CCP) of management, prophylaxis and biosecurity, including mutual interactions between them. It must be taken into account all, possibilities of direct and indirect spread of infectious agents and their incorporation into the real operating conditions of monitored cattle, pig and poultry farms.

In the field of management, main CCP are zootechnical records, veterinary records, health herd strategy, farm and stable environment, feed and water, excrements handling, hygiene and sanitation (disinfection, insect and rodent control, deodorization and deanimalization) and product quality (raw materials, food of animal origin). In the field of prophylaxis is very important an active surveillance (frequency of disease occurrence, morbidity, mortality), passive surveillance (monitoring of post-infection antibodies), health status of the herd/flock (farm dynamics of diseases), vaccination schedule, an effectiveness of vaccination, continuous assessment of antimicrobial consumption, vaccination schedule correction (every 6 months) and analysis of production and reproduction indicators.

And in the field of biosecurity: animals (farm and domestic animals), wild animals (game, wild birds and mammals), persons (staff, vets, consultants), transport (between and within the farm), feed and water (quantity, quality, structure), technological systems (housing, feeding, watering, lighting, ventilation, faeces removal), material (tools, instruments) and air (distance between farms and stables).

DISCUSSION

Many diseases are climate-sensitive and changes in environmental conditions and temperatures may lead to an increase of many bacterial, viral, parasitic, fungal, and vector-borne diseases spread among humans, animals and plants. Increased climate change and reduced biodiversity, will force pathogens to evolve and adapt rapidly (Hoberg and Brooks, 2015). Bacteria have evolved diverse mechanisms to survive in environments full of antibiotics. Temperature is both a key factor that affects the survival of bacteria in the presence of antibiotics and an environmental trait that is drastically increasing due to climate change. Antibiotic-resistant bacterias are now frequently isolated from most farmed animals (Argudin et al., 2017). Therefore, it is important to understand links between temperature changes and AMR selection (Rodríguez-Verdugo, 2020).

Increased prevalence of disease could result in an increase in the improper use of antimicrobial drugs, which could exacerbate AMR.

Good breeding practice principles play a very important role in improving of the health status of animals (Postma et al., 2016). The basis of these procedures is, to ensure an adequate level of immunity after birth, optimization of the breeding environment including minimization of animal stress, and following of the developed herd health plan.

The fundamentals of prophylaxis are based on an adequate level of innate and acquired immunity and implementation of a vaccination schedule. Vaccination in compliance with the vaccination schedule provides optimal protection of animals against selected disease agents (Hoelzer et al., 2018). Those multiple factors contribute to AMR development, it is advisable to combine the vaccination with other approaches such as increasing the level of hygiene and nutrition, rapid diagnosis of the causative agent by a veterinarian, including the responsible targeted use of antibiotics, etc. (O'Neill, 2016).

The level of biosecurity of the herd is associated with the amount of antimicrobials used (Raasch et al., 2018). Although the introduction and, above all, the ongoing biosecurity principles observance requires certain financial costs, in the long term it represents an investment for the breeder in the future, manifested not only in the improvement of the health of the herd, including an increase of the level of welfare. But at the same time it also leads to a reduction of morbidity and mortality rates, and so it reduces the amount of antimicrobials used on those farms, with the subsequent improvement of production and reproductive indicators. Finally, the economic profitability of breeders (Teagasc, 2018).

CONCLUSION

The direct impact of climate changes on livestock results in reduction of feed intake, milk production and reproductive efficiency, as well as the changes in mortality and immune system function. Indirect effects derived from the influence of climate on feed production, water availability and pest/pathogen populations. The climate crisis exacerbates the threat of drug-resistant infections and spreading of antimicrobial resistance between animal and human population. Environmental temperature is closely related to bacterial processes and infections. As environment temperatures rise with the climate change, bacterial infections may increase in frequency and may spread to higher altitudes and latitudes where they were previously absent. Therefore, it is necessary to pay attention to the preventive measures in the field of management, prophylaxis and the biosecurity of farm animals.

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MACROALGAE AS A SOURCE OF NUTRIENTS AND BIOACTIVE COMPOUNDS FOR ANIMALS

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ABSTRACT

Marine macroalgae, also called seaweeds, have great nutritional values and bioactive properties. Thus, they have been considered as a potential source of feed and food in the future. The nutritional and bioactive properties of macroalgae vary with macroalgae types, species, and growing seasons. Such variations can significantly affect the suitability of macroalgae as food or feed ingredients. In addition, high mineral contents in macroalgae also pose critical challenges while aiming to utilize their biomasses as nutrient sources. Our studies aimed to test the hypotheses that a) the potential of macroalgae as a sustainable nutrient source is affected by their species- and season-specific variabilities, b) macroalgal chemical composition and digestibility can be optimized by specific post-harvest hydrothermal pre-treatments, c) the content of macroalgal bioactive compounds can induce health-promoting outcomes in animals. Macroalgae demonstrated differences in nutritional compositions with higher crude protein (CP) and minerals in the spring than those harvested in the autumn. However, the total polyphenol (TPC) content in brown macroalgae was lower in the spring than in autumn. The macroalgal TPC was negatively associated with feed fermentability during *in vitro* rumen fermentation while including (20% dry matter) as feed additives. Interestingly, TPC-rich brown species, the *F. vesiculosus*, and *A. nodosum*, mainly from autumn harvest, reduced up to ~60% of *in vitro* CH₄ production but also suppressed feed degradation by inhibiting the cellulolytic bacteria. High-temperature blanching effectively depressed certain minerals (Na, K, and I) and arsenic in brown macroalgae improving their safety as animal feed ingredients but did not improve digestibility. Two important macroalgae, *A. nodosum*, and *F. vesiculosus*, when included in a high-fat (HF) diet, reduced ~40% body fat mass compared to the HF diet alone. Additionally, including these macroalgae in the HF diet improved cecal microbial composition, i.e., reduced obesity-linked bacteria and increased short-chain fatty acid-producing bacteria in mice.

These studies suggest that certain macroalgae species can be utilized as environment-friendly animal feed ingredients or health-promoting food additives. However, efficient post-harvest

processing techniques are required to remove critical and toxic minerals in harvested macroalgae biomass without losing digestible fractions. Moreover, efficient macroalgal biorefinery approaches can be beneficial to extract high-value bioactive compounds from macroalgae to create a novel value chain of whole macroalgal biomass.

HEAVY METAL REMOVAL FROM WATER BY NATURAL MATERIAL

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ABSTRACT

The work deals with the process of adsorption of Cu, Zn and Ag on natural zeolite. It is focused on sorption experiments, which were used to determine the equilibrium and adsorption capacity of Cu²⁺, Zn²⁺ and Ag⁺ ions. Natural zeolite ZeoCem Micro 20 was used as adsorbent. The results were evaluated using three adsorption isotherms. The samples were analysed by AAS. The maximum sorption capacity was determined from the Langmuir isotherm. The highest sorption capacity was $23.0947 \pm 0.9238 \text{ mg.g}^{-1}$ for silver adsorption. It was followed by zinc adsorption of $14.7710 \pm 0.5908 \text{ mg.g}^{-1}$. The lowest sorption capacity was $1844 \pm 0.5674 \text{ mg.g}^{-1}$ for copper adsorption. It follows that silver had a significantly higher sorption capacity than copper and zinc. The sorption capacities of copper and zinc were similar.

INTRODUCTION

Water is the most important natural substance on our planet. Without water, many organisms, including humans, would die out. Water creates a hydrosphere in which countless aquatic organisms live. It is also a habitat for terrestrial animals and plants. Water is an irreplaceable source of nutrients for humans. In addition, with the development of industry and agriculture, people have discovered many new ways of using water. Unfortunately, this industrial development also brought with it negatives in the form of pollutants and toxic substances accumulating in the waters. Industrial wastewater often contains heavy metals, ammonia and other dangerous substances (Szymula et al., 2021). The substances in question have a negative effect on living organisms. Currently, there are many methods of wastewater treatment, but these methods are often expensive and have their negatives. Adsorption appears to be a suitable and highly effective method of wastewater treatment. In adsorption, the most important thing is to choose the right adsorbent. Zeolites, bentonites, activated carbon, clay minerals and many biomaterials are most often used as effective adsorbents. Zeolites were often used in studies as

adsorbents of heavy metals and other substances. Heavy metals are one of the most dangerous substances found in water. They are harmful to humans, animals and plants.

In industrial wastewater many toxic metals, including copper, zinc and silver can be found. There are quite a few ways to remove copper, zinc and silver from water, but adsorption seems to be one of the most suitable. Zeolites, which are unique due to their specific physical and chemical properties, appear to be a suitable material for the adsorption of copper, zinc and silver.

The aim of the study was to assess the use of zeolite as an adsorbent for copper, zinc and silver.

METHODS

Zeolites are naturally occurring aluminosilicates, with an open framework crystal structure. They are used in different applications, e.g. adsorbents, surfactants, for removal of cations from polluted wastewater and industrial wastewaters. Natural zeolites can easily remove heavy metals from contaminated water. They are relatively cheap, safe, and environmentally friendly. The zeolite was obtained from the quarry in Nižný Hrabovec (SK), one of the largest deposits and cleanest zeolite areas in Europe (Zeocem, 2023).

Sorption experiments

The equilibrium experiments were carried out with a series of flasks containing 100 mL (V) of metal ion solution of different initial concentrations ($C_0 = 0.001$ to 1 g.L^{-1}) prepared from copper, zinc and silver nitrates and a fixed dosage of sorbent ZeoCem Micro 20 ($C_a = 1 \text{ g.L}^{-1}$) which were agitated for 2 hours in a rotary shaker at 200 min^{-1} , with a temperature control at 25°C , what was sufficient for the metal ions adsorption to reach an equilibrium. The initial pH of the solution was not modified.

After equilibration and sedimentation of suspensions the samples were settled and the solutions were analysed for metal content by AAS as described below. The amounts of metal adsorbed q_e (mg.kg^{-1}) in each flask were determined from the difference between the initial metal concentration C_0 and metal concentration at equilibrium C_e (g.L^{-1}) in the solution and were calculated according to equation:

$$q_e = \frac{(C_0 - C_e)V}{m_a} \quad (1)$$

m_a (g) is the weight of adsorbent, V (L) is the volume of solution.

All the experiments were performed in triples and the result was taken as the average value of each experiment.

Data analysis

Adsorption isotherms are a critical piece of information on optimization of the use of adsorbents. Isotherm models provide an adequate description of Mn (II) adsorption equilibria on zeolites. The following isotherms were used:

- Freundlich (1906):

$$q_e = K_f C_e^{1/n} \quad (2)$$

K_f ($\text{m}^{3/n} \cdot \text{kg}^{-1/n}$) is adsorption capacity, n is intensity (1); the isotherm represents sorption taking place on a heterogeneous surface with interaction between the adsorbed molecules (Albadarin et al., 2011),

- Langmuir (1916):

$$q_e = \frac{q_m a_L C_e}{1 + a_L C_e} \quad (3)$$

q_m ($\text{kg} \cdot \text{kg}^{-1}$) is maximum sorption capacity, a_L ($\text{m}^3 \cdot \text{kg}^{-1}$) is adsorption energy; the isotherm represents sorption taking place on a homogenous surface within the adsorbent (Günay et al., 2007),

- Redlich-Peterson (1959):

$$q_e = \frac{K_R C_e}{1 + a_R C_e^\beta} \quad (4)$$

K_R ($\text{m}^3 \cdot \text{kg}^{-1}$) and a_R ($\text{m}^{3\beta} \cdot \text{kg}^{-\beta}$) are constants, β (1) is exponent; the isotherm is used as a compromise between the Langmuir and Freundlich systems (Albadarin et al., 2011).

The concentrations of metals in solutions were measured by the atomic absorption spectrometer (AAS) iCE 3300 Thermo Scientific (USA) with deuterium correction background. The samples were settled and the clear solution was used for the analysis. The samples were diluted, if necessary, using distilled water.

RESULTS

The adsorption isotherms of Cu^{2+} , Zn^{2+} and Ag^+ on zeolite are presented in Figure 1, Figure 2 and Figure 3, respectively.

Figure 1 Cu^{2+} adsorption isotherms

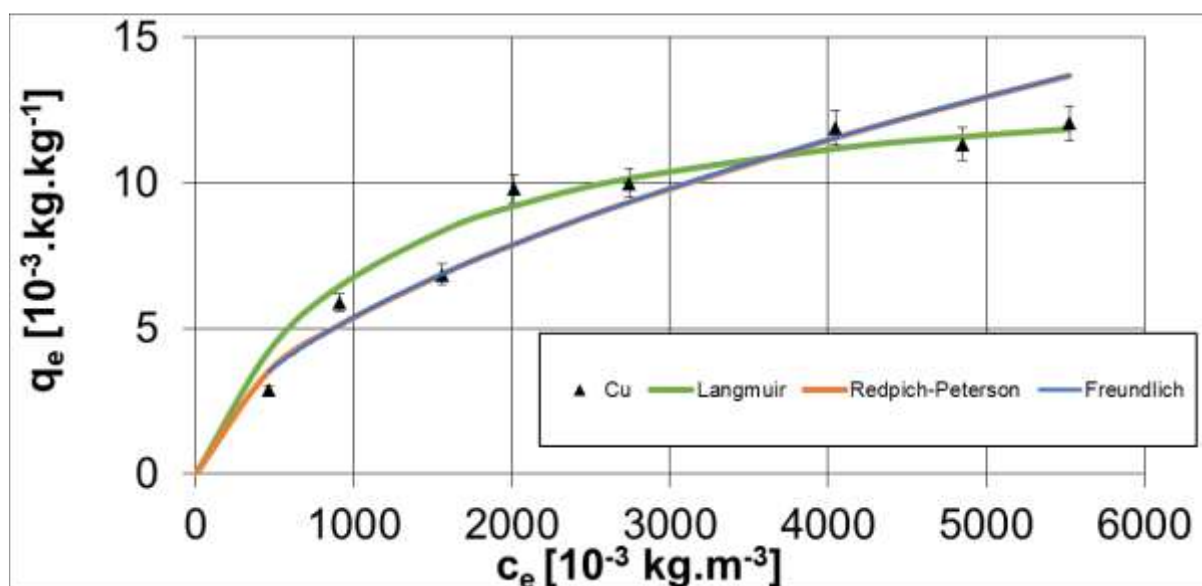


Figure 2 Zn^{2+} adsorption isotherms

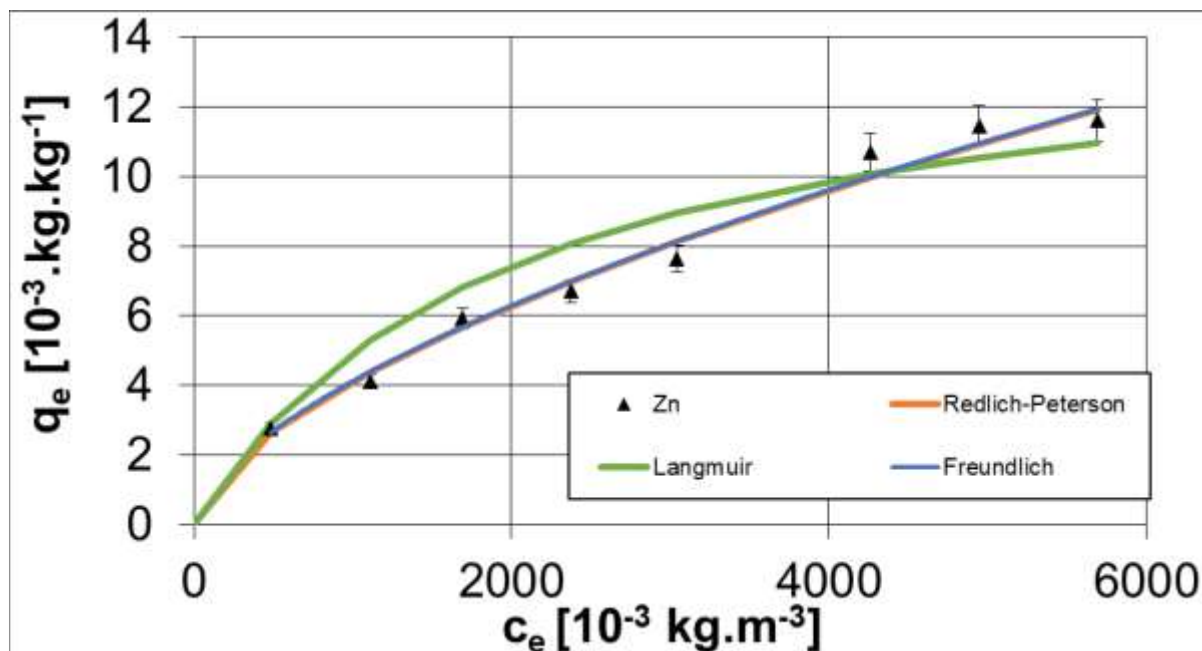
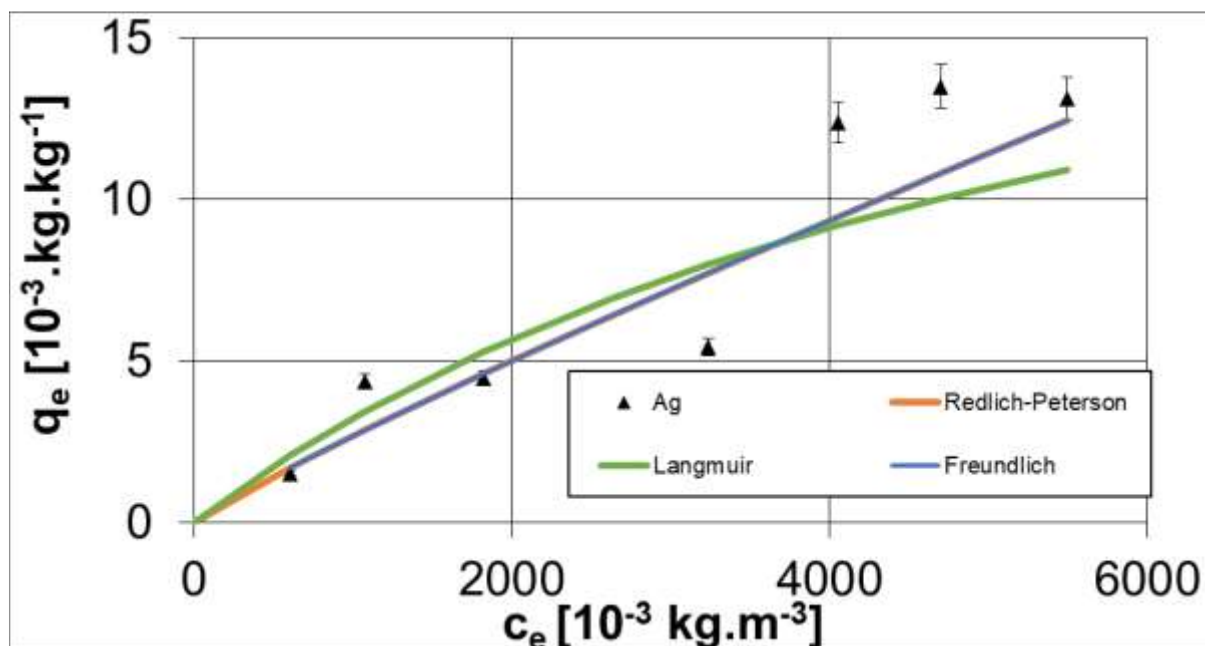


Figure 3 Ag⁺ adsorption isotherms

On the graphs presented in Figure 1 – Figure 3, the overlapping of two isotherms can be observed – it is the Freundlich adsorption isotherm and the Redlich-Peterson adsorption isotherm. The parameters of the isotherms are in.

Table 1 Adsorption isotherms parameters

Isotherm	Parameter	Cu ²⁺	Zn ²⁺	Ag ⁺
Freundlich	k_f	0.9854±0.0394	0.9761±0.0390	0.9080±0.0363
	n	1.8332±0.0733	1.6337±0.0653	1.1086±0.0443
	R^2	0.8955	0.9837	0.8382
Langmuir	q_m	14.1844±0.5674	14.7710±0.5908	23.0947±0.9238
	a_L	0.0009±0.0000	0.0005±0.0000	0.0002±0.0000
	R^2	0.9854	0.9761	0.9080
Redlich-Peterson	k_R	0.6830±0.0273	0.9452±0.0378	1.0000±0.0400
	b_R	5.3457±0.2138	15.5242±0.6210	189.0478±7.5619
	β	0.4572±0.0183	0.3894±0.0156	0.0983±0.0039
	R^2	0.9465	0.9918	0.9155

In the case of Cu²⁺ sorption on M-20, the equilibrium is best described by the Langmuir isotherm based on the highest correlation coefficient value of 0.9854. It was followed by the Redlich-Peterson isotherm with a correlation coefficient of 0.9465. The last one was the Freundlich isotherm, which had the smallest correlation coefficient value of 0.8955.

For Zn²⁺ sorption on M-20, the equilibrium is best described by the Redlich-Peterson isotherm based on the highest correlation coefficient value of 0.9918. It was followed by the Freundlich isotherm with a correlation coefficient of 0.9837. The last one was the Langmuir isotherm, the isotherm that had the smallest correlation coefficient value of 0.9761.

For Ag^+ sorption on M-20, the equilibrium is best described by the Redlich-Peterson isotherm based on the highest correlation coefficient value of 0.9155. It was followed by the Langmuir isotherm with a correlation coefficient of 0.9080. The last Freundlich isotherm was the isotherm that had the smallest correlation coefficient value of 0.8382.

The maximum sorption capacity q_m was estimated from the Langmuir isotherm. Silver had the highest sorption capacity of $23.0947 \pm 0.9238 \text{ mg}\cdot\text{g}^{-1}$. It was followed by zinc $14.7710 \pm 0.5908 \text{ mg}\cdot\text{g}^{-1}$. Copper had the lowest sorption capacity of $14.1844 \pm 0.5674 \text{ mg}\cdot\text{g}^{-1}$. It follows that silver had a significantly higher sorption capacity than copper and zinc. The sorption capacities of copper and zinc were approximately the same.

DISCUSSION

The Table 2 shows that synthetic and modified zeolites have significantly larger maximum sorption capacities than natural zeolites. The highest maximum sorption capacity was demonstrated by fly ash and NaOH synthesized zeolite with $310.0 \text{ mg}\cdot\text{g}^{-1}$.

Table 2 A comparison of sorption capacities of different Cu sorbents

Sorbent	q_m [$\text{mg}\cdot\text{g}^{-1}$]	Temperature [$^{\circ}\text{C}$]	Initial pH	Source
M-20	23.09	25	N/A	this work
magnetic nano-zeolite	59.90	25	9	Zhang et al., 2020
zeolite from Kamchatka	1.46	20	low	Belova, 2019
Faujasite type zeolite	94.46	25	5.6	Chen et al., 2020
NaP zeolite from waste lithium-silicon-powder	62.30	25	6.0	Pu et al., 2020
fly ash and NaOH synthesized zeolite	310.00	25	5.5	Vavouraki et al., 2020
NaX nano-zeolite	111.84	25	6.5	Yurekli, 2019
FAU-type zeolites from coal fly ash	57.80	room	N/A	Joseph et al., 2020

Based on Table 3 the maximum sorption capacity of ZeoCem Micro 20 for Zn^{2+} ions was lower than that of synthetic zeolites. The highest maximum sorption capacity was demonstrated by Na-X zeolite with $332.51 \text{ mg}\cdot\text{g}^{-1}$.

Table 3 A comparison of sorption capacities of different Zn sorbents

Sorbent	q_m [mg·g ⁻¹]	Temperature [°C]	Initial pH	Source
M-20		25	N/A	this work
Na-X zeolite	332.51	N/A	5.0	Panek et al., 2021
natural zeolite	7.57	20	unmodified	Kozera et al., 2020
Zeolite 3A	31.11	20	unmodified	Kozera et al., 2020
Zeolite 10A	34.30	20	unmodified	Kozera et al., 2020
Zeolite 13X	11.07	20	unmodified	Kozera et al., 2020
FAU-type zeolites from coal fly ash	36.77	room	N/A	Joseph et al., 2020

Based on Table 4 the highest maximum sorption for Ag⁺ ion sorption capacity was demonstrated by pomelo peel with 332.51 mg·g⁻¹. The Table 4 also shows that biosorbents are also important silver adsorbents.

Table 4 A comparison of sorption capacities of different Ag sorbents

Sorbent	q_m [mg·g ⁻¹]	Temperature [°C]	Initial pH	Source
M-20	23.09	25	N/A	this work
spent coffee grounds biochar	49.00	N/A	N/A	Islam et al., 2022
sunflower husk	22.90	21	5	Tomczyk et al., 2020
wood waste	19.10	30	6	Naga Babu et al., 2021
pomelo peel	137.40	30	6	
biofuel residue	90.06	20	5	Yao et al., 2015

CONCLUSIONS

The aim of the study was to assess the adsorption of Cu²⁺, Zn²⁺ and Ag⁺ ions onto natural zeolite. The zeolite was from Zeocem JSC. Zeocem Micro 20, which has an increased selective sorption, was used in the study. Based on the correlation coefficients, the most appropriate isotherm to describe the equilibrium for individual ions was selected. In the case of Cu²⁺ sorption on ZeoCem Micro 20, the equilibrium was best described by the Langmuir isotherm based on the largest correlation coefficient value of 0.9854. For Zn²⁺ sorption on ZeoCem Micro 20, the equilibrium is best described by the Redlich-Peterson isotherm. The correlation coefficient of the isotherm was 0.9918. For Ag⁺ sorption on ZeoCem Micro 20, the equilibrium is best described by the Redlich-Peterson isotherm. The correlation coefficient of the given isotherm was 0.9155. The Redlich-Peterson isotherm prevailed, which was suitable for describing the equilibrium for Zn²⁺ and Ag⁺. The least suitable isotherm to describe the equilibrium for Cu²⁺, Zn²⁺ and Ag⁺ was the Freundlich isotherm. The maximum sorption capacity was determined from the Langmuir isotherm. Silver had the highest sorption capacity

of $23.0947 \pm 0.9238 \text{ mg.g}^{-1}$. It was followed by zinc $14.7710 \pm 0.5908 \text{ mg.g}^{-1}$. Copper had the lowest sorption capacity $1844 \pm 0.5674 \text{ mg.g}^{-1}$. It follows that silver had a significantly higher sorption capacity than copper and zinc. The sorption capacities of copper and zinc were similar.

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THE SPREAD OF INVASIVE PLANT SPECIES: PROBLEMS AND POSSIBLE SOLUTIONS

INVÁZNE BOTANICKÉ DRUHY- PROBLÉMY SPOJENÉ S ICH ŠÍRENÍM A NÁVRHY NA RIEŠENIE

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V poslednej dobe predstavujú invázne druhy rastlín závažný celosvetový problém. V prírode sa správajú veľmi agresívne a potláčajú rast pôvodných druhov rastlín. Takýmto spôsobom je ohrozovaná biodiverzita druhov v krajine.

V posledných troch desaťročiach bol zaznamenaný nárast počtu invázných botanických druhov. Môže to byť spôsobené najmä rozširovaním obchodných sietí, ale aj fenoménom globálnej zmeny klímy, ktorá môže vytvárať vhodnejšie podmienky pre určité druhy na regionálnej úrovni (Ružek a Noga, 2015)

S nárastom medzinárodného obchodu, rastie aj množstvo zavlečených druhov rastlín objavených v Európe, najmä od začiatku 19. storočia. Dnes je do Európy každoročne novo zavlečených približne 6 druhov, ktoré nie sú pôvodné v žiadnej časti Európy. Naopak, v priemere asi 5 pôvodom európskych druhov je každý rok objavených v oblasti kontinentu, mimo ich pôvodného areálu výskytu (Lambdon, a kol., 2008).

Invázne rastliny boli najčastejšie dovezené ako okrasné alebo medonosné. Väčšina z nich nemá veľké nároky na živiny a preto sa začali z parkov a výsadiieb rýchlo šíriť do okolia a obsadzovať nové plochy. Najväčšia početnosť zavlečených druhov rastlín v Európe, sa nachádza na poľnohospodársky využívaných plochách. Ľudia si nebezpečenstvo invázných rastlín často neuvedomujú, pretože rastú väčšinou na miestach, kde kryjú aj nevzhľadné zákutia. Práve aj tieto miesta sú ohniskom ich šírenia do okolia (Sabo a Turisová, 2019). Významnou migračnou trasou sú aj cestné komunikácie.

Problematika invázných rastlín je v súčasnosti veľmi aktuálna aj na Slovensku. Legislatívne upravuje problematiku nepôvodných a invázných druhov rastlín zákon č. 543/2002 Z. z. o ochrane prírody a krajiny v znení neskorších predpisov. Sú zaradené do prílohy č. 1 v nariadení vlády Slovenskej republiky č. 449/2019 Z. z., ktorým sa vydáva zoznam invázných nepôvodných druhov vzbudzujúcich obavy Slovenskej republiky. Viazu sa naň ustanovenia zákona č. 150/2019 Z. z. o prevencii a manažmente introdukcie a šírenia invázných

nepôvodných druhov a zmeny a doplnenia niektorých zákonov. Boli sem zaradené druhy, ktoré spôsobujú najväčšie problémy, resp. ktoré majú najväčší negatívny vplyv na naše pôvodné druhy a ich biotopy a najviac menia krajinu.

Na území sa vyskytujú aj ďalšie nepôvodné druhy rastlín, ktoré síce neboli zaradené do legislatívnych zoznamov inváznych druhov, ale napriek tomu sa na našom území správajú invazívne alebo majú potenciál stať sa inváznymi, t. j. tiež majú negatívny vplyv na pôvodné druhy a biotopy Slovenska. Tieto druhy sú zaraďované do tzv. vedeckých zoznamov a z rôznych dôvodov zatiaľ neboli zaradené do legislatívnych zoznamov. Tiež sa monitorujú a zaznamenáva sa ich výskyt a ďalšie šírenie.

V dôsledku klimatických zmien nachádzajú invázne druhy rastlín aj na Slovensku stále lepšie podmienky. Hlavným faktorom, ktorý umožňuje rýchly nástup a získanie dominantného postavenia inváznych druhov je narušovanie pôdneho krytu. Obnažené plochy osídľujú porasty zlatobyle obrovskej (*Solidago gigantea*) či zlatobyle kanadskej (*Solidago canadensis*). Pohánkovec japonský (*Fallopia japonica*) vytvára na miestach svojho výskytu husté, porasty. Sumach pálkový (*Rhus typhina*) je okrasnou drevinou a pomocou podzemných poplázov sa šíri a obsadzuje nové stanovišťa. Agát biely (*Robinia pseudoacacia*) vrasť do prirodzených lesných spoločenstiev alebo vytvára samostatné porasty a zhoršuje kvalitu pôdy. Hviezdnik ročný (*Stenactis annua*) osídľuje synantropné stanovišťa, ale rastie aj pozdĺž komunikácií, v pobrežných krovinách či lúčnych biotopoch. Slnečnica hľuznatá-topinambur (*Helianthus tuberosus*) sa šíri hlavne v pobrežných porastoch, ale aj na antropogénnych stanovištiach pozdĺž ciest či na ruderalných plochách. Pavinič päťlistý (*Partenocissus quinquefolia*) je drevitá liana, často splaňuje a rozširuje sa hlavne do brehových porastov a cestných okrajov, ale problémy spôsobuje aj v intravilánoch miest. Netýkavka žliazkatá (*Impatiens glandulifera*) prenikla zo záhrad aj do voľnej prírody a šíri sa hlavne v brehových porastoch. Boľševník obrovský (*Heracleum mantegazzianum*) sa považuje za najväčší invázny druh. Ambrózia palinolistá (*Ambrosia artemisifolia*) je nebezpečná karanténna a alergénna burina. Americké astry (*Aster* sp. agg.) niekedy označované ako astry novobelgické rastú hromadne na opustených plochách, rumoviskách, skládkach, pobrežných krovinách a na okrajoch ciest.

Invázne rastliny majú vysoký reprodukčný potenciál. Dokážu sa rýchlo šíriť vegetatívnym spôsobom alebo vytvárajú veľké množstvo semien s vysokou klíčivosťou. V prípade ich masového rozšírenia významne menia charakter biotopov a ohrozujú pôvodné druhy rastlín a vytvárajú homogénne monocenózy. Prinášajú so sebou tak nielen ekologické, ale aj zdravotné a hospodárske problémy. Niektoré sú známe ako silné alergény (zlatobyľ kanadská, zlatobyľ obrovská, ambrózia palinolistá) iné vyvolávajú rôzne kožné poranenia (boľševník obrovský).

Keď sú už rastliny svojim masovým výskytom dominantné nad pôvodnými druhmi, tak je namieste mechanické ničenie, vytrhávanie, kosenie a v prípade už homogénneho porastu je nevyhnutné použiť aj chemický postrek respektíve kombináciu oboch metód. Následná rekultivácia plôch je pozitívnym výsledkom manažmentu (Cvachová a Gojdičová, 2003).

Včasný manažment a vhodný spôsob odstraňovania, ktorý je popísaný v prílohe č. 2 a 3 Vyhlášky MŽP SR č. 450/2019 Z.z. je veľmi dôležitý. Práve touto vyhláškou sa ustanovujú podmienky a spôsoby odstraňovania invázných nepôvodných druhov. Ďalej postupy usmerňuje Vykonávacie nariadenie Komisie (EÚ) č. 2016/1141 z 13.júla 2016. Ním sa prijal zoznam invázných nepôvodných druhov vzbudzujúcich obavy únie podľa Nariadenia Európskeho parlamentu a Rady (EÚ) č. 2014/1143 z 22. októbra 2014 o prevencii a manažmente introdukcie a šírenia invázných nepôvodných druhov. Toto nadobudlo účinnosť ešte v roku 2015

Riešenie negatívnych dôsledkov spojených so šírením invázných, nepôvodných, druhov rastlín nie je úlohou len rezortu životného prostredia, ale je to výzva i pre ostatné rezorty vrátane pôdohospodárstva, zdravotníctva, lesného hospodárstva, záhradníctva, sadovníctva, parkovníctva, samozrejme životného prostredia, ale aj klimatológie a vodohospodárstva. Predpokladá to rozdelenie povinností a kompetencií a prijatie konkrétnych opatrení. Súčasťou je aj aktívne zapojenie a podpora širokej verejnosti.

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**ROLE OF VERTICAL VS DIAGONAL PROGRAMS TO CONTROL, ELIMINATE
AND ERADICATED INFECTIOUS DISEASES IN THE ERA OF GLOBAL
CHANGES AND THE PATH TOWARDS RESILIENT AND SUSTAINABLE
INTEGRATED HEALTH SYSTEMS**

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The text of the contribution was not sent by the deadline.

LECTURES

NON-INFECTIOUS CLAW DISEASES AND HEAT STRESS IN DAIRY COWS

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ABSTRACT

The observations were performed on a Holstein-Friesian (HF) dairy farm in Eastern Slovakia. The clinical signs of heat stress were recognised on July 8, 2015. The claw examination done three months after the heat stress episode was compared with three examinations: twelve and two months prior to the heat stress and ten months afterward. The temperature-humidity index (THI) on July 8, 2015 was 88. We found 15.2%, 18.6%, 28.1%, and 12.9% cows with sole ulcer in the autumn 2014, spring 2015, autumn 2015, and spring of the following year, respectively ($P < 0.05$). In conclusion, the results of our observations showed that the heat stress in dairy cows can be associated with an increase in the prevalence of the sole ulcer.

INTRODUCTION

Digital dermatitis, sole ulcers, and white line disease are the most frequently reported foot lesions related to the lameness of dairy cows. Consequently, the lameness belongs to the most frequent cause of the largest economic losses in the dairy industry (Ettema and Ostergaard, 2006). Regardless of whether foot lesions cause lameness, they have a high impact on animal welfare and farm economics. Sole ulcers are dominating representatives of non-infectious claw diseases causing lameness and occur when claw horn formation is disrupted. The underlying tissue then becomes inflamed and sometimes exposed. Cows are particularly vulnerable around the time of parturition; changes in hormone levels cause increased vascular permeability, increasing the risk of oedema and ischemia in the hoof, while concurrent weakening of connective tissue causes the pedal bone to drop and compress the corium, further disrupting claw horn formation. Heat stress is defined as the sum of external forces acting on an animal that causes an increase in body temperature and evokes a physiological response. The temperature-humidity index (THI) has been commonly used to estimate the effect of heat stress on production and reproduction. There is a general agreement that significant effects are observed at a mean daily THI of around 72 (West, 2003). Documented physiological heat stress coping strategies used by dairy cows include: increased respiration rate, panting, sweating,

reduced milk yield, and reproductive performance. The duration of elevated temperatures has an inverse relationship with feed intake. Therefore, it is not surprising that heat stress is considered a major risk factor for lameness, but whether this association is a consequence of increased standing times or due to alterations in nutrient metabolism caused by a decrease in dry matter intake (DMI) is not known (Cook et al., 2007). Claw horn lesions, such as sole ulcer, are believed to develop from increased pedal bone mobility induced by changes in the corium at calving and potentially from nutritional insults such as subacute ruminal acidosis. The main aim of this study was to evaluate the effects of heat stress on the occurrence of the sole ulcer in dairy cows.

MATERIALS AND METHODS

The observations were performed on a Holstein-Friesian (HF) dairy farm in Eastern Slovakia. All dairy cows were milked twice daily (at 06:00 and 14:30 h) and had free access to water and were fed a total mixed rations (corn silage, corn, soybeans, minerals and vitamins), according to their lactational stage. No specific measures to protect the animals against the heat stress were taken on the farm. The clinical signs of the heat stress (increased respiration rate, panting, and sweating) were recognised in several animals during the afternoon milking on July 8, 2015. The claws of dairy cows were controlled three month (October 2015) after the heat stress event and the results were compared with results of the claw controls performed twelve and two months prior to the heat stress (October 2014, May 2015), and ten months after it (May 2016). The weather data of air temperature and relative humidity in the region on the day of the heat stress were obtained from the Slovak Hydrometeorological Institute to calculate temperature-humidity index. The THI was calculated as per the equation proposed by the National Research Council of the United States [18]: “ $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$ ” where T is the outdoor ambient temperature in °C, and RH is relative humidity expressed in %. Statistical analysis of differences in sole ulcer prevalence was performed by running a chi-squared test. P values < 0.05 were considered significant.

RESULTS

Comparisons of the four claw health controls revealed a highest prevalence ($P < 0.05$) of sole ulcers in the examination three months after (October 2015) the animals had been challenged by heat stress (Table 1). The local outdoor ambient temperature and humidity on July 8, 2015 were 35.6 °C and 61.3%, respectively. The calculation of THI resulted in the value of 88.

Table 1. Prevalence of sole ulcers in dairy cows challenged by the heat stress

	October 2014	May 2015	*October 2015	May 2016	χ^2
Cows total	211	210	209	212	NS
Sole ulcer (n)	32a	39a	59b	27a	$P < 0.05$

*a, b – prevalence differs ($P < 0.05$); NS – not significant; * Heat stress July 8, 2015*

DISCUSSION

Claw lesions are commonly categorized according to their aetiology into infectious and non-infectious lesions. Non-infectious lesions affect the claw horn, and their occurrence is associated with metabolic and hormonal events around calving that weaken the foot suspensory apparatus, lower the Body Conditioning Score (BCS), toe overgrowth, exposure to hard flooring, and thickness of the digital cushion. The prevalence of sole ulcers can differ according to the parity of the cows. Thus, the prevalence of sole ulcers was 4.2 and 27.8% for parity 1 and parity >1, respectively (Bicalho et al., 2009). Sole ulcer prevalence in the present study varied between 10 and 20% in the sessions which were not related to the heat stress event. The higher number of the sole ulcer detected three months after the heat stress in our study indicate a causative effect of the organ and metabolic changes triggered by the body reaction to high ambient temperature and humidity. It has been suggested that sole ulcers are a consequence of subclinical laminitis. Laminitis is an important predisposing cause of claw disorders in cattle. Inflammation of the corium results in the activation of tissue matrix metallo-proteinases (MMPs) that weaken the collagen fibre bundles that make up the suspensory apparatus within the claw. Coincident with this is the release of horn growth and necrosis factors that contribute to the inflammatory process and accelerate claw horn growth. The vascular disturbances associated with laminitis preclude the normal diffusion of nutrients and oxygen into the living-cell layers of the epidermis destined to become claw horn. It has been suggested that “claw horn disruption” may be a better term for the condition of laminitis, because it more accurately reflects the nature of the anatomical and physiological lesions associated with laminitis (Moragues et al., 2021). The pathogenesis of laminitis is believed to be associated with a disturbance in the micro-circulation of blood within the corium which leads to weakening of the suspensory apparatus within the claw and thus permits downward displacement and rotation of the third phalanx (P3). The result is compression of the corium and supporting tissues that lie between P3 and the sole which predisposes to the development of sole ulcers (Lischer et al., 2002). Rumen acidosis is considered to be a major predisposing cause of laminitis and presumably mediates its destructive effects through various vasoactive substances (endotoxins, lactate, and possibly histamine) that are released into the blood stream in coincidence with the

development of rumen acidosis. The SubAcute Ruminant Acidosis (SARA) is more common than the acute form of this disease. Rumen pH was lower in cows exposed to the higher temperatures and those fed the higher concentrate diets (Potterton et al., 2012). In addition, behavioural changes observed and traditionally associated with heat stress are confounded by changes in locomotion score that typically occur over the late summer months. Increases in claw horn lesion development in the late summer may be associated with the increase in total standing time per day due to heat stress (Cook et al., 2007). THI < 68 is accepted to be outside the thermal danger zone for cows. Mild signs of heat stress are observed at THI of 68 to 74, and a THI \geq 75 will cause drastic decreases in production performance. Therefore, THI of 88 observed in the present study speaks clearly for a severe stressful event in the examined animals.

CONCLUSION

Results of our observations show that the heat stress in dairy cows can be associated with increase in the prevalence of the sole ulcer. The findings may be useful in stressing a need to take measures to prevent the heat stress in dairy cows.

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GENETIC DIVERSITY OF *STRONGYLOIDES* INFECTING DOGS

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ABSTRACT

Strongyloides stercoralis was detected in fecal samples from African feral dogs and in a dog from the Czech Republic. Phylogenetic analysis of partial *cox1* revealed that found *S. stercoralis* strains belonged to the lineage A, which infects dogs, humans, and other non-human primates. The sequences of 18S rDNA supported these results. We diagnosed disseminated strongyloidiasis with fatal consequence in a dog from the Czech Republic. Appropriate use of coproscopic and molecular methods is important for *Strongyloides* diagnostics. Metagenomic analysis of model parasite-multi-host systems is a key to uncovering the genetic diversity required to understand the disease transmission.

INTRODUCTION

Rhabditid nematodes of the genus *Strongyloides* are important soil-transmitted nematodes in humans, domestic animals, and wildlife, especially in tropical rural areas (Schär et al., 2013; Hasegawa et al., 2016; Jaleta et al., 2017). The genus *Strongyloides* includes at least fifty species from which *Strongyloides stercoralis* is the most important for public and animal health. The nematode is a generalist parasite affecting a range of hosts, including carnivores, humans, and especially captive non-human primates (NHPs) (Bradbury et al, 2021) and causes

strongyloidiasis, disease with mild gastrointestinal symptoms but also potentially lethal disseminated systemic infection (Streit et al., 2020). Within *S. stercoralis*, two lineages have been described: the zoonotic lineage A and the likely canine lineage B (Jaleta et al., 2017). In our project, we explored the genetic diversity of *Strongyloides* species in feral dogs (a potential reservoir of infection) that share their habitat with other susceptible hosts (humans, NHPs) in three African countries and in a dog from the Czech Republic.

MATERIAL AND METHODS

Individual samples from feral dogs (N=365) were collected in the surroundings of the Volcanoes National Park in Rwanda, Bwindi Impenetrable National Park in Uganda, and in Dzanga-Sanga Protected Areas in the Central African Republic. Total DNA was extracted and used for qPCR detection of *Strongyloides* (Verweij et al., 2009). Selected positive samples for *Strongyloides* were amplified and high-throughput sequenced (HTS) on the Illumina MiSeq platform. For comparison, fecal sample from an ill dog imported to the Czech Republic from Slovakia was used for DNA amplification via conventional PCR followed by Sanger sequencing. The hypervariable region (HVR-IV) of the 18S rDNA gene and a selected portion of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*) were targeted by both approaches (Barratt et al., 2019).

RESULTS

Strongyloides stercoralis was detected in samples from African feral dogs. Based on *cox1*, all sequences were assigned to a highly supported clade corresponding to zoonotic lineage A *sensu* Jaleta et al., 2017. Sequences of 18S rDNA HVR-IV supported these results, haplotypes A and E with known zoonotic potential were found. The prevalence of *Strongyloides* varied among the location and was affected by the presence of other potential *Strongyloides* hosts sharing habitat with dogs. In addition, haplotype A of *S. stercoralis* was detected in the dog from the Czech Republic and confirmed disseminated strongyloidiasis with fatal consequences.

DISCUSSION

Regular monitoring of *Strongyloides* infections, including the genetic identification and genotyping, should be an important component of health management. However, genetic analyses are not routinely performed (Nosková et al., 2023). Flotation-based coproscopic methods are insufficient for detection of *S. stercoralis* because the oval eggs containig so-called U-larva, typical for other *Strongyloides* species, are not present in feces (Hasegawa and Pafčo,

2018), and Baermann larvoscopy should be implemented to detect typical L1 rhabditiform larvae instead. Based on our findings, we propose that feral dogs may serve as a reservoir of *S. stercoralis* to humans and NHPs and that *S. stercoralis* can negatively impact canine health. Haplotype A appears to be the causative agent of disseminated strongyloidiasis in dogs (Unterköfler et al., 2022), as evidenced by our case. Utilizing appropriate coproscopic methods can aid in the prevention of disseminated strongyloidiasis.

CONSLUSION

Haplotype from the zoonotic lineage A of *S. stercoralis* was detected in African dogs and in a dog from the Czech Republic. Circulation of this apparently common zoonotic haplotype should simultaneously be reflected in public health protection. Strongyloidiasis continues to be a global challenge for both human and veterinary medicine across the world.

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ASSESSING THE ROLE OF SALT LICKS IN PARASITE TRANSMISSION AND SPILLOVER BETWEEN DOMESTIC SHEEP AND WILD REINDEER IN NORWAY

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ABSTRACT

Wild reindeer in Norway live on natural pastures in mountain areas, which they share with an increasing number of grazing domestic sheep. Some areas have noted lower calf numbers and slaughter weights of wild reindeer during the last two decades, and changes in prevalence and burden of parasites may be one factor causing the decreasing trends in these areas.

Identifying Chronic Wasting Disease (CWD) in reindeer in Nordfjella in 2016 has highlighted the significance of saltlicks as potential transmission hotspots, with concerns about pathogen dissemination among animals frequenting these sites. Saltlicks are strategically placed salt blocks used to supplement the nutritional needs of grazing domestic ruminants, and their presence also facilitates easier monitoring of animals.

Saltlicks are intensively utilized by various ruminant species, both wild and domesticated, effectively functioning as congregation spots within natural pastures. To evaluate the impact of saltlicks on parasite transmission, soil samples from areas surrounding saltlicks and control spots in three wild reindeer areas in Norway were collected. These samples were subjected to qualitative and quantitative analysis to detect the presence of parasites.

Furthermore, the study involved the collection of fecal and visceral samples from both wild reindeer and domestic sheep. These samples underwent thorough analysis to identify the presence and abundance of parasites.

Analysis of the collected samples suggests parasite spillover between domestic sheep and wild reindeer, underscoring the potential for pathogen transmission between these sympatric species. In conclusion, this study sheds light on the role of saltlicks in facilitating parasite accumulation and potential spillover between domestic sheep and wild reindeer. The findings provide valuable insights that can help inform future strategies and approaches for effectively managing natural pastures shared by both sheep and wild reindeer, taking into account the dynamics of parasite transmission and spillover.

HOW CONAZOLE FUNGICIDES AFFECT VITAL BIOMACROMOLECULES: A STUDY ON MOLECULAR LEVEL

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ABSTRACT

Monitoring the impact of conazole fungicides on the structure and stability of important bio-macromolecules may contribute to reducing the use of these fungicides as well as considering and adjusting the tolerances of fungicides and their residues in food. In this contribution, we have studied the interactions between chosen conazoles as the ligands and mammalian transport proteins represented by human serum albumin and bovine serum albumin, respectively on the molecular level. Biophysical methods of optical spectroscopy combined with molecular modeling showed specific binding sites in the macromolecule structures for conazoles incorporation. Moreover, the mode and strength of interaction represented by association constants were determined.

INTRODUCTION

Studies of interactions between pesticides and target mammalian proteins are important steps toward an understanding a pesticide's toxicity. We have interested in fungicides and have chosen three conazoles widely used in agriculture as crops protection products: tebuconazole (TB), epoxiconazole (EPX), and prothioconazole (PTC).

TB is the active ingredient used in Orius 25 EW, which protects a number of crops such as grapes, rice, fruits, and vegetables because of its broad-spectrum antifungal activity. Its antifungal activity is due to its ability to inhibit the P450 enzyme, which blocks the conversion of lanosterol to ergosterol causing disruption of the fungal wall (Di Renzo et al., 2015). However, the inhibition potency of TB is not limited to fungi; it may also inhibit other P450-mediated activities resulting in various adverse effects (Robinson et al., 2012). The US EPA

has classified this fungicide as Group C-Possible Human Carcinogen (U.S. Environmental Protection Agency, 2014).

EPX and PTC are the active ingredients used in Opal 7.5 EC (EPX) and Prosaro 250 EC (PTC), respectively. They actively stop the production of new fungi spores and inhibit the biosynthesis of ergosterol. Inhibition of lanosterol-C14-demethylase (CYP51) leads to the accumulation of methylated sterols in the fungal membrane, thereby impairing its function (Goetz et al., 2006). Serum albumins (SA) are the most abundant plasma proteins and contribute to a significant number of transport and regulatory processes. Human serum albumin (HSA) and bovine serum albumin (BSA), respectively are monomeric proteins composed of 585 (HSA), 583 (BSA) amino acids with total molecular weight of 66 kDa. They are organized into three main domains (I, II, III), where each can be divided into two subdomains (A and B). BSA differs of HSA by a number of tryptophan amino acids, HSA contains only one tryptophan molecule (Trp214) while BSA two ones (Trp134 and Trp213). These amino acids are supposed to be binding sites for many ligands; moreover, they are identifiable by spectroscopic methods.

Recently, several studies have been carried out to examine the toxic effects of pesticides at the protein level (Silva et al., 2004; Cui et al., 2006), but information about the possible impact of conazoles on plasma proteins is still limited (Wang et al., 2011; Zhang et al., 2013).

MATERIAL AND METHODS

TB, EPX, and PTC with 99% purity as well as HSA, BSA (fatty acid free, globulin free, purity no less than 99 %) were purchased from Sigma Aldrich, Darmstadt, Germany. The pesticides were dissolved in spectroscopic grade 100 % ethanol at concentration 10^{-3} mol/L. Stock solutions of HSA and BSA (concentration 5×10^{-4} mol/L) were prepared in Tris-Cl⁻ (0.05 mol/L Tris + 0.1 mol/L NaCl) buffer, pH 7.4, and conserved in the dark at 4 °C.

Fluorescence measurements were performed on a SHIMADZU RF 5301 PC spectrofluorimeter (Shimadzu, Kyoto, Japan) in a 1 cm quartz cuvette. Excitation wavelength was set at 295 nm and fluorescence was collected from 300 to 500 nm using 5 nm/5 nm slits (HSA) and 3 nm/3 nm slits (BSA). Fixed concentrations of HSA and BSA (2×10^{-6} mol/L, 2.5 mL) with various concentrations of EPX and PTC were added to the sample cell by means of titration (to give a final volume 80 µL). After each titration, the sample was stabilized for 10 min. Measurements in all experiments were taken at three different temperatures (298, 303, 310 K).

Molecular docking: We used HSA and BSA crystallographic data from the Brookhaven Protein Data Bank. The species selected were 1AO6 (HSA) and 4F5S (BSA) (Sugio et al., 1999), as these describe the proteins in their unbound state with sufficient precision. The molecular

structures of EPX and PTC were taken from the PubChem database. For docking, we used AutoDockVina (Lee and Wu, 2015). Based on experimental results we believe that the rigid docking that is supported in AutoDockVina provides sufficiently good results to reality. Blind docking was first performed for initial interaction site targeting with the result of most energetically stable results being in the vicinity of the warfarin binding site with other binding energy falling by at least 10% or more. In the investigation, we also included other binding sites which are mentioned in the literature (Trott and Olson, 2010). Our main focus was thus on investigating TB, EPX, and PTC activity at the warfarin binding site. 2D representations were done as described in (Stierand et al., 2006).

RESULTS AND DISCUSSION

Fluorescence measurements were carried out to investigate whether TB, EPX, and PTC interact with HSA and BSA, respectively. The fluorescence spectra of conazole/albumin complexes in several ratios (not shown) gave us the knowledge about binding constants and thermodynamic parameters they are important for determination of an interaction mode in case if the conazoles incorporate into the HSA, BSA macromolecule structure. Measured and calculated data for the conazole complexes with HSA are summarized in Table 1.

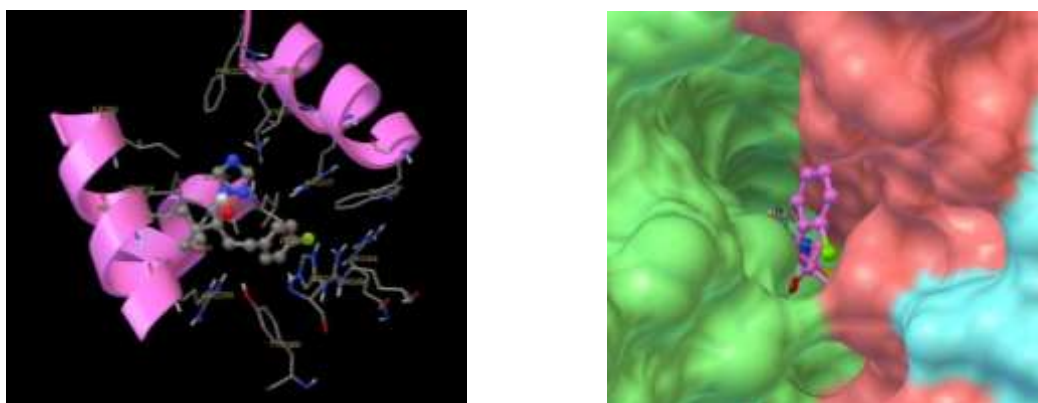
Table 1. Summarization of binding and thermodynamic parameters for TB, EPX, PTC interaction with HSA

<i>Complex</i>	<i>T (K)</i>	<i>K_A (L/mol)</i>	<i>n</i>	<i>ΔG (kJ/mol)</i>	<i>ΔH (kJ/mol)</i>	<i>ΔS (J/mol.K)</i>
<i>TB/HSA</i>	298	$8.51 \times 10^3 \pm 0.09$	1.01 ± 0.06	-22.68 ± 0.001	-56.96 ± 0.02	-115.98 ± 0.07
	303	$4.85 \times 10^3 \pm 0.02$	0.98 ± 0.02	-22.10 ± 0.001		
	310	$3.46 \times 10^3 \pm 0.02$	0.96 ± 0.09	-20.99 ± 0.002		
<i>EPX/HSA</i>	298	$6.22 \times 10^4 \pm 0.04$	0.98 ± 0.01	-26.51 ± 0.001	-105.74 ± 0.01	-265.88 ± 0.05
	303	$4.85 \times 10^3 \pm 0.02$	0.98 ± 0.02	-22.10 ± 0.001		
	310	$3.46 \times 10^3 \pm 0.02$	0.96 ± 0.09	-20.99 ± 0.002		
<i>PTC/HSA</i>	298	$5.75 \times 10^5 \pm 0.03$	1.11 ± 0.01	-32.95 ± 0.05	-64.39 ± 0.07	-105.50 ± 0.09
	303	$3.80 \times 10^5 \pm 0.01$	1.07 ± 0.01	-32.42 ± 0.04		
	310	$2.08 \times 10^5 \pm 0.01$	0.98 ± 0.01	-31.68 ± 0.05		

Dissociation constants in the Table show us that TB binds with HSA weakly in comparison with EPX, PTC. The negative values of ΔG indicate that these binding processes are spontaneous. The negative values of ΔH and ΔS suggest that the hydrogen bond and van der Waals forces play a major role in the interaction (Congdon et al., 2003).

To find a site of the conazole molecules incorporation into albumin macromolecular structure we have used theoretical models based on the molecular docking method (Trott and Olson, 2010). We identified subdomain IIA (warfarin's binding site) as the binding site of TB to BSA (Figure 1a). The amino acids surrounding this conformation are: Tyr149, Arg194, Arg195, Trp213, Arg217, Leu218, Phe222, His241, Arg256, Leu259 and Leu263. We can notice that TB is not bound in the cavity through any bonds, but its anchoring is supported by the existence of electrostatic forces. In Figure 1b, the second PTC molecule's binding site within BSA is shown in 3D representation. The binding site for PTC in the BSA molecule is located at the junction of domains I and III. In BSA, a π -cation bond between PTC and His145 with a binding energy of -28.05 kJ/mol occurs.

Figure 1. 3D representation of TB incorporation into BSA (left). The binding site on domain split for PTC/BSA interaction (right)



CONCLUSION

Investigation of the interaction of conazole fungicides with serum albumins is essential for the determination of their function in biological systems. TB, EPX, and PTC were found to interact with BSA and HSA in vitro under simulated physiological conditions. The binding and thermodynamic parameters confirmed structurally - related binding modes, which is in good agreement with the molecular modeling results. The displacement of probes from BSA and HSA after the addition of the fungicides indicated that Sudlow site I (subdomain IIA) was their probable binding site to BSA and HSA with the exception of the PTC/BSA complex, where we suggest that PTC binds onto the surface of BSA. In PTC/HSA and PTC/BSA complexes, we observed another more probable binding site due to conformational changes, giving better access for PTC to subdomain IIA.

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EXPOSURE OF HONEY BEES TO COMMONLY USED PESTICIDES MODULATES THEIR IMMUNE RESPONSE

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ABSTRACT

Honey bees are under constant stress from the environment which affects their health. In this study, we focused on the effect of pesticides on bee health. Following the application of fluvalinate we observed a significant increase in relative gene expression of antimicrobial peptides, on the other hand, the therapeutic dose of amitraz and formic acid caused a significant decrease in relative gene expression. We conclude that pesticides are able to modulate the immune response of bees causing either constant stimulation or making them more susceptible to diseases.

INTRODUCTION

Bees are among the most endangered species of animals by global changes. Whether it is the chemicals used for the protection of crops against pests that bees come in contact with during foraging, the pharmaceuticals used for the treatment of the bees themselves (Sánchez-Bayo et al., 2016), or the emerging predators of bees with the most recent being the Asian hornet (Requier et al., 2019). A lot of focus and resources have been therefore allocated to the management of bee health in recent years.

In this work, we focused on the effect of common pesticides that are used for the protection of crops and the treatment of *Varroa destructor*, a mite parasite of bees.

MATERIAL AND METHODS

Two experiments studying the sublethal effects of commonly used acaricides were conducted in this work; we assessed the effect of chronic exposure of bees to tau-fluvalinate and single exposure to amitraz and formic acid. In both cases, the long-living winter bees that are responsible for the winter survival of colonies were used as experimental animals.

For fluvalinate testing, 55 bees from each group were reared in cages and given fluvalinate in two concentrations combined with syrup or just pure syrup. Tau-fluvalinate is of low water solubility, so acetone was used as a solvent in both tested concentrations and the solvent control group. This experiment lasted 10 days, and samples were collected on the last day. For the second test, five colonies were treated with 60% formic acid using the sponge method with the amount of 2 ml of formic acid per frame. Five colonies were also treated with amitraz, using the fumigation method, performed by burning the strip with 7mg of amitraz inside the closed hive. Samples were collected 2 and 4 weeks after the application of acaricides.

The bees' intestinal tracts were harvested under aseptic conditions ($n = 15/\text{group}$) and washed in PBS. Following the manufacturer's instructions, the total RNA of guts was isolated by Purezol™ reagent (BioRad, USA). The purity and concentration of total RNA yield were measured using a spectrophotometer Nanodrop 8000 (Thermo Scientific, USA) at 260/280 nm. QuantiTect Reverse Transcription Kit (Qiagen, Germany) was used cDNA synthesis following the manufacturer's instructions. cDNA was then used for qPCR. Each reaction consisted of 20ng template cDNA, 5 μL of iQ™ SYBR® Green Supermix (BioRad, USA), 0.5 μM of forward and reverse primer for a total volume of 10 μL . β -actin was used as a reference gene and primer sequences are in Table 1. The thermal program included initial denaturation at 95 °C for 5 minutes, amplification including 40 cycles in 3 steps: denaturation at 95 °C, annealing at 59 °C, extension at 72 °C, each step 30 s with a final extension at 72 °C for 5 minutes, followed by melting curve analysis to confirm amplification of the specific product. Relative gene expression was calculated by the $2^{-\Delta\Delta\text{Ct}}$ method for each antimicrobial compound separately.

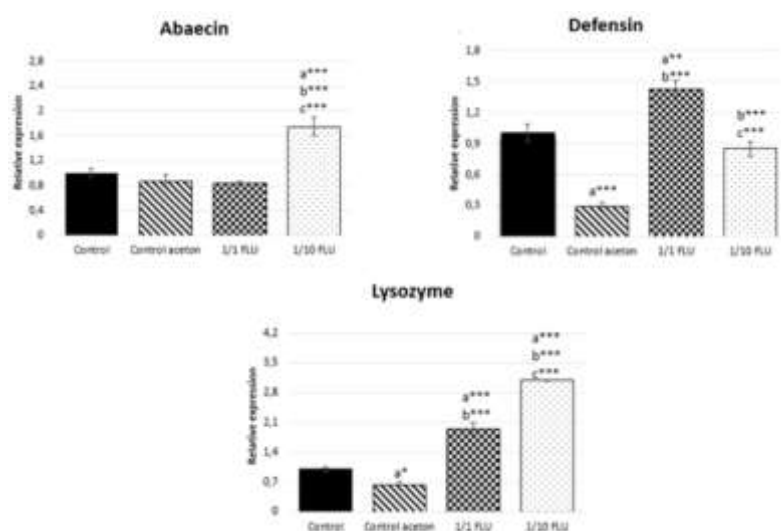
Table 1. List of primer sequences used in the study

Genes	Primer sequences	
	Forward primer (5' → 3')	Reverse primer (3' → 5')
β-actin	TTGTATGCCAACAACACTGTCCTTT	TGGCGCGATGATCTTAATTT
Abaecin	CAGCATTCGCGTATGTACCA	GACCAGGAAACGTTGGAAAC
Defensin-1	TGTCGGCCTTCTCTTCATGG	TGACCTCCAGCTTTACCCAAA
Lysozyme-2	CCAAATTAACAGCGCCAAGT	GCAATTCTTCACCCAACCAT

RESULTS

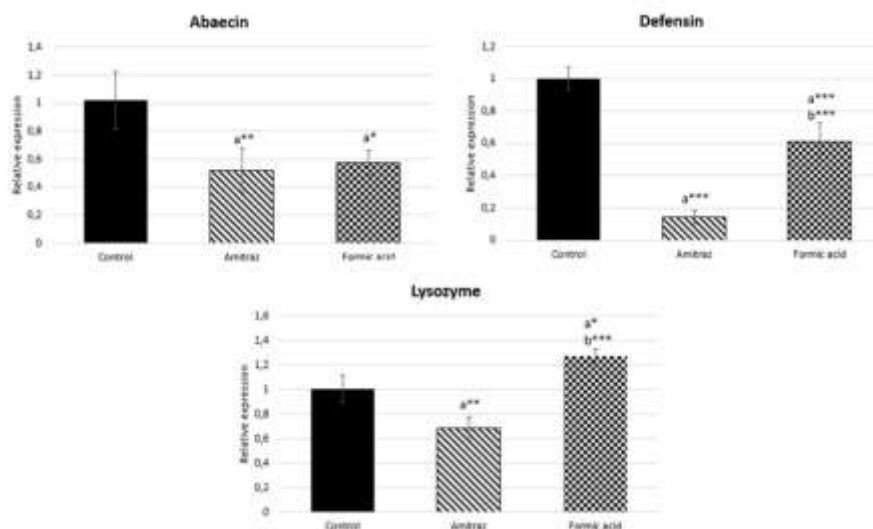
Continuous consumption of synthetic acaricide fluvalinate caused a significant increase in relative gene expression of antimicrobial peptide (AMP) abaecin in bees. Interestingly only a 10% concentration caused this change, with no change over control observed in other groups. Defensin, another antimicrobial peptide, had increased expression in the group with a maximum dosage of fluvalinate compared to the control. A 10% concentration of fluvalinate did not cause an increase in this case, but acetone itself caused a decrease in gene expression. Lastly, with lysozyme, we observed an increase in gene expression in both fluvalinate concentrations in comparison to the control, and again acetone caused a decrease in gene expression of this molecule (Figure 1).

Figure 1. Relative gene expression of antimicrobial peptides following fluvalinate exposure



Application of both amitraz and formic acid caused a decrease in relative gene expression of abaecin and defensin. In the case of defensin, amitraz caused a more significant decrease in expression over control. Lysozyme expression was significantly decreased after the application of amitraz and significantly increased with formic acid (Figure 2).

Figure 2. Relative gene expression of antimicrobial peptides following exposure to amitraz or formic acid



DISCUSSION

AMP are an important part of bees' defense against attacking microbial pathogens. We observed an increase in gene expression of AMP in bees exposed to fluvalinate and this stimulation can prove effective in fighting pathogens but constant stimulation can lead to exhaustion of bees. Our results are in contrast to the results of Garrido et al. (2013), which measured the expression of abaecin and defensin in bees that were treated with acaricides and found no increase over control in 24 hours post-exposure suggesting that there is a time delay in change of the expression. On the other hand, the application of acaricides in common doses according to standard procedure decreases the expression of the same molecules, posing a threat to defense against microbes. In the study of Flores et al. (2021), there was a positive correlation between the infestation of bees with mites and deformed wing virus load and gene expression of AMP, meaning that the decrease we see in the case of amitraz and formic acid could be the result of lowering *Varroa* infestation.

CONCLUSION

Commonly used pesticides have a dual effect on bees' health. They can either stimulate the immune system or cause suppression affecting the immunity of individual bees as well as the social immunity of the whole colony making it more susceptible to diseases.

ACKNOWLEDGEMENT

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POSTER EXHIBITION

SUSTAINABLE APPROACH IN TOXICOLOGY RESEARCH

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ABSTRACT

Over the past few decades environmental and ethical global awareness ensured many gradual changes that affected all industries. Chicken embryos in the first stages of their development provide a popular alternative to traditional animal testing. Newly identified venomous snakes bring the opportunity for identification of previously undescribed active components that might be medically valuable. Based on the results of our bioassay, venom from rare endemic snake species *Naja peroescobari* contains components with the ability to affect and damage vessels of chicken chorioallantoic membrane (CAM). Administration of venom solutions with concentration of 4000 mg/ml and 400 mg/ml onto CAM vessels resulted in hyperaemia, haemorrhage and formation of blood clots.

INTRODUCTION

The modern scientific approach places more emphasis on the ethical and environmental aspects of research, which represents a major challenge especially for the medical and cosmetic industries. Legislative limitations on the use of animal models encourage the search for alternative methods that would ensure results comparable to standardly performed tests and at the same time allow either the complete omission of experimental animals, or at least a partial reduction of the number of animals used and the limitation of ethically questionable methods to the most necessary procedures.

The specific position of the chicken embryo as a model organism results from its unique properties. Embryonic development is similar to mammals, but takes place independently of the metabolic processes of the mother's organism, while the inability to perceive painful stimuli in the early stages is one of major advantages, due to which they have an exemption from legislative for research use.

Biotesting makes it possible to monitor the effects of biologically active substances directly in a living organism. Animal venoms contain a number of active ingredients, the combination of which ensures a complex effect. Medical interest in venomous snakes focus on two main points: Snake bite treatment ensured mostly by antivenom production, and snake venom components with medically valuable properties. Newly identified and rare venomous snakes species are challenging for both – correct antivenom choice for treatment of the snake bites and identification of active components previously unknown. In this work we present bioassay monitoring the effects of venom obtained from rare endemic African snake species *Naja peroescobari* on vasculature of chorioallantoic membrane.

METHODS

Fertilized chicken eggs (Lohmann brown, Párovské háje, Nitra, SR) were cleaned with 70 % ethanol and incubated under standard conditions (37.5 °C; 60 % humidity). On 4. embryonic day (ED) 2 ml of egg white was removed from each egg in order to provide enough space for further manipulation with CAM. On 9. ED blunt end and paper membrane of each egg was removed, and based on the testing group, 50 µl of corresponding tested solution was applied directly onto CAM vessels. Each experimental group consisted of 4 eggs. In control group 50 µl of sterile saline solution was applied instead of venom solution. Directly after the administration of tested solutions onto CAM, all resulting changes and effects were recorded in 5 minute post-application interval. We focused mostly on three main effects – hyperaemia, haemorrhage and clotting, using Luepke's grading system (Table 1) for evaluation of irritation potential in HET-CAM test.

Table 1 Luepke's grading system for calculation of irritation potential (HET-CAM)

Time interval for manifestation of selected vascular effects	Score		
	Hyperaemia	Haemorrhage	Clotting
< 0.5 min	5	7	9
0.5 – 2 min	3	5	7
2 – 5 min	1	3	5

Luepke (1985)

The photos taken are documenting the CAM right before the application of 50 µl of tested solution, 30 seconds after application, 120 seconds after application and over 240 seconds after application. The three evaluated effects were assigned score according to the time of their

occurrence within a 5-minute post-application time interval. According to the resulting sum of average score of all three effects, the irritation potential of the venom solutions was assessed as negligible, slight, moderate or strong (Table 2).

Table 2 Irritation potential regarding the cumulative score

Cumulative Score	Irritation Potential
< 1.0	Negligible
1.0 – 4.9	Slight
5.0 – 8.9	Moderate
9.0 – 21.0	Strong

Luepke (1985)

RESULTS

Administration of two venom solutions with concentrations 4000 mg/ml and 400 mg/ml onto CAM vessels resulted in hyperaemia, haemorrhage and formation of blood clots (Figure 2,3), which means that venom obtained from *Naja peroescobari* contains components affecting vessels, inducing all vascular changes included in Luepke's grading system. The effects were concentration dependant, because we observed delayed onset of effects after administration of venom solution with lower concentration 400 mg/ml. After administration of higher venom concentration of 4000 mg/ml, the onset of targeted effects started within first minute post-application. Cumulative score calculated for venom solution with concentration 4000 mg/ml was 18 and for concentration 400 mg/ml the cumulative score was 12.5 (Table 3). According to Luepke's grading system both values of cumulative score are in the range of values rated as strong.

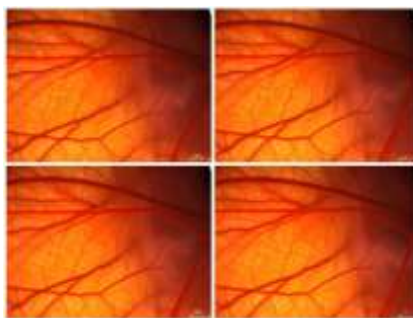
Table 3 Summary of cumulative scores calculated based on effects of Naja peroescobari venom

Venom	c [mg/ml]	Hyperaemia	Haemorrhage	Clotting	Cumulative score	Irritation potential
Control	N/a	0	0	0	0	0
<i>Naja peroescobari</i>	4000	5	6	7	18	Strong
	400	3.5	5.25	3.75	12.5	Strong

Over the past few decades environmental and ethical global awareness ensured many gradual changes that affected all industries. Chicken embryos in the first stages of their development provide a popular alternative to traditional animal testing. According to Polláková (3) the vascular network of fertilized chicken eggs, chorioallantoic membrane, provides a useful model for imaging vascular injury induced by snake venoms.

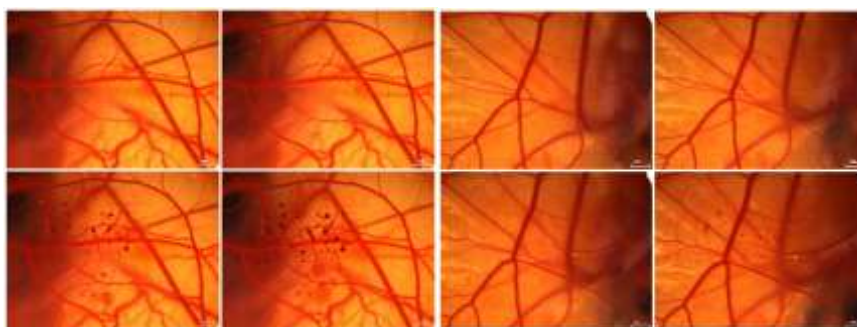
Newly identified venomous snakes bring the opportunity for identification of previously undescribed active components that might be medically valuable. Based on the results of our bioassay, venom from rare endemic snake species *Naja peroescobari* contains components with the ability to affect and damage vessels of chicken chorioallantoic membrane (CAM). Administration of venom solutions with concentrations of 4000 mg/ml and 400 mg/ml onto CAM vessels resulted in hyperaemia, haemorrhage and formation of blood clots.

Figure 1 Results of HET-CAM test of control



Source 2 Author's

Figure 2,3 Vasoactive effects of Naja peroescobari venom with concentration of 4000 and 400



Source 1 Author's

DISCUSSION

The composition of snake venoms provides the function of killing the prey, while the quantitative and qualitative representation of individual components varies at the level of the genus, within one species, and in certain cases even during the life of one individual. In addition to the dominant representation of components with an effect on the nervous system, the venoms of snakes of the genus *Naja* may contain smaller amounts of components with haemotoxic,

cardiotoxic and cytotoxic effects as well, which might not be as interesting from the point of view of envenomation as for their potential medical use. Recent evidence suggests that some elapid snake venoms contain anticoagulant toxins which may help neurotoxic components spread more rapidly. (1) Venoms of „spitting cobras“ from genus *Naja* contain components affecting vessels, possessing the ability to induce snake venom ophthalmia if in contact with eye. Rare endemic snake species *Naja peroescobari* used to be classified as species *Naja melanoleuca*, but was reclassified as separate individual species based on the results of the latest molecular analyses. Therefore we assume that the venom composition of both snakes might share some similarities. *Naja melanoleuca* venom contains 57.1 % three-finger toxins, 12.9 % phospholipases A₂, 9.7 % metalloproteinases, 7.6 % cysteine-rich secretory proteins and 3.8 % Kunitz-type serine proteinase inhibitors.(2) With the exception of three-finger toxins, the components of the other 4 groups have proven effect on different parts of cardiovascular system. If the composition of *Naja peroescobari* venom was at least partially similar, as it was previously assumed to be the same species as *Naja melanoleuca*, it could explain the effects we observed on CAM vasculature.

CONCLUSION

Venom from the rare endemic African snake species *Naja peroescobari* possesses vasoactive properties and ability to induce hyperaemia, haemorrhage and formation of blood clots, probably due to the presence of components affecting vessels and cardiovascular system. After these initial findings, we assessed the importance of further proteomic analysis of the *Naja peroescobari* venom that would allow the identification of the components responsible for the haemotoxic effects observed on chorioallantoic membrane, as well as other components affecting different physiological functions not manifested in HET-CAM test.

ACKNOWLEDGEMENTS

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SPECIES DIVERSITY OF TICKS (IXODIDAE) AND THE OCCURRENCE OF THE HYPERPARASITIC WASP IXODIPHAGUS HOOKERI (HYMENOPTERA: ENCYRTIDAE) IN SELECTED MODEL LOCATIONS OF EASTERN SLOVAKIA

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ABSTRACT

Work is aimed at the study of occurrence and seasonal dynamics of *Ixodes ricinus* ticks on the model site Hrhov in 2012 – 2021. Our goal was to determine the occurrence of the hyperparasitic wasp *Ixodiphagus hookeri* in *I. ricinus* ticks. A total of 37,871 ticks were collected at the site with the sympatric occurrence of 7 tick species. In 2012, the activity of *I. ricinus* on vegetation was highest in April (481 ticks), and in 2021 ticks were most active in September (133 ticks). The overall prevalence of wasps in *I. ricinus* ticks from the Hrhov location was 7%. Its occurrence was compared with the occurrence in ticks collected at four selected recreational sites of urban and suburban areas in Košice city, where the prevalence ranged from 6.7-20%.

INTRODUCTION

After mosquitoes, ticks represent the second most numerous group of epidemiologically significant blood-sucking arthropods (Stanko et al., 2021). From the point of view of protecting the health of people and animals, it is important to focus on the regulation of the population of ticks. The use of biological agents for tick control may represent a suitable alternative to acaricides. Numerous studies report the resistance of some tick populations to chemicals and their toxic effects on humans and animals (Buczek et al., 2021; Collatz et al., 2011). There is an assumption that the presence of endoparasites in habitats with the presence of ticks can reduce their abundance, thereby influencing the dynamics of diseases caused by tick-borne pathogens in the foci (Buczek et al., 2021). *Ixodiphagus hookeri* (Howard, 1908) is a parasitoid

wasp that parasitizes various species of ticks, including species of genera *Haemaphysalis*, *Dermacentor* and *Ixodes* which are present in the territory of Slovakia. *I. hookeri* lays eggs in the bodies of unfed tick nymphs. In temperate countries, where hosts of the parasitoid wasp are unavailable in winter, it lays eggs in the bodies of nymphs, where they remain in the diapause (Plantard et al., 2012). The embryonic development of eggs is induced by blood circulation in the body of the nymphal tick after blood sucking on the host. Wasp larvae feed on the internal tissue of the tick. Adults bite a hole in the nymph's body, and they fly through, causing death of their host. Thus, parasitized ticks die before the start of their own reproduction, and therefore *I. hookeri* is considered a suitable candidate for biological control of ticks in the environment (Collatz et al., 2011; Takasu et al., 2003). Frequent and high infestations of ticks by wasps in different parts of the world indicated the possibility of using *I. hookeri* as a form of biological control of ticks in nature. The first attempts of this alternative form of fighting with ticks were carried out at the end of the 20s and 30s of the 20th centuries (Řeháček 1998). Successful rearing of the parasitoid wasp in laboratory conditions has led to several attempts at mass release of *I. hookeri*. Methods such as releasing laboratory-raised wasps directly into nature, distributing parasitized inactive nymphs to places with a high incidence of ticks, placing parasitised nymphs of ticks in test tubes covered with gauze through which the wasps can fly out, in locations with a high incidence of tick hosts were used (Řeháček 1998)

METHODS

Ticks were collected by the flagging method during the years 2012 to 2021 at the Vysoký vrch (Hrhov) model site. The model site in the cadaster of the Hrhov village, in the Slovak Karst, is an important touristic area with a significant diversity of wild animals (deer, wild boar). The location is used for hunting purposes and at the same time as pastures for farm animals. The location belongs to the National Park of Slovak Karst. Sympatric occurrence of 7 species of ticks was recorded on the site: *Ixodes ricinus*, *Ixodes trianguliceps*, *Ixodes frontalis*, *Dermacentor reticulatus*, *Dermacentor marginatus*, *Haemaphysalis inermis*, and *Haemaphysalis concinna*. Temperature and relative humidity of the environment were measured year-round at the location, using data-loggers located at the base of trees. To compare the occurrence of the parasitoid wasp (*I. hookeri*), ticks were also flagged in recreational areas of the Košice city: Park Anička, Bankov, Čičky and Kavečany - observation tower. Before the DNA isolation itself, the ticks were determined into species and developmental stage according to the key (Siuda, 1993). DNA was isolated by the alkaline hydrolysis method, using a 1.25% ammonium hydroxide solution. *I. ricinus* ticks were examined for the presence of the

hyperparasitic wasp (*I. hookeri*) using *cox1* (cytochrome c oxidase 1). PCR products were separated using gel electrophoresis. After the electrophoretic separation, the result was visualized under UV light using a UV transilluminator. Positive PCR products were subsequently purified and sent for sequencing. The sequences were edited in the MEGA-X program and compared with homologous nucleotide sequences in the gene bank.

RESULTS

During the vegetation period in the years 2012 – 2021, a total of 37,871 ticks were collected by the flagging method at the model site Hrhov, of which 14,934 ticks belonged to the species *I. ricinus*. The highest percentage representation was nymphs 62.97% (9393 ticks) and larvae 23.22% (3467 ticks), males 7.65% (1144 ticks), females 6.23% (930 ticks). The seasonal dynamics of *I. ricinus* was monitored at the location; in 2012, the activity of *I. ricinus* ticks on vegetation was highest in April (481 individuals). In 2021 (133 individuals), the seasonal activity was highest in September, but due to the restrictions during the covid pandemic from 2021, it was not possible to collect regularly throughout the year. A total of 703 *I. ricinus* ticks (303 adults, 300 nymphs and 100 larvae) were examined for the presence of *I. hookeri* at the model site Hrhov. We selected a representative sample, 30 ticks for each year, over a 10-year period. The hyperparasitic wasp was recorded only in *I. ricinus* nymphs with a prevalence of 3-17%, the highest prevalence was in 2019 (16.7%). For the initial screening, a representative sample of ticks collected in 2019 at four model locations in Košice was selected: park Anička, Bankov, Čičky Majer and Kavečany - observation tower, which are popular recreational locations in Košice. The overall prevalence of *I. hookeri* was 13.3%, while it ranged from 6.7 to 20% in individual locations.

DISCUSSION

In recent decades, there has been an increasing amount of information and studies that confirm the important role of ticks as vectors of many groups of viral, bacterial, and protozoan pathogens causing serious diseases in humans as well as domestic and farm animals (Stanko et al., 2021). The model site Hrhov, where the collection and study of the seasonal dynamics of ticks, the presence and prevalence of the hyperparasitic wasp took place, is of considerable hunting and economic importance. Locations within the agglomeration of the Košice are important recreation and relaxation zones in the urban and suburban areas of the city. Localities differ in the way of land use as well as in the occurrence and species diversity of ticks. To reduce the number of *Dermacentor variabilis* ticks, in 1926 the wasp was transferred from

France to the state of Massachusetts on the island of Naushon Island (USA), where it had not previously been found. *I. hookeri* has been shown to be successfully implemented in *D. variabilis* and *I. scapularis* populations on this island. Up to 27% of *I. scapularis* nymphs from this locality contained wasp eggs. It was found that ticks from this site had a lower prevalence of pathogens *Borrelia burgdorferi* and *Babesia microti*, compared to ticks from other sites where the presence of wasps was absent (Hu and Hyland, 1998).

CONCLUSION

It has been shown that parasitoid wasps have the potential for biological control of ticks. Wasps can participate in the regulation of the occurrence of ticks and thus the pathogens they carry and transmit. Through long-term research at several locations in the protection zone of the NP Slovak Karst, as well as at several locations in the Košice, we have confirmed the parasitism of *I. ricinus* ticks by the wasp *I. hookeri*.

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OCCURENCE AND SEASONAL DYNAMICS OF *IXODES RICINUS* TICKS AND SELECTED DNA- AND RNA- INFECTIONS IN THE RECREATION AREA OF VOLOVSKÉ VRCHY

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ABSTRACT

The work is aimed at the study of the occurrence and seasonal dynamics of *Ixodes ricinus* ticks at the model site Jahodná, in eastern Slovakia. A total of 1,223 ticks from the genus *Ixodes* were collected by flagging the vegetation. The peak of the tick activity was recorded in June 2016. In 2017, the occurrence of ticks on vegetation peaked in May (238 individuals).

The role of ticks in the maintenance and transmission of tick-borne pathogens was monitored. The prevalence of *Babesia* spp. and *Anaplasma* spp. in ticks was $\pm 10\%$ for a two-year period. The prevalence of *Borrelia* spp. was higher in 2016 ($\pm 9\%$) and the MIR (minimum infection rate) of tick-borne encephalitis virus ranged from 1-6%.

INTRODUCTION

As the result of global changes, the spread of tick-borne pathogens occurs in non-endemic areas. At the same time, endemic regions are expanding, which increases the risk of co-infections. In Europe, the most numerous and epidemiologically significant tick species is *Ixodes ricinus*. It is a vector of a wide range of viral, bacterial, and protozoal pathogens that are responsible for infectious diseases (Sonenshine and Roe, 2014). Tick-borne encephalitis virus (TBEV) affects the CNS and is one of the most important human viral diseases in Europe. In recent decades, the incidence of the disease has increased significantly. More than 13,000 cases are reported annually in endemic areas, but it also appears in new regions (Ergunay et al., 2016). In Slovakia, an increasing trend in the incidence of the disease has been observed. Since 2009, there has been a significant increase in 2013 and 2016, when the total number of persons with tick-borne encephalitis was 174 (Dubinský et al., 2015). In 2020, 186 cases of the disease were reported, which represents the highest number of recorded cases in the last 20 years (EPIS, UVZSR).

Lyme disease is among the most common tick-borne diseases in the Northern Hemisphere and Europe. The disease is caused by gram-negative bacteria from the genus *Borrelia*. They cause a multisystem infectious disease (Blažeková et al., 2013; Dubinský et al., 2015). The infection rate of *I. ricinus* ticks, the causative agent of Lyme disease in Slovakia, is highly variable in space and time. It varies from 5 to 45% and these differences in prevalence are observable in different regions of Slovakia. This variability is influenced by several biotic and abiotic factors, which also effect on morbidity of residents in different parts of the country (Dubinský et al., 2015; Stanko and Slovák, 2019). Anaplasmosis is a tick-borne infectious disease caused by bacteria from the genus *Anaplasma*. The causative agent of the disease in wild and domestic animals and humans is *Anaplasma phagocytophilum*, which attacks white blood cells - granulocytes (neutrophils) in peripheral blood and eosinophils, causing granulocytic anaplasmosis. The prevalence of *A. phagocytophilum* in *I. ricinus* ticks varies depending on environmental conditions and the occurrence of reservoir hosts (Blažeková et al., 2013; Pet'ko et al., 2015). *Babesia microti* and *B. divergens* are the causative agents of human babesiosis. *Babesia* occurs quite often in animals. The infection is manifested by hemolytic anemia and hemoglobinuria (Hildebrandt et al., 2007).

METHODS

The collection of ticks was carried out during the years 2016 and 2017 at the model site Jahodná by flagging the vegetation. Ticks were determined to species and developmental stage according to the key (Siuda, 1993). They were divided into pools of 5-10 ticks and frozen at -80°C. RNA was isolated from ticks using the QIAGEN Rneasy kit. Subsequently, the samples were examined for the presence of the TBE virus using Real Time PCR. The remaining RNA was transcribed into cDNA. cDNA samples were examined for the presence of *Borrelia* spp., *Babesia* spp. and *A. phagocytophilum* by PCR. PCR products after electrophoretic separation were visualized under UV light.

RESULTS

During the years 2016 and 2017, a total of 1223 ticks from the genus *Ixodes* were collected. Ticks were collected in 100 meters transects from 600 meters a.s.l. to the upper limit of occurrence (700 m.a.s.l.). In 2016, ticks were collected from the end of April to the end of September. A total of 149 ticks were collected at an altitude of 600 meters - 67.79% nymphs (101 ticks), 19.46% (n=29) males and 12.75% (n=19) females. At an altitude of 700 m, altogether 308 ticks were collected - 257 nymphs (83.44%), 25 males (8.11%) and 26 females

(8.44%). In 2017, a total of 766 ticks were collected from the end of March to the middle October. As in 2016, a greater number of ticks were flagged at an altitude of 700 meters (541 ticks). The gender representation was as follows: 54 females (9.98%), 56 males (10.35%), 79.67% nymphs, which represents 431 ticks. In 2017, the most ticks were flagged in May and the least in August. The aim of the work was to determine the occurrence and seasonal dynamics of *I. ricinus*. At the monitored site, the seasonal activity in 2016 was the highest in June. During the vegetation period, the number of flagged nymphs was significantly higher than adults. In May 2017, a single-peak curve of seasonal activity in nymphs (238 ticks) on vegetation was recorded. In the following month, the activity was lower (153 ticks) and the lowest was observed in August. Seasonal activity of adults was highest in May and June with a sharp decline in July (6 individuals). Subsequently, it remained constant until the end of the vegetation period. Using PCR methods, 1152 ticks in pools (5 adults or 10 nymphs) were examined for the presence of pathogens. An overview of the number of examined pools collected in 100 m high transects and MIR values for individual infectious agents in *I. ricinus* at the model site Jahodná are in Table 1.

Tab. 1 Prevalence of tick-borne pathogens in Ixodes ricinus ticks during 2016 and 2017 at the model site Jahodná

year	MASL	Prevalence of Infection (MIR) %				
		number of examined pools	<i>Babesia</i> spp.	<i>Borrelia</i> spp.	<i>Anaplasma phagocytophilum</i>	TBEV
2016	600	19	10.5	5.33	0	5.26
	700	35	8.57	11.42	11.42	5.71
Total		54	9.26	9.26	7.41	5.56
2017	600	25	8	4	4	0
	700	63	11.11	3.17	4.76	1.69
Total		88	10.23	3.41	4.55	1.14

DISCUSSION

The model site Jahodná where ticks were collected is of considerable touristic importance. According to the literature, the seasonal activity of *I. ricinus* in our conditions has two peaks, it starts in early spring and the first peak occurs in the period when the temperature and humidity of the environment reach optimal values (90% humidity, 20 °C). The second peak is reached in the autumn months again under optimal conditions of abiotic factors (Dubinský et al., 2015). During the two-year period, we recorded a single-peak course of seasonal activity of *I. ricinus*,

which is related to global climate change and local abiotic and biotic factors at the monitored site. The highest activity was recorded in 2016 and 2017 in May and June. As a result of temperature changes and the increase of the average annual temperature, the autumn peak of tick activity gradually disappears, and the limits of their occurrence move to higher areas. The tick-borne encephalitis virus is among the most important viruses transmitted by ticks. The disease is widespread almost throughout Europe and Slovakia. The MIR of the tick-borne encephalitis virus during 2 years at the model site was 4.7%. It is reported in the literature that the virus occurs in about 1 ‰ (1/1000) of ticks in the environment (Peťko et al., 2015). Our results confirm that the prevalence of TBEV in ticks in micro-foci may be much higher. The infection rate of ticks with *Borrelia* in Europe and on the territory of the Slovak Republic ranges from 5-45%. The MIR value found by us for the two-year period was 8.4%, which corresponds to the published results. More than 65,000 cases of the disease are reported annually within the EU (ECDC, 2014).

CONCLUSION

The aim of the work was to determine the occurrence, seasonal dynamics, and prevalence or MIR of selected pathogens in ticks from genus *Ixodes* at the model site Jahodná. Seasonal dynamics of ticks on vegetation during both monitored years had a single-peaked curve. The monitored location is among the recreationally attractive. The recorded MIR values of *Borrelia* spp., *A. phagocytophilum* and TBEV were relatively low. However, it does not reduce the necessity of prevention when visiting the given location. The epidemiological situation at individual localities can change in connection with changing altitude, season within the year and during several consecutive or monitored years.

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PARASITES OF FROGS (*OCCIDOZYGA LIMA*) AS INDICATORS OF CHANGES IN THEIR NATURAL HABITATS

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ABSTRACT

The small, semi-aquatic frog species *Occidozyga lima* is naturally endemic to tropical Asia, where they fulfil various important roles in the ecosystem. Wild-caught frogs are regularly imported to Europe. Upon arrival, they often still harbour the parasitic fauna present at the region of origin. Veterinary diagnostics revealed that 86.36% of 22 frogs were infected with trematodes. Adult specimens of three different genera completed their complex life cycles in a final frog host. The presence of metacercaria in different soft tissues indicates that *Occidozyga lima* also serves as an intermediate host for trematodes. All required intermediate hosts must be present at the habitat of origin to reach the mentioned developmental steps. Therefore, the frogs and their trematodes may serve as indicators for the intactness of natural habitats.

INTRODUCTION

Worldwide, ranoid frogs fulfil an important role in wetland ecosystems, as they serve as natural food sources for many other animals and also prey on various animals of lower levels in the food web (Duellman and Trueb, 1994). Unique anatomical and physiological features make amphibians valuable bio indicators for environmental change and habitat pollution, yet their population numbers are progressively declining worldwide (Venturino et al., 2003). Semi-aquatic ranoid frogs spend most of their natural life in littorals of inland waters and serve as final hosts for various parasites with complex life cycles (Kuzmin et al., 2020; Shinad and Prasadan, 2017; Chikhlyayev and Ruchin, 2014, Saglam and Arikan, 2006). The presence of adult trematodes is evidence of the successful completion of all steps of the parasitic life cycle. It, therefore, indicates the availability of suitable intermediate host species in the ecosystem of the frog habitat (Shinad and Prasadan, 2018). Many intermediate host species are invertebrates, such as insects, crustaceans, bivalve molluscs, or gastropods. They require several developmental steps before they serve as hosts in a parasitic life cycle or become prey for

amphibians. Aquatic snails, for example, serve as an obligate intermediate host for various trematodes. At the same time, their biodiversity is globally declining due to anthropogenic effects, molluscicides, and habitat loss (Lu et al., 2017; Lydeard et al., 2004). Most dragonfly nymphs are another example of common obligate hosts in trematode life cycles. These aquatic carnivores rely on the availability of zooplankton, other insect larvae, or tadpoles for weeks, months, or years before they become flying adult specimens (Corbet, 1999). Therefore, the regional ecosystem in which the parasitic life cycle occurs has to remain intact for a considerable amount of time until adult trematodes can be recovered from a final frog host. The small, semi-aquatic, insectivorous dicroglossid frog species *Occidozyga lima* is naturally endemic to tropical Asia, especially Malaysia and the Philippines, where the frogs inhabit swamps, flooded agricultural fields, and other permanent or temporary freshwaters (Flury et al., 2021). These frogs are regularly imported to Europe for the pet trade and exhibited at zoos. Upon arrival at a collection, they often still harbour the parasitic fauna present at the region of origin. Veterinary examinations carried out during the critical quarantine time may then reveal the presence of such parasites and help to learn more about the intactness of original frog habitats.

METHODS

Over a period of four months (16th January 2023 to 16th April 2023), a total of 22 Asian green puddle frogs (*Occidozyga lima*) arrived at the pathology department at the Federal Institute of Consumer Safety in Stendal, Germany. Twenty of the frogs were newly imported. All of them were wild-caught adult specimens and had died per acute during the regular quarantine at a private hobby collection. For each frog, a dissection, bacteriological examination, parasitological examination, and histopathological evaluation were carried out. Upon arrival, a sterile skin swab was collected, the body length was measured with a manual forensic ruler, and the body weight was measured using a Sartorius digital precision scale (accuracy 0.001 g). Each frog was then placed on a single-use plastic Petri dish. Photos were taken from the ventral aspect, dorsal aspect, caudal aspect, and every macroscopically visible lesion using a Nikon D50 digital reflex camera. The dissection was done in dorsal recumbency, starting with a midline skin incision from the caudal pelvis to the angulosplenic portion of the mandibular rami. The skin of each ventromedial thigh was then further cut from the pelvic midline to the level of the stifle joint to expose the underlining skeletal musculature. The same was done for the forelegs, cutting the skin from the sternal midline to the mid of the humerus. Another photo was taken before the coelomic cavity was cut open with scissors, and a sterile swab was

collected for bacteriology. The abdominal wall was opened along the midline, from the pelvis to the caudal end of the xiphisternum and directly caudally to each foreleg from the sternum to the vertebral column. Then the xiphisternum and sternum were removed to expose the heart and lung, and another photo of the situs was taken. New, sterile dissection instruments were used to remove a piece of liver, lung, and kidney for bacteriology. A piece of lung, urinary bladder, coelomic serosa, and skeletal musculature was placed on a microscope slide. Then the intestine was removed, cutting the caudal to the pylorus and around the terminal rectum. The small intestine was placed on a microscope slide, and so was the large intestine. Each native organ sample was gently covered with a cover slip and examined light microscopically, using a Hund 600 microscope at 100x and 200x magnifications and phase contrast. When parasites were detected, the affected organ sample was put in a wet chamber until the end of the dissection and then examined in greater detail using a Leica DM4 microscope connected to a Leica c-mount camera and Leica LAS software. After that, parasites were placed in 70% ethanol in 2 ml screw-cap tubes and stored at 4 °C in the refrigerator until molecular genetic analysis. The remaining frog body was preserved in 10% formalin for at least 24 h. For histopathology, forelimbs and hind limbs, including skeletal musculature, bones, joints, and skin, were chosen. The liver, lung, kidney, genital tract, stomach, larynx, and vertebral column, including the spinal cord, head, eyes, and brain, were embedded in paraffin. Three to five-micrometer sections were stained with haematoxylin and eosin. A native smear preparation of intestinal content was air dried and stained according to the laboratory's protocol for the modified Ziehl-Neelsen staining by Henriksen to examine for *Cryptosporidium* oocysts.

RESULTS

Of the 22 examined frogs, 15 were females, and seven were males. The mean body length was 2.85 cm (range: 2.30 cm – 3.1 cm), and the mean body weight was 1.93 g (range: 0.85 g – 2.65 g). In total, 76 trematode specimens were found in 86.36% (n=19) of frogs. 59.21% (n = 45) of them were morphologically identified as *Pleurogenoides* sp., all of them were found in the small intestine of infected frogs, with a maximum infection intensity of 12 specimens per frog, a mean intensity of 2.75 specimens per frog and a prevalence of 77.27% (n = 17) among all examined frogs. Out of the 76 trematodes, 13.15% (n = 10) were *Diplodiscus* sp., the infection intensity was up to three specimens per frog with a mean intensity of 1.43 and the prevalence among all examined frogs was 31.82 (n = 7). *Diplodiscus* sp. inhabited the small and large intestines of frogs. 5.26 % (n = 4) of all trematodes were lung flukes belonging to the genus *Haematoloechus*. They were found in 13.64% (n = 3) of all examined frogs and the mean

infection intensity was 1.33 specimens. Out of all trematodes, 22.37% ($n = 17$) were metacercariae. The mean infection intensity was 2.83 per frog, and the prevalence was 27.27% of all examined frogs. Metacercaria were found in skeletal muscle and connective tissue at various locations in the frog organism. Among all parasitized frogs, 42.10% ($n = 8$) had mono infections, 47.37% ($n = 9$) were infected with two different trematodes simultaneously, and 10.53% ($n = 2$) harboured three different trematodes at the same time. None of the frogs carried all four types of trematodes identified during this study. Among the frogs infected with only one type of trematode, 31.58% ($n = 6$) had only *Pleurogenoides sp.*, and 5.25% ($n = 1$) had either exclusively *Diplodiscus sp.* or metacercaria. None of the frogs carried *Haematoloechus sp.* only. One frog had lived in captivity for at least ten months and harboured one *Pleurogenoides* specimen and one *Haematoloechus* specimen. Another frog had lived in captivity for at least five months and was still infected with one *Diplodiscus* specimen. The *Pleurogenoides sp.* reached a mean length of 788.64 μm , *Haematoloechus* reached 8.13 mm, and *Diplodiscus* grew one to two millimetres long. None of the frogs was diagnosed with Cryptosporidia. Several of the trematodes were found in histological sections.

DISCUSSION

The prevalence of trematode infections in the examined puddle frogs is high. All detected adult trematodes were sexually mature specimens and produced eggs. Therefore, *Occidozyga lima* is a common and true final host in which *Diplodiscus sp.*, *Pleurogenoides sp.*, and *Haematoloechus sp.* can complete their life cycles. *Pleurogenoides sp.* and *Haematoloechus sp.* rely on aquatic snails as first intermediate hosts and require insect larvae or crustaceans as second intermediate hosts (Chikhlyayev and Ruchin, 2014, 2022; Brinesh and Janardanan, 2014; Janardanan and Prasadana, 1991; Schell, 1965). *Diplodiscus sp.* require only one intermediate host, usually an aquatic snail species such as a planorbid, viviparid, or ampullariid snail (Besprozvannykh et al., 2018; Cichy and Żbikowska, 2016; Murty, 1973). Only when all obligate hosts are present in an ecosystem, the parasite can reach sexual maturity in a final host. The number of *Pleurogenoides sp.* detected in frogs is the highest, followed by metacercaria, *Diplodiscus*, and *Haematoloechus*. Infections with several parasites at the same time are common, indicating that these frogs originate from an ecosystem with a certain biodiversity. Asian puddle frogs (*Occidozyga lima*) are small frogs, mostly reaching body lengths of three centimetres or less. Considering the availability of food sources for a parasite in a given organ system within a host organism and the body size of the host and parasite in relation, that may explain the differences in detected infection intensities. The

complex trematode life cycles cannot be completed in artificial environments lacking the necessary biodiversity. However, all three trematode genera, that occurred as reproducing adults in *Occidozyga lima*, were also detected in frogs that had lived for several months in captivity. In contrast, none of these frogs harboured intact metacercariae.

CONCLUSION

The numerous adult, reproducing trematode specimens of three different genera, often co-infecting the same final frog host, and the additional metacercariae indicate high biodiversity in the natural frog habitat. The smallest trematodes were abundant, and large lung flukes were less common. Specimens of all three trematode genera utilizing frogs as final hosts have rather long life spans and can even be detected several months after the frog has left its original habitat. Further research on the life cycles and parasite-host interactions in the context of natural ecosystems is required for more detailed insights into this topic.

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DIAGNOSIS OF THE HEREDITARY EYE DISEASE COLLIE EYE ANOMALY (CEA) CAUSED BY MUTATION IN THE *NHEJ1* GENE IN SHEPHERD DOGS USING PCR METHOD

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ABSTRACT

Collie Eye Anomaly (CEA), also called Collie Scleral Ectasia Syndrome, is a hereditary disorder predominantly affecting collie breeds. The main clinical sign is choroidal hypoplasia and the formation of colobomas. In affected dogs we find a 7.8 kb deletion in the 4th intron of the *NHEJ1* gene. In this research we studied 40 dogs belonging to sheepherding breeds. Using a simple PCR method followed by electrophoretic separation, we were able to accurately determine the genotypes of the individuals. On the basis of the findings, it was possible to design the types of crosses between the parents with the expected lowest proportion of newborn affected offspring.

INTRODUCTION

Anomaly of the eye of the collie (CEA) is a hereditary eye disorder in dogs. It was first described in 1953 (Magrane 1953). The primary symptom of the disease is characterized by regional hypoplasia of choroids that represent a highly vascularized layer of the eye that supplies blood and nutrients to the retina (Bedford, 1982, Dostál et al., 2010). The defect can be ophthalmoscopically detectable, during diagnostic window in the age of 6 to 12 weeks of life, when the pigmentation of the eye fundus isn't fully developed. Defects of the sclera characterized by colobomatous lesions may also occur and present as pits within or engulfing the optic nerve head or in the adjacent fundus (Chang et al., 2010). The disease is caused by a 7.8 kb deletion in intron 4 of the *NHEJ1* canine gene located on 7. chromosome. This deletion has been described in sheep-herding breeds that are related to the collie lineage and also in some other dog breeds. A genetic test based on this association can distinguish three genotypes: normal, carrier and affected (Marelli et al., 2021).

METHODS

Samples of biological material were collected from the examined individuals. Two types of collection were performed, where blood was taken and in the second method, buccal mucosal swab was performed. Blood was collected into sterile tubes with the addition of the anticoagulant ethylenediaminetetraacetic acid (EDTA). The *vena cephalica antebrachii* was most commonly used for collection. Buccal mucosal swabs were performed with brushes according to the given procedure. To avoid contamination of the sample, the subject must not have consumed any food or water in the previous 15 min. Swabs were performed for 5 seconds with a periodic direction change of movement over the mucosa bilaterally, using two brushes to obtain a sufficient number of epithelial cells.

The obtained samples were labelled, stored sterily and transported to the laboratory for examination. A commercial ReliaPrep™ Blood gDNA Miniprep System (Promega, Wisconsin USA) was used to isolate DNA from blood. The same kit was also used for buccal swabs with minor modifications in the isolation protocol due to the different nature of the sample. The sample incubation interval in the water bath was extended from the original 10 minutes to 30 minutes to ensure thorough lysis of the epithelial cells. The DNA samples obtained were analyzed spectrophotometrically to determine the quality and quantity of nucleic acids. Samples were subjected to PCR reactions using a commercial primer set (Eurofins Genomics, Germany) as described by Parker et al. (2007). The kit contains two pairs of primers, namely primers for amplification of the intact stretch of the gene (*NHEJ1*-F17, 5'-TCTCACAGGCAGAAAGCTCA-3' and *NHEJ1*-R17, 5'-CCATTCATTCCTTTGCCAGT-3') as well as primers for the amplification of the mutated segment with deletion (*NHEJ1*-F20, 5'-TGGGCTGGTGAACATTTGTA-3' and *NHEJ1*-R23 5'-CCTTTTTGTTTGCCCTCAGA-3'). These primers were added to the PCR reaction mixture at 0.625 µl at a concentration of 100 pM. The mixture was run in a thermocycler where, after an initial denaturation at 95°C (2 min), 35 repetitions of denaturation at 95°C (40s), hybridization at 50°C (50s) and elongation at 72°C (90s) were continued. PCR was terminated by final synthesis at 72°C (5 min). Amplicons were electrophoretically separated in 1.5% agarose gel, at an electrical voltage of 75V for 60-65 min.

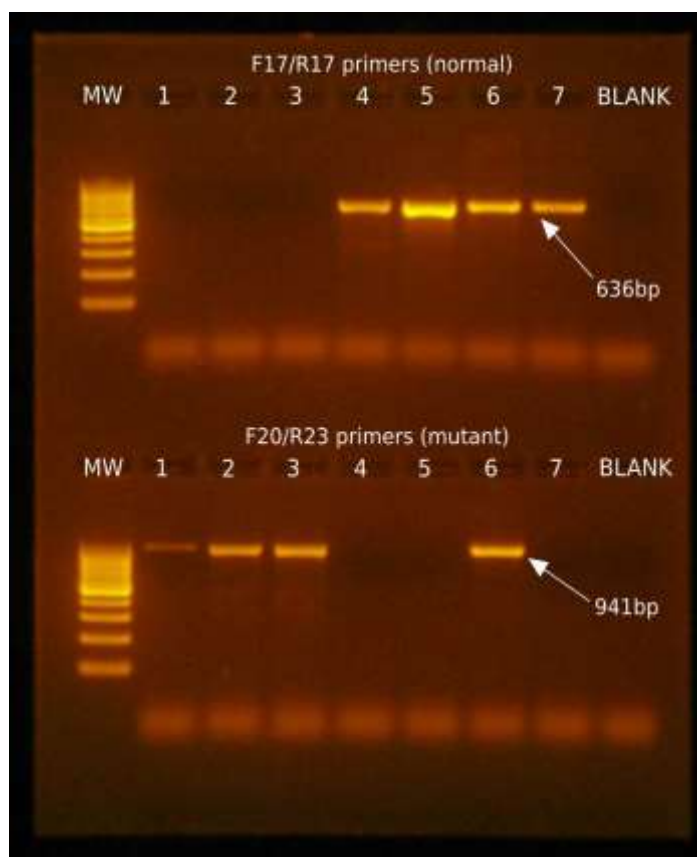
RESULTS

The amplicons obtained by PCR reaction and their subsequent separation on agarose gel were analysed and the genotypes of the examined individuals were determined on the basis of the PCR reactions. Since this is a monogenic type of inheritance, after examination of the individual

using both pairs of primers, we can unambiguously determine the genotype of the individual. In healthy individuals (genotype dominant homozygotes - AA) we found fragments only when using primers F17/R17. Affected individuals are genotypically recessive homozygotes (aa), and therefore we found fragments only using primers F20/R23. If the individual is a carrier of the disease (heterozygote - Aa), we found amplicon fragments for both primer pairs.

In this research, we studied a group consisting of 40 shepherd dogs. Specifically, 20 Australian Shepherds, 7 Longhaired Collies, 4 Border Collies, 4 Shetland Sheepdogs and 5 German Shepherds. In 31 individuals, no mutation in the *NHEJ1* gene was confirmed and the amplicon fragments appeared on agarose gel when using primers F17/R17, which amplify the intact section of the gene (i.e. the section without mutation). This confirmed the phenotypic finding in these individuals as they showed no changes ophthalmologically. Samples collected from a family of long-haired collies, consisting of 6 individuals with ophthalmologically confirmed CEA were examined by the same method. Characteristic fragments of 941 base pairs (bp) were found in the whole family, using primers F20/R23 (Fig. 1).

Figure 4 Electrophoretic separation of amplicons on agarose gel. For the first three samples (1-3), fragments 941bp were produced only using primers F20/R23, which clearly confirms the presence of the mutation in the gene. In samples 4, 5 and 7, fragment p s were produced only using primers F17/R17, characteristic of healthy individuals. In sample number 6, the presence of fragments at both primer pairs characterized the heterozygous state of the individual who is a latent carrier of the disease.



In 3 dogs belonging to different breeds (Australian Shepherd, Shetland Sheepdog and Border Collie), amplification and fragment formation occurred in both primer pairs. This confirms the heterozygous state of the gene, where a normal gene is found on one chromosome of the homologous pair and a mutated gene on the other.

DISCUSSION

After evaluating the results of the individual analysis, we came to the conclusion that 31 dogs from the tested group were negative. In 6 dogs that had ophthalmologically confirmed occurrence of this disease, the disease was also clearly confirmed genotypically, since in these dogs we identified the presence of a mutation responsible for the development of this condition. Although these individuals have the disease, their visual impairment is not so significant, and therefore we can conclude that they have a mild course of the disease. This fact was also confirmed in other study, where only small amount of dogs suffer to defective vision or blindness (Palanova, 2015). We can also confirm the transmission of the disease from parents to offspring since these individuals come from the same family. Therefore selective breeding should be considered in all breeds, where incidence isn't very high, to exclude the mutant allele from the population. In breeds, where frequency of the allele is too high, we cannot exclude all affected dogs and carriers. In these breeds, we should introduce a breeding management. For example, we can breed a healthy individual with carrier and test the offspring before another breeding. This will slowly increase frequency of healthy allele in population (Dostal et al., 2010).

CONCLUSION

In this study, we confirmed the presence of the mutation in gene *NHEJ1* in 6 dogs with ophthalmologically diagnosed CEA. We also identified 3 dogs from studied group which are phenotypically healthy, but they carry a mutated gene that can pass onto the offspring. This is the reason for continuing the research and persevering in the study of this disease and educating the owners about it as well.

ACKNOWLEDGEMENTS

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EFFECT OF INCREASING TEMPERATURE ON MICROBIAL CONTAMINATION IN CATTLE REARING

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ABSTRACT

The aim of our study was to investigate the effect of environmental temperature to microbial contamination in dairy cow houses. The physical parameters of the air were measured. Air samples were also taken and subjected to microbiological testing. The values of temperature, relative humidity, and air flow corresponded to suitable living conditions for the animals. The concentration of airborne microorganisms varied seasonally, higher contamination of the air was recorded in warmer seasons in comparison to cooler seasons. For reducing the biological risk, it is necessary to know the behaviour of microorganisms during seasonal changes on the farm to implement the necessary protective measures. The global changes and increase in temperature can negatively influence the health, welfare, and productivity of dairy cows.

INTRODUCTION

Global climate change affects growth rates, production, reproduction, performance, morbidity, and mortality in livestock. The basic climatic factor influencing livestock production is the thermal environment, which is a combination of ambient temperature, relative humidity and air movement (Cheng et al., 2022). It is estimated that climate change could increase global average temperatures by up to 4 °C within a few years, significantly affecting livestock production systems as well as animal and human health (Quintana et al., 2020). The effect of high ambient temperature and humidity leads to heat stress in dairy cows, which is manifested by a disturbance of their standard physiological, production conditions and can cause the death of individuals (Feliciano et al., 2020). Increased ambient temperature and decreasing humidity also result in more intensive growth of airborne microorganisms, which, combined with poor hygiene on the farm and in the milking parlour, leads to increased contamination of the milk produced. High relative humidity also promotes the growth of several

species of moulds, bacteria, and mites and, in combination with reduced sunlight, contributes to the increased survival of microorganisms (Quintana et al., 2020).

METHODS

The experiment was conducted on a cattle farm in the eastern part of Slovakia, whose breeding is intended for dairy production. Samples were collected in a barn with an established free housing system for dairy cows in boxes with litter. Sampling of bioaerosol was carried out by the aeroscopic method using the MAS-100 Eco instrument with the appropriate Petri dish placed. Multiple samples of airborne microorganisms were collected from a single sampling site. The inoculated plates were incubated under the following conditions: Total count of bacteria - Meat pepton agar - 24 h / 37°C; Coliforms bacteria - Endo agar - 24 h / 37°C; Moulds - Sabouraud agar - 72 h / 25°C. After subtraction, the counts of microorganisms were adjusted using correlation tables, converted to \log_{10} CFU/m³ and the standard deviation was calculated.

The physical parameters of the air, including ambient temperature and humidity were detected by a thermo-hydrometer and the air velocity was measured by an anemometer, both Testo instruments. Mean values and standard deviations were calculated from the measured data.

RESULTS

The average measured physical air parameters for each season are shown in Table 1. The obtained values of ambient temperature, relative humidity and velocity correspond to the weather at different times during the year. The high relative humidity values in autumn were due to the rainy season.

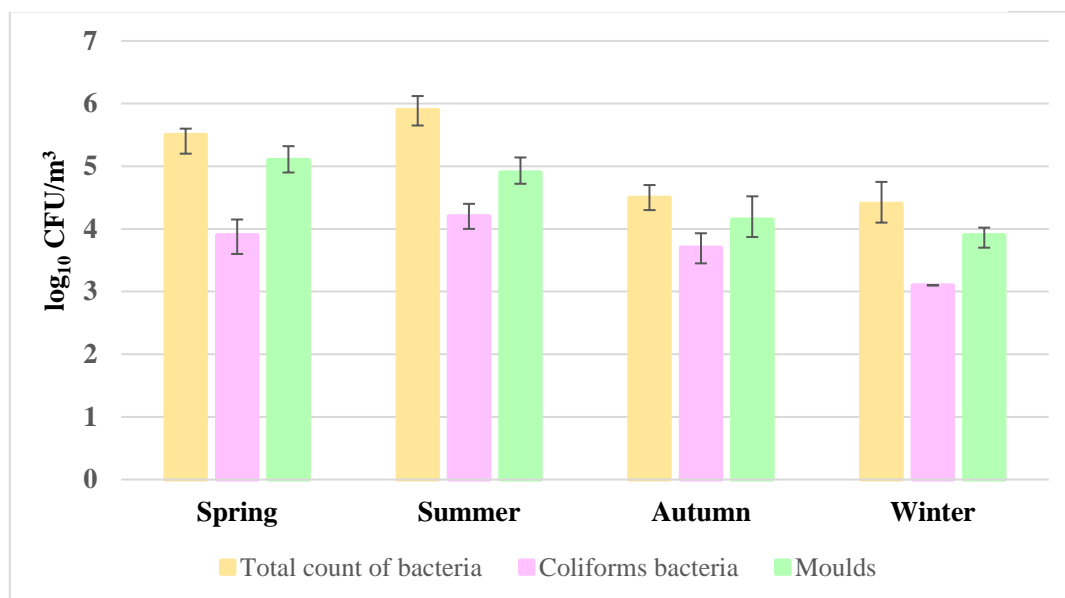
Table 1 Physical air parameters

	T (°C)	RH (%)	v (m/s)
Spring	18.66 ± 0.74	57.94 ± 1.82	0.53 ± 0.88
Summer	24.5 ± 0.92	63.57 ± 1.10	0.45 ± 0.76
Autumn	12.84 ± 0.24	94.86 ± 0.94	0.27 ± 0.63
Winter	6.84 ± 0.34	70.04 ± 0.98	0.15 ± 0.68

The average concentrations of airborne microorganisms at different times of the year are shown in Figure 1. There were no significant differences in the concentrations of individual microorganisms over the seasons. In general, lower numbers of microorganisms are detected in colder seasons compared to warmer seasons. These seasonal variations were also observed

in this study. From the summer to the winter of that year, all groups of microorganisms showed a progressively decreasing trend. Although there is a positive correlation between increased humidity and fungi, at the same time, no increase in mould was detected, despite the prolonged wet and rainy weather in autumn.

Figure 5 Concentrations of airborne microorganisms



DISCUSSION

The measured and calculated average physical air parameters in the different seasons of the year represent suitable living conditions for the animals. As stated by Quintana et al. (2020), the recommended ambient temperature for dairy cows ranges from -5°C to 22°C. In our work, the temperatures in the three seasons fall within this temperature range. The exception is a relatively hot and dry summer when the average temperature was approximately 2.5°C above the recommended range. Suitable values for relative humidity on dairy farms are between 55% and 75% for dairy cows (Bureau, 2001), which corresponds to spring, summer, and winter. The elevated RH value (above 94%) was caused by prolonged autumn rains. The minimum requirement for airflow velocity should be 0.2 m/s, and our results meet this claim.

The results of laboratory air analyses confirm a decrease in the number of microorganisms related to the gradual change of seasons. Microbial populations in bioaerosols are highly complex systems that vary with seasonal changes. Our results from laboratory air analyses confirm a decrease in microorganism concentrations associated with a gradual change in seasons. The same results were obtained by Sanz et al. (2015), where up to almost twice as many bacterial isolates were detected in the hot season as in the cold season on a dairy farm. Popescu et al. (2011), in turn, found a positive correlation between the increase in temperature

and the increase in the number of bacteria in the environment both in the morning and in the evening. Even low concentrations of micro-organisms create a risk to animal and human health, which makes it necessary to observe protective measures.

CONCLUSION

The authors of several studies that have been carried out confirm that climate change will affect animal health and welfare. The obtained results show that, in particular, two factors, temperature and relative humidity, affect the concentrations of bioaerosol, which is a diffuse system containing biotic (bacteria, viruses, fungi and their metabolites) and abiotic components. Stables, in particular, present ideal conditions for increased production of bioaerosol. Airborne microorganisms have a negative impact on the health of housed animals and farm workers, and they are a potential source of environmental contamination. This study forms the basis for further research to identify and analyse the risk to animal health for farmers and farm workers, as well as for people living near farms.

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PERCEPTION OF ECO-FRIENDLY ACTIVITIES AT THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

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ABSTRACT

The University of Veterinary Medicine and Pharmacy in Košice (UVMP) signed up to the global environmental campaign “Race to Zero for Universities and Colleges” in 2021. In order to gauge interest of UVMP students and employees in environmental topics, a questionnaire consisting of 29 questions were prepared. Response of 657 respondents have shown that UVMP’s employees and students are interested in the environment, its current state and protection. The respondents also proposed a variety of measures for improving the environment at the university. The results of the questionnaire have been used to analyse the environmental awareness at UVMP and will help prepare an action plan in line with UVMP’s Race to Zero commitment.

INTRODUCTION

Climate change is considered as one of the biggest problems in the world today. The University of Veterinary Medicine and Pharmacy in Košice (UVMP) is a research public university, recognized in Slovakia and internationally, which educates experts in all three levels of higher education in the field of veterinary medicine, food hygiene and pharmacy based on the latest scientific knowledge in accordance with the "One World, One health" concept. Creation and protection of the living environment topics are taught at all levels of university studies.

UVMP signed up to the global environmental campaign “Race to Zero for Universities and Colleges” in 2021. By joining the initiative UVMP committed to support a three-point agenda: 1) mobilising more resources for climate change research, 2) a commitment to achieve carbon neutrality by 2030 or no later than 2050 and 3) development of environmental and sustainability education in curricula as well as student and community programmes. In order to fulfil these commitments, the Race to Zero signatories must draw up an action plan within one year of joining the initiative (<https://www.educationracetozero.org/about-race-to-zero-for-universities-and-college>).

In 2021, a Green University Working Group was established at UVMP. The working group wanted to find out whether the university staff and students are interested in promoting individual and institutional environmental responsibility.

A number of studies have been conducted on perception of climate change, environmental awareness and/or behaviour of general public (Lee et al., 2015; Papoulis et al., 2015; Vogiatzi et al., 2018; Krkoška Lorencová et al., 2019; Drimili et al., 2020, 2020a; Persson et al. 2021; Gareiou et al., 2022). However, much less research on this topic has been carried out among university students and staff. In Slovakia, evaluation of environmental awareness of university students has recently been carried out by the University of Presov in Presov, Slovakia. The study showed that while the students' environmental awareness within the cognitive and emotional factors was high, the environmental awareness within behavioural factor was only moderate. (Novotný et al., 2021). Another survey on environmental awareness and attitudes of university students from Hatay, Turkey during the 2011-2012 academic year showed that the environment was not the most important problem for university students on a local or national scale and the level of awareness was found to be higher than the level of attitude in the university students. Gender was not found as an effective parameter on environmental attitude (Bozdogan, et al. 2016). A more recent survey, which assessed nursing department students' attitudes towards environmental problems on sample of 1047 students in three universities in Turkey between December 2017 and March 2018, showed that nursing students had positive attitudes towards environmental problems, and mean scores were found to be higher in female students and students who were sensitive to behaviours about environment (Bakan et al., 2020). In view of this, the present study surveys the opinion of the university staff and students at University of Veterinary Medicine and Pharmacy in Košice, Slovakia concerning their environmental consciousness and awareness of the problem of climate change.

METHODS

The questionnaire was prepared in Slovak and English. It was emailed to UVMP staff (500), PhD students (108), students (1640) and international students (390). The questionnaire was promoted and made available on UVMP's website (<https://www.uvlf.sk/>) and on social networks (Facebook, Instagram, Twitter). In addition to gauging interest in environmental topics at the university, the questionnaire served as a data collection tool to analyse the current state of the environment at UVMP and to identify shortcomings to be remedied. The questionnaire topics included perception of the climate crisis, UVMP's relaxation zones, waste management, engagement in eco-friendly activities, support and care for local fauna and

alternatives to car use. Additional topics were covered by the respondents under two open-ended questions. The questionnaire consisted of 29 questions (list of survey questions is available from the authors), which the respondents answered anonymously using Google Forms from 1 to 10 December 2021. Two questions (28-29) were open-ended and the remaining ones were single-answer multiple choice questions, some of which were Likert scale questions. The respondents were also able to comment on the questions and provide their observations and suggestions. The variables included in the questionnaire were sex (male/female), age (18-25, 26-35, 36-55, 56-65, over 65), occupation (student, teacher/researcher, technical staff/administrative staff/labourer) and language of responses (Slovak/English). The questionnaire evaluation presented in this paper is a first-stage evaluation and offers only basic data analysis without applying the above-mentioned variables to questions 4-29. A more detailed data analysis will be done at a later stage. The results will be used to prepare UVMP's Race to Zero Action Plan.

RESULTS AND DISCUSSION

The questionnaire was filled out by 657 respondents, 540 women (82.2%) and 117 men (17.8%). From the total number of 657 respondents, 566 (86.1%) spoke Slovak language and 91 (13.9%) spoke English language. Most of the respondents, 446 (67.9%), were aged 18-25 years. There were 97 (14.8%) respondents aged 26-35 years, 75 (11.4%) respondents aged 36-55 years, 35 (5.3%) respondents aged 56-65 years and 4 (0.6%) respondents aged over 65 years. 522 students (79.5%) participated in the survey, of whom 431 (82.6%) were studying in Slovak and 91 (17.4%) in English. The questionnaire was completed by 83 teachers and researchers (12.6%), 52 technical staff, administrative staff and labourers (7.9%). The respondents submitted almost 700 suggestions and observations.

We have grouped questions into 6 thematic areas: Perception of the climate crisis; UVMP's relaxation zones; Waste management at UVMP; Engagement in green activities; Support and care for local fauna and flora; Alternatives to car use. In this paper we present the results of three areas.

Perception of the climate crisis: More than 85% of respondents perceived the climate crisis as a real threat to their lives. Some considered it a threat to the lives of the next generation (generations). Respondents stated that educated professionals must have the main say in resolving the climate crisis. Drimili et al. (2018) on the sample of 500 respondents in Athens, Greece in 2017, stated that majority of them (85.2%) perceived the impacts of environmental problems on their daily life and also for future generations (95.8%).

Waste management at UVMP: Approximately 1/3 respondents stated that they produced a normal amount of waste paper, plastics and bio waste. About 70% of respondents stated they did not generate metal, electrical and hazardous waste at all. More than 43% of respondents were satisfied with waste sorting possibilities at the UVMP campus. More than 47% of respondents had reservations about waste sorting at UVMP. The basic requirement was to reduce waste generation and increase the proportion of sorted waste. Containers and bins for separated waste must be where the waste is generated. It is also necessary to replace plastics with biodegradable materials (including lunch packaging). Gareiou et al. (2022) investigated the opinion and behaviour concerning the current use of plastic, their recycling scenario and the potential for their replacement with more sustainable and biodegradable material in Greece and Lebanon. The data were collected using a structured questionnaire. From the survey it arose that from one third to half of the responders, the preference on products with biodegradable packaging is very low. This trend is more evident for the Lebanese case, rather than Greek one. However, females in Greece, when choosing products packaging, are more affected than males by whether the packaging is biodegradable.

Similarly, a recent survey on the attitudes of Athens residents towards recycling, composting and the operation of green centers showed that half of the respondents are involved in recycling, and the majority of the responders are willing to participate in future composting programmes and have a positive opinion about the operation of green centers (Drimili et al., 2020). The respondents in our survey suggested placing a container for disposal of toner cartridges and a collection point for batteries on UVMP premises. Other suggestions included saving paper, toners, using recycled paper, online learning materials and composting at the university campus and halls of residence.

Alternatives to car use: More than 46% of respondents go to school/work on foot, 5% use a bicycle and only 0.9% ride a scooter. Almost a third of respondents use a bicycle or scooter on their way to school or work at least occasionally (26.2%). 5% use a bike or scooter all year round. Respondents cite few bike paths in the city (28.4%), a small number of bicycle stands (22%), road traffic (20.2%), and fear of bicycle theft (10.9%) as the main reasons preventing them from riding bicycles or scooters for school or work. Other reasons are mainly lack of institutional support (e.g. insufficient parking facilities at university), great distance to the nearest shared bicycle or scooter, great distance between the place of residence and the university, bad weather, the absence of rules for bike and scooter traffic on pavements, age and health, missing anti-theft stands, and the need for a car to perform work duties. The strongest motivation for more frequent use of bicycles or scooters is better infrastructure (29.4%) and

safe storage during parking (22.2%). As many as 19.7% of respondents cannot and do not want to use a bicycle or a scooter on their way to school or work. Respondents suggested the introduction of a Car Free Day event. Cyclists proposed to increase the number of bike stands, build a shelter with a camera system and sockets for charging scooters or bicycles. Respondents also suggested the repair and better maintenance of pavements and roads in the area.

CONCLUSIONS

The results of the survey revealed that the staff and students of the UVMP in Košice who took part in the survey are interested in environmental topics and are aware of the threats posed by the climate crisis to their lives or the lives of future generations.

While it is satisfying that almost half of the respondents commute to work or school on foot, more could be done to promote other alternatives to car use such as bike or scooter by improving Košice city's cycling infrastructure and introducing anti-theft measures on the university campus.

The survey showed there is still room for improvement in the UVMP waste management practices, especially as regards waste reduction and waste sorting options.

It is very encouraging that the majority of the respondents would take part in eco-events organized by the university and more than a third would get involved in green projects involving the inhabitants of the city of Košice. These findings give further backing to the plans of UVMP's Green University Working Group to organise eco-friendly activities in future and engage the local government in these activities. In addition, an environmental club could be funded by university for students and staff, which could turn positive attitudes into behaviours and help materialise ideas for a greener and more sustainable university.

Only political regulations are not sufficient to make people live in a healthier environment. Therefore, it is recommended that society should be made conscious about the environment. In this regard, awareness-raising activities should be conducted for university staff and students, who have the potential to raise awareness in society due to their roles.

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THE IMPACT OF THE CLIMATE CHANGE ON THE HONEYBEE (*APIS MELLIFERA*) IN SLOVAKIA

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ABSTRACT

The current global climate change has a serious impact on natural systems with a negative impact on a large number of plant and animal species, including insects. The honeybee (*Apis mellifera*) represents one of the most important species of social insects that contributes significantly to the preservation of biodiversity, with important economic impact, including mostly the pollination of agricultural crops and also the production of honeybee products. Considering the importance of honeybees in the habitat, it is important to point out the consequences of changes and possible risks resulting from climate change, which, together with other biotic and abiotic factors, participate in reducing the number of honeybee colonies and declining of their health.

INTRODUCTION

Currently, there is no doubt that the Earth's surface is gradually warming, with the land warming more than the oceans and the changes being more intense from the equator to the poles. Due to the interaction of other factors, global climate change occurs on Earth, which has a serious impact not only on natural ecosystems, and their biodiversity, but also on the further development of human society (EEA, 2004). The impacts of global climate change on honeybees are not easy to monitor, as a number of factors influence their distribution and life manifestations. It is necessary to take into account not only climate changes, but also the transformation of the landscape and its habitats, their degradation or fragmentation as a result of various forms of anthropogenic activity. The honeybee (*A. mellifera*) is one of the most important animal species for human nutrition, as it pollinates up to 78% of the flowering plants on Earth and approximately 70% of the main agricultural crops. Unlike other pollinators, honeybees do not specialize in certain types of plants, they fly up to a distance of about 4.5 km from the hive, so one honeybee colony is able to pollinate an area of up to 6360 ha (Seeley, 2016). Honeybee (*A. mellifera*) pollinates most of agricultural crops intended for human consumption, their economic importance also lies in the production of honey and other

honeybee products, and the contribution of honeybees to the preservation of biodiversity is also significant. Over the past 50 years, the volume of crops dependent on honeybee pollination has tripled, which increases demands on the number of honeybee colonies (Klein et al., 2007). The decline of insect populations, including honey honeybees (*A. mellifera*), occurred mainly in Europe as a result of biotic and abiotic factors. COLOSS (Prevention of Honeybee Colony LOSSes) monitors honeybee colony losses every year, and the last published data from the winter of 2016/2017 showed a total loss of honeybee colonies in 30 countries of the world at the level of 20.9%, which is an alarming number (COLOSS, 2022; Brodschneider et al., 2018). The causes of honeybee colony losses can be divided into biotic and abiotic. Important abiotic factors include: uncontrolled or excessive use of pesticides, global climate change, loss of food habitats and lack of food for honeybees (Wagner, 2020; Al Nagggar et al., 2019). Biotic factors include different types of viruses, bacteria, protozoa and arthropods (DeGrandi-Hoffman et al., 2018; Lannutti et al., 2022). Collapses of honeybee colonies rarely have only one cause, mostly a combination of biotic and abiotic factors, and a synergistic effect is often observed (Marín-García et al., 2022).

Impact of climate change on the distribution of honeybee pathogens

Several representatives of prokaryotic and eukaryotic organisms can be mentioned as an example of the influence of the climate change on the geographical distribution of honeybee pathogens associated with the occurrence of non-native invasive species. The parasitic microsporidians *Vairiomorpha apis* and *Vairiomorpha ceranae* (formerly *Nosema apis* and *Nosema ceranae*) Apis responsible for nosematosis in honeybees represent the second most widespread biotic factor related to bee decline after the bee ectoparasitic mite of the family Varroidae (*Varroa destructor*). In the non-native invasive species *V. ceranae* was observed better adaptation to higher temperatures than in *V. apis*, and its geographical spread in the direction of the north-south gradient at the expense of the native species *V. apis*. In the past, *V. apis* was present in most honeybee colonies, but the global trend currently describes the retreat of this species, which is gradually being replaced by the related invasive species *V. ceranae* as a response to climate warming (Dainat et al., 2012; Huang et al., 2014; Hurná et al., 2022). Other examples of non-native pathogens infecting the honeybee are *Apicystis bombi*, a parasitic protozoan of the Apicomplexa phylum; representatives of the Trypanosomatidae order *Crithidia mellificae* and *Lotmaria passim*; and the small hive beetle *Aethina tumida* (Small Hive Beetle /SHB) (Lipa and Triggiani, 1996, Ravoet et al., 2015).

Among the non-native species of honeybee viruses, representatives of the Districtoviridae family (IAPV - Israeli acute paralysis virus; Kashmir honeybee virus/ KBV - Kashmir honeybee virus; ABPV-Acute honeybee paralysis virus; queen blackening virus /BQCV- Black Queen Cell Virus) and the Iflaviridae family (DWV-Deformed Wing Virus, ; slow honeybee paralysis virus/ SBPV- Slow honeybee paralysis virus) can be mentioned (Lanutti et al., 2022).

Impact of the climate change on the immune system of honeybees

When monitoring the current state of the honeybees are able to cope with biological stress only if their immunity is not affected by diseases, viral or parasitic infections (Conrad, 2010). Defense against stress is ensured by the immune system represented by cellular and humoral immunity, and supported by collective, so-called social immunity. Infections caused by pathogens, activates the production of various antimicrobial peptides, phenoloxidase cascade, phagocytosis or behavioral defense mechanisms (Evans et al., 2006). Honeybees fight pathogens by collecting antimicrobial substances from the environment in the form of propolis, which is considered a manifestation of social immunity (Simone et al., 2009). Propolis, which has significant antibiotic and antiviral properties, effectively contributes in strengthening the immune system. The use of propolis by honeybees, likely reduced the need to further develop their immune systems to the extent, that other insects did (Conrad, 2010). Expression of genes coding immune response, is dependent on availability of propolis components (Richard et al., 2008). A decrease in the diversity of plant habitats, from which honeybees obtain propolis in addition to food, results in a decrease in the immunity of the honeybee colony (Evans & Spivak, 2010).

Impact of the climate change on phenophases and nutritional stress of honeybees

Phenology, as an applied scientific discipline, uses bioindicators to capture changes in the landscape that are accompanied by weather extremes (drought, heat waves, torrential rainfall) during the onset of climate change. Long-term phenological data shows, that with the gradual warming of the atmosphere, the beginning of spring vegetative phenophases starts earlier by 1–5 days on average, which brings the risk of damage to flowers by late spring frosts (Gardianová, 2019). Also, the autumn vegetative phenological phases are recorded to start significantly later by 4–20 days (Škvareninová, 2014). The shift of the phenological manifestations of plants to an earlier period shows that depending on the phenological development of plants, the development of the honeybee colony also unfolds (Kubov, 2015).

The impact of climate change on bee colonies manifests itself in the form of nutritional stress, which occurs in the case of extreme weather fluctuations, a too long lay-free period, and a lower supply of flowering plants. Worsening of honeybee colonies survival during the winter and pre-spring period can be caused to significant temperature fluctuations which are an accompanying symptom of global climate change.

Impact of the presence of invasive plants on habitats and the honeybee

Biological invasions are part of significant global changes that challenge the maintenance of biodiversity and natural resources (Simberloff et al., 2013). Invasive plant species fundamentally affect and change the composition of existing vegetation (they displace native species from their natural habitats, thereby reducing biological diversity). They spread spontaneously, and thus cause economic damage, while some also pose a health risk for humans (pollen allergies, skin reactions). As examples of invasive plants, we have chosen the species canadian goldenrod (*Solidago canadensis*), japanese sedge (*Reynoutria japonica*) and giant hogweed (*Heracleum mantegazzianum*), which are currently among the most invasive plant species in Central Europe (Szymura and Szymura, 2015). These originally ornamental plants are quickly spread, which is due to their strong colonizing ability (Kabuce and Priede, 2010). Their roots produce substances with an allelopathic effect, that inhibits or suppress the growth of other plants. As a result, these invasive species create stable and widespread monocultures, with a high germination rate, which negatively affects the diversity of flowering meadows. This fact is particularly significant in intensively farmed areas, where there are fewer wild-growing flowering plants, which are often locally isolated in the formation of mass-grown crops. As a result, honeybees have a more monotonous diet than in the evolutionary past, which can have a significant impact on honeybee development, fitness and immunity. It is now known that a variety of different pollens is necessary for optimal growth and development of honeybees (Goulson et al., 2015).

CONCLUSION

Climate change in recent decades have a significant impact on beekeeping at various levels. Changes can be seen in honeybee behavior and physiology, quality of the plant environment, reduction in the capacity for the development of honeybee colonies, emergence of new pathogens and their geographical spread. However the main impact is considered to be a change in the distribution of flowering plant species on which honeybees depend. Factors such as increased stress associated with honeybee colonies relocating, honeybee diseases, extensive

use of pesticides in agriculture, electromagnetic radiation, lack of nutrients, mono-diet cultures are leading to a decrease in honeybee resistance (van Engelsdorp, 2009). The likely consequence of the climate change is the introduction of new invasive plant species, an increase in the distribution range of pathogens, and an overall change in the balance between honeybee, plant habitat and diseases (Le Conte and Navajas, 2008). Identifying the causes that cause mentioned facts is not easy and usually not even clear. However, the unprecedented acceleration of these processes currently speaks in a favor of the global climate change. Spread of invasive plants and invasive pathogens, and the overall global climate change, may not always be the primary stimulus, but its influence is evident. In any case, the search for the causal relationships of these processes is a challenge for the future.

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DETECTION OF CHROMOSOMAL DAMAGE AFTER MICONAZOLE EXPOSURE IN BOVINE PERIPHERAL BLOOD

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ABSTRACT

Conazoles are fungicides applied to control fungal growth in the agricultural industry and to treat fungal infections in humans and animals. The fungicidal properties of conazoles are due to their inhibition of ergosterol biosynthesis. Miconazole was evaluated for the ability to induce chromosomal aberrations (CAs) and mitotic indices (MI) in cultured bovine peripheral lymphocytes. The bovine cultures were treated with the fungicides at the concentrations ranged from 2.5 to 25 µg.ml⁻¹ for the last 48 of incubation. A statistically significant in chromosomal aberrations frequencies were found after the exposure to the fungicide at highest concentrations ($P < 0.05$ or $P < 0.01$).

INTRODUCTION

Conazole pesticides are a class of fungicides containing imidazole or triazole that are both applied as an antifungal agent in agriculture, as well as a therapeutic agent in human and veterinary medicine. Conazoles reduce fungal membrane integrity by inhibiting the cytochrome P450 enzyme lanosterol 14 α -demethylase (CYP51), which is required for ergosterol biosynthesis and maintenance of proper membrane fluidity and permeability in fungi (Zarn et al., 2003). In addition to fungal CYP51, conazoles also target mammalian and other vertebrate CYP51, which catalyzes the formation of the cholesterol precursor zymosterol (Hermesen et al., 2012).

The aim of our study was to evaluate the potential genotoxic/cytotoxic effects of miconazole using chromosome aberrations (CAs) in bovine peripheral lymphocytes *in vitro*. Chromosome aberrations are generally considered as biological endpoints to determine the level of genetic damage.

MATERIAL AND METHODS

Miconazole was solved in dimethylsulfoxide (DMSO) and used in the experiments. Mitomycin C (MMC, Sigma, St. Louis, MO, USA) at a concentration of $0,4 \mu\text{mol.l}^{-1}$ was used as a positive control agent. 0.5 ml of heparinised whole blood of healthy young bulls was added to 5 ml of chromosome medium RPMI 1640 supplemented with L- glutamine, 15 $\mu\text{mol/L}$ HEPES (Sigma, St. Louis, MO, USA), 15% foetal calf serum (BOFES, Sigma, Chemical Co. St. Louis, MO, USA), antibiotics (penicillin 250 U/ml and streptomycin 250 $\mu\text{g.ml}^{-1}$) and phytohaemagglutinin (PHA, 180 $\mu\text{g.ml}^{-1}$, Wellcome, Dartford, England). Lymphocyte cultures were incubated at 37 °C for 72 h and treated with 2.5, 5, 10, and 25 $\mu\text{g.ml}^{-1}$ of miconazole for the last 48 h of the incubation. One and half hour before the end of cultivation, colchicine (Merck, Darmstadt, Germany) was added to all control and experimental flasks at the final concentration of 5 $\mu\text{g.ml}^{-1}$. For the standard cytogenetic analysis the slides were stained with Giemsa solution. One hundred well-spread metaphases were analysed for the CA including chromatid, isochromatid breaks (CB, IB) and chromatid, isochromatid exchanges (CE, IE). Gaps (G) were examined separately. The mitotic index (MI) was calculated as the metaphase ratio of the total number of 1000 cells. A χ^2 test was employed to estimate the induction of CA, reduction of MI and for estimating the cell cycle delay.

RESULTS

The frequencies of chromosomal aberrations induced by miconazole in bovine lymphocyte cultures are seen in Table 1. A 48 h exposure to the miconazole showed statistically significant elevations in the mean of CAs in each donor at concentrations ranging from 10 to 25 $\mu\text{g. ml}^{-1}$ ($P < 0.05$ or $P < 0.01$) as well as at concentration of 5 $\mu\text{g.ml}^{-1}$ in donor 1 ($P < 0.05$). A reduction in the MI frequencies with a statistically significance was seen after the application of the highest concentration ($P < 0.05$).

DISCUSSION

In our study, clastogenic effect of miconazole in bovine cultures was indicated. Similar results in the increase of chromosomal aberrations were documented by Hassan (1997) after treating of mouse bone-marrow cells and primary spermatocytes with both acute and subacute treatments with miconazole. On the contrary, miconazole was not genotoxic when tested *in vitro* in a bacterial reverse mutation (Ames) assay or in an *in vivo* mouse bone marrow micronucleus test (Center for drug Evaluation and Research, 2010).

Several studies demonstrated potential genotoxic effects of other conazoles. Authors Koleničová et al. (2020) studied epoxiconazole for its potential genotoxic and cytotoxic effects on bovine peripheral blood using alkaline and neutral comet assays. They exposed the lymphocytes to epoxiconazole at concentrations of 2.5, 5, 10, 25, 50 and 100 $\mu\text{g} \cdot \text{ml}^{-1}$ and they showed that epoxiconazole caused dose-dependent elevation of DNA damage. Other researchers, Hamdi et al. (2019) reported harmful effect of epoxiconazole on cell viability, inducing cell cycle arrest, cytoskeleton disorganization and DNA damage in F98 glioma cells.

CONCLUSION

Our results indicated ability of mikonazol to induce clastogenic/genotoxic and/or cytotoxic effects in bovine peripheral lymphocytes.

Table 1: Induction of CA in bovine peripheral lymphocytes exposed to Mikonazol for 48 h

Dose	Metaphase number	G	Types of CA				% Breaks (\pm SD)	% MI
			CB	IB	CE	IE		
Donor 1								
Control	100	5	1	1	-	-	2.0 \pm 0.14	3.1
Mikonazol ($\mu\text{g} \cdot \text{ml}^{-1}$) 48 h								
2.5	100	6	2	4	-	-	6.0 \pm 0.238	3.0
5	100	3	4	3	-	-	7.0 \pm 0.255*	3.0
10	100	2	6	4	-	-	10.0 \pm 0.3**	2.2
25	100	5	4	3	-	-	7.0 \pm 0.255*	1.6*
MMC (0.4 μM)	100	10	14	7	-	-	21.0 \pm 0.407***	0.6***
Donor 2								
Control	100	4	-	2	-	-	2.0 \pm 0.14	2.7
Mikonazol ($\mu\text{g} \cdot \text{ml}^{-1}$) 48 h								
2.5	100	8	3	2	-	-	5.0 \pm 0.218	2.8
5	100	4	5	1	-	-	6.0 \pm 0.238	2.5
10	100	3	2	8	-	-	10.0 \pm 0.3**	2.2
25	100	2	5	2	-	-	7.0 \pm 0.255*	1.6*
MMC (0.4 μM)	100	11	12	8	1	-	22.0 \pm 0.414***	0.4***

*, **, *** -statistical significance ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively: χ^2 test), CB, IB-chromatid, isochromatid breaks, CE, IE-chromatid, isochromatid exchanges

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FUNGICIDE MICONAZOLE IS UNLIKELY TO CAUSE CHANGES IN METHYLATION OF THE BOVINE *GSTP1*, *GSTA4* AND *ACHE* GENES IN BOVINE LYMPHOCYTES PROLIFERATING *IN VITRO*

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ABSTRACT

DNA methylation is a fundamental epigenetic process associated with the regulation of gene expression. It is not stable, but is sensitive to factors such as diet, environment, or age. In the present study, we investigated potential changes in the methylation of *GSTP1*, *GSTA4*, and *AChE* genes in bovine lymphocytes cultured *in vitro* with the fungicide miconazole, which is widely used for the treatment of fungal infections not only in cattle. We used methylation-specific PCR for this purpose and found that miconazole at 1.25, 2.5, 5, 10, 25, and 50 µg/ml was unlikely to cause changes in the methylation status of these genes.

INTRODUCTION

DNA methylation is a fundamental epigenetic process involved in the regulation of gene expression. Within the eukaryotic genome, it is present in cytosines that are mainly part of CpG dinucleotides and while most methylated CpGs remain methylated throughout development, CpGs present in so-called CpG islands (CG-dense regions) are hypomethylated. DNA methylation is not stable and the methylation status of the genome is the result of the interaction of genetic, epigenetic, and environmental factors (Dhar et al., 2021).

Pesticides are a chemically diverse group of substances used in agriculture to protect crops and control food production. Miconazole, a fungicide from the imidazole group, is used to treat a variety of fungal infections. This drug inhibits 14 α -demethylase, a key cytochrome P-450 enzyme involved in ergosterol synthesis in the fungal cell membranes. Ho et al. (2021) revealed that miconazole showed antitumor effects by promoting apoptosis in bladder cancer cells. In addition to its medicinal effects, the genotoxic effects of miconazole have been reported (Hassan, 1997).

Glutathione-S-transferases (GSTs) are a group of multifunctional enzymes whose main function is to protect the cell against oxidative stress and several toxic molecules.

Acetylcholinesterase (AChE) is an enzyme that plays an important role in the cholinergic synapses of the central and peripheral nervous systems by hydrolyzing the neurotransmitter acetylcholine.

In the present study, we evaluated the methylation status of *GSTP1*, *GSTA4*, and *AChE* genes in bovine lymphocytes cultured *in vitro* with miconazole, to determine whether this fungicide can induce changes in the methylation of these genes. Methylation-specific PCR (MSP) was used for this purpose.

METHODS

Blood collection and *in vitro* cultivation of lymphocytes with miconazole. Blood was collected from two bulls (Slovak-spotted cattle, 5—6 months), and *in vitro* culture of lymphocytes with miconazole was carried out as described in a previous study (Halušková et al., 2019). The above procedures complied with the national and institutional rules for working with animals (Decision of the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, the Slovak Republic No. EKVP/2023-06). Lymphocytes were cultured for 24 h with the following concentrations of miconazole: 2.5, 5, 10, 25, and 50 µg/ml. The cultivation was repeated once more with the duplicates of each sample, while a sample with 1.25 µg/ml of miconazole was also incorporated. DNA isolation, bisulfite modification and MSP

For DNA isolation and bisulfite modification, we used commercially available kits: Relia PrepTM Blood gDNA Miniprep System (Promega) and MethylEdgeTM Bisulfite Conversion System (Promega). The *GSTP1* gene MSP analysis, including the design of primers for both the unmethylated and methylated genes, was performed as previously described (Halušková et al., 2019). Primers for the unmethylated and methylated *GSTA4* and *AChE* genes were designed using the online available software MethPrimer (<https://www.urogene.org/cgi-bin/methprimer/methprimer.cgi>). The amounts of reagents in the 25 µL PCR reactions and the conditions of electrophoretic analysis for the *GSTA4* and *AChE* genes were used as it was previously described for the *GSTP1* gene (Halušková et al., 2019). The PCR conditions for these two genes were as follows: I) 95 °C, 2 min II) 95 °C, 40 sec; 63 and 60 °C for the *GSTA4* and *AChE* gene, respectively, 30 sec; 72 °C, 1 min III) 72 °C, 5 min. A non-template sample (NTS) was included in each amplification reaction.

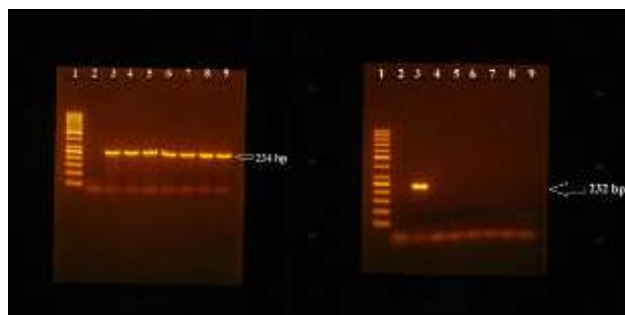
RESULTS

We evaluated the methylation status of bovine *GSTP1*, *GSTA4*, and *AChE* genes in lymphocytes cultivated without and with appropriate concentrations of miconazole. Standard commercially available bovine DNA, both unmethylated and methylated, was also analyzed.

MSP analysis showed that the relevant bands to be amplified with primers designed for unmethylated genes, specifically a 209 bp band for the *GSTP1* gene, a 234 bp band for the *GSTA4* gene (Fig. 1A), and a 231 bp band for the *AChE* gene, were present in the amplification profiles obtained with DNA isolated from lymphocytes cultured without and with the appropriate concentrations of miconazole, as well as in the amplification profile obtained with standard bovine DNA. On the contrary, MSP analysis with primers designed for methylated genes showed that the corresponding bands to be amplified with these primers, specifically, a 200 bp band for the *GSTP1* gene, a 232 bp band for the *GSTA4* gene (Fig. 1B) and a 227 bp band for the *AChE* gene, were not present in any of the same samples analyzed. The expected bands were present only in samples where fully methylated standard bovine DNA was used as the template DNA (Fig. 1B). So our analysis showed that all genes were unmethylated in all samples studied, except for the latter.

Fig. 1: MSP analysis of the GSTA4 gene in bovine lymphocytes cultivated in vitro with miconazole:

A – electrophoretic analysis of amplification mixtures obtained with primers designed for unmethylated GSTA4 gene and with template DNA isolated from lymphocytes of animal 2 cultivated without and with the following amounts of miconazole: 1 – molecular weight marker (GeneRuler 50 bp DNA ladder, Thermo Scientific), 2 – NTS (non-template sample), 3 – commercially available standard bovine DNA, 4 – control DNA from lymphocytes cultivated without miconazole, 5 – 2.5 µg/ml of miconazole, 6 – 5 µg/ml of miconazole, 7 – 10 µg/ml of miconazole, 8 – 25 µg/ml of miconazole, 9 – 50 µg/ml of miconazole. B – electrophoretic analysis of amplification mixtures obtained with primers designed for the methylated GSTA4 gene and with template DNA isolated from lymphocytes of animal 1 cultivated without and with the following amounts of miconazole: 1 – molecular weight marker (GeneRuler 50 bp DNA ladder, Thermo Scientific), 2 – NTS (non-template sample), 3 – fully methylated commercially available standard bovine DNA, 4 – control DNA from lymphocytes cultivated without miconazole, 5 – 2.5 µg/ml of miconazole, 6 – 5 µg/ml of miconazole, 7 – 10 µg/ml of miconazole, 8 – 25 µg/ml of miconazole, 9 – 50 µg/ml of miconazole.



A

B

DISCUSSION

Miconazole is a frequently used triazole antifungal agent for treatment of mycotic infections in cattle, so we studied its effect on methylation of the selected detoxification genes and the acetylcholinesterase gene. The only study found in literature dealing with the effect of pesticides on the methylation status of bovine genes is the work of Pallota et al. (2019). They found methylation changes in the *XIST* gene promoter in bovine spermatozoa under the influence of the organophosphate pesticide chlorpyrifos. We found that the fungicide miconazole probably did not induce changes in the methylation of *GSTP1*, *GSTA4*, and the *AChE* genes. However, in the future it will be necessary to analyze more CpG dinucleotides in the regulatory sequences of these genes, e.g. using bisulfite sequencing. In addition, the effect of combinations of various pesticides with antimycotic drugs such as miconazole can be studied.

CONCLUSION

The antimycotic fungicide drug miconazole was unlikely to induce methylation changes in bovine *GSTP1*, *GSTA4* and *AChE* genes in *in vitro* proliferating bovine lymphocytes.

ACKNOWLEDGEMENTS

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EVALUATION OF THE CYTOTOXIC AND GENOTOXIC POTENTIAL OF AN ACETAMIPRID-BASED NEONICOTINOID INSECTICIDE

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ABSTRACT

Pesticides are used globally in crop production. Many of them are able to affect not only target but also non-target organisms. For some of them, they can affect hormonal balance, immunity, and reproductive capacity, but among the most serious possible side effects are the genotoxic potential of pesticides. The genotoxic properties of agrochemicals can be investigated using a spectrum of *in vitro* and *in vivo* methods recommended by the OECD, including the comet assay. Using the alkaline and neutral comet test, we evaluated the ability of the acetamiprid-based neonicotinoid to induce breaks in the DNA molecule in cultured bovine cells. The obtained results suggested the genotoxic potential of the tested insecticide.

INTRODUCTION

In modern agriculture, various types of agrochemicals - fertilizers and pesticides – are applied to achieve high crop yields. The widespread global use of pesticides brings not only positives, but also negatives: endocrine disruption, reduced fertility, reduced immunity, cancer, allergies and neurodegenerative diseases. They can contaminate soil, water, and air, remain in the crops, and may enter the food chain, thereby posing a threat to human beings (Sharma et al., 2019) and other living organisms. Such organisms also include cattle, which can meet various types of xenobiotics through grazing, water, and feed (Ferre et al., 2020).

The goal of our study was to observe the potential genotoxic effect of neonicotinoid insecticide based on acetamiprid in cell cultures of cattle. For this purpose, we used the alkaline and neutral comet test, which is part of a set of tests used in genetic toxicology (Beedanagari, 2017).

METHODS

Blood was collected sterilely from two healthy bull donors (Slovak spotted cattle, 5-6 months old). Histopaque®-1077 (Sigma-Aldrich, St. Louis, MO, USA) was used for lymphocyte isolation. The acetamiprid-based neonicotinoid insecticide formulation MOSPILAN® 20SP (20.2% acetamiprid, CAS 135410-20-7 and 2.4% benzene sulfonic acid, CAS 90194-45-9) (Nippon Soda Co., Ltd., Japan) was dissolved in pure water to prepare amounts of 2.5; 5; 10; 25 and 50 µg.mL⁻¹ that were added to 48h lymphocyte cultivation for the last 2h. Hydrogen peroxide (H₂O₂ (300 µM) was a positive control. Medium RPMI 1640 (supplemented with 25mM HEPES/L-glutamine) (GE Healthcare HyClone Lab, Utah, USA) with bovine fetal serum (Sigma-Aldrich, St. Louis, MO, USA), phytohemagglutinin (PHA-L, 20 µg.ml⁻¹, PAN Biotech, Germany) and antibiotics/antimycotics (Sigma-Aldrich, St. Louis, MO, USA) were used for 48h cultivation of lymphocytes. The percentage of cell viability was determined using trypan blue dye. The procedure described in the paper of Koleničová et al. (2020) was used for slide preparation and alkaline as well as neutral comet assay.

RESULTS

The percentage of cell viability for lymphocytes used in alkaline comet test was 90%. After exposure of proliferating lymphocytes to the preparation of acetamiprid-based neonicotinoid (Mospilan), and performing the alkaline comet assay we noted statistical significance for DNA damage ($p < 0.05$; $p < 0.01$; $p < 0.001$) caused by amounts of 5 to 50 micrograms per ml. These results suggested a dose-dependent (genotoxic) effect of the tested insecticide formulation. We performed also neutral comet assay to determine the ability of the Mospilan to induce double-stranded breaks in the DNA molecule. The cell viability was 78.7%. As with the alkaline test, we observed statistically significant values of DNA damage from 5 to 50 micrograms per ml in the neutral comet assay ($p < 0.05$; $p < 0.01$).

DISCUSSION

As indicated by Sharma et al. (2019), pesticides are applied to increase crop productivity; however, in due course of time, they are accumulated in plant parts, water, soil, air and biota. Extensive use of pesticides contaminates soil and water, remain in the crops and finally enter food chain, thereby posing threat to the human beings and other organisms. One of the most important damages that must be monitored after exposure of organisms to pesticides is damage to the DNA molecule in their cells. DNA damage is considered to play an important role in the

initiation step (first step of three major steps) of carcinogenesis process (Beedanagari, 2017). Therefore, to increase the specificity and sensitivity of detecting DNA damage a combination of *in vitro* and *in vivo* genotoxicity assays is recommend to use (referred to as “battery approach,”) to predict human carcinogenicity potential of a test chemical under investigation. One such test is the comet assay, which is of great benefit in testing the effects of xenobiotics at the DNA level (Costa and Teixeira, 2014; Langie et al., 2015). This is valuable not only in humans but also in the spectrum of living organisms.

CONCLUSION

Results obtained using the *in vitro* alkaline and neutral comet assay indicated that the acetamiprid-based insecticide has the potential to induce DNA damage in exposed bovine cells.

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THE ANALYSIS OF ANTIBIOTIC CONTAMINATION OF WATER FROM DIFFERENT SOURCES IN THE EUROPEAN UNION

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ABSTRACT

The presence of antibiotics in aquatic ecosystems poses a risk to One Health. This study presents the analysis of 23 water samples from rivers, lakes, streams, ponds, pools, ports, water reservoirs and seas obtained in Slovakia, Germany, Turkey, Portugal and Croatia. The method STAR was used, which is based on the inhibition of the test bacteria strain. The presence of antibiotics was detected in four water samples: 1. Slovakia, river - macrolides; 2. Slovakia, reservoir - tetracyclines and quinolones; 3. Croatia, sea - tetracyclines and 4. Turkey, sea - sulphonamides and quinolones.

INTRODUCTION

In recent years, the presence of pharmaceuticals in drinking water, surface water, groundwater, wastewater, sewage sludge, soil, etc. has been detected worldwide. Their properties pose a risk to organisms and their health when exposed chronically (Li et al., 2023).

Antimicrobial agents enter the environment through urine and excreta or through improper disposal of waste. In addition, various chemicals, including antibiotics, could enter the environment as part of wastewater from the pharmaceutical industry (Polianciuc et al., 2020).

Wastewater contaminated by antibiotics and their by-products is treated in wastewater treatment plants (WWTP) through a combination of processes to eliminate the physical, chemical or biological pollutants and reduce the impact of the wastewater on the environment and human health. However, the presence of antibiotics in both metabolised and unmetabolised forms, large amounts of diverse bacterial populations (commensal, pathogenic and environmental bacteria) and suitable growth conditions (temperature, pH, high nutrient concentration, etc.) make WWTP or other water sources (rivers, lakes, ponds, etc.) an ideal environment for the development of antibiotic-resistant bacteria (Kumar and Pal, 2018; Nnadozie and Odume, 2019).

To protect the environment and ensure the purity and quality of water, the aim of this study was to analyse water samples from different sources for the presence of antibiotic residues.

METHODS

Water samples: For the analysis of antibiotic residues, 23 water samples were collected from different sources and different countries (Table 2). The water samples were stored in a freezer at -18 °C until analysis. Immediately before analysis, the samples were naturally thawed.

STAR: The STAR method, the agar diffusion test, is used to determine the residues of inhibitory substances based on the inhibition of the growth of bacterial strains (Table 1). Briefly, agar media were prepared according to the manufacturer's instructions with the addition of bacterial spores and paper discs (diameter 9 mm, Albet LabScience, A 2668 090, Albet-Hahnemühle, Dassel, Germany) were placed on the surface of the agar. 30 µl of the sample was added in triplicate to the disc, respectively. Negative (demineralised water) and positive (antibiotic standard) controls were used. The petri dishes were incubated. After incubation, the size of the inhibition zone (IZ) was measured with a digital calliper (Mitutoyo, Kawasaki, Japan) with an accuracy of 0.01 mm. The Microsoft Office Excel 2016 programme was used to calculate the average values of the IZ sizes and the deviations of the values from these average values (\pm standard deviation / SD).

Table No. 1, Specifications of bacterial strains, their sensitivity to antibiotics, test agars and incubation conditions for the STAR method

Bacterial strain	Producer	Incubation	Sensitivity	Agar medium	Producer
<i>Bacillus cereus</i> ATCC 11778	CCM, Brno, CZ	30°C/18h	Tetracyclines	Test agar pH 8.0	Merck10664 Darmstadt, DE
<i>Bacillus</i> <i>stearothermophilus</i> var. <i>Calidolactis</i> ATCC 10149	Merck1.1149, Darmstadt, DE	55°C/15h	Beta-lactams and sulphonamides	Diagnostic Sensitive Test agar	Oxoid CM261, Basingstoke, GB)
<i>Bacillus subtilis</i> BGA	Merck10649, Darmstadt, DE	30°C/18h	Aminoglycosides	Antibiotic medium 11, pH 8	Difco259310, Detroid, USA
<i>Escherichia coli</i> ATCC 11303	CCM, Brno, CZ	37°C/18h	Quinolone	Test agar pH 8.0	Merck10664, Darmstadt, DE
<i>Kocuria rhizophila</i> ATCC9341	CCM, Brno, CZ	37°C/24h	Beta-lactams and macrolides	Test agar pH 8.0	Merck10664, Darmstadt, DE

RESULTS

Of all 23 water samples examined, inhibitory substances were detected in four samples. Inhibition zones were detected for *B. cereus*, *B. stearothermophilus*, *E. coli*, and *K. rhizophila* (Table 2).

Table No. 2, The results of the analysis of antibiotics in water samples with indication of origin and source

Nº	Origin	Country	Source	Inhibition zones [mm ± SD]				
				B.C.	B.S.	B.St.	E.C.	K.R.
1.	Agathase	DE	Lake	-	-	-	-	-
2.	Althmühl	DE	River	-	-	-	-	-
3.	Bardejov	SK	Well	-	-	-	-	-
4.	Brzotín	SK	River	-	-	-	-	0,58 ± 0,07
5.	Bukovec	SK	Reservoir	-	-	-	-	-
6.	Čaňa	SK	Reservoir	0,96 ± 0,23	-	-	1,05 ± 0,16	-
7.	Hornád	SK	River	-	-	-	-	-
8.	Hrhov	SK	Pond	-	-	-	-	-
9.	Janík	SK	Pond	-	-	-	-	-
10.	Komárovce	SK	Well	-	-	-	-	-
11.	Košice	SK	Pool	-	-	-	-	-
12.	Košice	SK	Well	-	-	-	-	-
13.	Levice	SK	Well	-	-	-	-	-
14.	Levoča	SK	Well	-	-	-	-	-
15.	Poproč	SK	Stream	-	-	-	-	-
16.	Prešov	SK	Well	-	-	-	-	-
17.	Rijeka	HR	Port	-	-	-	-	-
18.	Rovinj	HR	Sea	1,54 ± 0,19	-	-	-	-
19.	Ružín	SK	Reservoir	-	-	-	-	-
20.	Tajo	PT	River	-	-	-	-	-
21.	Trebišov	SK	Well	-	-	-	-	-
22.	Side	TR	Sea	-	-	0,64 ± 0,15	2,81 ± 0,38	-
23.	Širava	SK	Reservoir	-	-	-	-	-

DE – Germany, SK – Slovakia, HR – Croatia, PT – Portugal, TR – Turkey, B.C. – *Bacillus cereus*, B.S. – *Bacillus subtilis*, B.St. – *Bacillus stearothermophilus*, E.C. – *Escherichia coli*, K.R. – *Kocuria rhizophila*.

DISCUSSION

Water quality and safety is assessed from physical, chemical, microbiological and biological points of view. Human and veterinary medicines belong to the so-called chemical risks. The European Union (EU) considers water quality as one of its environmental priorities sustainability, ecological balance and human health. However, current EU water quality legislation does not include requirements for many pollutants, including antibiotics, due to a lack of information and knowledge about their toxicity and occurrence in the environment (Danner et al., 2021).

Case studies of water bodies such as rivers, lakes and reservoirs, as well as WWTP, have revealed widespread contamination with tetracycline, sulphonamide, fluoroquinolone, and

macrolide antibiotics at concentrations ranging from nanograms to milligrams per litre (Liu et al., 2019; Wang et al., 2020; Zhi et al., 2019).

The results of this study indicate the presence of macrolide antibiotic residues through the formation of IZ on the *K. rhizophila* plate (sample 4, Slovakia, river), tetracyclines on the *B. cereus* plate (sample 6, Slovakia, reservoir; sample 18, Croatia, sea), sulphonamides on the *B. stearothermophilus* plate (sample 22, Turkey, sea) and quinolones on the *E. coli* plate (sample 4, Slovakia, river; sample 22, Turkey, sea).

CONCLUSION

In this study, 23 water samples from lakes, rivers, streams, wells, reservoirs, seas, ports and pools (in Slovakia, Croatia, Turkey, Germany and Portugal) were analysed for the presence of antibiotic residues using the STAR method. The results obtained show the presence of tetracyclines, macrolides, sulphonamides and quinolones in four samples. One of the main causes of the occurrence of antibiotic residues in the aquatic environment and the emergence of antibiotic resistance can be attributed to the high consumption of antibiotics worldwide, which is often accompanied by reckless use. The inadequate removal of micropollutants in wastewater during its purification also contributes to this.

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THE EFFECT OF AMBIENT TEMPERATURE ON THE CONTENT OF AFLATOXIN M1 IN RAW COW'S MILK

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ABSTRACT

Mycotoxins are produced naturally by the growth of moulds and are harmful to both animals and humans. Climate change is predicted to have an impact on the occurrence of mycotoxins in cereal grains and feedstuffs, and thus also in milk, as temperature and humidity are crucial factors for fungal growth. In this study, 36 samples of raw cow's milk were analysed for the presence of aflatoxin M1 (AFM1) using the AflaM1 Scan (Zeulab S.L., Zaragoza, Spain). The results obtained showed an extremely high prevalence of AFM1 in the milk with an overall compliance of less than 16.6%.

INTRODUCTION

Mycotoxins are naturally produced by fungi that cause a toxic reaction when they enter higher vertebrates naturally in low concentrations. There are 350-400 known mycotoxins. The most common mycotoxins of concern to humans and animals include aflatoxins, citrinin, ochratoxins, fumonisins, patulin, zearalenone, nivalenol, deoxynivalenol, fumonisins and ergot alkaloids (Awuchi et al., 2022). As temperature and humidity are important parameters for fungal growth, climate change is expected to influence the occurrence of mycotoxins (Pereira et al., 2019).

Aflatoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* were the first identified source of mycotoxin contamination in ruminant feed (Pereira et al., 2019). Aflatoxin M1 (AFM1), a hydroxylated metabolite of aflatoxin B1, is found in milk and dairy products from animals that have ingested contaminated feed (Guo et al., 2019). Due to this fact, AFM1 is included in the list of substances that are regularly monitored based on the National Residue Monitoring Plan (Shahbandeh, 2023).

The European Union sets maximum residue limits (MRLs) for aflatoxins in raw milk samples. A limit of 0.05 ppb (µg/kg) has been introduced for the manufacture of dairy products. This

limit is significantly lower for products such as infant formula and follow-on formula (0.025 ppb ($\mu\text{g/kg}$)) (Commission Regulation (EC) No. 1881, 2006).

Since the presence of mycotoxins in milk is a risk factor for One Health, the aim of this study was to analyse the level of AFM1 in milk collected from the farm over the course of one year.

METHODS

Milk samples: A total of 36 samples of raw cow's milk were collected three times a month during the year from farms in eastern Slovakia. The milk samples were stored in a freezer at -18 °C. Immediately before testing, the samples were heated to 40°C for 20 minutes and homogenised.

AflaM1 Scan: AflaM1 Scan (Zeulab S.L., Zaragoza, Spain) is a competitive immunochromatographic rapid test for the quantification (30-200 ppt (ng.kg^{-1})) of AFM1 in raw cows, sheep and goat milk. The presence of AFM1 is inversely proportional to the intensity of the resulting test line and quantification requires a reader IRIS. The test was used according to the manufacturer's instructions. Briefly, 200 μl of the milk sample was placed in a tube containing freeze-dried contents, mixed well with a pipette and incubated at 40°C for 3 minutes. The test strip was then inserted into the test tube and incubated at 40°C for a further 7 minutes. After incubation, the strip was placed in the cassette and the results were read using the device IRIS in conjunction with the mobile application IRIS.

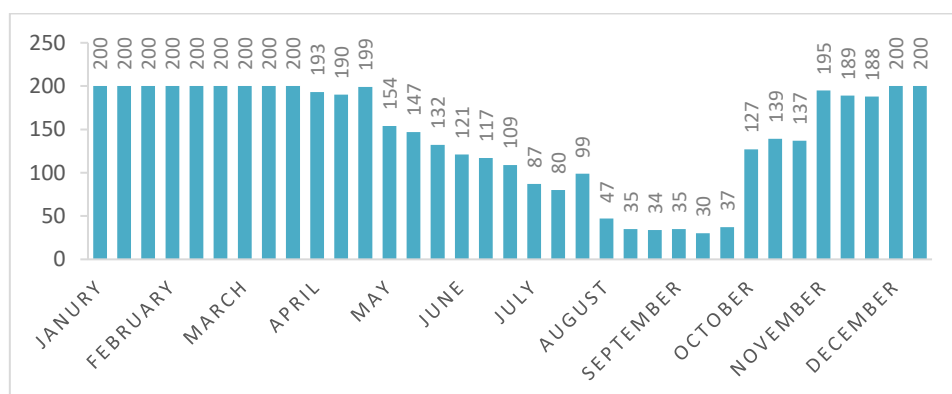
Interpretation of results based on quantification of AFM1 content in the sample: < 30 ppt - 35 ppt = compliant; 36 - 49 ppt = suspect, please confirm; 50 - 64 ppt = non-compliant, please confirm; 65 - > 200 ppt = non-compliant.

IRIS: IRIS (Zeulab S.L., Zaragoza, Spain) is an automatic device for the analysis (autotest) and/or interpretation of the result (strip reading) of Zeulab's rapid tests. The device evaluates the presence of contaminants in the matrix by measuring the absorbance, which is expressed in numerical values.

RESULTS

Of all 36 samples tested for the presence of AFM1, 30 (83.3%) samples were tested as non-compliant (positive) and only 6 (16.6%) samples were tested as compliant (negative). Of the total 30 samples that were tested as non-compliant (positive), up to 12 (40 %) samples contained AFM1 at a concentration above the maximum detection limit of the AflaM1 scan test used (> 200 ppt (ng.kg^{-1})).

Figure No. 1, Graphical representation of the AFM1 content in raw cow's milk samples over the course of a year



The numerical values are given in ppt [ng.kg-1]

The concentrations of AFM1 in the milk samples during the year are shown in Figure 1. The AFM1 content decreased from May to October. The highest levels of AFM1 were measured from December to March.

DISCUSSION

The presence of aflatoxins in feed or milk is a growing concern these days. They can have many harmful effects on human and animal health.

The main findings of this study were that aflatoxins are widespread in raw cow's milk at farm level. Of the 36 samples tested over the course of a year, only 6 samples were within the legal limits for the European Union and were therefore classified as compliant. This corresponds to an overall conformity of 16.6 %. The results of the non-compliant samples ranged from 80 ppt to > 200 ppt. The results showed a high prevalence of AFM1 in milk samples collected from November to the end of April (≈ 200 ppt). Levels decreased from May to the end of October, with the lowest levels in July and August.

When we compare these results with previous studies conducted around the world, the prevalence is exceptionally high. A 2015 study of raw milk in the Iranian city of Sarab found a 40% match (Kamkar, 2005). In another study in Pakistan, AFM1 levels in milk were analysed monthly. The values in October ranged from 0.27 $\mu\text{g/L}$ to 0.45 $\mu\text{g/L}$ with a mean value of 0.345 $\mu\text{g/L}$. In February, the mean value was 0.466 $\mu\text{g/L}$. 99.4% of Pakistani samples exceeded the recommended limit of 0.05 $\mu\text{g/L}$ or 0.05 $\mu\text{g/kg}$ (Hussain and Anwar, 2008).

The time of year can also influence the occurrence of AFM1. The observed levels are higher in the winter months. This is due to the diet of the housed animals. Cattle are usually fed forage and concentrates during winter housing. In contrast, cattle graze on fresh pastures during the

summer months and the risk of aflatoxin contamination is lower (Lee et al., 2021). Some animals may be stalled during the summer months, but even then storage conditions for grain and feed should be better and provide less humid conditions for mould growth.

The risk of aflatoxin contamination is considered higher in regions with tropical or subtropical climates. Extreme temperatures and droughts can be factors for the growth of *Aspergillus* spp. in crops and thus increase the risk of aflatoxin contamination (Warnatzsch et al., 2020).

CONCLUSION

In this study, 36 raw cow's milk samples were tested for the presence of AFM1. The results showed an agreement of only 16.6%. The majority of the samples were at the upper end of the scale (> 200 ppt). From the results, we can conclude that temperature and humidity are important environmental factors for mould growth. Further research may need to be conducted in this area. If milk samples are taken in a number of countries in the European Union and worldwide to describe the ongoing problem on a larger scale.

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OBSERVATION OF INCIDENCE OF *NOSEMA* SPP. IN SLOVAKIA

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ABSTRACT

Nosematosis is a diarrhoeal disease of bees caused by microsporidia of the genus *Nosema*. There are 3 species known to parasitise bees, but only two, *Nosema apis* and *Nosema ceranae*, have a worldwide distribution.

Our aim was to investigate 426 crushed bee corpse abdomens, microscopically positive for *Nosema* spp., using molecular methods. Samples were collected from three ecological regions of Slovakia. Using molecular methods, we demonstrated positivity in 415 samples. After sequencing the positive PCR products and comparing the sequences (BLAST) with the sequences stored in the gene bank, we identified *Nosema ceranae* species in all positive samples. Finally, we determined the prevalence of *Nosema ceranae* in three ecoregions of Slovakia.

INTRODUCTION

Bees play an important role in the pollination of agricultural crops, plants and as a result play a key role in maintaining the biodiversity of ecosystems (Lopes et al., 2022). They provide humans with healthful products, honey, pollen, propolis, beeswax and royal jelly (Truong et al., 2023). Despite their usefulness, bee populations have declined significantly globally in recent decades, threatening the agricultural industry (Lopes et al., 2022). A combination of factors climate, exposure to pesticides, heavy metals from the environment, starvation from lack of grazing, predators and pathogens, contribute to these losses (Panek et al., 2018).

One of the most common worldwide pathogens that contributes to the weakening and death of honey bee colonies globally includes microsporidia of the genus *Nosema* causing a disease called nosematosis (Takashima, Ohari and Itagaki 2021). There are 3 known species of this microsporidian parasite parasitizing the digestive tract of bees (Urbieta-Margo et al., 2019). *Nosema apis* Zander (Fries 1993) *Nosema ceranae* (Fries et al., 1996) and the newest *Nosema*

neumannii (Chaemurot et al., 2017). These microsporidial species differ from each other in spore morphology, epidemiology and clinical manifestations (Lourenco et al., 2021).

Nosema apis and *Nosema ceranae* are widespread globally (Higes et al., 2006), while *Nosema neumannii* is so far only found in bee populations in Uganda (Chemurot et al., 2017).

Bees become infected by consuming infectious spores. When ingested the spores attack the epithelium of the digestive tract and cause its destruction. Afterwards, malabsorption and maldigestion manifest and alter the overall physiology of the organism, resulting in death of the bees (Gisder et al., 2010).

Using microscopic diagnostics of the digestive tract, spores can be detected (Duquesne et al., 2021) and using the molecular method of PCR, it is possible to determine the specific species of this microsporidian pathogen (Truong et al., 2023).

Nosema infection is increasingly associated worldwide with sudden CCD colony collapse disorder (Samie et al., 2021).

METHODS

Samples of winter honey bee carcasses were taken by queen bee breeders in breeding colonies for the annual compulsory examination for the presence of nosematosis. Samples were taken in January and February 2022. A set of 30- 50 bee cadavers from each hive were sent by the beekeepers to the Institute of Apiculture in Liptovský Hrádok, where microscopic diagnosis of *Nosema* spp. Subsequently, the positively diagnosed samples were sent to the Department of Biology and Physiology at the University of Veterinary Medicine and Pharmacy in Košice for further diagnosis using molecular methods.

Microscopically positive samples of crushed bee abdomens were isolated for genomic DNA using an isolation kit (AmpliSens, Russia). Using multiplex PCR with primer pairs for both *Nosema apis* (321 bp amplicon) and *Nosema ceranae* (218 bp amplicon) species simultaneously followed by visualization using ELFO and a transilluminator, we evaluated the positivity of the samples and determined the species according to the length of the amplicon. Then we sent the samples for sequencing and compared the PCR product results (BLAST) with the sequences stored in the gene bank.

Subsequently, we evaluated the prevalence in three ecoregions of Slovakia.

Table n. 1. : Primers for diagnostics species of *Nosema* spp.

Species	Primer	Sequence	bp
<i>Nosema apis</i>	APIS FOR	5'GGGGCCATGTCTTTGACGTACTATGTA 3'	321 bp
<i>Nosema apis</i>	APIS REV	5'GGGGGGCGTTTAAATGTGAAACAACATG 3'	321 bp
<i>Nosema ceranae</i>	MITOC FOR	5'CGGCGACGATGTGATATGAAAATATTAA 3'	218 bp
<i>Nosema ceranae</i>	MITOC REV	5'CCCGGTCATTCTCAAACAAAAAACCG 3'	218 bp

RESULTS

After sequencing the positive PCR products and comparing the sequences (BLAST) with the sequences stored in the gene bank, we confirmed the presence of *Nosema ceranae* species (218 bp) in all samples.

After identification of individual samples according to ecoregions, we found that 104 samples were positive in the eastern ecoregion of the country, 118 samples in the central ecoregion, and most of the 193 positive samples were from the western ecoregion of Slovakia.

DISCUSSION

This survey was made in 2022 to determine the prevalence of *Nosema* spp. in Slovakia and to identify the species responsible for the disease.

Samples collected in January and February 2022 were first examined microscopically and then by molecular methods. The detection of nosematosis species was performed using molecular methods, which are not only able to detect the presence in the sample but also to determine the species of the pathogen (Valenčáková et al., 2015). In all 415 positive samples examined by molecular methods, we detected *Nosema ceranae* species, which corresponds to a size of 218 bp (Sulborska et al., 2019). The last study of *Nosema* spp. occurring in Slovakia was carried out at our site in 2021, where we diagnosed *Nosema ceranae* species in all examined samples (Hurná et al., 2022). In the 2009 and 2010 studies in Slovakia, *Nosema ceranae* was predominant, but *Nosema apis* was also detected (Staroň et al., 2012). *Nosema ceranae* is displacing *Nosema apis* in the *Apis mellifera* population (Klee et al., 2007). A gradually increasing proportion and displacement of *Nosema apis* from more temperate and subtropical climatic regions of the world has been documented in many regions of the world.

The result of this study is related to the gradual displacement of *Nosema apis*, a species of *Nosema ceranae* globally. (Pacini et al., 2016). Many European countries, such as Hungary, Italy, Bulgaria, Belgium, Spain, Portugal, UK, France, Israel (Jabal Urbiel et al., 2022) Serbia, Croatia, Bosnia and Herzegovina, Montenegro, Greece and Macedonia (Matović et al., 2020), report increased prevalence of *Nosema ceranae*. Global trade in bee products and materials, but especially queen bee trade may be a source of infection in some countries (Truong et al., 2023).

CONCLUSION

In our research, we focused on the examination of samples by molecular methods and the associated accurate diagnosis of *Nosema* spp. species in Slovakia, together with the determination of the prevalence of *Nosema* spp. in three ecoregions of Slovakia.

The aim of our work was to examine all microscopically diagnosed positive samples by molecular methods, to verify the accuracy of the microscopic method of diagnosis, to determine the species of *Nosema* spp., and to observe the prevalence in 3 ecoregions of Slovakia.

We found that molecular diagnostics is an accurate and efficient method for diagnosis and species differentiation of pathogens. *Nosema ceranae* was present in all positive samples. After identifying the positive samples, we found that the highest number of positive samples were from the western ecoregion of the country and the lowest from the eastern ecoregion, which may be related to the intensity of beekeeping in the areas as well as increased international trade.

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ENDOPARASITES INFECTION SPREAD IN EAST SLOVAK LOWLAND

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ABSTRACT

This study aimed to determine the prevalence of endoparasites in human population, dog faeces, soil samples, and identify potential risk factors associated with the endoparasites infection spread in East Slovak Lowland. In general, 10.5% of human stool samples and 43.6% of dog excrements were positive. Altogether, 52.0% of soil samples were positive for at least one endoparasites species. Several potential risk factors were investigated, of which population density, the number of Roma and presence of sewage with tap water had an impact on the spread of endoparasites.

INTRODUCTION

The prevalence of intestinal parasitic diseases in Slovakia is relatively low. However, they are easily dispensable in marginalised groups. East Slovak Lowland (ESL) belongs to one of the lesser developed region what is also associated with a higher unemployment rate and lower living standards. This district is ethnically mixed, and areas are linked with problems caused by marginalized groups (Ravaz, 2020). Above mentioned circumstances, with the addition of socio-economic situation, poverty, and coexistence with environmental pollution may also pose a risk to society as a whole. It is therefore advantageous to monitor the epidemiological situation as a precaution and to know the risk factors of disease transmission in the area.

METHODS

Human stool samples were collected from inhabitants aged between 0-80 years old from ESL. In total 428 stool samples were collected. All the samples were examined either by a commercially available concentration kit (Paraprep L, Mondial, France) or with SAF concentration method.

Totally 803 dog excrements were collected randomly from public places and coprologically examined for the presence of endoparasite developmental stages by a flotation methods with Shaeter's flotation solution of sucrose and the Faust flotation solution (Jurášek et al., 1993).

Similarly, 148 soil samples were collected and multiple soil samples (300 - 500g) were taken from surface level (5cm) from one area. The samples were surveyed by flotation-sedimentation method according to Kazacos (1983).

The results of parasitological examinations were processed in software MS Excel. The importance of factors were calculated with correlation coefficient, which was converted into a percentage value (Markechová et al., 2011).

RESULTS

In the 426 examined human samples, we detected helminth eggs in 10.6% samples. Eggs of *Ascaris lumbricoides* (8.9%) and *Trichuris trichiura* (4.5%) were the most overall prevalent. We did not confirm the presence of developmental stages of endoparasites in any of the samples obtained from the majority population. We detected endoparasite only in a minority population, where up to 15.8% of examined samples were positive for the presence of helminth eggs (Table 1).

From 803 dog faeces from public places endoparasite developmental forms were found in 43.6% of samples (Table 2.). The most frequently observed endoparasites species were eggs of *Toxocara canis* (22.7%), *Trichuris vulpis* (14.1%) and eggs of the family Ancylostomatidae (10.1%). The infection prevalence rates with eggs of *Toxascaris leonina*, *Ascaris* spp. and *Capillaria aerophilla* varied from 1.4% to 5.6%. *Ascaris* spp. eggs, which are not typical parasites of dogs, occasionally occurred also in faeces collected from towns and villages (Table 2), and these findings are quite alarming.

Overall, 52% soil samples from public places in the ESL were found positive by microscopic examination for endoparasite developmental stages. The prevalence of endoparasites in soil from the towns was 12%, while the prevalence in soil from the villages and settlements was 72.5 %. The most frequent developmental forms of the endoparasites found in soil were eggs of *Ascaris* spp. (37.2%), *Toxocara* spp. (29.1%) and *Trichuris* spp. (29.1%). The eggs of *T. leonina* and Ancylostomatidae occasionally occurred (Table 3).

In total, we investigated 8 various factors (soil type, number of inhabitants, number of Roma, the availability of drinking water, the accessibility to sewage systems and the presence of sumps in villages and settlements) to identify the potential of risk factors associated with the endoparasites infection spread in ESL (Figure 1). We confirmed usage of sumps and usage of

sewage in village does not affected the endoparasites spread in ESL. The target line was established and considered significant if the factor has a correlation share above 10% (Figure 1).

Table 1. Results of parasitological examination in human samples

		N	P	OR	95% CI
<i>Ascaris lumbricoides</i>	Majority	142	0		
	Minority	284	45	-	-
<i>Trichuris trichiura</i>	Majority	142	0		
	Minority	284	45	-	-

n - number of examined samples; *p* - number of positive samples; *f.* - family; 95 % CI - 95 % Confidence Interval of OR; OR - Odds Ratio; *- reference group for calculating the OR; "-"- unable to calculate due to lack of data

Table 2. Parasitological evaluation of dog's excrement from public places

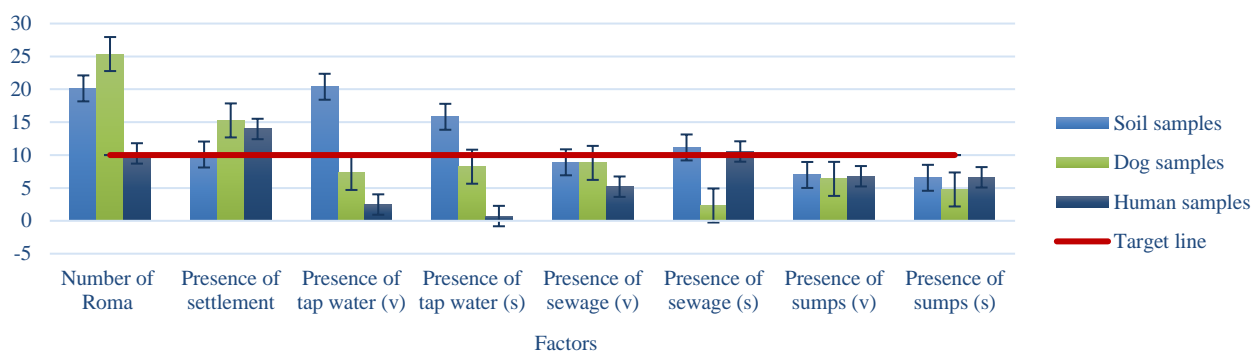
		N	P	OR	95% CI
Overall	Villages	137	32	0.36	0.23-0.55
	Towns*	624	284		1.79-4.19
	Settlements**	42	34	5.98	2.73-13.10
<i>Toxocara canis</i>	Villages	137	14	0.37	0.20-0.66
	Towns*	624	146		1.49-4.80
	Settlements**	42	22	4.13	2.20-7.75
f. Ancylostomatidae	Villages	137	5	0.27	0.10-0.68
	Towns*	624	76		1.45-9.23
	Settlements**	42	0	0	-
<i>Toxascaris leonina</i>	Villages	137	1	0.20	0.02-1.50
	Towns*	624	2		0.66-37.19
	Settlements**	42	8	7.54	3.14-18.10
<i>Trichuris vulpis</i>	Villages	137	12	0.60	0.31-1.13
	Towns*	624	86		0.88-3.14
	Settlements**	42	15	3.75	1.93-7.31
<i>Ascaris spp.</i>	Villages	137	4	6.22	1.37-28.14
	Towns*	624	3		0.03-0.72
	Settlements**	42	5	14.55	4.40-48.04
<i>Capillaria aerophilla</i>	Villages	137	4	0.43	0.15-1.24
	Towns*	624	40		0.80-6.45
	Settlements**	42	1	0.411	0.05-2.95

n - number of examined samples; *p* - number of positive samples; *f.* - family; 95 % CI - 95 % Confidence Interval of OR; OR - Odds Ratio; *- reference group for calculating OR; **- reference group for calculating the OR in settlements were towns and villages are combined; "-"- unable to calculate due to lack of data

Table 3. Results of parasitological examination in the soil

		N	P	OR	95% CI
Overall	Towns*	50	6		0.015-0.11
	Villages	98	71	23.66	8.49-65.94
<i>Toxocara</i> spp.	Towns*	50	1		0.003-0.20
	Villages	98	42	36.75	4.87-277.01
f. Ancylostomatidae	Towns*	50	3	-	
	Villages	98	0	-	
<i>Toxascaris leonina</i>	Towns*	50	2		0.035-45.69
	Villages	98	1	0.24	0.02-2.79
<i>Trichuris</i> spp.	Towns*	50	1		0.003-0.20
	Villages	98	42	36.75	4.87-277.01
<i>Ascaris</i> spp.	Towns*	50	0	-	
	Villages	98	55	-	

n - number of examined samples; *p* - number of positive samples; *f.* - family; 95 % *CI* - 95 % Confidence Interval of *OR*; *OR* - Odds Ratio; * - reference group for calculating the *OR*; "-" - unable to calculate due to lack of data

Figure 1. Comparison of correlation coefficients between each factor and number of positive samples in ESL Error bars represent SE (standard error), (*s* – settlements; *v* – village)

DISCUSSION

In the samples of the majority population, which lives in standard hygienic conditions, we, like Pipiková et al. (2017) found no developmental stages of endoparasites. A very low prevalence of endoparasites (0.6%) in the majority population in eastern Slovakia was recorded by Papajová and Šoltys (2020). Poor hygiene, lifestyle, coexistence with animals without adequate veterinary control and environmental pollution greatly increased the risk of contracting a parasitic disease. In dog samples we recorded higher incidence of endoparasites in dogs from settlements than from urban locations. Similar results are reported in their work by Pipiková et al. (2017). Similarly highest incidence of endoparasite eggs was in soil samples from areas with low hygiene standards are reported by Papajová and Šoltys (2020). The aforementioned authors also confirmed the occurrence of eggs of *Ascaris* spp. only in soil samples from locations with a predominance of marginalized population groups. The analysis showed that out of the 8 studied factors 5 of them (number of inhabitants, number of Roma, usage of water supply in

village and settlements and usage of sewage in settlements) had some influence on the spread of parasites on ESL.

CONCLUSIONS

Endoparasite diseases are constant and unrelenting problem in ESL. Thus endoparasites representing a public and environmental health problem. Our analysis showed that number of people, number of Roma, usage of tap water and sewage were the most important factors in the transmission of parasitic diseases.

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IMPACTS OF CLIMATE AND HOST FACTORS ON *CULICOIDES* IN SLOVAKIA

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ABSTRACT

In this study, we report the abundance, species composition and host preference of biting midges (*Culicoides*) during 2 seasons (2021 – 2022) in the Riding Complex of the UVMP in Košice and in Game Park of Rozhanovce. In total, we trapped 15,678 *Culicoides*. Extremely dry conditions, especially in 2022, significantly affected the species composition and abundance of midges in the horse breeding and in the forest ecosystem. *C. obsoletus*/*C. scoticus* was the dominant species at both trapping sites, with a prevalence of 96.8% in the horse breeding and 91.7% in the Game Park. The most common hosts on which the biting midges sucked blood were humans (44.4%) in the horse farm and red deer (*Capreolus capreolus*) (70%) in the Game Park.

INTRODUCTION

Increasing environmental temperature contributes to increased *Culicoides* dispersal and modification of their life cycles (Okulewicz et al., 2017) in many areas. Biting midges (*Culicoides*) have been the subject of extensive research in almost every country in Europe in the last decade, especially in terms of transmission especially animal pathogens of veterinary importance. Climate change brings the changes in their biodiversity. In Slovakia, biting midges are active from early spring (March) to late autumn (November), while their life cycle and activity significantly influenced by environmental temperature and airflow (Kočíšová et al., 2020; Kočíšová et al., 2021); the moisture content of the breeding substrate is of partial importance for larval development. In Slovakia, the average annual temperature has increased by up to 2.51 °C over the last 60 years, according to the SHMÚ. In Košice, 2022 was the fourth warmest year in the last 20 years, with an average annual temperature of 10.9 °C and a temperature range during the summer months of 21.8-23.5 °C. In 2021, the average annual temperature was 10 °C (19.1-23.5 °C). High temperatures and a lack of precipitation in the summer months with a cumulative deficit of up to 200 mm caused extreme drought in Košice

and the surrounding area in 2021-2022 (SHMÚ 2023) and thus a change in the species composition and abundance of biting midges.

MATERIAL AND METHODS

We trapped the biting midges (*Culicoides*) with special traps CDC 1212 (John Hock Company, USA) in the Riding Complex of UVMP in Košice and Game Park in Rozhanovce during two seasons (2021 – 2022) from May to September. We carried out the trapping in horse and wild game pastures, as well as directly in the stables and in the pheasant aviaries. In total, we conducted 38 trappings during 2 seasons (2021 – 2022), from which we recovered 15,678 *Culicoides*. We diagnosed biting midges by basic morphological signs with a Zeiss-Stemi DV-4 magnifying glass using a rapid diagnostic key (Lehman et al., 2012) and an interactive key (Mathieu et al., 2012). We used a portion of the mitochondrial cytochrome b (Cyt b) gene to identify host blood in biting midges (Radrová et al., 2013).

RESULTS AND DISCUSSION

In the Riding Complex we made 12 trappings in 2021 capturing 3,597 midges classified into 11 species the highest abundance was recorded for *C. obsoletus/C. scoticus* (86.6%/ 3,116 individuals). In 2022, we also made 12 captures of which however 6 captures were negative probably due to high temperatures and drought. We recovered only 4 species of *Culicoides*, similarly the highest activity of *C. obsoletus/C. scoticus* (96.83%/1165 pcs). Of the total 4,797 midges sorted during the two seasons, 187 females were with fresh blood. For host blood diagnosis, we examined 21 inoculated females by PCR analysis of the cytochrome b gene. Up to 8 samples were diagnosed as the dominant human host (38.1%). In one sample, we identified blood of *Equus caballus* (4.76%) and *Oryctolagus cuniculus* (4.76%).

In the Rozhanovce Game Park we have trapped 10,881 biting midges during two seasons. In 2021 we conducted 8 trappings and captured 10,745 *Culicoides* of which the most abundant species was *C. obsoletus/C. scoticus* (91.7%/9857 pcs) out of 10 morphologically determined species. In 2022, we recovered only 102 *Culicoides* from 6 traps of which the most abundant species was *C. obsoletus/C. scoticus* (87.3%/89 pcs) from 4 morphologically determined species. Of the total number of midges, 80 females were found with fresh blood in their abdomen, of which 10 were subjected to PCR analysis for host blood diagnosis. Two samples were negative in 7 samples (70%) we diagnosed red deer (*Capreolus capreolus*) and in one sample (10%) common pheasant (*Phasianus colchicus*) as the host.

The highest abundance of Biting midges was recorded in species of the subgenus *Avaritia* (*C. obsoletus*/*C. scoticus*). These species are morphologically similar to each other. In females, morphological diagnosis is impossible (Zittra et al., 2020), so molecular identification methods must be used for better species identification. These species are the main vectors of pathogens of mainly viral origin in the Palaearctic region and are also among the most abundant in Europe (Bartsch et al., 2009), as confirmed by our research. At present, there are still few studies investigating the occurrence and host preference of *Culicoides* in forest ecosystems. However, wildlife may play an important role as reservoirs in the transmission of various pathogens via *Culicoides* vectors.

CONCLUSIONS

From the perspective of the Centres for Disease Control and Prevention's (CDC) public health and climate change strategy, long-term xenomonitoring of pathogens, hosts, and vectors is key to the registration of human, domestic, and wildlife disease cases, as they often spread to new geographic areas. Emerging insect-borne pathogens of parasitic origin are among the most important aetiological agents of diseases facing humanity in the early 21st century and are becoming a major problem. Determination of species composition and detection of host blood are crucial to control the spread of new invasive *Culicoides* species and subsequent potential transmission of pathogens. In the face of global warming and rising temperatures in our area.

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CAN CLIMATE CHANGES AFFECT THE OCCURENCE OF NEW HOOKWORMS SPECIES IN SLOVAKIA?

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ABSTRACT

Climate changes may affect the occurrence frequency, intensity and geographical distribution of parasites. The *Ancylostoma* and *Uncinaria* genera belong to the Ancylostomatidae family, referred to as hookworms. Our research confirmed the presence of the *Uncinaria stenocephala* genus in dogs and wild foxes in Slovakia. This species occurs specifically in regions with moderate climate whereas the occurrence of the *Ancylostoma* spp. species is limited primarily to tropical and subtropical regions. The aforesaid indicates that climate changes might affect the occurrence of newly emerging hookworm species in Slovakia, in particular the *Ancylostoma* spp., which impose risk to health of animals as well as humans.

INTRODUCTION

The occurrence and spread of hookworms in Europe is associated with extreme weather conditions and seasonal changes in micro - and macroclimate (Okulewicz, 2017). According to a territorial study on climate changes in Slovakia, global warming may manifest itself in our country in an increase in average air temperatures by 2–4 °C by year 2075 (<https://www.shmu.sk/sk/?page=1379>). It is highly probable that such changes will have negative effects on the occurrence of newly emerging species of hookworms not only in Europe, but also in our country.

Infections caused by hookworms are globally spread among the populations of dogs, cats and wild animals, and impose a high risk for public health. With regard to humans, the most important species are *Necator americanus* and *Ancylostoma duodenale* as they cause an anaemic disease of the small intestine in tropical and subtropical regions where the weather is wet since their free-living stages need temperatures above 21 °C for their optimal development (Hawdon and Wise, 2020). Important anthroponotic species also include *A. ceylanicum* and *A. braziliense*. As for carnivores, the species that most frequently occur in them include *Ancylostoma caninum*, which occur in dogs, foxes and other Canidae, and *Uncinaria*

stenocephala, which occur in dogs, cats, foxes, Canidae and Felidae, it is the most wide-spread ancylostomiasis in the temperate climate zone (Svodobová et al., 2013). Hookworms are usually transmitted orally through the ingestion of the filariform larvae that live freely in soil, but can also be transmitted percutaneously. Hookworm species are a cause of the Cutaneous Larva Migrans (CLM) syndrome in humans (Robertson and Thompson 2002).

Climate changes, increasing average temperatures and surface temperatures in conjunction with pathogens and diseases were proclaimed to be the most dangerous threat to the global health in 21st century (Del Guicide et al., 2019). According to García-Rodrigo et al., 2017, global warming and “tropicalisation of the European climate”, associated with high temperatures and humidity, may contribute to the creation of optimal conditions in Europe for the spread of tropical species, such as *A. duodenale*, *A. braziliense* and *A. caninum*. In terms of climate conditions, the *Ancylostoma* spp. species prefer for their development warmer environments than the environments preferred by *Uncinaria stenocephala* (Okulewicz, 2017).

METHODS

In the presented research, 103 faecal samples were examined in order to diagnose hookworms in dogs from shelters, dogs kept in households, hunting dogs, as well as foxes. The basic coprological examination was carried out by applying the flotation method with the aim of confirming the presence of eggs of the Ancylostomatidae family. Four foxes were subjected to a parasitological dissection of their gastrointestinal tract; in one of them, 5 adult individuals of *Uncinaria stenocephala* were detected and subsequently confirmed based on the coproculture and PCR diagnostics. Two positive samples (faeces of a lactating female and faeces from a fox in which eggs of the Ancylostomatidae family were confirmed) were used to create coprocultures for the subsequent identification of larvae of this family. Larvae of various stages were morphologically identified using the available key published in an article by Gibbs et al., 1961. The coproculture is a method that is used for cultivating and subsequent identification of rhabditiform and filariform larvae of *Uncinaria stenocephala*. The definitive identification of the species was carried out using a one-step nested PCR that analyses the gene encoding cytochrome c oxidase subunit I (cox1).

RESULTS AND DISCUSSION

Global climate changes are currently evident all over the world and, apparently, its pace is rising. The complicating factors include unfavourable weather conditions, rising sea level and wild fires caused by draught. Throughout their life cycle, parasites depend on the outdoor

environment and such changes will therefore affect their chances for survival, the dynamics of their populations, the enlargement of the transition areas or an increase in their pathogenicity, as well as the disturbance of synchronisation between a host and a parasite (Petney et al., 2021). Our research has confirmed a 17% prevalence of species of the Ancylostomatidae family in the set of examined animals. In 2022, Šmigová et al. confirmed a lower percentage—4.3%. In Spain, the 2020 prevalence of species of the Ancylostomatidae family in a group of stray dogs was 35.6% (Cerrilo et al., 2020). A Polish study confirmed that the occurrence of the Ancylostomatidae family is only a half of the figure observed in Spain, i.e. 15.4% (Karamon et al., 2018).

In order to confirm the *Uncinaria stenocephala* species in the examined animals, we applied, in addition to the flotation method, also the coproculture method for the diagnostics and morphometric identification of larvae, as well as dissection of their intestines and the PCR method, by which we detected the *U. stenocephala* species from the eggs and adults found during the dissection of foxes. The first more detailed study was conducted in Slovakia in 2022 by Štrkolcová et al.; the study confirmed the *U. stenocephala* species in dogs in 9.6%. In a Danish study conducted by Al-Sabi et al. in 2013, the presence of cardiac, pulmonary and intestinal parasites based on a post – mortem analysis in foxes was analysed in two regions in Copenhagen and in Southern Jutland. These authors have specifically identified the species and confirmed the prevalence of *Uncinaria stenocephala* in 84.3% of foxes in Copenhagen and in 60.4% of foxes in Southern Jutland. However, the epidemiological importance of *U. stenocephala* has not been completely clarified due to insufficient amount of available publications. As a result, further molecular studies are required to shed light on the potential zoonotic potential of *U. stenocephala* (Štrkolcová et al., 2022).

With regard to the occurrence of ancylostomiasis caused by zoonotic species in Europe, several cases of autochthonous disease *Larva Migrans Cutanea* (CLM) have been reported. In Poland, patients were diagnosed after they returned from Thailand and Madagascar; however, in Southern Europe (Italy, Spain, France, Great Britain and Germany), over 20 cases of CLM have been reported for the last 25 years. In many cases of the CLM syndrome in humans in Europe, concrete species have not been identified (Garcia-Rodrigo et al., 2017; Del Guicida et al., 2019). In Europe, those cases mostly include parasitary skin diseases brought from tropical and overseas countries. Nevertheless, such observations may change our attitude since we regard them as typical tropical diseases with parasites located exclusively in tropical regions. Such an increase in CLM cases in Europe may be attributed to global warming; however, due to a low

number of cases reported per year, a definite stance may hardly be provided (Del Giudice et al., 2019).

CONCLUSION

At present, spreading of parasites is mainly facilitated by global warming. It may affect the spread of parasites to new regions beyond their original geographies, their adaptation to new hosts and modification of their developmental cycles. Such changes may also affect the occurrence of newly emerging species of hookworms in Europe, including Slovakia, which fact is of crucial importance for the control of global burden of parasitic diseases.

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CHANGES OF PARAMETERS OF SURFACE WATER BY POLLUTION BY AGRICULTURAL ACTIVITIES

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ABSTRACT

The objective of this study was to monitor the changes of parameters of surface water (rivers) in the area Košice basin, eastern Slovakia with the aim to assess contamination of water by potential sources in this area. Samples for physico-chemical and microbiological examination were collected at 6 sampling points from 2014 up to March 2015, covering all four seasons. Surface water in the area close to both farms was polluted with organic substances (COD_{Mn}), however they did not exceed the limit set for surface water. At many samplings we detected in surface water presence of total coliforms, *E. coli* and occasionally also faecal enterococci indicating faecal pollution that could eventually affect ground water in individual wells. Our investigations showed that protection zones of water sources were not always sufficient. There were considerable variations in the quality of surface water during the year but no clear relationship between microbial contamination and seasons was observed.

INTRODUCTION

Different uses of water affect both the quality and the quantity of the water available and the management of water pollution and water resources play an important role at both national and international level. Availability of good quality surface water is affected also by global climate changes that cause shortages and overexploitation in some places and flooding in other places with all related consequences including decreased safety of food and potential disease transfer. Anthropogenic pressure on the environment leads to decrease in water quality but there is some limit which cannot be exceeded or else global ecological balance will be disturbed. There are many man-made pollutants that can contaminate surface water, such as leaching of nitrates and pesticides into surface water as a result of rainfall, soil infiltration and surface run off from

agricultural land. Such sources cause considerable variations in the contaminant load of water over time (Adams and Foster, 1992; Fawell and Nieuwenhuijsen, 2003).

The main source of organic pollution of rivers is the organic matter derived from diverse human activities. This involves domestic and industrial sewage, wastes from agriculture and animal production, food processing facilities and other. Many toxic organic compounds are non-biodegradable, or are degraded slowly, so they persist in the ecosystem, some affect even kill fish and other aquatic organisms; some are nuisances, giving water and fish an offensive taste or odour. Acidification of inland waters by acidifying compounds of sulphur and nitrogen affects quality of water and causes damage to aquatic ecosystems, especially to fish. Freshwater eutrophication is another worldwide problem. Eutrophication (excessive growth of phytoplankton and filamentous algae resulting in increased turbidity, production of toxins, diurnal changes in dissolved oxygen) is caused by enrichment of water with nitrogen and phosphorus. Phosphorus emissions arise predominantly from domestic and industrial effluents, but the share of agriculture is not insignificant. Rivers are recipient for rain water from relevant catchment areas but also of wastewater (treated and untreated) and infiltration from landfills. Removal of some pollutants is very difficult and expensive, therefore prevention of such pollution is preferred. Partial solution of this problem is based on zones of protection of water sources (Act No. 29/2005 Coll.; Act No. 322/2003 Coll.). In Slovakia, the Act No. 29/2005 Coll. on hygiene protection zones of water sources specifies the above mentioned zones of protection as zones of the Ist, IInd and IIIrd degrees. In the Ist degree zone all activities not related to the operation of water source are banned. Only authorised individuals are allowed to enter this zone. Less strict bans on activities apply to the zones of IInd and IIIrd degree.

Water may be polluted by various pathogens – bacteria, viruses, protozoa and helminths. The spectrum of pathogenic and potentially pathogenic micro-organisms spread by water is extensive. The most frequent are the causative agents of intestinal diseases (typhoid, paratyphoid, salmonellosis, tuberculosis, brucellosis, tularaemia, leptospirosis, cholera, amoebic dysentery).

The aim of the study was to monitor changes in the quality of surface water (rivers) in the area flowing close to animal farms and villages oriented on agricultural production with the aim to identify potential sources of its contamination.

MATERIALS AND METHODS

Monitoring of quality of surface water was focused on determination of physico-chemical parameters and bacterial counts indicating quality and potential pollution of water sources. The

quality of the investigated surface water was evaluated on the basis of the Slovak Republic Government Regulation (No. 269/2010 Coll.). Chemical examination of surface water included determination of pH, electrical conductivity, dissolved oxygen, chemical oxygen demand (COD_{Mn}), chlorides, nitrates, iron and phosphates.

The pH was determined according to STN ISO 10523 by means of a pH-meter HACH and a WATERPROF pH Tester 30. Conductivity was determined by a conductometer WTW InoLab Cond 720 (Germany). Dissolved oxygen was determined electrochemically using an oxygen probe LDO HQ Series Portable Meters supplied by HACH and chemical oxygen demand by oxidation with KMnO_4 according to STN EN ISO 8467. Determination of Ca^{2+} and Mg^{2+} was carried out by titration according to Horakova et al. (2003), chlorides were determined by titration according to STN ISO 9297 by titration and nitrates with ion-selective nitrate electrode WTW (InoLab pH/ION 735P, Germany). Iron was determined by powder HACH Method 8025 Color True at 465 nm. Orthophosphates were determined colorimetrically using HACH DR 2800 analyser and a procedure recommended by HACH.

We determined colony forming units (CFU) of bacteria cultivated at 22°C (BC22) and 37°C (BC37) (heterotrophic count) according to STN EN ISO 6222. Coliform bacteria (CB) and *E. coli* were cultivated according to STN EN ISO 9308-1 using Endo agar (HiMedia, India) and incubation for 24 hours at 37°C and 43°C, respectively, and the characteristic colonies were counted. Determination of counts of faecal enterococci (FE) was carried out according to STN EN ISO 7899-2. All samples were examined in duplicate.

RESULTS AND DISCUSSION

Results of physico-chemical examination are presented in Tables 1 and microbiological in Table 2.

Our monitoring showed that levels of all parameters determined in samples of surface water were below the limits specified by relevant regulation in all seasons except for concentrations of N-NO_3^- - which exceeded legislative limits at all sites. Chemical oxygen demand (COD) is an important water quality parameter. Higher COD levels in surface water mean a greater amount of oxidisable organic material, which will reduce dissolved oxygen (DO) levels. A reduction in DO can lead to anaerobic conditions, which is deleterious to higher aquatic life forms.

Table 1 Results of physico-chemical examination of surface water

Chemical parameters	Legislative limits	Min – Max
pH	6.0 - 8.5	6.2 - 8.0
TDS (105°C)	1000 mg/l	98.6-544.3 mg/l
TDS (550°C)	640 mg/l	2.2-166.9 mg/l
Conductivity	110 mS/m	21.5-106.9 mS/m
Dissolved oxygen	> 5mg/l	6.4-8.8 mg/l
COD _{Mn}	15 mg/l	2.2-15 mg/l
Chlorides (Cl ⁻)	200 mg/l	< 28 mg/l
Nitrate nitrogen (N-NO ₃ ⁻)	5 mg/l	3.16-9.03 mg/l
Iron (Fe)	2 mg/l	< 1.6 mg/l
Phosphates (PO ₄ ³⁻)	1 mg/l	< 0.3 mg/l

TDS – dissolved solids; COD_{Mn} – chemical oxygen demand

Runoff is the key mechanism of pathogen transport to surface waters. During a rain event, the partitioning of flow between surface runoff and infiltration through the soil depends upon a number of factors. Storm intensity and duration, soil hydraulic characteristics (e.g., permeability, antecedent moisture and temperature), land slope, and soil cover have all been shown to influence runoff and therefore pathogen transport (USEPA. 2000). If rainfall intensity exceeds the capacity of the soil to infiltrate water, overland flow occurs, and microorganisms can be carried rapidly in surface runoff (Tyrrel and Quinton, 2003).

To be available for transport in runoff, pathogens are released from the manure, most of them remain associated with the faecal deposit (Fridrich et al., 2014; Rosen, 2000).

Table 2 Average results of microbiological examination of surface water

Season	Samples	E.coli	CB	FE	BC22	BC37
		Legislative limits according SR Government Regulations No. 296/2010				
		20 CFU/1ml	100 CFU/1ml	10 CFU/1ml	5000 CFU/1ml	CFU/1ml
Spring	2a	92	>300	0	>300	95
	2b	45	58	0	206	117
	2c	6	57	1	110	100
	2d	0	32	0	90	70
	2e	100	>300	12	>300	>300
	2f	12	89	13	208	121
Summer	2a	67	>300	0	168	65
	2b	60	74	45	0	>300
	2c	28	108	8	0	200
	2d	7	9	7	0	136
	2e	72	80	11	0	>300
	2f	29	61	19	0	280
Autumn	2a	88	98	0	99	150
	2b	110	136	0	≥300	>300
	2c	15	87	0	0	150
	2d	180	200	0	220	>300
	2e	170	210	0	220	>300
	2f	18	78	5	12	65
Winter	2a	96	160	0	>300	56
	2b	415	115	0	>300	>300
	2c	35	150	2	0	229
	2d	0	90	0	0	>300
	2e	10	210	0	0	>300
	2f	5	99	1	0	225

CB – coliform bacteria (total coliforms); FE – faecal enterococci; BC22 – bacteria cultivated at 22°C; BC37 – bacteria cultivated at 37°C; CFU – colony forming units

For surface water there is a limit only for KM 22 (5000 CFU/1 ml), which was not exceeded in any sample. The limit set for CB in surface water was exceeded several times in all seasons at all sampling sites but most frequently in autumn and winter that can be related partially to application of manure. The limit set for CB in surface water was exceeded several times in all seasons at all sampling sites but most frequently in autumn and winter that can be related partially to application of manure. In the same sample there were detected also *E.coli* colonies (5-415). Determination of *E.coli* and faecal enterococci (FE) indicated some faecal contamination of surface water practically at all sampling sites. There were considerable variations with regard to seasons (Fridrich et al., 2014).

CONCLUSION

Our examinations showed relatively good quality of surface water with respect to determined physico-chemical parameters, even at sampling points where some pollution from point sources was expected. Some faecal contamination of surface water was detected practically at all sampling sites. Importance of water protection zones was confirmed also by our results as surface water exposed to the lowest potential contamination showed best microbial quality.

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A RETROSPECTIVE OVERVIEW OF MOSQUITOES OCCURRENCE IN SLOVAKIA IN THE PERIOD OF CLIMATE CHANGE

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ABSTRACT

Mosquitoes are one of the most important vectors and temporary ectoparasites of humans and animals. In some countries they are found in such abundance that they represent a serious health, economic and social problem. A variety of environmental factors influence directly or indirectly the distribution and occurrence of both mosquitoes and pathogens. One such key factor that influences the dynamics of pathogen transmission by mosquitoes is ambient temperature. It directly influences the development and survival of pathogens in mosquitoes, animals, humans and reservoir hosts. It also influences mosquito activity (number of host bites and capacity). In addition, temperature can determine mosquito survival and distribution (including the seasonal diapauses of invasive species in temperate regions, which is necessary for their establishment in such climates), abundance (hatching rates and adult mortality) and seasonal range. Precipitation also positively influences mosquito distribution and abundance during the season. Current and future climate changes are risk factors for the spread not only of mosquitoes but also of other dipterans vectors and the pathogens they transmit. It is therefore essential to identify and monitor these factors that could act as predictors of the risk of mosquito spread as well as pathogen transmission. These predictions are key to the development of strategies to reduce the spread of new or emerging and re-emerging diseases. For these reasons, the aim of this paper is to present a past and current overview of mosquito prevalence in Slovakia and to indicate factors that may potentially influence the risk of spread of mosquito-borne pathogens in Slovakia.

INTRODUCTION

Research on mosquitoes in Slovakia has a long tradition of more than two hundred years (Rumi, 1807). The species composition of Slovak mosquitoes was compiled comprehensively by Országh and Okály (1978). Later, in the first half of the 20th century, mosquitoes of the genus *Anopheles* were mainly studied in connection with the endemic occurrence of malaria in eastern

Slovakia (Makar and Mihalyi, 1943; Korbel, 1952; Kunst and Novák, 1956). An important extension of knowledge about the mosquitoes of Slovakia was contributed by Kramář (Kramář et al., 1952), who described 17 species of mosquitoes. In the 1960s, the most important works for the knowledge of Slovak mosquitoes were those describing the occurrence of mosquitoes in the Danube Lowland, where 28 species of mosquitoes were found (Trpiš, 1962). In the 1970s, 37 species of mosquitoes were described in eastern Slovakia and 11 species in the Tatra Mountains (Trpiš, 1960; 1965). In the early 1980s, mosquito research was taken up by Labuda and his team (1983), and later in the 1990s and early 21st century, the mosquito fauna was monitored by several scientific teams (e.g. Halgoša and Petrus, 1997; Jalili et al., 1999; Országh et al., 2001; Országh et al., 2006; Minář et al., 2007; Bocková et al., 2013; Bocková and Kočišová, 2016). The occurrence and spread of mosquitoes and the pathogens they transmit has been significantly affected in recent decades by changes in climatic conditions, which are mainly manifested by a warming environment, closely linked to changes in hydrological conditions (rainfall and variability of rainfall, air humidity, evaporation, soil moisture, etc.). Extreme weather fluctuations, such as the occurrence and spread of mosquitoes and their pathogens, are also a major factor in the spread of mosquitoes in the past few decades. Intense rainfall, frequent flooding, early onset of spring, hot or tropical days in summer, long warm autumn and late onset of winter, result in the creation of suitable conditions for the mass breeding of mosquitoes, the invasion of new species and, consequently, the occurrence of diseases atypical of the Central European climate zone. In the last 10 years, species have been introduced into Europe from East and South-East Asia (*Aedes aegypti*, *Ae. albopictus*, *Ae. j. japonicus* and *Ae. koreicus*), but also from America (*Ochlerotatus atropalpus* and *Oc. triseriatus*). Until 4 years ago, 49 species of mosquitoes belonging to 6 genera were known in Slovakia; currently, the mosquito fauna of Slovakia has been expanded by the invasive species *Ae. koreicus* (Oboňa et al 2020) and *Ae. j. japonicus* (Čabanová et al., 2021).

MATERIAL AND METHOD

Our research team conducted mosquito trapping within eastern and south-western Slovakia. In 2019-2022, we have made 42 traps (2021 pcs). For the purpose of determining the species composition and abundance of mosquitoes, we divided the sites into urban (Košice - recreation centre Anička, Botanical Garden, Zoo, Riding school UVMP in Košice) and suburban (Čaña, Čermel', Nižný Klátov, Ružín, Krompachy, Jasová-district Nové Zámky). Mosquitoes were captured using an entomological net by sliding it in the air during mosquito flight, in the early evening, especially around water source (Ružín, Čaña, and Krompachy). We transferred the

specimens caught in this way into plastic containers with a small amount of paper cotton wool moistened with ethyl acetate. At the sites in Košice, Nižný Klátov and Jasová, we trapped adult mosquitoes with CDC Miniature Light Trap type traps, using CO₂ attractant compressed in a metal bomb with gradual release during the night hours (approximately from 18:00 to 8:00). The trapped mosquitoes were transported to the laboratory with a net placed in a PVC box, where they were transferred into plastic containers and stored in a freezer box, at -18 °C. Female mosquitoes were diagnosed under a stereo microscope (ZEISS-STEMI DV4). Identification of some morphologically difficult to distinguish mosquito species was done by PCR, for which we selected a section of the mitochondrial cytochrome c oxidase subunit I (CO1) gene and used specific primers for its further amplification. Visualization, purification and sequencing of PCR products were performed on electrophoresis using 1% agarose gel stained with GoodView™ Nucleic Acid Stain and the visualization itself is displayed using a long-wavelength UV lamp, where amplification bands are illuminated upon a positive reaction. Positive PCR products were sent for purification and sequencing to SEQme's dedicated laboratory in the Czech Republic. All sequences were edited in GeneTool for comparison with known sequences using BLAST-analysis in the NCBI database.

RESULT AND DISCUSSION

We trapped a total of 2,021 mosquitoes of 17 species (*Anopheles maculipennis* s.l., *An. plumbeus*, *An. atroparvus*, *Culex pipiens* complex, *Cx. torrentium*, *Cx. modestus*, *Aedes annulipes*, *Ae. cantans*, *Ae. caspius*, *Ae. cataphyla*, *Ae. cinereus*, *Ae. excrucians*, *Ae. geniculatus*, *Ae. punctor*, *Ae. sticticus*, *Ae. vexans*, *Culiseta anulata*). The highest prevalence was recorded for *Culex pipiens* complex (43.5%) and *Aedes vexans* (21.5%) in the recreational areas of Ružín, Čaňa and Sereď. *Ae. excrucians* (9.5%) was the most frequently caught in Jasová (Nové Zámky). The number of mosquitoes in the nets was significantly affected by unfavourable climatic conditions, especially high temperatures during the summer season and low humidity, when many breeding sites dried up. For example, in 2022, the mean annual temperature in Košice was 11 °C, with a seasonal mean monthly temperature of 21.9 °C in June (deviation from normal of 2.5 °C), 22.4 °C in July (deviation from normal of 1.8 °C) and 23.5 °C in August (deviation from normal of 2.8 °C) (SHMÚ 2023). Cumulative atmospheric precipitation in the summer months of 2022 was below the long-term normal, ranging from 55 mm in August to 70 mm in June. Compared to the so-called pre-Covidian period, when we trapped 63,434 mosquitoes, 22 species in localities of eastern Slovakia, which is almost 97

times more than in last 4 year seasons. Seasonal changes in adult population dynamics reflect the different climatic conditions of local sites (Bocková and Kočíšová, 2016). In recent years, we have witnessed a linear increase in human and animal diseases whose causative agents are transmitted by insect vectors. This situation is attributed to a number of biotic, abiotic and anthropogenic factors, which have led to the introduction of new invasive mosquito species into Slovakia. This raises the question of how suitable Slovakia's habitat is for the occurrence of such vectors. As mentioned in the introduction, the occurrence of invasive mosquito species has been confirmed in Slovakia. In 2020 *Aedes koreicus* in the city of Prešov (Oboňa et al., 2019). This mosquito is native to Korea, Japan, northern and southern China (Hohmeister et al., 2021). In Europe, it has so far been reported in certain areas in Belgium (2008), Italy (2011), the Swiss-Italian border and Slovenia and European Russia (2013), southern (2015) and central Germany (2016), Hungary (2016), Austria (2018) (Ganushkina et al., 2016; Negri et al., 2021) and the Netherlands (2022) (Teekema et al., 2022). A year later, *Ae. japonicus japonicus* was also confirmed to occur at three locations across Slovakia (Čabanová et al., 2021). *Ae. japonicus* is a complex of four morphologically similar subspecies, but only *Ae. j. japonicus* has spread from its native range of Japan, Korea, south-eastern Siberia and China, including Hong Kong and Taiwan (Kampen and Werner, 2014). In Europe, this subspecies has so far been found in Belgium (2002), Switzerland and Germany (2008), Slovenia and Austria (2011), Hungary and the Netherlands (2012), Croatia and France (2013), Italy and Liechtenstein (2015), Bosnia and Herzegovina and Serbia (2017), Spain and Luxembourg (2018), and Romania (Horváth et al., 2021; Janssen et al., 2020; Koban et al., 2019; Smits et al., 2021). These two invasive Asian mosquitoes can tolerate colder temperatures quite well and should therefore be expected to spread further. Compared to the species *Ae. albopictus*, which is native to tropical south-east Asia, this mosquito has adapted well to the climatic conditions of the warmer, more southerly areas of Europe. The occurrence of 4 females of *Ae. albopictus* were recorded in eastern Slovakia (Bocková et al., 2013), but no larvae were found and in subsequent years no adults were found. The limiting factor for the survival of this mosquito is winter temperatures. The egg survival limit of the European population of *Ae. albopictus* during the winter diapause is the average monthly temperature of the winter months of +1.0 °C; sub-zero temperatures are unsuitable for survival.

CONCLUSION

The emergence and spread of invasive mosquito species is becoming a serious public health problem in Europe. Given the ability of the Asian invasive species *Ae. koreicus* and *Ae. j.*

japonicus to adapt to European climate change we expect their spread to continue in Slovakia. At the same time, we can also expect the spread of *Ae. albopictus* further into Central Europe. It is for these reasons that continuous xenomonitoring of both mosquitoes and the potential hosts.

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AVIAN MIGRATION AND SPREAD OF INFECTIOUS DISEASES

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ABSTRACT

Disease is an important factor in the evolution of avian migration. Research suggests that pathogen densities and disease may influence the evolution of migratory behaviour. Migratory birds are important to public health because they carry emerging zoonotic pathogens, either as a reservoir host or by dispersing infected arthropod vectors. In addition, bird migration provides a mechanism for the establishment of new endemic foci of disease at great distances from where an infection was acquired. Understanding the biology and conducting effective conservation of migratory species requires knowledge of migratory connectivity, epizootiology, the geographic linkage of individuals, or populations between phases of the annual cycle.

INTRODUCTION

Several pathogens are associated with avian migrants, including viruses, bacteria, fungi and protozoa. Migratory birds can move over large distances in short time and have the potential to act as an extraordinary vector for certain pathogens. As a consequence, migratory birds can be responsible for the emergence of pathogens in new areas, some of which is zoonoses and act as a threat to human health. Migratory birds are known to be a problem to poultry farming, contributing to transmission and spread of infectious diseases affecting poultry. In some occasions, infectious diseases have led to significant decline in populations of the migratory birds itself. The combination between the highly energetic costs of migratory flight and infectious diseases is unfavourable and can have severe consequences, even in cases of mild or subclinical infections.

Bird migration and dispersion of diseases

During migration, a large number of birds gathers at breeding, wintering and stopover locations. This induces both intraspecies and interspecies transmission of pathogens between birds and other animals. Interspecies transmission is especially important and problematic when it occurs

between wild and domestic birds (Dhama et al., 2008). Avian influenza (AI) is one of the most problematic disease transmitted by migratory birds. It can have great impact on domestic birds, and in many cases cause flock mortality. Free-range poultry is especially vulnerable for infections brought by migratory birds. Domestic free-range ducks are likely to attract other ducks of the same kind, while chicken and turkey are most likely to get in contact with granivorous birds. Avian influenza viruses is transmitted in water. Thus, migratory birds with stopovers in wetlands or other aquatic environments are particularly susceptible (Jourdain et al., 2007). Migratory birds mainly carry Low pathogenic AI, which has the potential to mutate into High pathogenic AI (HPAI) after transmission between wild birds and poultry. Migratory birds can carry both LPAI and HPAI, and as a result can cause severe outbreaks in domestic poultry worldwide. Huge economical losses are associated with AI in poultry, and H5N1 acts as a threat to human health (Dhama et al., 2008).

Many arboviruses are also suspected to be spread by migratory birds. It is documented that birds can carry ticks. Hence, migrants have the potential to carry infected ticks over large areas and thus spread tick-borne infectious diseases such as TBE and Lyme disease (Waldenström et al., 2007).

Mosquito-borne arboviruses, including West Nile virus (WNV) and Usutu virus (USUV), can also be spread by migratory birds. After the induction of an infection by a mosquito, the viremic titers of birds will be temporarily maintained high. It allows the transmission of viruses from infected birds to a new ornithophilic mosquito. Thus, the potential of spread of WNV and USUV depends on the time these viremic titres remains high (Colpitts, 2016).

Avian pox, another emerging disease, was first detected in central Europe in 2005 in great tits. The virus was first introduced into Austria and Hungary, and later also observed in Czech Republic, Slovakia and Germany, with the majority of cases during winter (Literák et al., 2010).

Pathogens impact on migratory birds

Migratory birds can carry pathogens which can be transmitted between individuals at wintering and breeding grounds, but also at stopover places. Severe infections can develop, which can lead to mortality and in some cases decline of populations. Another consequence of infectious diseases in migratory birds, is its impact on the migratory flight (Jourdain et al., 2007).

The combination between infectious diseases and the energetic cost of migration often results in migratory culling. Migratory culling is the removal of infected individuals from a flock of migratory birds due to delayed onset of migration or failure to complete the flight. Typically, bird migration is associated with enhancing pathogen spread. However, migratory culling is an

example on how bird migration can lower the infection risk by removing the infected individuals from a population (Satterfield et al., 2018).

Although migration has the potential to act as a method to reduce the circulation of pathogens by migratory culling, there are examples of events where infectious diseases has led to a significant decline in populations of migratory birds. The epidemics of trichomonosis in 2006 strongly affected wild birds in Britain. During the epidemics, the population of greenfinches and chaffinches declined significantly. Greenfinches were highest affected, resulting in a 36% decline of the breeding populations due to trichomonosis in the regions of greatest incidence. A 21% decline was identified in the breeding population of Chaffinches (Newton, 2013).

Newcastle Disease in double-crested cormorants in North America has served for another significant decrease in population numbers. Double-crested cormorants are medium distance migratory birds of North-America, and the only known species in which ND has caused a large-scale mortality (Kuiken, 1999).

Other infectious diseases cause sporadic infections within populations of migratory birds, such as avian pox and salmonellosis. The great tit, which spread avian pox to central Europe, were infected at the breeding grounds in Scandinavia before migrating south. Avian pox is known to be more aggressive in great tits compared to other species. Normally, the disease tends to cause mild infections and rarely death. The most common consequences are increased mortality and reduced ability to raise young. Thus, a slight decrease in the population number can occur (Newton, 2013). Salmonellosis is another, more common source of sporadic mortality in wild birds (Thomas et al., 2007).

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BATS AS BIOLOGICAL DRIVERS OF PATHOGEN SPREAD IN A CHANGING ENVIRONMENT

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ABSTRACT

Bats are of immense epidemiological importance. In recent years, increased attention has been paid to their reservoir function in viral and bacterial diseases. The order Chiroptera is characterized by a wide range of morphological, ecological and behavioural features. Bats are suitable reservoir species for an ever-increasing number of infectious disease agents.

INTRODUCTION

Bats represent a host group for many zoonotic and potentially zoonotic pathogens. Environmental changes induced by human activities have resulted in increasing rates of exposure of bats to pathogens. And this is likely to increase the intensity and potential for the spread of infectious pathogens in the future. Ecological drivers of the spread and emergence of pathogens in new hosts, including humans, involve a complex mixture of processes, and understanding these complex factors can aid in predicting spread. In particular, only once the ecology of the pathogen and host is known will it be possible to fully evaluate the impact of anthropogenic changes. In order to comprehensively understand the interplay between host and pathogen ecology, interdisciplinary approaches are needed. In the group of reservoirs of pathogens with sylvatic circulation, bats occupy a specific place. With their unique and diverse life biology, their ability to fly, their often highly gregarious social structure, their long lifespan and low fecundity, bats are destined to be an important link in epizootic processes.

General biology of bats

Bats belong to the class Mammalia. They form a separate taxonomic order Chiroptera, which is subsequently subdivided into 2 suborders, Yangochiroptera and Yinpterochiroptera (Tsagkogeorga et al., 2013). Bats account for approximately 20% of all recent mammalian

species (Lei and Dong, 2016). There are currently over 1,400 species of bats (Welch and Leppanen, 2017).

Cosmopolitan bats of the suborder Yangochiroptera are generally characterized by smaller physical size, weaker eyesight, and a specific anatomical structure of the larynx. Due to the anatomy of the larynx, they are able to emit ultrasonic echolocation signals for the purpose of spatial orientation and foraging (Springer, 2013).

The suborder Yinpterochiroptera comprises the family Pteropodidae, it is a herbiferous squid distributed in the tropical climates of the American, Australian and Asian continents. Calones are characterized by excellent eyesight at a level comparable to that of primates. With the exception of three species of the genus *Rousettus*, fruit bats have not evolved the ability to echolocate during evolution (Springer, 2013).

In contrast to bats possessing a laryngeal-type biosonar, fruit bats of the genus *Rousettus* generate echolocation pulses using the tongue (Springer, 2013). In addition to fruit bats, bats of the families Rhinolophidae, Hipposideridae, Megadermatidae and Rhinopomatidae have been placed in the suborder Yinpterochiroptera based on molecular genetic studies (Tsagkogeorga et al., 2013).

The average lifespan of bats is approximately 3.5 times longer compared to other mammals of similar size. Females of most species give birth to a maximum of 1 young per year (Wilkinson and South, 2002).

In direct correlation with the diverse species spectrum of bats, their trophic specialization is also diverse. Bats consume insects, plant fruits, other small vertebrate species, flowers and pollen. A total of 3 species of haematophagous vampire bats in the family Phyllostomatidae feed on the blood of animals and humans (Kunz, 1982).

Bats as pathogen reservoirs

More than 200 viruses are associated with bats. Almost all of them belong to RNA viruses, probably due to their great ability to adapt to changing environmental conditions and bat macro-organism through higher genetic variability (Hayman et al., 2013; Moratelli and Calisher, 2015). Bats are considered natural reservoirs of a wide range of zoonotic microorganisms, especially viruses. These viruses include various species of the genus *Lyssavirus*, *Henipavirus*, *Coronavirus*, and *Filovirus* (Calisher et al., 2006; Omatsu et al., 2007).

Although many of the emerging infectious diseases associated with bats are viral in origin, bacterial pathogens also play a role in circulating in bat populations (Mühldorfer, 2013). Bats are susceptible to several infectious agents causing infections in humans and domesticated

animal species, such as enteric pathogens (*Salmonella typhimurium*, *S. typhi*, *S. enteritidis*, *Shigella*, *Yersinia*, and *Campylobacter* spp.) (Mühldorfer et al., 2011). Bats are also susceptible to vector-borne bacterial pathogens of *Bartonella*, *Borrelia* spp. and *Rickettsia* species (Corduneanu et al., 2018). There are also a number of studies confirming the circulation of several species of the genus *Leptospira* in bats (Dietrich et al., 2015).

In addition to viruses and bacteria, pathogenic fungi also find application in the pathogenesis of infectious diseases in wild animals, including bats. The fungus *Pseudogymnoascus destructans* causing white-nose syndrome has had a devastating effect on bats in North America. The fungus *Histoplasma capsulatum*, occurring in association with guano in caves and other roosts of bat colonies, also has zoonotic potential (Holz et al., 2018).

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PHARMACEUTICAL WASTE IN THE ENVIRONMENT AND ITS IMPACT ON HUMAN AND ANIMAL HEALTH

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ABSTRACT

"One Health" is a concept of international multidisciplinary collaboration that brings together people, animals, and the environment with the aim of maintaining and improving the health of all beings on the planet. This approach emphasizes the interdependence of humans, animals, and the environment, and its main idea is that a healthy world is one in which healthy people and healthy animals live in a healthy environment.

INTRODUCTION

In recent years, a combination of human activities, ecological damage, and socio-economic factors, including illegal hunting, wildlife farming, and illegal trade, have contributed to the direct interaction between humans and wildlife (Wu et al., 2022). The most significant factor in the rise of infectious diseases caused by wildlife is changes in land use. These changes affect the behavior, distribution, and abundance of disease vectors, thereby increasing the potential for disease transmission (Fang and Song, 2021). Agriculture, deforestation, and urbanization have altered the structure and function of ecosystems, species distribution, and biodiversity (Li et al., 2022).

With the development of international trade, legal and illegal trade of wildlife has also increased, diversifying the pathways of contact between humans and wildlife. Stressed animals excrete more viruses and are more susceptible to infections. Even seemingly healthy wildlife can harbor pathogens that cannot be eliminated through meat cooking (Fang and Song, 2021). Livestock diseases can threaten animal product supplies, livelihoods, and people's economy.

Antimicrobial-resistant bacteria spread through communities, food, healthcare facilities, and environments such as soil or water (Centers for Disease Control and Prevention, 2021).

Drugs in the environment

Medications used by humans and animals often find their way into the environment, particularly into rivers, lakes, and even drinking water. They are most commonly introduced into the environment through human and animal urine and feces, as well as through the disposal of medications in sinks and toilets. In both cases, the pharmaceuticals end up in wastewater treatment plants (WWTPs), which are not designed to remove these substances from wastewater. As a result, pharmaceuticals and their metabolites can penetrate surface water and subsequently contaminate groundwater and other components of the environment. A significant portion of active ingredients also enters the environment through the pharmaceutical industry and improper medication disposal, such as throwing medications into the trash (Lyons, 2014). In addition to the transfer of pharmaceuticals into ecosystems through WWTP effluent, the use of reclaimed water from WWTPs for agricultural irrigation can contribute to their transfer. When growing crops or raising livestock for meat using such water, pharmaceuticals can be transported into the food chain. For example, triclosan was found to accumulate in the roots of lettuce, spinach, black pepper, and cucumbers through this route (Snopková et al., 2021).

Environmental studies have demonstrated the presence of a large number of pharmaceuticals in the water system, including groundwater, surface water, drinking water, and even soil. These include: Antibiotics (such as clarithromycin, erythromycin, roxithromycin, lincomycin, sulfamethoxazole, trimethoprim, sulfadimethoxine, sulfamethazine, sulfathiazole, ciprofloxacin, norfloxacin, chloramphenicol, tetracyclines), Antifungals and antivirals (such as miconazole, fluconazole, acyclovir, penciclovir, lamivudine, oseltamivir), Analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) (such as diclofenac, ibuprofen, naproxen, ketoprofen, phenazone, salicylic acid, paracetamol), Antiepileptics (such as carbamazepine, primidone), Antidepressants (such as fluoxetine, olanzapine, paroxetine), Beta-blockers (such as metoprolol, propranolol, betaxolol, bisoprolol, nadolol, atenolol), Diuretics (such as furosemide, hydrochlorothiazide) and ACE inhibitors (such as enalapril, captopril), Blood lipid regulators (such as simvastatin, bezafibrate, clofibrate acid), Oral contraceptives: estrogens (such as 17 α -ethinylestradiol, mestranol), Cytostatics, contrast agents, and antiemetic domperidone, Warfarin and antidiabetic drugs (such as metformin, glibenclamide), Omeprazole and H₂ antagonists (such as cimetidine, famotidine), Bronchodilators and sympathomimetics (such as ephedrine, pseudoephedrine), Narcotics and psychotropic substances (such as morphine, codeine, methadone, cocaine, 6-acetylmorphine, amphetamine, methamphetamine,

11-nor-9-carboxy- Δ -9-tetrahydrocannabinol, bromazepam, diazepam, lorazepam, lormetazepam, pentobarbital, etc.).

The occurrence of all the mentioned pharmaceuticals has been found in countries such as Germany, Austria, Italy, Spain, Greece, Croatia, Denmark, the United Kingdom, the Netherlands, Switzerland, China, Brazil, the United States, Canada, Mexico, South Africa, Iraq, Saudi Arabia, India, Belgium, France, Portugal, Poland, and Romania (Toma and Crisan, 2018).

Impact of pharmaceutical waste on human and animal health

Endocrine disruptors and hormones can interfere with sexual development in humans as they are highly active compounds that interact with hormonal systems. They can be found in the air, food, water, as well as cosmetics, cleaning products, medical materials, and devices. Persistent exposure to these chemicals can amplify or even become the main cause of diseases such as type 2 diabetes, obesity, cardiovascular diseases, and certain types of cancer. Endocrine disruptors include well-known phthalates, polychlorinated biphenyls, diphenyl ethers, as well as estrogens and triclosan, which can be found in toothpaste, cosmetics, and cleaning products. Estrogen is not only a reproductive hormone but also affects almost all tissues in the body and is associated with the development of diseases such as cancer, endometriosis, obesity, insulin resistance, as well as cardiovascular, autoimmune, and neurodegenerative diseases (Encarnação et al., 2019).

The negative effects of medications on wildlife can be confirmed based on two past cases: reproductive system disorders in fish caused by estrogens and the decline of vulture populations due to diclofenac. Diclofenac had an extremely negative impact on vulture populations in Southeast Asia. This medication was used to treat livestock, and the vultures consumed the carcasses of these animals.

Another example is the effect of female sex hormones on fish reproduction. Several sex hormones, such as 17- α -ethinylestradiol used in contraceptive pills, have been identified in the aquatic environment. Fish are highly sensitive to this medication, and even low concentrations can alter the ratio between female and male sexes in favor of females due to a lack of sexual differentiation in males (Kümmerer, 2010).

Antimicrobial resistance

Since the discovery of penicillin in the 1920s, antibiotic treatment has significantly improved the health and well-being of people, and to some extent, has extended the average human lifespan. Additionally, antibiotics are widely used in animals for prevention or growth promotion. However, the misuse of antibiotics in both human and veterinary medicine has led to bacterial resistance against multiple drugs (Li et al., 2022).

Causes of antimicrobial resistance

The main cause is considered to be the excessive and unjustified use of antibiotics in human and veterinary practices, as well as the inadequate development of new molecules for anti-infective drugs. Epidemiological studies have confirmed a direct relationship between antibiotic consumption and the development or spread of resistant bacterial strains. Surveys conducted by the Centers for Disease Control and Prevention in 2013 demonstrated that treatment indications, antibiotic selection, and therapy duration were inadequate in 30-50% of cases, and only 7.6% had a causative microbe identified. Another important factor is the misuse of antibiotics in the food industry. It is estimated that up to 80% of total antibiotic sales are intended for animals. Mass administration of antibiotics contributes to the emergence of resistant bacteria in food-producing animals, which can then be transmitted to humans through the food chain (Podracká, 2017).

CONCLUSION

In the context of the looming climate crisis, the increasing risks of disease emergence and spread, antimicrobial resistance and food insecurity, the implementation of a common health approach is more important than ever before. Public awareness and education are crucial for advancing this approach and safeguarding our collective health and the planet. Medicines and their metabolites primarily enter the environment through human and animal urine or feces, as well as through the disposal of drugs down sinks and toilets. A significant portion of active substances also enters the environment through the pharmaceutical industry and improper disposal of medications, such as throwing them in the trash. Pharmaceutical waste, like any other type of waste, pollutes the environment, but besides environmental risks, this waste can also pose a risk to human and animal health.

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SELECTED TICK-BORNE BACTERIAL ZOOSES AND IMPACT ON HEALTH

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ABSTRACT

The main goal of this contribution is to highlight the relation to the large group of diseases that are transmitted through arthropods and their worldwide distribution, with a closer look at the zoonoses. The importance of knowledge increases as globalization and climate change increase the likelihood of transmission, as both cause favorable conditions for the transmission of bacteria and viruses. Increases in reservoir abundance, climate change, changing habitat structures, socio-political changes, and the import of animals for welfare reasons, trade, and traveling are considered potential factors for the introduction of pathogens and vectors to new areas. This contribution highlights bacterial zoonoses in animals and humans and their impact on health.

INTRODUCTION

Ticks and their role in disease transmission

A large number of pathogens circulate unnoticed in nature. Ticks play an important role as they represent a significant threat to the health of animals and humans worldwide. They pose a risk due to their function as vectors, transmitting various zoonotic viral and bacterial pathogens that can cause severe clinical symptoms and even death when transmitted to animals or humans (Jahfari and Sprong, 2016). In the temperate and cool regions of Europe, the most significant vector in terms of public health is *Ixodes ricinus*. It is followed by *Dermacentor reticulatus* and *Dermacentor marginatus* (Vayssier-Taussat et al., 2015).

Lyme disease

Lyme disease is a severe infectious illness (Steere et al., 2001). It is caused by the gram-negative bacterium belonging to the genus *Borrelia* and the species *Borrelia burgdorferi* sensu lato (Mead et al., 2015). Ticks become infected with *B. burgdorferi* during their larval stage and the bacteria persist in them throughout their entire life cycle (Neelakanta et al., 2007). Lyme disease is transmitted from ticks to hosts during blood feeding, typically after 36 hours of tick attachment. The disease usually starts with nonspecific clinical symptoms similar to the flu. Spirochetes

spread to various organs (skin, heart, joints, and the central nervous system) through the bloodstream and colonize different tissues, leading to the development of systemic diseases. A typical clinical sign is erythema migrans, a red-bordered rash with a white center (Rebman et al., 2015). The highest treatment efficacy is achieved in the early stage of the disease. Antibiotics such as doxycycline, azithromycin, clarithromycin, penicillin, and amoxicillin are the primary choice for treatment (Jarčuška, 2007).

Anaplasmosis

Anaplasmosis is caused by bacteria of the genus *Anaplasma*, belonging to the order Rickettsiales, which emerged from the fusion of the families Anaplasmataceae and Rickettsiaceae (Dumler et al., 2001). Anaplasmas are obligatory intracellular microorganisms and Gram-negative bacteria. They can reside and multiply in vertebrate reservoirs for many years. Bacteria from the genus *Anaplasma* cause diseases in domestic animals and humans (Rymaszewska and Grenda, 2008). The main symptoms of anaplasmosis include fever, headache, myalgia, and malaise. Severe illness is more frequently reported in older and immunocompromised patients, but it can also affect immunocompetent individuals and may lead to hospitalization or death if appropriate treatment is not provided promptly. Generally, patients show significant improvement within 24-48 hours after starting antimicrobial treatment with doxycycline (Prusinski et al., 2023).

Q fever/coxiellosis

Coxiella burnetii is an obligate intracellular Gram-negative bacterium belonging to the family Coxiellaceae and has been isolated from a wide range of animals worldwide. It causes Q fever in humans and coxiellosis in animals. Q fever/coxiellosis is considered an emerging disease with significant impacts on public health, animal health, and the economy. Rarely, this disease can be fatal. In humans, it is often accompanied by overall weakness. In up to 60% of infected individuals, the infection can be asymptomatic (Gonzalez-Barrio and Ruiz-Fons, 2019). Although the highest risk of *C. burnetii* infection in humans is associated with interactions with infected livestock, the frequency of sporadic cases of Q fever caused by free-living wildlife is increasing (Flint et al., 2016).

Rickettsiosis

Bacteria belonging to the family Rickettsiaceae have been isolated on every continent in the world except Antarctica (Le Van et al., 2020). Representatives of the family Rickettsiaceae cause zoonoses that are among the oldest known vector-borne diseases. They are obligate intracellular bacteria with various biological properties and are classified into two main genera, *Orientia* and *Rickettsia* (Sachman-Ruiz and Quiroz-Castañeda, 2018).

The genus *Orientia* consists of the species *Orientia tsutsugamushi*, which causes scrub typhus, a form of Japanese river fever that has been known since the fourth century BC, and *Orientia chuto*, which also causes disease in humans (Goldman and Green, 2015).

The genus *Rickettsia* includes two major groups: the typhus group, which includes *Rickettsia typhi*, causing endemic typhus (murine typhus), and *Rickettsia prowazekii*, causing epidemic typhus. The second group is the spotted fever group, which includes many other species of *Rickettsia*, such as *Rickettsia rickettsii*, *Rickettsia akari*, and *Rickettsia felis* (Blanton and Walker, 2016).

The clinical presentation in the spotted fever group can range from mild symptoms to life-threatening conditions. The most common symptoms experienced by patients include fever, nausea, vomiting, and a rash at the site of the tick bite (Gaywee et al., 2007).

Tularemia

The causative agent of tularemia is *Francisella tularensis*. It is a gram-negative, aerobic, facultative intracellular bacterium. Taxonomically, it is divided into four known subspecies: *F. tularensis* subsp. *tularensis*, *F. tularensis* subsp. *holarctica*, *F. tularensis* subsp. *mediasiatica*, and *F. tularensis* subsp. *novicida* (Kingry et al., 2014).

The most relevant subspecies for clinical practice are *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica*, also known as type A and B, respectively (Maurin and Gyuranecz, 2016). *Tularensis* subsp. *tularensis* is mainly found in North America and causes a severe form of the disease, being particularly virulent (Celli et al., 2013). In Europe, the majority of infections are caused by a less virulent strain of *F. tularensis* subsp. *holarctica* (Tully and Huntley, 2020).

The disease almost always manifests as an acute febrile illness with high fever, lymphadenopathy, and ulcerative lesions. Symptoms appear 3-4 days after a rapid feverish illness, accompanied by malaise, fatigue, shivering, and headaches (Strehl et al., 2014). Ulceroglandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal tularemia are the most common forms of the disease (Maurin and Gyuranecz, 2014).

The choice of the appropriate antibiotic depends on the severity of the disease and the patient's age. Streptomycin or gentamicin, taken orally for 7-14 days, have the lowest inhibitory concentration (WHO, 2007).

CONCLUSION

As the climate change are affecting the temperature and humidity, affecting the weather, and consequently affecting and changing the habitats of all livings on earth, the importance of One

Health approach is undeniable. A well-functioning cooperation between the human health, animal health and environmental health is critical in facing the risk of new pandemics caused by vector-borne zoonotic diseases, to preserve the human and animal health as well as restrict the global economic impact from such pandemics.

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THE RELATIONSHIP BETWEEN THE WATER CONSUMPTION AND MILK PRODUCTIVITY IN DAIRY COWS

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ABSTRACT

Water is one of the most important nutritional factors in food animals. Obviously, the demand for drinking water depends on many factors including the level of productivity. That is why the main purpose of the work was to study the relationship between the water consumption and milk productivity in dairy cows. There was established the positive relationship the water consumption with the quantity of the obtained milk ($r=0.58$) and high, middle and low dependability accordingly to the fat ($r=-0.59$) and protein ($r=-0.26$) content and milk density ($r=-0.04$). The conclusion was made that regulation and monitoring the water consumption by dairy cows may be an important factor of the economical and veterinary wellbeing of dairy farms.

INTRODUCTION

Water is an important component of the nutrition of all living beings, including productive farm animals. The quantity and quality of drinking water used often determine the quantity and quality of the products obtained, the level of animal morbidity, and the indicators of the economic activity of the farm. According to Morgan (2011) on modern high-performance dairy farms, cases of acute poisoning or death of animals as a result of water consumption are relatively rare. However, the author also mentions, that chronic poisoning of animals with small doses of substances of water origins that are toxic to a living organism can be of great importance from the point of view of animal health and the economic well-being of farms. In this regard, we believe that monitoring the quantitative and qualitative indicators of consumed drinking water is an important tool for ensuring the veterinary and economic well-being of the dairy herd. An analysis of the results of scientific research indicates the important role of ensuring an adequate water balance in cattle. The need for drinking water varies depending on

the age, physiological state and productive qualities of animals. In this regard, Brew et al. (2011) studied the quantitative characteristics of water consumption in heifers and fattening bulls. It was found that animals with a live weight of 276 ± 67.0 kg ($n=146$) and with a daily gain of 1410 g/day drank 30.0 ± 8.6 liters of water per animal per day. The authors also found that the local Brahman calves consumed less water than the imported British breed and noted a positive correlation ($p < 0.05$) between the amount of water drunk, growth and the amount of feed consumed. The results of research conducted by Lucas et al. (2008) indicate that the amount of water drunk by cows decreases during calving, during illness, at elevated general body temperature or air humidity. Overall, the authors concluded that monitoring water intake is an important indicator of the health and reproductive status of individual cows.

In this regard, the aim of the work was to study the relationship between the amount of water drunk and milk production indicators in dairy cows.

MATERIALS AND METHODS

The studies were carried out on a dairy farm of the central biogeochemical zone of Ukraine. While keeping on an open walking area, a group of cows ($n = 205$) in the summer-autumn period for 76 days recorded milk productivity indicators (total amount of whole milk received, its fat content, density and protein content) and the amount of water drunk by cows. The amount of water drunk was monitored using a KV-1.5 meter installed in the water supply network of a livestock farm. While keeping on an open walking area, cows drank water from a metal container 8.0 m long, 1.0 m wide and 0.5 m high. Statistical processing of the obtained research results was carried out using a personal computer and a spreadsheet Microsoft Excel. The resulting digital material was processed by the method of variation statistics using Student's and correlation criteria. Animal studies were carried out in accordance with the rules of the "European Convention for the Protection of Animals Used in Scientific Research" (Strasbourg, 1985).

RESULTS AND DISCUSSION

The results of the studies are presented in table 1. On average, each cow drank 37.1 liters of water per day during the study period; the average daily milk yield per cow was 15.1 liters.

Table 1. Dependence of indicators of milk productivity on the water consumption by cows

Statistical indexes	Amount of water drunk, m ³ /day	Total milk yield, kg/day	Milk fat content, g/L	Milk protein content, g/L	Milk density
Lim	4.0–12.0	2680.0–3539.0	3.27–4.12	2.89–3.04	1026.2–1028.1
M±m	7.6±0.27	3100.1±20.43	3.73±0.022	2.96±0.004	27.27±0.040
r	—	0.58	-0.59	-0.26	-0.04

Note. r - in relation to the daily amount of water drunk.

During a correlative analysis of cows' milk productivity indicators, depending on the amount of water drunk, a high positive dependence on the amount of milk received ($r = 0.58$) and a high, medium and low negative dependence on fat content ($r = -0.59$), content protein ($r = -0.26$) and density ($r = -0.04$) of milk. Therefore, the obtained research results show that with an increase in the amount of water drunk, the milk productivity of cows increases significantly. At the same time, the fat content of the obtained milk decreases, the protein content tends to decrease, and the density of the milk does not change. This conclusion is consistent with the data of other authors. For example, S. Ammer et al. (2018) found that an increase in the amount of water consumption during lactation corresponds to an increase in the level of milk production of cows. According to the authors, the half-life of water and the need for water in cows 24 days before calving, 24 and 42 days after calving was 7.5, respectively; 3.7 and 2.9 days and 42; 66 and 87 liters per day. The study done by Appuhamy et al. (2016) offers a set of empirical models that can assist in determining drinking water needs of dairy farms. Doelman et al. (2008) cows in the second month of lactation were given 2.5 g/l histidine with water. As a result, the amount of water drunk increased (from 85.1 to 92.1 l/day) and at the same time milk productivity increased (by 1.7 l/day).

Taking into account the given data, some authors offer different methods of stimulating cows to consume more water. In particular, it was found (Jensen et al., 2021) that at a water temperature of 3, 10, 17 and 24 °C and an external air temperature of 15.3 °C (10.2–23.7 °C), the amount of water drunk by cows was, respectively, 75.6, 76.7, 76.9 and 71.5 liters per day. Based on these research results, it was concluded that the high temperature of drinking water is an important deterrent to its consumption by cows and, accordingly, the reason for the decrease

in their milk production. Other authors (Golher et al., 2015) noted that the degree of water mineralization does not affect the milk production of cows. In the case of a choice between these two types of water, the authors noted only a tendency towards the overwhelming use of water with a high level of mineralization. Therefore, the analysis of the above research results indicates that the regulation of the level of drinking water consumption by cows is an important factor in increasing the milk productivity of cows and ensuring the veterinary well-being of the herd.

CONCLUSIONS

When studying the relationship between indicators of milk productivity of cows and the amount of water consumption, a high positive dependence on the amount of milk obtained ($r = 0.58$) and a high, medium and low negative dependence on fat content ($r = -0.59$), protein content ($r = -0.26$) and density ($r = -0.04$) of milk respectively. Regulation of drinking water consumption by cows is an important factor in improving the milk production of cows and ensuring the veterinary welfare of the herd. We believe that further study of the features of the qualitative and quantitative provision of dairy cows with drinking water is an important task of modern veterinary and zooengineering science.

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DETECTION OF THE INFLUENCE OF XENOBIOTICS ON PIG CHROMOSOMES

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ABSTRACT

The genetic potential of pigs can have a major impact on the productivity and profitability of pig farming. Environmental pollution with xenobiotics, such as pesticide residues and human or veterinary drugs increases the genetic risk for all living organisms, including humans. The genotoxic effect of xenobiotics is considered the most serious. Pigs are exposed to pesticides in a variety of ways, including direct exposure during ectoparasite elimination, spraying during crop protection, and feeding on contaminated feed. Genotoxicity testing plays an important role in biological monitoring and assessment of carcinogenic risks associated with pesticide exposure. Tests should include *in vitro* and *in vivo* testing of the potential of pesticides to induce gene mutations and/or chromosomal aberrations. From the first identified chromosomal rearrangements and abnormalities, it was found that the pig is susceptible to a number of chromosomal abnormalities that lead to impaired fertility to complete infertility. In order to reduce the presence of chromosomal rearrangements in pig herds, cytogenetic screening is needed to detect animals carrying chromosomal aberrations.

Xenobiotics in the environment

The genetic potential of pigs can have a major influence on the productivity and profitability of a pig enterprise. Litter size, growth rates and carcass quality are influenced by the combination of genetics, nutrition, health, environment and management (Mullan, 2017). A number of new chemical substances are introduced into practice every year. From the point of view of their toxicity, it is also necessary to study effects that do not manifest themselves immediately, but with long-term exposure in small doses can cause more permanent changes in the genetic material and various health problems (Kovalkovičová et al., 2005). With the current widespread use of xenobiotics in animal and plant production, the problem of deteriorating hygienic

conditions and the health status of farm animals is becoming more relevant than ever before (Kumar and Panneerselvam, 2008).

With the development of the society, the xenobiotics have brought high potential risk to human and animal (Yanzhu et al., 2017). Environmental pollution with xenobiotics increases genetic risks for all living organisms, including humans. Xenobiotics such as residues of pesticides, human or veterinary drugs can have synergistic adverse effects on farm animals. The most serious is the genotoxic effect of xenobiotics, which is usually monitored by evaluating the induction of chromosome aberrations and damage to the DNA molecule in the form of single-strand breaks (SSB) and double-strand breaks (DSB). Double-strand breaks in DNA are considered to be the most important DNA damage contributing to genome instability and tumorigenesis. In addition to toxicity, which is significantly qualitatively affected by the dose, another characteristic of xenobiotics is their accumulation in the organism. Their chronic effect is also very dangerous, which manifests itself in so-called clinically unmanifest effects, such as allergies, embryotoxicity, teratogenicity, mutagenicity, immunopathological changes, carcinogenicity, etc. (<http://www.nrl.uvm.sk>[21.2.2013])

Pesticide use has become part of everyday life; pesticide contamination is a global problem. Pesticides are chemicals used in the agricultural and livestock industries to control harmful organisms, including insects, weeds, and parasites to improve farm productivity. Pesticides include insecticides, herbicides, fungicides, nematicides, and others, depending on the target organisms. However, the widespread and excessive use of pesticides also exposes humans and animals who are the non-target species (Changwon, 2021). Pigs are exposed to pesticides in various ways, including direct exposure to eliminate ectoparasites, spray drift during crop protection, and consumption of contaminated feed. Fortunately, the pesticides currently used and to which pigs are exposed accumulate to a lesser extent. However, the prolonged use of agricultural pesticides allows toxicants to be continuously exposed to pigs, and several studies have investigated the mechanism of action of pesticides *in vitro* and *in vivo* in pigs (Knold et al., 2002; Bazulic et al., 2002). Genotoxic potential of pesticides is a principal risk factor for long-term health effects (Ahluwalia and Kaur, 2021).

A new class of insecticides with wide use in veterinary and plant production are neonicotinoid insecticides, which selectively act on nicotinic acetylcholine receptors (nAChR) in the central nervous system of insects (Zhao, 2018). Neonicotinoids have been found to be ubiquitous in the environment, drinking water and food. Available toxicological data from animal studies indicate to their possible genotoxicity and cytotoxicity with consequences such as impaired

immune system function or impairment animal growth and reproduction. Poisoning in pigs from neonicotinoides may arise from incorrect application, pigs eating contaminated feed or accidental exposure (Houghton, 2018). There are reports of neonicotinoid poisoning (imidacloprid and acetamiprid) in animals and humans (Mohamed et al., 2009). It is important to evaluate the genotoxic and cytotoxic actions of these new agricultural pesticides to contribute with toxicological data and regular use without polluting the environment and without leaving their residues in water and food sources with their possible risk on the organism health (Imamura et al., 2010).

The extensive use of the antifungals, mainly azole antifungals, is generally associated with higher discharge of these drugs into the environment causing increased concentrations of their residues. Medical antifungal drugs are used in the management of topical and systemic human fungal infections, such as the infections caused by the different fungal agents (Nett and Andes, 2016). Antifungal drugs are also widely used in animals such as horses, dogs, cattle and sheep, pigs, and birds to treat fungal infections caused by dermatophytes, *Aspergillus*, yeasts, and mucorales (Rochette et al., 2003). Besides their use in human and animals, they are also known to be used in agriculture (Dalhoff, 2018; Trösken et al., 2005). Antifungal drugs are known to distribute into different environmental compartments once they are released into the environment. The presence of antifungal residues in the environment also poses a potential risk of toxicity to non-target organisms (Chen et al., 2014).

Genotoxicity testing

Genotoxicity testing plays an important role in the biomonitoring and assessment of the carcinogenic risks associated with pesticide exposure (Mountains et al., 2000). Tests should include *in vitro* and *in vivo* assays to detect the pesticide potential to induce genetic mutations and/or chromosomal aberrations (Araldi et al., 2015). Cytogenetic changes in cultured peripheral blood lymphocytes, such as chromosome aberrations (CA), sister chromatid exchanges (SCE) and frequency of micronuclei (MN), have long been used as biomarkers of genotoxic exposure and the early effect of genotoxic carcinogens (Albertini et al., 2000; Adad et al., 2015). Pavanello and Clonfero (2000) also include the evaluation of DNA damage using the comet test to these analyses.

Chromosomal aberration analysis

Chromosomal aberrations (CAs) are an important tool for assessment of exposure to pesticides (Ahluwalia and Kaur, 2021). From the first chromosome rearrangements and abnormalities

identified, it has been clear that the pig is susceptible to a number of chromosome abnormalities resulting in impaired fertility to total infertility (Donaldson et al., 2021).

Reciprocal translocations are the most prevalent chromosome rearrangement known to occur in the pig, representing 84% of all observed structural rearrangements (Ducos, 2007). Balanced structural chromosome rearrangements are a leading cause of fertility losses in pigs, particularly reciprocal translocations, with carriers experiencing average litter size losses of 40% (ranging between 10–100%) relative to the herd average (Pinton et al., 2004).

The incidence of translocations among breeding boars ranges from 0.5% to 1.5%. Carriers of translocations have litters with 3-5 piglets less than herd average (King et al., 2019). Conversely, Robertsonian translocations are very rare (Ducos et al., 2008). The higher incidence of Robertsonian translocations in cattle, as opposed to pigs (like in humans) for which reciprocal translocations are more common, may be due to the difference in the type of chromosomes. In cattle, autosomes are acrocentric; therefore, Robertsonian translocations are more likely to occur (like in mice). In pigs ($2n=38$), however, 12 pairs of autosomes are meta-centric and six pairs are acrocentric (<https://onlinelibrary.wiley.com/doi/pdf/10.1111/age.12581>).

Chromosome rearrangements, including balanced reciprocal translocations, are expected to occur frequently in swine herds throughout the world, being proposed to occur spontaneously in 1/200 live piglets (Ducos et al., 2007; King, 2019). Carriers of rearrangements, if permitted to breed, may then pass on the rearrangement to approximately 50% of their successful offspring, increasing the prevalence of chromosome rearrangements in swine herds over time (Ducos et al., 1998). In order to reduce the presence of chromosome rearrangements in swine herds, is needed cytogenetic screening allows for the detection of animals carrying chromosomal aberrations and to avoid using them as breeders (Ducos et al., 2008).

From the very beginning of animal cytogenetic research, cattle and pigs attracted the attention of cytogenetics (Basrur, et al., 2008). The development and implementation of laboratory and genomic techniques for use in clinical cytogenetics has played an important role in swine breeding for the last forty years (Donaldson et al., 2021). In the pig, the most common technique for molecular cytogenetics analysis is fluorescent *in situ* hybridization (FISH) and primed *in-situ* labelling (PRINS; Danielak-Czech et al., 2016).

The advent of the fluorescence *in situ* hybridization (FISH) technique, initially applied to human chromosomes, noticeably expanded cytogenetics research and investigations applied to

domestic animals due to the possibility of revealing specific chromosome regions, entire chromosomes, or chromosome arms according to the choice of probe (Iannuzzi et al., 2021).

One of the great advantages of the FISH technique is that it can be applied to interphase cell nuclei, meiotic preparations (sperm and oocytes), embryos, and elongated chromatin fibers, in addition to metaphase chromosomes, thereby allowing more complete cytogenetic investigations of animal cells (Transk et al., 1990). Cytogenetic screening of livestock is essential to detect animals with chromosomal aberrations and exclude them from breeding programs, thus preserving the genetic quality and value of livestock.

Comet assay

Among the available genotoxicity tests, the Comet Assay is recognized due to its robustness, sensitivity and statistical power to evaluate Deoxyribonucleic acid (DNA) breaks, which can be considered as hallmarks of mutagenicity (Araldi et al., 2015). The alkaline Comet Assay can evaluate cellular single and double-stranded DNA breaks induced by pesticide exposition and determine the cytotoxic effect of pesticides by assaying for apoptosis-associated fragmentation of nuclear DNA.

CONCLUSION

Xenobiotic contamination is a worldwide problem. Not only is the ecosystem affected, but also the health of animals and people. Many of them are capable of disrupting the endocrine balance of the body, which has adverse consequences for animal health; such as reduced fertility, weakened immunity, behavioral disorders or the development of cancer. Therefore, it is important to know the potential genotoxic effect of applied substances, including pesticides, neonicotinoids, and azole fungicides. Considering their possible impact on non-target organisms, which can be farm animals, companion animals and also humans, research on the negative impact of xenobiotics is highly topical and in demand. Their harmful effect on the body can have carcinogenic, teratogenic and mutagenic effects.

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LITERATURE ON REQUEST

THE MOST SERIOUS NEGATIVE EFFECTS IN NATURE FOR THE HONEY BEE - DISEASES

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ABSTRACT

Monitoring the health status of bee colonies is important primarily due to the subsequent targeted eradication of the disease. Currently, bees are endangered, their population is decreasing. Not only in Slovakia, but also in other countries of the world, among the most serious diseases of bees are Varroosis apium, American foulbrood and Nosematosis apium. This article has an informative nature with the aim of pointing out the most widespread diseases of bees.

INTRODUCTION

The honey bee (*Apis mellifera*) is an integral part of our nature. It is not only an insect that ensures the pollination of a whole range of plants, but provides man with a wide range of its products. Among the most famous are honey, royal jelly, propolis and poison.

In recent years, bee populations have fallen by a third in many parts of the world. Among the most common diseases causing the death of bee colonies are American foulbrood, or the increasing incidence of parasites such as the bee mite (*Varroa destructor*). Likewise, chemical spraying that kills bees or causes their genetic mutations and the cultivation of monocultures, which leads to a reduction in the diversity of the food supply, also have a negative effect.

RESULTS

VARROOSIS APIUM

Varroosis apium is a parasitic disease of bees and bee brood caused by the mite *Varroa destructor*. It belongs to dangerous infections and is subject to mandatory reporting. In Slovakia, reporting is not mandatory due to widespread occurrence.

Clinical symptoms of the disease appear only after a long time, at first 2-3 years after the infection of the bee colony. After 4-5 years of infection, the bees are so attacked that they die during the winter period. The characteristic symptoms of the disease are detected in young bees. Bees with imperfectly developed legs or wings (or missing ones), possibly with a shortened abdomen, etc. hatch from the attacked fruit. Healthy bees carry out such affected bees and throw them in front of the hive, where they die (Shimanuki, 2000).

Currently, there are no effective biological and zootechnical methods of combating *Varroa destructor* that could replace the chemical substances used so far. Without treating bee colonies with chemical drugs, it is not possible to maintain the breeding of European honey bee breeds.

Medicinal products divided according to use:

- Fumigation – preparations based on amitraz are used;
- Contact drugs - these are drugs with a long-term effect and include drugs based on fluvalinate and flumethrin;
- Evaporation – this includes organic acids, especially formic acid and oxalic acid;
- Essential oils - eucalyptol, menthol, methyl salicylate, thymol.

Figure 1: Occurrence of *Varroa destructor* in the world (Toporčák, 2022)



AMERICAN FOULBROOD (*HYSTOLYSIS INFECTIOSA PERNICIOSA LARVAE APIUM*, *PESTIS APIUM AMERICANA*)

American foulbrood is one of the diseases that are subject to reporting, prevention and control in trade according to Act no. 39/2007 on veterinary care.

The causative agent of American foulbrood is the sporulating gram-positive bacterium *Paenibacillus larvae* (Genersch et al., 2006). It produces oval spores and these spores are protected against the external environment by a multi-layered envelope (Fries and Camazine, 2001). Larvae become infected with *P. larvae* spores through food. Larvae at the age of 8 to 24

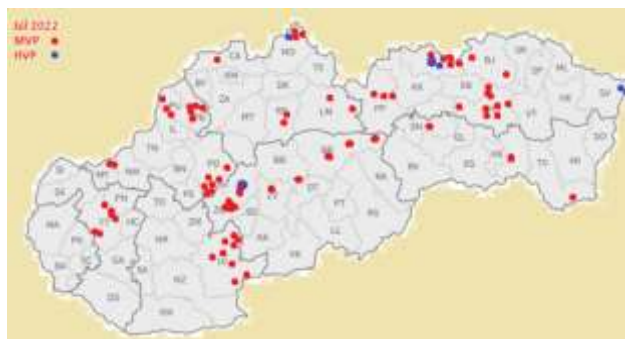
hours are the most susceptible. After infection, the larvae die of general sepsis. The cell cappings are darkened, sunken and sometimes perforated. The body of the larva is perfectly decomposed, including the chitinous skin. The dried larva sticks firmly to the bottom wall of the cell (Yue et al., 2008).

We follow the current National Program for the Eradication of American foulbrood and European foulbrood. Positive colonies are destroyed by burning.

Table 1: Incidence of American foulbrood and European foulbrood in Slovakia in January and February 2023 (ŠVPS, 2023)

Sumárny Výkaz nákaz za SR										
Obdobie od január/2023 do február/2023										
	Nové ohn.	novom	priebeh mes.					Zanik ohn.	<koniec mes.>	
			Počet zvierat v ohnisku						Zamor. ohn.	Poč.zv. v ohn.
			vním.	chorých	abynut.	utrát.	odpor.			
B452 api Mor včelieho plodu	0	0	1221	132	0	138	0	2	71	1083
B453 api Hniloba včelieho plodu	0	0	117	0	0	0	0	0	9	117

Figure 2: Incidence of American foulbrood in Slovakia in 2022 (Toporčák, 2023)



NOSEMATOSIS APIUM

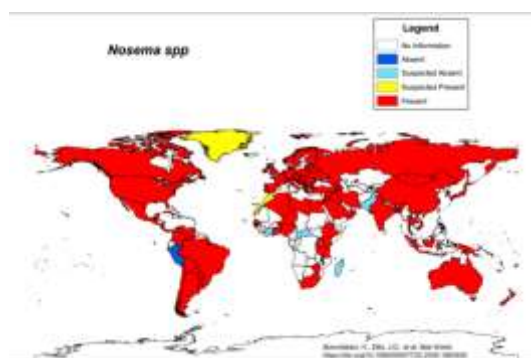
Nosema infection of bees is a parasitic disease of adult bees and is no longer classified as a dangerous notifiable bee infection. It is the most widespread disease of bees.

Nosema disease is caused by the element *Nosema apis*. *Nosema ceranae* was first discovered in 1996. The main difference between the two nosemas is the speed at which they cause the death of a bee colony. Bees die within 8 days after being attacked by *Nosema ceranae* spores, which is much faster than *Nosema apis*.

Adult bees become infected with nosema spores through contaminated water or food. Subsequently, the spores reproduce in the epithelial cells of the bee's stomach and produce large numbers of new parasites and are finally excreted in the faeces (Higes et al., 2008).

The basic measures for nosema infection include collecting and burning dead and sick bees, spinning honey, diluting it with water and 15 min. boiling (that's how we can use such honey for bees). We melt older combs into wax, light combs can be disinfected with vapors of organic acids (formic acid, acetic acid). We disinfect the hives and frames with heat. The beehives are not treated because the medicines used in the world are antibiotics and they cannot be used in beehives in the EU.

Figure 3: Incidence of *Nosema spp.* in the world (Boncristiani, Elis, 2020)



CONCLUSION

Bees are an unthinkable part of the ecosystem. Bees, like other animals, suffer from various diseases that need to be known and properly fought against. This article deals with the most important diseases of bees such as varroa, nosema and American foulbrood.

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IMPACT OF CLIMATE CHANGE ON CALF REARING MANAGEMENT AND ANTIMICROBIAL USE

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ABSTRACT

The aim of this study was to determine the influence of season on the morbidity of pre-weaned dairy calves and its influence on the consumption of antibiotics. The five-year study was performed on the one farm in 414 calf housed in the hutches. Live weight, morbidity, mortality and antibiotic use by calves were monitored in every season. All statistical analyses were performed in TIBCO Statistica. Calves born in autumn had a significantly lower birth weight, weaning weight and weight gain. However, the effect of season on morbidity, mortality and antibiotic use on calves was no statistically significant.

INTRODUCTION

The technology of dairy calf housing is one of the most important factors affecting the health, growth and welfare of calves. There are estimated 60% of dairy calves in Europe and 75% of calves in the Czech Republic are housed in outdoor individual hutches during the milk feeding period (Marcé et al., 2010; Doležal and Staněk, 2015).

The biggest problem with calf housing in hutches is high temperature and solar radiation intensity during the summer period. The inside temperature in hutches in the summer months very often exceeds critical values +25 °C or +26 °C (Hahn, 1997; Collier et al., 2019). As environmental temperature rises, the energy intake of calves decreases because of lower dry matter intake while the energy expenditure on maintenance and metabolism increases to remove the heat load. Consequently, the calves under the heat stress sacrifice a fraction of growth energy for heat regulation, which explains their decreased growth performance (Purwanto et al., 1993; Nonaka et al., 2008). Calves exposed to high temperatures have reduced their resistance to disease and increased mortality (Nonaka et al., 2008; Tao et al., 2012; Marrero et al., 2021).

Because of the ongoing climate change and potential global warming, the interest in heat stress-related aspects of the animal well-being is increased (Kovács et al., 2018).

The aim of this study was to determine the influence of season on the morbidity of pre-weaned dairy calves and its influence on the consumption of antibiotics.

MATERIAL AND METHODS

The five-year study was performed on the one farm in 414 calf (from birth to 60 days of age) which were housed in the individual hutches immediately after their birth. Nutrition, drinking (from buckets) and bedding were the same for all calves throughout the monitoring period.

The calves were divided into four groups according to the season of birth (spring – from 20.3 to 20.6., summer- from 21.6. to 21.9., autumn – from 22.9. to 20.12., winter – from 21.12. to 19.3.). The calves were weighed in weekly intervals and their health were recorded once a day. Records of calf treatment with antibiotics were obtained monthly from the treatment diary. Digital registration data loggers were installed both in the Stevenson screen and in all individual hutches, where they recorded the air temperature on hourly intervals throughout the year.

The qualitative parameters (morbidity, mortality, antibiotics use) were evaluated by nonparametric tests, quantitative parameters (live weight, average daily gain) using analysis of covariance in the TIBCO Statistika (13.5.0.17, TIBCO Software Inc.) software package. The Scheffe test was used to compare averages between the tested groups.

RESULTS

The calf growth and their health are affected by many factors, among which are mainly the level of the breeding environment, housing system, feeding, level of management etc.

Table 1. The live weight of calves depending on their season of birth

Season of calf birth	N	Birth weight [kg]	Live weight at weaning [kg]	Average daily gain [kg]
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
spring	88	44.1 \pm 4.4 A	85.8 \pm 7.6 B	0.694 \pm 0.113
summer	114	42.7 \pm 6.2	84.9 \pm 8.4 C	0.702 \pm 0.098 a
autumn	110	41.3 \pm 6.2 A	80.9 \pm 9.1 B,C,D	0.661 \pm 0.110 a,E
winter	102	42.9 \pm 5.6	85.8 \pm 10.0 D	0.715 \pm 0.124 E

Statistical significance: A,B,C,D,E ($p < 0.01$); a ($p < 0.05$)

The birth weight of calves varied from 35.1 to 49.3 kg. The calves born in autumn had statistically significantly ($p < 0.01$) lower birth weight by 2.8 kg than the calves born in spring (Table 1).

The weaning weight of calves ranged from 71.8 to 95.8 kg. The calves born in autumn achieved demonstrably ($p < 0.01$) the lowest live weight at weaning in 60 days of age compared to the calves born in other seasons (Table 1).

The average daily gains of the calves were in the range of 0.551 to 0.839 kg. The calves born in autumn had a significantly lower average daily gains by 0.054 kg respectively by 0.041 kg in compared to the calves born in winter ($p < 0.01$) or summer ($p < 0.05$) (Table 1).

The highest morbidity and mortality were found in calves born in summer and autumn (Table 2). While the calves born in winter were non-significantly more treated with antibiotics compared to the calves born in other seasons (Table 2).

Table 2. Morbidity, mortality and proportion of calves treated antibiotics

Season of calf birth	Morbidity [%]	Diarrheal diseases [%]	Respiratory disease [%]	Diarrheal + Respiratory diseases [%]	Mortality [%]	Proportion of antibiotic use [%]
spring	20.9	8.5	1.4	11.0	2.3	23.7
summer	28.3	14.8	0.5	12.9	7.0	40.8
autumn	27.5	15.1	2.2	10.2	6.4	46.0
winter	23.4	13.5	1.4	8.5	4.9	51.8

A non-significant higher occurrence of diarrheal diseases was observed in calves born in summer and autumn (Table 2). A non-significant higher incidence of respiratory diseases was recorded in calves born in autumn. The coincidence of diarrheal and respiratory diseases was found in calves born in summer (Table 2).

DISCUSSION

The pre-weaning period is vital in the development of calves on dairy farms and improving daily liveweight gain is important to both financial and carbon efficiency; minimising rearing costs and improving first lactation milk yields. Increased environmental temperature within the first month of life increases the daily live weight gain by (0.012 kg/d per 1 °C) (Hyde et al., 2021).

Consistent with many studies, we have proved that the growth of the calves is influenced by the season in which they were born. In contrast to our results, Broucek et al. (2006) demonstrated that the calves born during the hot summer period housed from day 5 until weaning at 56 days of age in hutches have significantly lower live weight and lower average daily gains, lower starter intake and drink more water compared to the calves born in autumn. Chester-Jones et al. (2017) also proved in their study that all calves born in autumn and winter had greater body weight, average daily gain and starter intake.

Calf-hood disease is a serious problem in many dairy operations that can have substantial effects on heifers' survival and productivity, and has economic and welfare impacts. The most common diseases affecting the calves during the milk feeding period are diarrhoea and respiratory diseases (Cho and Yoon, 2014). Factors associated with the increased risk of diarrhoea and respiratory diseases occurrence include season of birth, failure of transfer of passive immunity and temperature in pre-weaning housing (Windeyer et al., 2014).

The calves exposed to the prenatal heat stress have impaired passive transfer of immunoglobulins from ingested colostrum, and this subsequently worsens their state of health. The calves exposed to the heat stress in the postnatal period, experience a change in the development and function of the immune system as a result of disruption of the production of own T and B lymphocytes and subsequent reduction of their resistance to disease, their mortality increases (Nonaka et al., 2008; Tao et al., 2012; Marrero et al., 2021). These findings support our results because the highest morbidity and mortality were found at the calves born in summer and autumn.

CONCLUSION

We have proved the influence of calf birth season on their body weight and growth. The calves born in summer and autumn had tendency to have greater morbidity and mortality while higher antibiotics use was found at the calves born in winter. The negative effects of the heat stress as one of the climate change manifestation, can be limited in calves primarily by adjusting their housing (e.g. use of hutches made of suitable materials; use of special hutches; suitable location and orientation of hutches, their ventilation and shading) and management (e.g. ensuring sufficient fresh water and fresh starter; increasing the amount of milk, feed, or milk replacer; appropriate timing of interventions and zootechnical activities).

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CLIMATE CHANGE AND ZOOSES IN THE CONCEPT OF "ONE HEALTH"

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ABSTRACT

People live in close contact with animals. Zoonotic diseases can naturally be transmitted from vertebrates to humans, as well as from humans to vertebrates. More than 60% of human pathogens have a zoonotic origin. This includes a wide range of bacteria, viruses, fungi, parasites, and other pathogens. Factors such as climate change, urbanization, animal migration, wildlife trade, travel, vector biology, and anthropogenic factors influence the emergence and repeated occurrence of zoonotic diseases. In this contribution, we evaluated the etiology of two zoonotic diseases occurring in Slovakia and their impact on human health. The implementation of "One Health" measures is recommended for effective prevention and control of zoonoses.

INTRODUCTION

More than 200 zoonotic diseases are known, representing a significant portion of new and existing human diseases (WHO, 2020). It is stated that approximately 60% of all human pathogens and 75% of emerging infectious diseases originate from animals (Mohammadpour et al., 2020). A particular problem is represented by infectious diseases of companion animals, especially dogs and cats, and recently, other exotic animals. Various stray and abandoned animals, overpopulated urban pigeon populations, and other wild birds as well as rodents become problematic (Švrček et al., 2008). Zoonoses pose a significant public health problem and a direct risk to human health (Grace et al., 2012). Currently, the World Health Organization (WHO) as well as the World Organisation for Animal Health (OIE) promote the concept of "One Health." The aim of linking human and veterinary medicine is health protection. By protecting animal health, we protect human health, especially in the case of diseases transmitted from animals to humans (Mojžišová, 2018). Owning companion animals can be associated with several advantages however, domestic animals can serve as the main source of zoonotic pathogens. One study indicates that more than 75% of households have a close contact with domestic animals (e.g., sleeping in bed with owners, licking faces) (Stull et al., 2015). Dogs are the main source of zoonotic infections and transmit several diseases to humans. Zoonotic

diseases can be transmitted to humans through infected saliva, aerosols, contaminated urine, faeces, and direct contact with dogs, scratches, or bites. Pathogens are excreted into the environment, increasing the risk of human infection (Ghasemzadeh and Namazi, 2015). Zoonotic diseases associated with companion animals (especially dogs and cats) include bacterial infections (campylobacteriosis, leptospirosis, salmonellosis, Lyme disease, brucellosis, pasteurellosis, staphylococcosis, streptococcosis, tularemia, yersiniosis, ehrlichiosis, etc.), viral infections (rabies, pseudorabies, norovirus, influenza, etc.), parasitic infections (giardiasis, roundworms, toxoplasmosis, etc.). Global climate change is currently observed worldwide and seems to be accelerating gradually. Climate change can influence the re-emergence of zoonoses, vector biology, host dynamics, pathogen virulence, distribution and migration of wildlife, socio-economic status, and the use of water and soil (Rahman, 2020).

Leptospirosis

Leptospirosis is a zoonotic disease caused by strictly aerobic gram-negative spirochetes belonging to the genus *Leptospira* (Levett et al., 2006). In dogs, clinical signs of leptospirosis are most commonly attributed to infection with serovars *Icterohaemorrhagiae*, *Canicola*, *Grippotyphosa*, *Pomona*, and *Bratislava* (Greene et al., 2006). The bacteria are transmitted in the proximal renal tubules and are excreted in urine for several months or even throughout the animal's life, leading to direct or indirect infection of other animals and humans (Stokes and Forrester, 2004). The survival of *Leptospira* in the environment is variable and depends on the presence of water, soil, heat, and humidity. Stagnant water provides a suitable environment where *Leptospira* can remain infectious for more than six months (André-Fontaine, 2006). Dogs younger than six months are particularly susceptible to severe hepatic failure (Langston and Heuter, 2003). Most human infections caused by pathogenic *Leptospira* manifest with increased body temperature, multisystemic disease associated with acute kidney failure, liver damage, meningitis, and pancreatitis (Haake and Levett, 2014). Vaccination is necessary for the control of leptospirosis in dogs. The first vaccine dose should be administered no earlier than the 9th week of life. The purpose of vaccination of dogs is to prevent leptospiremia, to reduce the severity of clinical signs, to prevent the shedding of leptospires in urine, thus reducing the risk of human infection. Important preventive measures include rodent control and avoiding contact with reservoir species (Schreiber et al., 2005).

In one study, cases of human leptospirosis in Slovakia between 1997 and 2006 were monitored, and a total of 310 cases were reported. The main serological types detected were *Grippotyphosa*, *Icterohaemorrhagiae*, *Pomona*, and *Tarassovi*. Nine deadly cases were also reported (Bakoss et al., 2012).

Rabies

Rabies represents a significant public health problem worldwide. It causes over 70,000 human deaths annually (Dietschold et al., 2008). The causative agent of rabies is a neurotropic RNA virus from the family Rhabdoviridae, genus *Lyssavirus*. Lyssaviruses have an affinity for nerve cells and infect peripheral nerve endings (Schnell et al., 2010). Rabid dogs pose the greatest danger (Rupprecht et al., 2002). The virus is primarily transmitted through animal bites, as it is present in saliva. After entering through peripheral wounds (skin, muscles, subcutaneous tissue), lyssaviruses spread centrally to the central nervous system (Gillespie et al., 2003). The incubation period for rabies in animals and humans is variable, ranging from 14 to 90 days. Rabies typically manifests as an acute illness (2-14 days) and is almost always fatal. In some cases (approximately 20%), rabies infection causes muscle weakness and ascending paralysis without an excitatory phase, leading to the paralytic form of rabies, which is nearly always fatal (Davis et al., 2015). According to the Act No. 39/2007 Coll., in accordance with § 17 par. 5 of the law, every owner or keeper of a carnivorous animal species is obliged to ensure vaccination against rabies according to the vaccination schedule recommended by the vaccine manufacturer (ŠVPS 2021).

In September 2022, the first case of rabies was confirmed in a badger (a wild animal) in the Humenné district, near the border with Poland and Ukraine. Additional positive cases were confirmed at the National Reference Laboratory in Zvolen, where results were positive for a deceased dog and a deceased red fox found in the hunting grounds of Rebjáková in the Rovné nad Udavou cadastral area. The area is located within a 10-kilometer defined buffer zone established in connection with the positive case in the badger on September 30, 2022 (ŠVPS 2023).

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DYNAMICS OF TICK-BORNE ENCEPHALITIS AND LYME DISEASE INCIDENCE IN THE REGIONS OF SLOVAKIA IN 2001 – 2020

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ABSTRACT

The study analyses the dynamics of tick-borne encephalitis (TBE) and Lyme disease (LB) incidence in the regions of Slovakia in the first two decades of the 21st century in 5-year cycles. The distribution of TBE is highly focal, with increase in incidence in the mountainous regions of central and western Slovakia with a moderately warm to colder climate and a slightly decreasing incidence trend in the lowlands of southern Slovakia with a warm and dry climate. For LB, the distribution of disease is widespread rather accross the area than focal, and the trend of the incidence in the districts did not follow that one of TBE. As the vector of both diseases is the tick *Ixodes ricinus*, the results suggest that TBE virus and LB bacteria respond to different environmental factors.

INTRODUCTION

Since the second half of the last century, we have observed an general increase in the average air temperature in Slovakia and, at the same time, changes in the distribution of the tick *Ixodes ricinus*, especially an increase in populations in the colder mountain areas where it was previously occurred only sporadically (Medlock et al., 2013). Associated with these changes are changes in the incidence of the human diseases it transmits, particularly tick-borne encephalitis (TBE) and Lyme disease (LB). Significant changes are evident in TBE, where the modus of cases registered by the Public Health Office of the Slovak Republic in 1980 – 1984 was in the altitude range of 100-200 m with the highest case at 550 m above sea level, 20 years later, in 2000 – 2004 in the range of 200-300 m above sea level with a maximum at 862 m above sea level (Lukáš et al., 2010). This trend is general across Europe (Medlock et al., 2013).

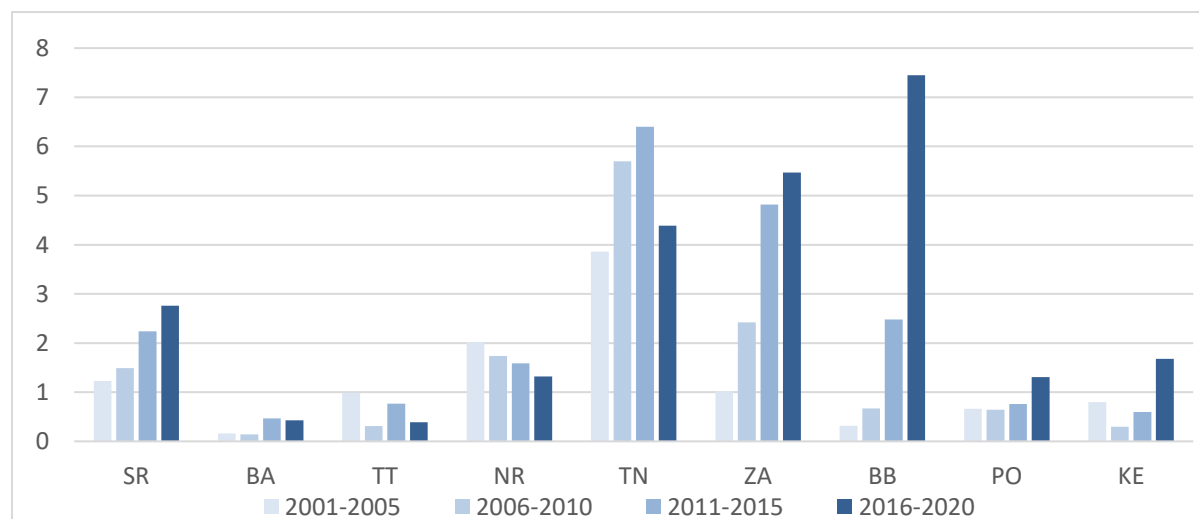
METHODS

Data on the incidence of TBE and LB in the districts of Slovakia (SK) in the years 2001 – 2020 were obtained through the Epidemiological Information System of the Office of Public Health of the Slovak Republic <https://www.epis.sk/>. Incidence values in individual regions of Slovakia in 5 annual averages with emphasis on climatic region are compared. The Bratislava (BA), Trnava (TT) and Nitra (NR) regions are predominantly in the warm and dry climatic region, the Žilina (ZA) region is predominantly in the moderately warm and humid region, the Trenčín (TN), Banská Bystrica (BB), Prešov (PO) and Košice (KE) regions have a comparable representation of the warm moderately humid and moderately cold region.

RESULTS

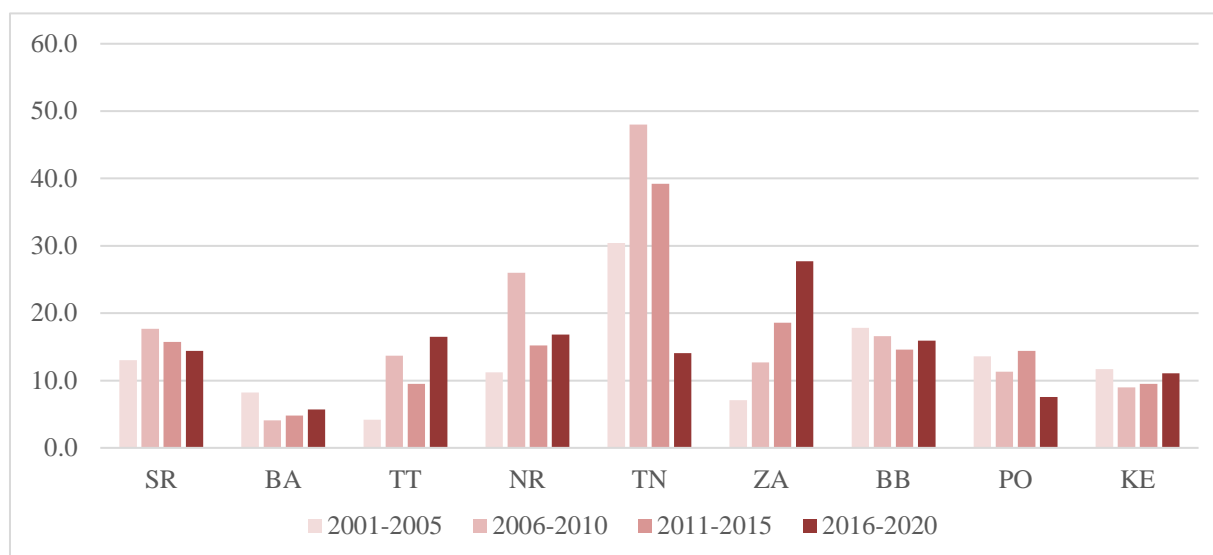
The incidence of TBE in Slovakia (Figure 1) had a significant and steady and more than two-fold increasing trend over the 5-year cycles, 1.2-1.5-2.2-2.8. The highest incidence in the first half of the first decade was in the TN region, (3.9) about half of that in the NR region (2.0), slightly below the Slovak average in the TT and ZA regions (equally 1.0), below the SK average was in the KE, PO, BB and BA regions (from 0.8 to 0.2). The incidence of TBE increased significantly in the next three pentads, with the final increase in the second half of the second decade being approximately twofold in BA, PO and KE, 5.5-fold in ZA and up to 25-fold in BB region. In contrast, a steady decline in incidence was recorded in the NR region (from 2.0 to 1.3) and an uneven decline in the TT region (from 1.0 to 0.4). In TN region, after an initial increase to 6.4 in the first half of the second decade, there was a significant decrease to 4.4 cases per 100,000 inhabitants.

Figure 1: Dynamics of TBE incidence in districts of Slovakia in 2001-2020 in 5-year cycles.



The trend of LB incidence during the first decades of the 21st century is different from that of TBE (Figure 2). The average incidence in Slovakia over 5 year cycles initially trended upwards, from 13 to 17.7 followed by a steady decline to 14.4. The highest incidence of LB at the beginning of the millennium was again in the TN region (30.4), followed by the BB region (17.8), close to the average in the PO (13.6), KE (11.7) and NR regions (11.2), around half of the average in BA (8.2) and ZA (7.1), and the lowest incidence was in the TT region (4.2). When comparing the trend in 5-year cycles in the cooler ZA and, paradoxically, in the warm TT region, there was a more significant increase in incidence (equally 3.9-fold). In the BB and PO region there was a slight decrease in incidence, in the other regions (BA, NR and KE) the dynamics of incidence was more (NR) and less fluctuating (BA, KE).

Figure 2. Dynamics of LB incidence in regions of Slovakia in 2001-2020 in 5-year cycles.



DISCUSSION

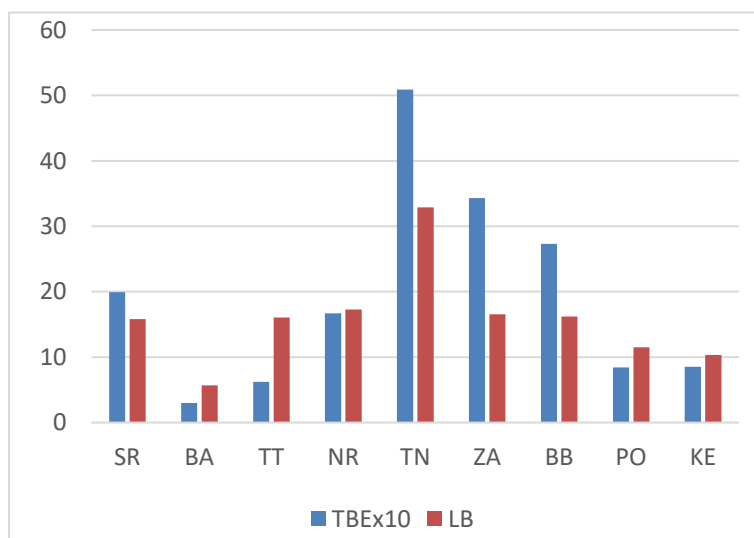
Despite the fact that both TBE and LB share the same vector, the *Ixodes ricinus* tick, and even the majority of reservoir hosts, the distribution and trend of TBE and LB in humans in Slovakia in the first two decades of the 21st century is significantly different.

For TBE, a gradual increase in the number of cases per 100,000 population is evident, especially in counties that lie in areas with approximately equal proportions of moderately humid warm to moderately cool climates (TN, ZA, BB, PO, KE). Conversely, in counties with a predominantly warm and dry climate (NR, TT), there has been a gradual decline in incidence, which is explained by the fact that the *I. ricinus* tick prefers the slightly warm and slightly humid climate of mountainous areas.

However, the dynamics of LB incidence in Slovakia in 5-year cycles over two decades is different. A gradual increase in the incidence of LB as in TBE occurred only in the ZA region, and a similar pattern with an initial increase followed by a significant decrease was observed for both diseases in the TN region. The most pronounced increase in TBE incidence in the BB region was accompanied by a slight steady decline in LB, less intensely in the PO region. In contrast, the downward dynamics of TBE incidence in TT and NR region with warm and dry climate was upward for LB. In KE and BA region the fluctuation of TBE and LB values was insignificant.

There is also a noticeable difference in the distribution of TBE and LB in the regions of Slovakia in the 20-year average (Figure 3). TBE cases were more frequent in central and western Slovakia in BB, ZA and TN regions (2.7-5.1) than in TT and NR regions (0.6-1.6) and in the east in PO and KE regions (0.8), but for LB the average incidence was fairly even in all regions (10.3-17.3), which is close to the Slovak average (15.8), except at the extremes in TN (32.9) and BA (5.7).

Figure 3. Average incidence of TBE and LB in 2001-2020.



This implies that in a relatively small area of Slovakia the conditions for tick life and also the survival of tick-borne encephalitis virus and *Borrelia burgdorferi* s.l. are probably different and the climatic conditions of the area are not determinant. This raises new questions as to what other biotic or abiotic factors influence the

ecology of ticks, or the ecology of their hosts and transmitted pathogens.

CONCLUSION

The trend of TBE and LB in the first decades of the 21st century was different. TBE spiked focally in the ZA and BB counties, declined slightly in the lowland counties, and with no significant changes in eastern Slovakia. LB is more widespread and evenly distributed with a fluctuating trend, indicating different environmental influences.

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CHANGES IN DRINKING WATER QUALITY DUE TO ANIMAL HUSBANDRY

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ABSTRACT

The aim of this study was to monitor the quality of ground water supplied to animal farms and 2 villages in the area of Košice basin, eastern Slovakia. Samples for physico-chemical and microbiological examination were collected at 12 sampling points in all four seasons. The physico-chemical evaluation focused on parameters that indicate pollution of water resulting from human activities and farming. Examination of potable water used on agricultural farms showed some possibility of contamination of sources by runoff and inappropriate manipulation with excrements.

Our investigations showed that protection zones of water sources were not always sufficient. Quality of ground water supplied for mass consumption complied with legislative regulations except for BC 22 in summer and autumn. Water from individual wells contained occasionally presence of total coliforms, *E.coli* and enterococci and higher heterotrophic counts.

INTRODUCTION

Human activities interfere in many ways with natural water cycle and affect the society-water relationship. Constantly increasing human population and its expectations regarding the standard of living increase demands on exploitation of existing resources including water (Cotruvo et al., 2004). Different uses of water affect both the quality and the quantity of the water available and the management of water pollution and water resources play an important role at both national and international level. Availability of good quality potable water is affected also by global climate changes that cause shortages and overexploitation in some places and flooding in other places with all related consequences including decreased safety of food and potential disease transfer.

Many infectious diseases of animals and humans are water-borne. The agents of these diseases are transferred by ingestion of water contaminated with human or animal faeces that contain

pathogenic bacteria, viruses and parasites (protozoa, eggs of parasites). Monitoring of safety of water sources is based on determination of parameters that indicate pollution caused by sewage, animal excrements, storage of waste, animal manure and artificial fertilisers, and other (Fawell and Nieuwenhuijsen, 2003; Cotruvo et al., 2004). An important tool that helps to eliminate pollution of water sources is the Directive 2010/75/EU on integrated prevention of pollution and control that applies to industrial and agricultural installations with large pollution potential. The WHO Guidelines for Drinking-Water Quality (WHO, 1996; 2008; 2011) serve as a guide for the setting of national regulations and standards for water safety in support of public health. The requirements on the quality of water used for human consumption are determined by the Slovak Republic Government Regulation No. 496/2010 Coll.

Determination of microbiological safety of drinking water has traditionally been carried out by monitoring the counts of bacteria that serve as indicators of faecal contamination. Coliform bacteria (CB) and *E. coli* in drinking water indicate faecal contamination due to insufficient protection of water source, inadequate water treatment, hygiene protection and distribution or secondary contamination. Faecal streptococci or Enterococci (EC) are indicators of faecal contamination and general contamination. Their presence is an indicator of fresh faecal contamination and thus of serious shortcomings in protection of water sources and water safety. Despite the fact that ground water is filtered when passing through the soil, it is susceptible to microbial contamination, particularly with viruses, and requires periodical checking and should be disinfected when used for mass consumption. To ensure microbiological safety of potable water various disinfection technologies are used. A sufficient quantity of good potable water cannot be ensured without protecting the water sources (Act No. 29/2005 Coll.; Act No. 322/2003 Coll.).

The aim of the study was to monitor changes in the quality of water obtained from ground water sources that was intended for mass consumption (farms, villages).

MATERIALS AND METHODS

Monitoring of quality of ground water in an agricultural area of eastern Slovakia focused on determination of physico-chemical parameters and bacterial counts indicating quality and potential pollution of water sources. Samples of water for examination were collected from May 2014 up to March 2015, to cover all four seasons.

Chemical examination of surface and ground water included determination of pH, electrical conductivity, dissolved oxygen, chemical oxygen demand (COD_{Mn}), chlorides, nitrates, iron

and phosphates. In addition, sum of calcium and magnesium and free chlorine was determined only in potable water and total dissolved solids (TDS) only in surface water.

The pH was determined according to STN ISO 10523 by means of a pH-meter HACH and a WATERPROF pH Tester 30. Conductivity was determined by a conductometer WTW InoLab Cond 720 (Germany). Dissolved oxygen was determined electrochemically using an oxygen probe LDO HQ Series Portable Meters supplied by HACH and chemical oxygen demand by oxidation with KMnO_4 according to STN EN ISO 8467. Determination of Ca^{2+} and Mg^{2+} was carried out by titration according to Horakova et al. (2003), chlorides were determined by titration according to STN ISO 9297 by titration and nitrates with ion-selective nitrate electrode WTW (InoLab pH/ION 735P, Germany). Orthophosphates were determined colorimetrically using HACH DR 2800 analyser and a procedure recommended by HACH. Potable water was examined for the presence of active chlorine by titration according to STN EN ISO 7393-3 by titration (Horakova et al., 2003).

We determined colony forming units (CFU) of bacteria cultivated at 22°C (BC22) and 37°C (BC37) according to STN EN ISO 6222. A pour-plate method was used and the counts of BC22 and BC37 were determined using meat-peptone agar and aerobic incubation at relevant temperature for 24 hours. Coliform bacteria (CB) and *E. coli* were cultivated according to STN EN ISO 9308-1 using Endo agar (HiMedia, India) and incubation for 24 hours at 37°C and 43°C, respectively, and the characteristic colonies were counted. Determination of counts of faecal enterococci (FE) was carried out according to STN EN ISO 7899-2.

RESULTS AND DISCUSSION

Results of physico-chemical examination are presented in Table 1. Examination of ground water showed that some limits specified by SR Government Regulation No. 496/2010 Coll. was exceeded while the levels of pH, conductivity, dissolved oxygen, COD_{Mn} and active chlorine (residual concentration after disinfection of water intended for mass consumption) corresponded with the requirements on potable water.

Table 1 Results of physico-chemical examination of ground water

Chemical parameters	Legislative limits	Min – Max
pH	6.0 - 8.5	6.6-8.6
Conductivity	110 mS/m	28.7-103.6 mS/m
Dissolved oxygen	> 5mg/l	5.7-8.6 mg/l
Ca ²⁺ + Mg ²⁺	1.1-5.0 mmol/l	0.9-5.2 mmol/l
COD _{Mn}	15 mg/l	0.1-2.1 mg/l
Chlorides (Cl ⁻)	100 mg/l	< 212 mg/l
Nitrates (NO ₃ ⁻)	50 mg/l	4-98 mg/l
Iron (Fe)	2 mg/l	< 0.1 mg/l
Active chlorine		do 0.8 mg/l
Phosphates (PO ₄ ³⁻)	1 mg/l	< 2.1 mg/l

COD_{Mn} – chemical oxygen demand

Results of microbiological examination of monitored waters are presented in Table 2. Our microbiological examination concentrated on indicators of faecal contamination that is associated with transfer of many disease agents. The BC22 counts were exceeded in individual sources, where less strict requirements were applied, highest counts were detected also summer but also in winter. The counts of BC37 were much exceeded in individual sources practically in all seasons. Limit for CB counts in potable water was exceeded only once in water for mass consumption (in summer). In the same sample there was detected also 1 *E.coli* colony. It is possible that some contamination occurred before or during the sampling (from water tap). The faecal enterococci must not be detected in any 100 ml sample of water. Samples of ground water intended for mass consumption were free of indicators of faecal pollution except for sample from sources in summer (1, 11 CFU) and autumn (3, 40 CFU). This water is periodically checked for its quality and disinfected before distribution to consumers (Rosen, 2000).

Table 2 Results of microbiological examination of ground water collected in individual seasons

Season	Sample	E.coli	CB	FE	BC22	BC37
		Legislative limits according SR Government Regulations No. 496/2010				
		0 CFU/100ml	0 CFU/100ml	0 CFU/ 100 ml	200 CFU/ 1 ml	50 CFU/1ml
Spring	1a	0	0	0	170	49
	1b	0	0	0	6	0
	1c	0	0	0	0	0
	1d	0*	2*	0*	20**	2***
	1e	0	0	0	26	6
	1f	2*	12*	0*	36**	156***
Summer	1a	0	0	0	250	4
	1b	1	17	0	180	0
	1c	0	0	0	110	0
	1d	4*	21*	5*	>300**	178***
	1e	0	0	0	122	100
	1f	11*	22*	34*	>300**	200***
Autumn	1a	0	0	0	270	70
	1b	0	0	0	45	35
	1c	0	0	0	5	3
	1d	40*	70*	0*	75**	121***
	1e	0	0	0	53	38
	1f	3*	8*	0*	101**	11***
Winter	1a	0	0	0	5	4
	1b	0	0	0	2	0
	1c	0	0	0	0	2
	1d	3*	15*	0*	300**	260***
	1e	0	0	0	42	1
	1f	5*	10*	0*	89**	56***

CB – coliform bacteria (total coliforms); FE – faecal enterococci; BC22 – bacteria cultivated at 22°C; BC37 – bacteria cultivated at 37°C.

The limits for sources intended for individual consumption – max. 30 persons or capacity – are as follows: *0 CFU/10 ml; **500 CFU/1 ml; ***100 CFU/1ml.

CONCLUSION

Chemical examination of the quality of ground water intended for mass consumption indicated that the required level of some parameters was exceeded (chlorides, phosphates, nitrates). Bacteriological safety of this water is ensured by regular monitoring and disinfection. The contamination of individual sources of drinking water was not very high and could be improved by their cleaning and disinfection. However, removal of sources of potential contamination would appear as the best choice.

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EVALUATION OF GENOTOXIC CHANGES IN CELL CULTURES AFTER EXPOSURE TO MICONAZOLE USING MICRONUCLEUS ASSAY

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ABSTRACT

Possible genotoxic effect of miconazole on bovine lymphocytes cultures was investigated using a micronucleus assay. The cells of whole blood were exposed to miconazole (2.5, 5, 10, 25 and 50 $\mu\text{g}.\text{ml}^{-1}$) for the last 24 h of cultivation. Miconazole showed statistically significant reduction in cytokinesis-block proliferation index (CBPI) at concentration of 5 to 50 $\mu\text{g}.\text{ml}^{-1}$ ($p < 0.001$). The micronuclei induction showed statistically significance only in donor no. 2 at concentration of 25 $\mu\text{g}.\text{ml}^{-1}$. No statistical significance was observed in the percentages of nucleoplasmic bridge (NPB) and nuclear bud (NBUD) for both donor. Statistically significance of apoptosis and necrosis was observed at concentrations of 25 and 50 $\mu\text{g}.\text{ml}^{-1}$ ($p < 0.5$, $p < 0.01$, $p < 0.001$).). In conclusions the miconazole was able to cause a genotoxic effect.

INTRODUCTION

Miconazole is the azole antifungal agent with a fungistatic to fungicidal effect that is used for the topical treatment of cutaneous and mucosal mycoses. This agent is distinguished from other azoles by possessing two mechanisms of action. The first mechanism is shared with other azoles and involves the inhibition of ergosterol synthesis. Another mechanism involves inhibition of peroxidases, which results in the accumulation of peroxide within the cell resulting in cell death (Hašek, 2013).

The micronucleus test is recommended worldwide for screening chemicals for genetic toxicity. The cytokinesis blocked micronucleus assay (CBMN) extended to the so-called "cytome assay" is a method for detecting chromosomal aberrations, genome mutations, cytostatic effects or cell cytotoxicity that occur during cell division (Franzke et al., 2020). Cells are blocked at the binucleated stage, in which we can measure micronuclei (MNi), nucleoplasmic bridges (NPBS) originating from asymmetric chromosome rearrangements and/or telomere end fusions, as well as nuclear budding (NBUDS), which represent the mechanism by which cells remove amplified

DNA and are therefore considered a marker of possible gene amplification (El-Zein et al., 2008).

METHODS

Blood collection, cell cultivation and processing were performed according to Šiviková et al. (2018). 24 h before the end of cultivation we added miconazole at concentrations: 2.5, 5, 10, 25 and 50 $\mu\text{g}.\text{ml}^{-1}$. For CBPI, apoptosis and necrosis, 500 cells were assessed. The occurrence of micronuclei, NPB and NBUDS were assessed in 1000 binucleated cells. Statistical analysis was performed by χ^2 square test (BNMN, NPB, and NBUD) and ANOVA+ Dunnett's post test (CBPI, apoptosis, and necrosis).

RESULTS

The results of 24 h *in vitro* exposure to miconazole on bovine lymphocytes are shown in tables 1-3.

Table 1 Results of micronuclei induction and CBPI changes after 24 h in vitro exposure to miconazole in bovine lymphocytes

miconazole $\mu\text{g}.\text{ml}^{-1}$	Donor 1		Donor 2	
	% BNMN \pm SD	CBPI \pm SD	% BNMN \pm SD	CBPI \pm SD
DMSO	0.7 \pm 0.084	1.529 \pm 0.022	0.5 \pm 0.071	1.394 \pm 0.026
2.5	0.9 \pm 0.094	1.487 \pm 0.038	0.6 \pm 0.077	1.640 \pm 0.036
5	0.6 \pm 0.077	1.487 \pm 0.012	0.8 \pm 0.089	1.287 \pm 0.029 ***
10	0.8 \pm 0.089	1.412 \pm 0.033 ***	0.9 \pm 0.094	1.242 \pm 0.033 ***
25	0.5 \pm 0.071	1.274 \pm 0.017 ***	1.4 \pm 0.118 *	1.131 \pm 0.030 ***
50	1.1 \pm 0.104	1.213 \pm 0.028 ***	ND	1.083 \pm 0.006 ***

DMSO –dimethylsulfoxid (negative control) 0.1 %; ND – not defined

, * - statistical significance ($p < 0.05$; $p < 0.001$), χ^2 square test (BNMN) and ANOVA+Dunnett's post test (CBPI)*

The micronuclei induction showed statistically significance only in donor no. 2 at concentration of 25 $\mu\text{g}.\text{ml}^{-1}$. CBPI decrease was detected from the concentration of 10 $\mu\text{g}.\text{ml}^{-1}$ in donor 1 and concentration of 5 $\mu\text{g}.\text{ml}^{-1}$ in donor no. 2 (Table 1). Frequency of NPB and NBUD changed slightly without statistical significance (Table 2). Apoptotic cells occurrence was increased in

significant manner at concentrations of 25 and 50 $\mu\text{g.ml}^{-1}$ in donor no. 2 only. Necrosis in higher incidence was seen at concentrations 25 and 50 $\mu\text{g.ml}^{-1}$ in both donors.

Table 2 Results of the NPB and NBUD occurrence after 24 h in vitro exposure to miconazole in bovine lymphocytes

miconazole $\mu\text{g.ml}^{-1}$	Donor 1		Donor 2	
	% NPB \pm SD	% NBUD \pm SD	% NPB \pm SD	% NBUD \pm SD
DMSO	0.1 \pm 0.032	0	0.1 \pm 0.032	0.2 \pm 0.045
2.5	0.1 \pm 0.032	0	0.2 \pm 0.045	0.1 \pm 0.032
5	0.2 \pm 0.045	0	0.1 \pm 0.032	0
10	0.2 \pm 0.045	0.1 \pm 0.032	0.2 \pm 0.045	0.2 \pm 0.045
25	0	0	0.2 \pm 0.045	0.1 \pm 0.032
50	0	0	0.3 \pm 0.055	0

DMSO –dimethylsulfoxid (negative control) 0.1 %; χ^2 square test

Table 3 Results of the apoptosis and necrosis occurrence after 24 h in vitro exposure to miconazole in bovine lymphocytes

miconazole $\mu\text{g.ml}^{-1}$	Donor 1		Donor 2	
	% apoptosis \pm SD	% necrosis \pm SD	% apoptosis \pm SD	% necrosis \pm SD
DMSO	2.933 \pm 0.116	2.733 \pm 0.306	2.400 \pm 0.4	2.633 \pm 0.153
2.5	2.600 \pm 0.2	3.200 \pm 0.2	2.8 \pm 0.2	2.867 \pm 0.416
5	2.267 \pm 0.416	2.467 \pm 0.416	2.2 \pm 0.529	3.000 \pm 0.2
10	2.733 \pm 0.416	2.533 \pm 0.231	2.993 \pm 0.116	2.733 \pm 0.306
25	2.800 \pm 0.721	3.533 \pm 0.306 *	3.267 \pm 0.306 *	3.533 \pm 0.306 **
50	3.867 \pm 0.306	4.133 \pm 0.306 ***	3.933 \pm 0.116 ***	4.267 \pm 0.116 ***

DMSO –dimethylsulfoxid (negative control) 0.1 %

, **, * - statistical significance ($p < 0,05$; $p < 0,001$), ANOVA+Dunnett's post test*

DISCUSSION

Miconazole - synthetic imidazole, is used for the treatment of superficial mycoses, cutaneous candidiasis, dermatophytosis, and other infections. It is generally applied topically to treat the diseases like ring worm, perioral candidiasis, jock itch, and athlete's foot. (Tayah and Eid, 2023).

We compared our results with research on other conazols due to the lack of miconazole testing results. Schwarzbacherová et al. (2017) tested Tango[®] fungicide (fenpropimorph and epoxiconazole) on bovine lymphocytes where no clastogenic/aneugenic effect was observed. On the other hand, significant decrease ($p < 0.01$) in CBPI was found. For instance, testing the

pure active agent epoxiconazole on bovine lymphocytes showed similar results; no aneugenic effect and decrease in CBPI (Šiviková et al., 2018).

Other pesticide formulations Orius® (Šiviková et al., 2013) and Prosaro® (Schwarzbacherová et al., 2015) containing triazole active agents (tebuconazole and tebuconazole plus prothioconazole respectively) were not able to induce micronuclei at significant levels in bovine lymphocytes *in vitro* but caused significant proliferation decrease after 24 and 48 h exposure. Yüzbaşıoğlu et al. (2008) they studied fluconazole on human lymphocytes. MN frequencies were significantly increased in a dose-dependent manner compared with the negative control. CBPI were not affected by treatments with fluconazole. According to these results, fluconazole is clastogenic and aneugenic in human lymphocytes, but these effects could not be observed in mice.

CONCLUSION

The fungicide miconazole acted possible genotoxic effect on bovine lymphocytes after 24 h exposure under *in vitro* conditions.

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A RARE CASE OF CANINE OCULAR DIROFILARIOSIS AS A CONSEQUENCE OF CLIMATE CHANGE AND MOSQUITOES ADAPTATION IN EASTERN SLOVAKIA

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ABSTRACT

Climate influences mosquito survival and reproductive rates, affecting environmental suitability, distribution, abundance and activity during the year, and the rate of development, survival and reproduction of dirofilarians in mosquitoes. The ability of heartworms to reproduce and evolve in hosts and mosquitoes is the result of a joint coevolution that affects the genetic makeup of both pathogen, vector and host. Their interaction is a dynamic process that is constantly ongoing and under pressure from changing external factors. At the Veterinary Clinic Elephant, a filarial nematode was surgically removed from the third eyelid of a dog in 2019 and subsequently identified morphologically and molecularly as *Dirofilaria (Nochtiella) repens* based on the 5.8S rRNA and cox1 mtDNA genes.

INTRODUCTION

It is currently estimated that up to 45% of the human and animal population in Europe are at risk of infection by nematodes transmitted by insect vectors (Otranto et al., 2013). *Dirofilariasis* was first described by Francesco Birag in 1626, who found worms, later named *Dirofilaria immitis* (Leidy, 1856), in the heart of a hunting dog from the Pad valley area (Italy). Currently, the situation of this disease, especially in canine carnivores, is alarming and has invaded the whole of Europe, Slovakia included. The disease has zoonotic potential and, although humans are considered an aberrant host in which the life cycle of the parasite is incomplete, the subcutaneous nodular form, of which *Dirofilaria repens* is the causative agent, is being diagnosed more and more frequently in humans (Capelli et al., 2018). According to ESDA data

(2021), more than 4,200 cases of infection caused by *D. repens* species have been registered in humans in Europe so far, and only 32 cases caused by *D. immitis* species. Most of these cases have been reported from Ukraine, the Russian Federation, Italy, France, Austria, Hungary and Romania. Outside Europe, however, *D. striata*, *D. tenuis*, *D. ursi* and *D. spectans* have also been isolated from humans. However, not every mosquito is capable of transmitting infectious microfilariae in natural populations, although experimental infection has been successful in many mosquito species, such as *Culex erraticus*, *Cx. modestus*, *Cx. nigripalpis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Aedes canadensis*, *Ae. caspius*, *Ae. excrucians*, *Ae. scapularis*, *Ae. sierrensis*, *Ae. sollicitans*, *Ae. stimulans*, *Ae. taeniorhynchus*, *Ae. trivittatus*, *Ae. vexans*, *Anopheles maculipennis*, etc. Available literature sources list more than 77 species of mosquitoes in the family Culicidae (genera: *Culex*, *Aedes*, *Anopheles*, *Mansonia*, *Coquillettidia*, *Psorophora*, and *Culiseta*) that are capable of transmitting heartworms (Azari-Hamidian et al., 2009; Riahi et al., 2021). The occurrence of many of these mosquitoes has also been confirmed in Slovakia (Jalili et al., 2000; Bocková and Kočišová, 2016). Eastern Slovakia in particular has an exceptional position in terms of mosquitoes occurrence in recent times. Their significant abundance coupled with a warm, moderately dry climate and favourable annual rainfall create ideal conditions not only for mosquitoes survival, but also for the life cycle of dirofilariae. In the presented case study, we report a case of the ocular form of canine dirofilariosis and present selected factors that influence the increased prevalence of this disease in Slovakia (Miterpáková et al., 2020).

MATERIAL AND METHODS

A Chihuahua dog was admitted to the Elephant Veterinary Clinic in 2019 with a suspected case of eye worm. The dog lived with its owners in the Nad Jazerom district of Košice and had no travel history. The clinic performed extirpation of an encapsulated lesion from the third eyelid (*palpebrae tertiae*) of the dog. The worm was morphologically diagnosed as a representative of the family Onchocercidae and placed in 70% ethanol. Subsequently, DNA isolation was performed at the University of Veterinary Medicine and Pharmacy in Košice using the Qiagen DNeasy® Blood and the Tissue extraction kit (QIAGEN, Germany) according to the manufacturer's protocol. To identify the parasite, we selected a section of the 5.8S ribosomal RNA gene and the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene. After analysis, we compared the obtained sequences with known sequences in the NCBI gene database in the BLAST program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

RESULTS AND DISCUSSION

In the reported case, we confirmed by molecular methods that the clinical picture - mild conjunctivitis, foreign body sensation - frequent scratching of the eye with the paw, itching, swelling and hypersensitivity was caused by encysted female *Dirofilaria repens* on the third eyelid. It was an autochthonous case of a rare ocular form of dirofilariosis in Košice. Differential diagnosis was necessary to distinguish other eye worms, e.g. *Onchocerca lupi* and *Thelazia callipaeda*. It is the canine thelaziasis that has an increasing tendency of occurrence in Slovakia in recent years (Miterpáková et al., 2022). Other autochthonous cases of the cardiac form of heartworm disease in dogs caused by *D. immitis* and in increased numbers by *D. repens* have also been confirmed in the districts of Košice and Košice - surroundings (Miterpáková et al., 2008, 2020). From a public health perspective, the most significant risk factor is the high rate of overpopulation of *D. repens* in dogs. It is untreated dogs with no clinical signs and high blood counts of microfilariae that are the main source of infection for the increasingly frequent cases of human dirofilariosis (Miterpáková et al., 2017). Therefore, monitoring the prevalence of canine dirofilariosis and subsequent treatment, especially for latent infection, is so important (Pazdzior-Czapula et al., 2018). Mosquitoes are an essential part of the life cycle and spread of dirofilariae, acting not only as vectors but also as essential intermediate hosts in which microfilariae mature into infective L3 larvae. The length of maturation is variable and depends on the ambient temperature conditions. Development in mosquitoes can take 8-10 days at 28-30 °C, 11-12 days at 24 °C and 16-20 days at 22 °C (Cancrini and Gabrielli, 2008). *D. immitis* and *D. repens* females develop for 120-180 and 189-259 days, respectively, and release microfilariae into the blood of the definitive host. According to a study by Genchi et al (2009), it was found that a minimum environmental temperature of 14 °C is required for the development of microfilariae in mosquitoes. The infective microfilariae then migrate to the mosquito's oral tract from where they are inoculated into the new host when the mosquito is sucked again. Transmission of dirofilariae is dependent on the presence of sufficient numbers of infected microfilariae, susceptible mosquitoes and a suitable climate that allows external incubation of the parasites in the vector (Genchi et al., 2009). However, successful transmission of the parasite is also fully dependent on the vector's ability to survive, as mosquitoes will die if the number of heartworms is high. Important attributes in mosquito bionomics for dirofilariae transmission are: the vector's host-seeking/preferring behaviour; the ability to move away from the hatchery; the geographical distribution and activity of the mosquitoes; the number of generations per year; the population size; and the seasonality of the mosquitoes (Genchi et al., 2009). We assume that the spread of dirofilariosis in Slovakia is also influenced by the

overpopulation of foxes and their synanthropic way of life makes foxes an important natural reservoir. In eastern Slovakia, the prevalence of *D. repens* in foxes ranges between 53 and 79 % (Hurníková and Miterpáková 2008; Hurníková et al., 2010). Thus, heartworm disease has become a problem that occurs every year, and the number of cases of the disease in dogs is steadily increasing. Previous studies have confirmed that one of the main vectors of both *D. repens* and *D. immitis* in Slovakia is *Aedes vexans* (Bocková et al., 2013, 2015). As we found *Ae. vexans* and *Cx. pipiens* are among the dominant mosquito species of eastern Slovakia (Kočišová et al., 2022).

CONCLUSION

It follows that dirofilariosis has become a common disease in Slovakia. Cases of worm migration to various non-traditional organs will increase in both dogs and humans. Dogs with a latent form of the disease and climatic changes affecting the occurrence of suitable vectors/mosquitoes will play a key role in its spread.

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EVALUATION OF THE EFFECT OF PURE ACETAMIPRID ON CELL CULTURES USING THE MICRONUCLEUS ASSAY

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ABSTRACT

Acetamiprid-based insecticides are used to many types of plants to protect them against various pests. In our study, we investigated genotoxic effects of the acetamiprid on the bovine peripheral blood lymphocytes *in vitro* after 24-hour exposure at concentrations of 5, 10, 25, 50 and 100 $\mu\text{g.ml}^{-1}$. Micronucleus (MN) induction was observed with statistically significant levels at the concentrations of to 25 $\mu\text{g.ml}^{-1}$ ($p<0.01$; $p<0.05$) for both donors. Cytokinesis-blocked proliferation index (CBPI) values decreased; statistically significant levels were obtained at the concentrations of 100 $\mu\text{g.ml}^{-1}$ ($p<0.001$) for donor no. 1 and at the concentrations of 25 $\mu\text{g.ml}^{-1}$ ($p<0.05$), 50 $\mu\text{g.ml}^{-1}$ ($p<0.01$) and 100 $\mu\text{g.ml}^{-1}$ ($p<0.001$) for the second one.

INTRODUCTION

The micronucleus test (MNT) is very common test used to assess genotoxicity of many pharmaceutical and agricultural chemicals. It is recommended for its ability to detect both structural and numerical chromosome aberrations (Kirsch-Volders et al., 1997). Acetamiprid is an oral and contact neonicotinoid insecticide, highly neurotoxic to insect larvae and adults. It binds to and blocks the nicotinic acetylcholine receptor on the postsynaptic membrane (Zhang 2000). It has a high potential for bioaccumulation and is highly toxic to birds and moderately toxic to aquatic organisms (Lewis et al., 2016).

In our study, we decided to assess the possible genotoxic (MN frequency) and cytotoxic effect (CBPI changes) of acetamiprid on bovine peripheral lymphocytes *in vitro*. Ruminants represent suitable biomarkers of pesticide exposure due to their feeding; there is a risk of their exposure and the presence of residues in animal products.

METHODS

Blood taking, cell cultivation and its processing was performed according to Galdíková et al. (2019). Cells were exposed to acetamiprid concentrations (5, 10, 25, 50 and 100 $\mu\text{g.ml}^{-1}$) *in vitro* for 24 hours. 1000 binucleated cells were scored for each concentration tested for micronucleus (MN) occurrence (chí square test) (Fenech et al., 2003). Cytokinesis-blocked proliferation index (CBPI) index was evaluated in 500 cells in a row (ANOVA +Dunett's post test).

RESULTS

The results of 24-hour acetamiprid exposure are shown in Table 1.

Table 1. Dependence of MN frequency and CBPI on acetamiprid concentration after 24 h treatment

acetamiprid $\mu\text{g.ml}^{-1}$	Donor 1				Donor 2			
	BNMN		%BNMN \pm S D	CBPI \pm SD	BNMN		%BNMN \pm S D	CBPI \pm SD
MN number	1	2			1	2		
control	2	0	0.2 \pm 0.022	1.183 \pm 0.003	2	0	0.2 \pm 0.024	1.113 \pm 0.012
5	10	1	1.4 \pm 0.009**	1.166 \pm 0.005	10	1	1.2 \pm 0.007**	1.076 \pm 0.003
10	9	1	1.1 \pm 0.006**	1.159 \pm 0.004	8	1	1.0 \pm 0.024*	1.077 \pm 0.006
25	6	1	0.8 \pm 0.015*	1.161 \pm 0.006	7	0	0.7 \pm 0.024*	1.061 \pm 0.003*
50	6	0	0.6 \pm 0.019	1.157 \pm 0.002	4	0	0.4 \pm 0.005	1.056 \pm 0.012**
100	3	0	0.3 \pm 0.022	1.098 \pm 0.004***	3	0	0.3 \pm 0.006	1.030 \pm 0.008***

($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

This exposure led to micronucleus induction with statistically significant levels at the concentrations of 5, 10 $\mu\text{g.ml}^{-1}$ ($p<0.01$) and 25 $\mu\text{g.ml}^{-1}$ ($p<0.05$) for first donor and at the concentrations of 5 $\mu\text{g.ml}^{-1}$ ($p<0.01$), 10 $\mu\text{g.ml}^{-1}$ and 25 $\mu\text{g.ml}^{-1}$ ($p<0.05$) for second donor. CBPI values decreased with increasing concentration of the acetamiprid. Statistically significant levels were obtained at the concentrations of 100 $\mu\text{g.ml}^{-1}$ ($p<0.001$) for first donor and at the concentrations of 25 $\mu\text{g.ml}^{-1}$ ($p<0.05$), 50 $\mu\text{g.ml}^{-1}$ ($p<0.01$) and 100 $\mu\text{g.ml}^{-1}$ ($p<0.001$) for the second one.

DISCUSSION

In our work, we used MNT to evaluate the genotoxic effects of the insecticide acetamiprid in its pure form by two parameters, the frequency of MN and the change in the CBPI proliferation

index. Our results showed that acetamiprid was able to induce micronuclei and cause a decrease in the cell proliferation.

Similar results were obtained by Kocaman and Topaktaş (2007) who exposed the human lymphocytes to pesticide Mosetam[®] (acetamiprid); the dose dependent micronuclei induction was found. They used the following concentrations for 24 and 48 treatment: 25, 30, 35 a 40 $\mu\text{g.ml}^{-1}$. Proliferation was decreased at two highest concentrations tested.

Others detected dose dependent micronuclei induction after acetamiprid treatment in CaCo-2 cells (Çavaş et al., 2012). The authors tested acetamiprid in concentrations: 25, 50, 100, 75, 150 and 300 $\mu\text{g.ml}^{-1}$. Next, only the highest concentrations (150 and 300 $\mu\text{g.ml}^{-1}$) caused statistically significant decrease of nuclear division index, which is similar to CBPI. In human pulmonal cells IMR-90, there were obtained similar results to previous (Çavaş et al., 2014), micronuclei induction and a decrease in the proliferation after acetamiprid treatment were seen. Furthermore, other neonicotinoid insecticides were able to cause changes in micronucleus induction and proliferation, e.g. imidacloprid in human lymphocytes (Demsia et al., 2007), thiacloprid in bovine peripheral lymphocytes (Galdíková et al., 2019) or clothianidin in mice erythrocytes (Calderón-Segura et al., 2015).

CONCLUSION

Induction of MN and decrease in CBPI proliferation index indicate to possible genotoxic effects of acetamiprid on bovine peripheral lymphocytes; further analysis are needed to confirm these results.

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OCCURRENCE OF *CRYPTOSPORIDIUM* SPP. AND *ENCEPHALITOZOON* SPP. IN SOIL SAMPLES IN SLOVAKIA

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ABSTRACT

Little attention is currently paid to the detection of the opportunistic pathogens we investigated from soils. They are a threat especially for children under five years old, who usually get sand or clay in their mouths while playing. In this work, we focused on the detection of *Cryptosporidium* spp. and *Encephalitozoon* spp. from soil samples from different regions of Slovakia. We proved the different genotypes of *C. parvum* by Nested PCR and *E. intestinalis* by Real-time PCR, which compared to traditional microscopy-based methods offer potential advantages such as increased sensitivity, higher specificity and easier interpretation.

INTRODUCTION

Agricultural land and soil around farms represent a significant source of infection due to contamination by microbial pathogens from agricultural runoff contaminated with livestock faeces. Bradbury et al. (2021) reported in their studies that *Cryptosporidium* spp. was detected in 5.7% of the investigated soils in Mississippi, USA, while animal husbandry near the sampling sites was an important factor influencing the results of this study. Public parks can also be a source of infection, and soil contaminated with microsporidia poses a risk to public health (Brusseau et al., 2005). Children are the most at-risk group, as they can accidentally ingest pathogens from contaminated soil while playing on playgrounds. (Dado et al., 2012). In China, microsporidial keratoconjunctivitis has been reported in pediatric and adolescent patients after exposure to mud while playing rugby (Kwok et al., 2013). Two studies from Singapore also reported an increasing incidence of microsporidial keratitis after contact with soil (Loh et al., 2009; Tan et al., 2013). In 2012, Dado et al. reported that 6% of the investigated parks in Madrid, Spain, were contaminated with *Cryptosporidium* spp. and suggested that children were mainly infected by accidental ingestion of contaminated soil. Based on previous studies, it can be said that the detection of parasites *Cryptosporidium* spp. and *Encephalitozoon* spp. from environmental samples is more than desirable as a prevention against diseases caused by these species.

METHODS

Collection and preparation of samples, DNA extraction

As the parasites studied by us have zoonotic potential and they are not transmitted only from animals to humans, but also to the environment, we tried to take soil samples as close as possible to cooperatives, farms, or areas with a lower standard of living for people, without sewage. Environmental soil samples were taken from six locations in Slovakia. From the east, they were the farm in Zemplínska Teplica, the farm in Košická Polianka, the farm in Veľké Raškovce, the Roma settlement in Jasov and the Roma settlement in Nálepko. From central Slovakia, we examined soil from the agricultural cooperative Bánová and from the Roma settlement Čelovce. The soil was always taken from several places so that it contained different components (sand, clay, animals...) After removing stones, plant remains and unwanted particles, each soil sample was stored at 4 °C until further processing. Before DNA extraction, According to the procedure of Claudel et al. we added 50 ml of saline solution (0.09% NaCl) to each soil sample and filtered through a large sieve. Subsequently, we homogenized the sample with a Precellys 24 device (Bertin Technologies, GmbH, Frankfurt am Main, Germany) 2 x 45 s at 6500 rpm. / min. For optimal homogenization, we poured glass beads with a diameter of 0.5 mm and zirconia beads with a diameter of 1.0 mm into the microtubes, added a soil sample and filled them with a lysis solution. The DNA isolation itself was carried out using the GF-1 isolation kit, which we used in this experiment due to its high oocyst capture capacity, while strictly following the manufacturer's instructions.

PCR amplification

To detect cryptosporidia from soil samples, we used a modified Nested PCR according to the procedure of Xiao (2010) aimed at amplifying the 60 kDa glycoprotein gene (gp60) of *Cryptosporidium* spp. The master mix (Solis BioDyne, Estonia) required for the PCR reaction (50 µl per sample) contained: 0.6 µl 5 U Taq DNA polymerase (FIREpol); 1.2 µl 0.1 µM primers specific for the genus *Cryptosporidium* (forward and reverse): GP_F1 (5'-ATGAGATTGTCGCTCATTATC-3'), GP_R1 (5'-TTACAACACGAATAAGGCTGC-3'), for the first reaction and GP_F2 (5'-GCCGTTCCACTCAGAGGAACC-3'), GP_R2 (5'-CACATTACAAATGAAGTGCCGC-3') for the second reaction; 32 µl H₂O for PCR; 5 µl Buffer with detergent; 4 µl MgCl₂; 1 µl dNTPs and 5 µl template. PCR reactions were carried out in a thermal cycler (XP Thermal Cycler Blocks), with a program consisting of an initial denaturation of 5 min. at 95 °C, 30 cycles of denaturation (30 seconds at 95 °C), annealing (45 seconds at 54/58 °C) and polymerization (1.5 minutes at 72 °C) and final polymerization (7

minutes at 72 °C). We analyzed the final 450 bp products (for primers targeting the gp60 gene) on a 1.5% agarose gel stained with GoodView-Nucleic Acid Stain in TAE buffer. The sequencing service verified positive samples using the Sanger sequencing method, and the final sequences were compared to homologous sequences deposited in GenBank using BLAST.

Detection of *Encephalitozoon* spp. from the examined soil samples, we performed by Real-time PCR, using the PCR mix SYBR Green. The total volume of the reaction mixture was 25 µl, which contained: 12.5 µl FastStart Universal SYBR Green Master (Roche); 0.5 µl Specific primers (0.3 µM): PMP1 (5'-CACCAGGTTGATTCTGCCTGAC) and PMP2 (5'-CCCTTCCGGAACCAAACCCTG); 4.5 µl H₂O for PCR and 7 µl template. PCR reactions were carried out according to Malčėková et al. (2013) in a thermal cycler (LightCycler 480 II, Roche). The program consisted of the following steps: incubation (2 minutes at 50°C), initial denaturation (10 minutes at 95°C), 40 cycles of denaturation (15 seconds at 95°C) and hybridization (1 minute at 60°C) and melting (melting temperatures 15 seconds at 95 °C). A sample whose melting point was the same or within ± 0.5 °C of the melting point of the positive control sample was considered positive. Positive PCR products were subsequently sent for sequencing from both directions. The sequences were compared with known sequences in the GenBank NCBI database.

RESULTS

Sequencing confirmed the positivity of the soils for the pathogens we investigated, specifically, the soil sample from the Roma settlement of Jasov contained *C. parvum* genotype IIAA15G2R1 and the soil sample from Čeloviece the same species, genotype IIAA18G1R1. Soil from the farm in Zemplínská Teplica showed positivity for *C. parvum* genotype IIAA18G1R1, as well as *E. intestinalis*.

DISCUSSION

Infection with cryptosporidia and microsporidia through soil is less widespread, but several cases are known, mainly in childhood (Dado et al., 2012; Deka, et al., 2022). The recommendation to include these pathogens in the monitoring network of local authorities was made by Panagiotou et al. (2022) in their studies, where up to 95% of tested farmers were positive for *Cryptosporidium* spp. or *Encephalitozoon* spp. In this work, we investigated the occurrence of cryptosporidia and microsporidia in soil samples collected near farms, cooperatives and in areas without sewerage, with a lower standard of living. We detected cryptosporidia and microsporidia from environmental samples (soil) together in three samples.

In the Roma settlements of Jasov and Čelovce, we identified the presence of *C. parvum*, genotypes IlaA15G2R1 and IlaA18G1R1. The presence of *C. parvum* genotype IlaA18G1R1 and *E. intestinalis* was confirmed by sequencing from a soil sample from the farm in Zemplínska Teplica.

CONCLUSION

Due to the frequent occurrence of *Cryptosporidium* spp. and *Encephalitozoon* spp. in the environment, it is key to educate people in the field of hygiene and to motivate them to improve hygienic conditions, to dispose of human and animal excreta appropriately, and especially to report diseases caused by these species to the competent authorities. We diagnosed all the examined soil samples using molecular methods, which are still among the most reliable techniques for proving the presence of pathogens in the examined samples. Nested PCR still remains a reliable standard for the diagnosis of cryptosporidia, Real-time PCR appears to be a suitable method for the detection of microsporidia.

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ARE THE CLINICAL SIGNS SPECIFIC ENOUGH FOR THE DIAGNOSIS OF HAEMONCHOSIS IN SHEEP?

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ABSTRACT

Haemonchus contortus (Strongylida, Trichostrongylidae) is a haemophagous nematode in the abomasum of small ruminants. A typical clinical symptom of haemonchosis is anemia. The effort to prevent the development of infection often leads to excessive deworming, which is the possible cause of resistance against medicinal products.

Our aim was to recommend to farmers a procedure for an appropriate herd deworming strategy. We visited sheep farms in the South Moravian, East Bohemian and Zlín regions and collected faecal samples from sheep of different age categories for EPG of strongylid eggs and PCR examination for detection of *H. contortus*. During sampling, body condition was assessed - Body Condition Score (BCS) and conjunctival color was assessed using the FAMACHA scale. We examined sheep in 8 farms, a total of 202 animals. Using PCR, *Haemonchus contortus* was detected in the faecal samples of 89 sheep (44%). Of the PCR *Haemonchus* positive animals, 73.33% of the young sheep were anemic by 2 years of age, while in the adult animals' anemia occurred in only 33.65%. Statistical analysis confirmed that anemia was highly significantly more common in young PCR *Haemonchus* positive individuals than in adults ($p = 0.00025$, $p < 0.01$). In severe infection with strongylid nematodes, the incidence of PCR *Haemonchus* positive sheep is statistically highly significant compared to moderate and mild infection. As the number of strongylid eggs increases, the number of PCR *Haemonchus* positive sheep increases ($p < 0.01$).

A herd deworming strategy in our conditions, focus primarily on young animals under 2 years of age. Deworming is required for individuals with varying degrees of anemia according to FAMACHA, while up to 2 years of age it is necessary to classify anemia from grade 3, in older sheep from grade 4. Coprological examination can determine the intensity of infection with strongylid nematodes and subsequently deworm animals with medium and strong intensity of infection. One from important principle is not to deworm the whole flock. Quality nutrition, especially protein deficiency, plays a significant role in the fight against parasites

INTRODUCTION

Haemonchus contortus (Strongylida, Trichostrongylidae) is considered the most pathogenic nematode of small ruminants. It measures around 5 cm, lives in an abomasum, where it feeds by sucking blood. As a result of haematophagous activity, depending on the number of helminths, anaemia develops. Although *H. contortus* is mainly found in the tropics and subtropics, it has also spread to the temperate zone. Survival of adverse temperature conditions during the winter period is made possible by the hypobiosis of the fourth larval stage in the mucous membrane of the abomasum. The eggs of *H. contortus* are similar to other strongylid eggs. Using PCR, *H. contortus* eggs in faeces can be confirmed or excluded. Frequent deworming, which is one of the possible causes of the development of resistance of parasitic disease causative agents to the active substances contained in antiparasitic product, can be fatal for the host animal with such a pathogenic nematode as *Haemonchus contortus*.

Aims of the study

Farmers often deworm their animals without a diagnosis confirmed by coprological examination. We investigated clinical symptoms (anaemia, nutritional status) in connection with coprological examination (EPG) and molecular biological methods (PCR) in order to recommend to farmers a procedure for an appropriate herd deworming strategy that would protect the health of the animals and minimize the risk of the possible development of resistance of parasites.

MATERIAL AND METHODS

We investigated sheep farms in the South Moravian, Zlín and East Bohemian regions. We collected faeces samples from 20 animals from the herd. Different age categories of sheep were included. Faeces samples were taken from the rectum separately in two sample containers for EPG and PCR examination. During sampling, body condition was assessed - Body Condition Score, BCS, scale 1 (cachexia) to 5 (obesity). The colour of the conjunctiva was evaluated using the FAMACHA scale, scale 1, 2, physiological colour, 3 dubious, 4, 5 anaemia. Faecal samples were processed for EPG according to Mac Master and scored as mild infection 0-500, moderate infection 550 to 2000 and heavy infection >2000 strongylid eggs in 1 g faeces. Faecal samples for PCR were processed according to Melville et al. (2014). Statistical analysis was performed using the Statsoft Inc. statistical software STATISTICA, version 12. The analysis compared the frequency of PCR positive and negative samples and the frequency of PCR positive young and adult animals in individuals with physiological conjunctiva colour and with anaemia. The frequency of PCR positive and negative samples was also compared depending on the age and

nutritional status (BCS) of the sheep. Analysis was performed using the chi-square test with Yates correction. The results of quantification of strongylid nematode infection were tested for normality using the Shapiro-Wilk test. Due to the non-normal distribution of the data, the Mann-Whitney U test was used to compare the intensity of infection between PCR positive and negative individuals and between individuals with physiological conjunctiva colour and with anaemia. The significance level is set at 0.05.

Results

We examined sheep in 8 farms, a total of 202 individuals. Using PCR, *Haemonchus contortus* was detected in the faecal samples of 89 sheep (44%). In the South Moravian region, it was detected in most samples (> 15 out of 20), on the other hand, in Zlín it was detected only in a maximum of 3 out of 20 samples. Statistical analysis did not show differences in the detection of *H. contortus* depending on the age of the sheep or depending on the nutritional status (BCS) ($p > 0.05$). The assessment of anaemia according to FAMACHA showed that in sheep with physiological colour of the conjunctiva (FAMACHA grade 1,2,3) there is a statistically significantly higher proportion of *Haemonchus* PCR negative individuals ($p < 0.05$) compared to anaemic individuals (FAMACHA grade 4,5), where the ratio of PCR positive and negative individuals is proportional. Of the *Haemonchus* PCR positive individuals, 73.33% of the young sheep (up to 2 years) were anaemic, while in PCR positive adult individuals, anaemia occurred in only 33.65%. Statistical analysis confirmed that anaemia was highly significantly more common in young PCR *Haemonchus* positive individuals than in adults ($p < 0.001$). In severe infection with strongylid nematodes, the incidence of PCR *Haemonchus* positive animals is statistically highly significant compared to moderate and mild infection. With increasing number of strongylid eggs, the number of PCR *Haemonchus* positive sheep increases too ($p < 0.01$). In individuals with proven strong infection with strongylid nematodes, there was a statistically significantly higher prevalence of individuals with symptoms of anaemia (FAMACHA 4.5) compared to mild infection ($p < 0.01$).

DISCUSSION

Haemonchus contortus is a pathogenic nematode that has spread in our sheep farms and is the cause of poor health of sheep. Using the PCR method, we detected the presence of the parasitic nematode *Haemonchus contortus* eggs, but not the intensity of infection associated with the development of anaemia. The overall health of the animals on the pasture can be affected by a wide spectrum of strongylid nematodes. In our samples, the prevalence of haemonchosis increases with intensity of parasitic infection caused by strongylid nematodes. Young

individuals are more susceptible to infection and health complications (Scott, 2007). We have shown that young sheep under 2 years of age show a higher sensitivity to infection and pathogenicity of *Haemonchus contortus*. The course of the disease with the development of anaemia in young animals is more severe than in adult animals. The basic clinical symptom of infection with blood-sucking nematode *Haemonchus contortus* is anaemia, the degree of which can be assessed according to the FAMACHA guide. Our results show, that this method can be used especially for young animals under 2 years of age. Coprologically proven severe parasitic infection indicates the risk of haemonchosis, because as the number of strongylid eggs increases, the prevalence of *Haemonchus* positive animals is significantly higher.

CONCLUSION

A herd deworming strategy in our conditions must focus primarily on young animals under 2 years of age. This group must be checked regularly depending on the given infection conditions on the pastures. Individuals with varying degrees of anaemia require deworming according to FAMACHA and it is ideal to detect anemia already at stage 3 (especially in young animals under 2 years of age). Coprological examination can determine the intensity of infection with strongylid nematodes and help to select animals with medium and strong intensity of infection for deworming. One from important principle for maintenance sensibilities parasites to used medicament is not to deworm the entire herd, but always leave a part of the sheep without the application of an antiparasitic. Quality nutrition plays a significant role in the fight against parasites, especially the lack of proteins contributes to the development of infection (Wallace, 1996)

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INFLUENCE OF TEMPERATURE ON THE EFFICIENCY OF WASTEWATER TREATMENT

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ABSTRACT

The main objective of this work was to evaluate the ambient temperature in the vicinity of a wastewater treatment plant in conjunction with the determination of chemical parameters of the effluent (pH, total phosphorus, total nitrogen, ammonia nitrogen, chemical oxygen demand) and microbial contamination (faecal coliform bacteria, coliform bacteria, faecal enterococci and mesophilic bacteria). The study found high variability in the microbiological and chemical content of the wastewater. The efficiency of the purification process in the analysed wastewater treatment plant was very high.

INTRODUCTION

Wastewater treatment plants are one of the major sources of release of faecal coliform bacteria, faecal enterococci, mesophilic bacteria, and antibiotic-resistant microorganisms, especially *E. coli*, into various environments worldwide, posing a health risk to humans and animals (Yang et al., 2022). As a result, rivers and small streams into which onsite wastewater treatment facilities discharge can also become an important source of nutrients, new pollutants, organic matter, and also the antibiotic-resistant bacteria mentioned above (Proia et al., 2016).

The general objective of biological wastewater treatment is to convert dissolved and particulate degradable elements into satisfactory end products. The biological process produces a large amount of biological wastewater sludge, which is composed of water, microorganisms, organic and inorganic components, e.g., total nitrogen (N_2), ammonium nitrogen ($N-NH_4$), and total phosphorus (P), as well as many pollutants such as significant metals, organic pollutants, and pathogens in sewage sludge. The wastewater treatment plant is affected by several factors, the most important of which is temperature. During the coldest and hottest seasons, there are tremendous challenges and it is difficult to understand what the optimal treatment of wastewater is, especially when the temperature varies from less than 4°C to more than 38°C (Alisawi, 2020).

This study provides an overview of the latest findings, current status and efficiency of wastewater treatment and suggests a direction for future developments.

METHODS

This study was based on an ambient temperature evaluation to determine the chemical parameters of the wastewater - pH, N₂, N-NH₄, P and chemical oxygen demand (COD). However, an evaluation of the detection of microbial contaminants of the wastewater (faecal bacteria, faecal enterococci, mesophilic bacteria and antibiotic-resistant enterobacteria) was also carried out. The samples examined from the influent and effluent of the wastewater treatment plant were taken in the summer season (from June to August). Individual species of enterobacteria from the water environment were identified using the Maldi ToF biotyper device. The isolated *E. coli* strains were found to have a minimum inhibitory concentration (MIC) of 20 antibiotics according to CLSI: VET01 (CLSI, 2018). By PCR analysis, ESBL genes (CTX-M group genes) were detected in the *E. coli* strains examined. Subsequently, the effect of ambient temperature on the efficiency of the wastewater treatment process and the spread of antibiotic-resistant *E. coli* in the environment was investigated.

RESULTS

The microbiological and chemical content of wastewater varies over the course of a year. The purification processes lead to the removal of organic substances (N₂, N-NH₄, P, COD, etc.), which can significantly affect the water quality of the rivers. The efficiency of the purification process in the studied wastewater treatment plant is very high, as shown in Table 1. Every chemical parameter determined in the treated water was within the required range, except for COD, which was above the limit (125 mg·l⁻¹). The COD in the influent reached a value of 861.6 mg·l⁻¹ and 298.3 mg·l⁻¹ in the effluent.

Identification of the effluent samples was confirmed using the Maldi ToF biotyper. Individual species of enterobacteria - mainly *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus faecalis* in the investigated samples were detected.

The highest level of antibiotic resistance among the *E. coli* strains tested was found to be 76% to ampicillin. The occurrence of *E. coli* resistant to cephalosporin - ceftiofur (58%) and ceftriaxone (31%) was also frequently detected. The ESBL phenotype was confirmed in 14% of *E. coli* strains. Genes (CTX-M group genes) were detected in 28% of the samples tested. These results showed an increased prevalence of enterobacteria with ESBL genes in the

wastewater treatment plant. However, increasing ambient temperature has an indirect effect on the occurrence of antibiotic resistance, but increases the spread of bacteria in the environment.

Table 1. Microbial and chemical evaluation of the efficiency of wastewater treatment

Parameters	Influent	Effluent	% of efficiency
Chemical parameters (limit)			
pH (6,0 – 9,0)	7.9	7.1	-
N₂/sum (40 mg·l⁻¹)	49.95 mg·l ⁻¹	3.56 mg·l ⁻¹	92.9%
N-NH₄ (30 mg·l⁻¹)	42.33 mg·l ⁻¹	2.5 mg·l ⁻¹	94.1%
P/sum (4 mg·l⁻¹)	352 mg·l ⁻¹	1.15 mg·l ⁻¹	99.5%
COD (125 mg·l⁻¹)	861.6mg·l ⁻¹	298.3 mg·l ⁻¹	65.35%
Microbiological parameters			
Faecal coliform bacteria	6.7·10 ⁴ CFU·ml ⁻¹	1.0·10 ³ CFU·ml ⁻¹	98,50%
Coliform bacteria	3.2·10 ⁵ CFU·ml ⁻¹	1.3·10 ⁴ CFU·ml ⁻¹	96,00%
Faecal enterococci	2.1·10 ⁵ CFU·ml ⁻¹	1.0·10 ³ CFU·ml ⁻¹	99,50%
Mesophilic bacteria	8.7·10 ⁵ CFU·ml ⁻¹	2.2·10 ⁴ CFU·ml ⁻¹	97,50%

DISCUSSION

Biological oxygen demand or COD is often used to evaluate and analyse organic pollutants in water systems and the efficiency of wastewater treatment plants. The level of COD in the examined wastewater treatment plant reached 694 mg·l⁻¹ in the influent and about 300 mg·l⁻¹ in the effluent (Hussain et al., 2013). In our study, similar values for COD were found in the influent (861.6 mg·l⁻¹) and in the effluent (298.3 mg·l⁻¹).

In the study of Ghimpusan et al. (2017), the average N-NH₄ removal was found to be 59-60%. While in our study, the amount of N-NH₄ was reduced from 42.33 mg·l⁻¹ to 1.15 mg·l⁻¹, which corresponds to 94.1% N-NH₄ removal.

Wastewater treatment processes are not specifically designed to remove all hazardous organic pollutants. These are taken up by aquatic organisms, which can pose a risk to the entire food chain (Rajasulochana and Preethy, 2016).

CONCLUSION

The main objective of this study was to evaluate the efficiency of wastewater treatment at chemical and microbiological levels with the detection of antibiotic resistant *E.coli*. Organic

and inorganic chemicals such as P and N₂ in the treated water entering the receiving water cause pollution and eventually eutrophication. Wastewater treatment leads to the removal of organic substances (N₂, P, BOD₅, COD, etc.) that can significantly affect water quality in rivers and streams. Every chemical parameter determined in the treated water was within the required range during the year. *Enterobacteriaceae* (*E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*) were detected in the samples tested from the influent and effluent of the treatment plant. The highest values of resistance of *E. coli* were found for ampicillin and cephalosporins.

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IMPACT OF AN AMBIENT TEMPERATURE ON A MILK PRODUCTION OF DAIRY COWS

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ABSTRACT

The main objective of this work was to evaluate the effects of high and low temperatures on milk production of dairy cows in Eastern Slovakia. We investigated and evaluated the milk efficiency of Holstein cows kept in a free stall barn with deep litter without additional cooling during the summer months. The decrease in milk yield was significant in summer between July and August, highest in May and June (40 ± 5.5 kg) and then decreased continuously from September to November (28 ± 5.5 kg).

INTRODUCTION

The general increase in global temperature and the incidence of extreme weather events with frequent heat waves is likely to increase in the Slovak Republic as well. Heat stress has a significant impact on milk production. The temperature and humidity index (THI) is often used to assess heat stress on dairy farms (Liu et al., 2019).

Dairy cows have difficulty coping with elevated temperatures of extreme heat, often resulting in lower milk yields and higher susceptibility to disease and other physical problems. Heat stress is a problem that could become a more critical issue for dairy farming in the future. While THI is an important environmental index and can also help determine the degree of heat stress, it does not reflect the physiological changes that dairy cows experience under heat stress. However, simultaneous measurement of THI and evaluation of physiological indices would be very useful to improve milk production. Extreme climate events could lead to serious problems in providing sufficient high-quality feed for dairy cows in the future and to an increase in costs for farmers who have to buy additional feed. A third aspect of climate change that could potentially be a problem for dairy cows is the increase in insect-borne diseases. Aspects of climate change can be beneficial, but can also introduce animal diseases that have not been seen before or have been rare. Milk production requires large amounts of water in feed production,

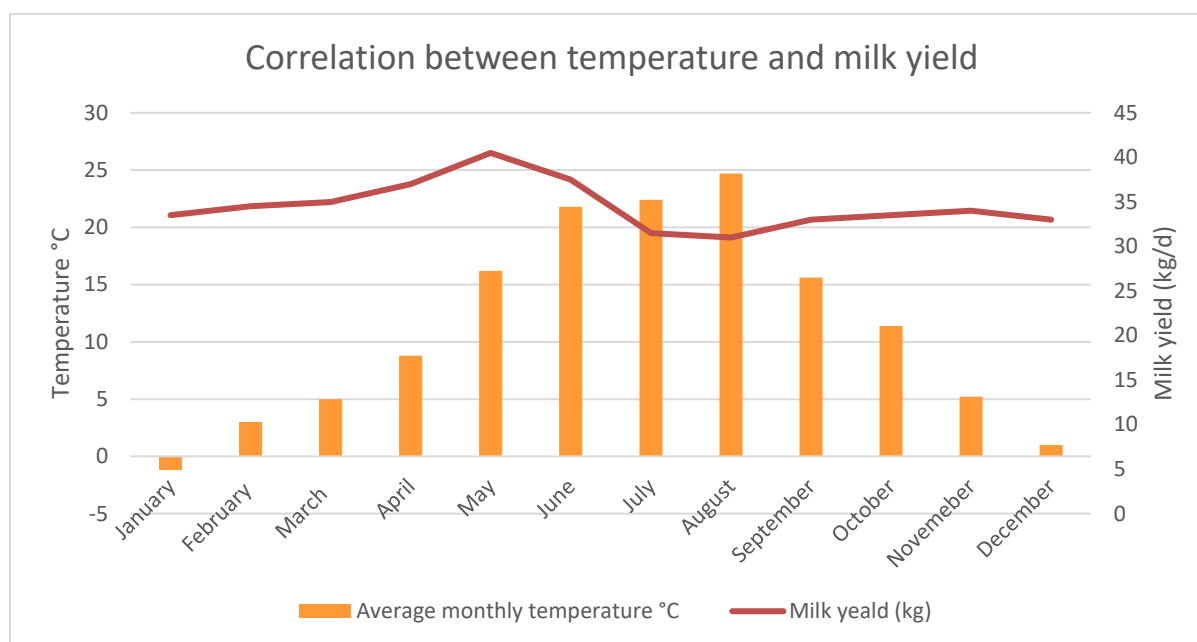
animal husbandry and milking. When water becomes a limited resource due to drought, milk production faces major problems (Liu et al., 2019; Karlsson et al., 2020; Moore et al., 2023).

METHODS

This study is based on the measurement of THI and evaluation of heat stress on milk production in dairy cows (Holstein-Friesian) on a farm in Eastern Slovakia. In our study, data (ambient temperature, relative humidity) were evaluated as one of the main factors influencing milk production in dairy cows. Meteorological data (temperature, relative humidity) in the barn were continuously recorded with electronic probes placed at the height of the animals and connected to a data logger. Testo digital thermometers and maximum-minimum thermometers were used to record the maximum and minimum temperatures. Testo digital hygrometers were used to measure and record relative humidity. The THI was used to measure and evaluate heat stress in order to quantify the relationship between climate and milk production. The THI is a combined measure of ambient temperature and relative humidity and is used to detect and quantify heat stress in dairy cows. However, in our study we assessed the influence of the average monthly ambient temperature in each month of the year on milk production.

RESULTS

The milking performance of Holstein-Friesian cows was compared during the year (Figure 1). Monthly average temperatures were measured from January to December throughout the year. The lowest monthly average temperatures were measured from December to February, when milk production was (33 - 35 kg/d). The highest milk production was recorded from May to June (40 ± 5.5 kg/d). Average daily temperatures were between 16 and 21.8°C. A significant decrease in milk production was observed in summer during the months of July and August, when daily average temperatures were the highest. The maximum average temperature in August reached 24.7°C, while the maximum daily temperature was up to 37°C on some days. Relative humidity varied between 60 and 85% during the year. In summer, the dairy cows' ability to release heat through the skin and evaporation is limited, so milk production decreases. Higher milk production was observed in dairy cows during the autumn months from September to November. The average milk yield was higher in autumn than in summer, reaching about 33 - 34 kg/d. This was a consequence of the decreasing ambient temperature in the cow shed and the lack of heat stress.

Figure 1. Correlation between temperature and milk yield

DISCUSSION

Heat stress is a non-specific physiological response of the animal to the thermal environment when the animal produces more heat than it can dissipate. Holstein-Friesian cattle are the most popular dairy breed in the world and also in the Slovak Republic. In summer, the ability of this breed to dissipate heat through skin evaporation is limited by the relatively low surface area to body weight ratio, underdeveloped sweat glands and short, dense hair on the body surface, which affects milk production. The decrease in milk production was significant between July and August (36.62 ± 6.62 kg/d vs. 32.26 ± 5.88 kg/d; $P < 0.01$). Milk yield was highest in May (41.55 ± 7.93 kg/d), as shown in a study by Novak et al. (2009). A similar situation occurred in our study when the highest milk yield was observed in May and June. In contrast, in the study by Marumo et al. (2022), a higher actual average daily milk yield was observed in winter (35.733 ± 0.481 kg/d) than in autumn (35.526 ± 0.481 kg/d), but this was not different from the yields in summer and spring. No significant difference was found between the actual average daily milk yield in summer (35.533 ± 0.481 kg/d) and spring (35.648 ± 0.481 kg/d).

CONCLUSION

Observation of changes in ambient temperature over the course of a year in relation to milk yield of dairy cows kept in a free stall barn on deep litter without additional cooling has an important position in the evaluation of milk efficiency of dairy cows. The results of our study show a decrease in milk production in summer in the months of July and August, when daily

average temperatures were highest and heat stress occurred in most dairy cows. However, milk production was highest in the months of May and June, when the microclimate on the cattle farm was favourable and pleasant. Average daily temperatures during these months ranged from 16 - 21.8°C and daily milk production was recorded to be 40 ± 5.5 kg/d.

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THE OCCURENCE OF SOIL-TRANSMITTED HELMINTHIASES AMONG HOSPITALIZED CHILDREN

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ABSTRACT

Children have a major role in transmission of soil-transmitted helminths (STH) infections. The objective of the present study was to assess the prevalence of STH among hospitalized children aged 0-18 years in Slovakia. The STH eggs were detected in 12.7 % of examined stool samples, at which *Ascaris lumbricoides* (12.5 %) and *Trichuris trichiura* (1.4 %) eggs were found. STH were detected only in children living in Roma settlements. Based on our results we can conclude that also the environment plays an important role in the transmission of STH in children.

INTRODUCTION

Soil-transmitted helminthiasis are a group of parasitic diseases caused by intestinal nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*) and the hookworms (*Ancylostoma duodenale* and *Necator americanus*). They are transmitted to humans by faecally-contaminated soil and are included in the World Health Organization list of Neglected Tropical Diseases (WHO, 2013). The impact that these infections have on health varies depending on the intensity of infection and the species, but soil-transmitted helminthiasis are regarded as the most important cause of physical and intellectual growth impairment (Bethony et al., 2006). School-age children are an important high-risk group for STH infections. STH impede physical growth and cognitive development of children. For this reason, school attendance and performance are affecting (Pasaribu et al., 2019; Safi et al., 2019).

The aim of this study was to monitor the occurrence of the most important soil-transmitted helminthiasis in children hospitalized in Košice, Prešov and Lučenec hospitals at whom an additional parasitological examinations were performed.

METHODS

In total, 513 paediatric hospitalized patients in Košice, Prešov and Lučenec aged between 0-18 years were examined coprologically during one year study. Morning stool was collected by hospital staff to the plastic containers (about 10-15g) after an informed consent was signed by parents and legal guardians. Containers were stored without any conservation in refrigerator and transferred to the laboratory for a diagnostics which was performed within 24-48 h. Samples were processed by commercially available kit (Paraprep L, Mondial, France) with subsequent microscopy (Papajová et al., 2021). Significance of differences between groups of children were determined using Student t-test at the levels of significance 0.05.

RESULTS

Totally 513 stool samples of hospitalized children were examined for the STH eggs presence. The most common clinical symptoms during hospital admittance of children were anaemia, gastroenteritis, diarrhoea, abdominal pain, nausea and/or vomiting, and fever of unknown origin. The overall prevalence of soil-transmitted helminths was 12.7 %. Only *Ascaris lumbricoides* (12.5 %) and *Trichuris trichiura* (1.4 %) eggs were found in the stool.

Table 1 Prevalence of soil-transmitted helminths among hospitalized children

Helminth species	Positive cases	%
<i>Ascaris lumbricoides</i>	64	12.5
<i>Trichuris trichiura</i>	7	1.4
Overall prevalence		12.7

The overall infection prevalence in hospitalized children is shown in Table 2. Hospitalized children were also divided according to the ethnicity and the environment in which they live. No children from majority population were found to be positive for the presence of STH. The STH infection prevalence among children from minority population living in the Roma settlements was 21.0 % (Table 2). The most prevalent helminths were *A. lumbricoides*. Ascarid eggs were found in 13.9% of the stool samples from children hospitalized in Košice, 3.6% of samples from Prešov and 3.2% of samples from Lučenec.

Table 2 Number of hospitalized children with soil-transmitted helminths according to hospitals and ethnicity

Hospital	Positive children	Total	Prevalence (%)
Lučenec	10	56	17.9
Košice	44	297	14.8
Prešov	11	160	6.9
minority	65	309	21.0*
majority	0	204	0

* Significance at the level $P < 0.001$

DISCUSSION

In our hospitalized children the prevalence of helminths was relatively high (12.7%). It can be due to the weakened immune system because helminths often belong among the opportune pathogens that attack the organisms of patients with serious respiratory disease (Shuji et al., 2006). Our results corresponded with those of Pipiková et al. (2017a) which studied the occurrence of intestinal helminth infections among the children living in Košice and Prešov counties. The most prevalent species was *A. lumbricoides* and *T. trichiura*. Similarly, Königová et al. (2010) confirmed the STH occurrence in 2.6% hospitalized children and the most common clinical symptoms were anaemia, bronchopneumonia, colitis and gastritis.

STH were confirmed only in hospitalized children from minority population living in the Roma settlements. Many of these settlements have polluted environment, low standard of personal and communal hygiene, lack of sanitation, waste pits or landfills, and remaining over occupancy in a small area where residents share their household with pets (Papajová and Šoltys, 2020; Šmigová et al., 2022; Ihnacik et al., 2022). Rudohradská et al. (2012) also reported that the faecal pollution of the environment represents for children patients a significant health risk. Similarly Pipiková et al. (2017b) studied the occurrence of parasitic infections among the children, dogs and its association with soil contamination in two villages with different hygiene level standards. Infections were present in both examined localities, but in the village with higher living standard, a better personal and communal hygiene level and better dogs care a lower occurrence of parasitic germs in soil was detected. High prevalence of protozoa and helminths was observed not only within canine population but also in children throughout the year in the village with lower hygiene and socio-economic standard.

CONCLUSION

Based on our results we can conclude that the STH infections still represent an actual health threat, especially for children. The poverty and poor hygiene contribute most to children health problems. The occurrence of STH depends on the contamination of the environment contaminated with animal and human feces. Eradication and public health programmes addressing hygiene standards need to be implemented by local governmental authorities.

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MEAT FROM IRRADIATED PIGS – HOW CAN BE USED?

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ABSTRACT

In the case of nuclear accidents (Fukushima), it is necessary to evacuate slaughter pigs from the nuclear accident vicinity as quickly as possible. In the course of the accident and especially during the transport, a whole-body or a partial external irradiation may happen, with a subsequent oxidative stress, which could be manifested by changes in the quality of the meat. In the experiment, pigs weighing 30 kg were exposed to a full body irradiation (⁶⁰Co) by a dose of 0.5 Gy. Samples from both, *longissimus thoracis et lumborum* and *semimembranosus* muscle were collected for analysis.

There were no statistically significant differences of monitored meat quality parameters among the control and experimental group.

INTRODUCTION

Over the past 50 years, the risk of farm animals exposure to ionizing radiation was represented mainly by highly unpredictable consequences of nuclear accidents (Chernobyl 1986, Fukushima 2011), impact of by-products of nuclear weapon tests, international terrorism and currently ongoing war conflicts.

External radiation dose, and radiation dose from internal contamination, may affect mainly farm animals in the immediate vicinity of the damaged nuclear facilities (Ohmori et al., 2014). Due to modern technologies in pig farming, it is possible for certain period of time, significantly reduce internal contamination of farmed animals by radionuclides. Nowadays, more and more emphasis is given on studying the effects of low doses of ionizing radiation on living organisms. In a case of a whole body exposure, the imaginary border between stochastic and deterministic effects of ionizing radiation on humans and pigs represents a dose of 0.5 Gy. Especially, the radical theory of ionizing radiation on living organisms presuppose the development of oxidative stress in irradiated individuals. This accelerates lipid peroxidation of polyunsaturated fatty acids liberating alkanes and alkane metabolites (Phillips et al., 2015). In particular, by this

oxidation-reduction process (redox state), the meat color and the levels of myoglobin, hemoglobin and cytochromes are affected (Bekhit and Faustman, 2005).

MATERIAL AND METHODS

In the experiment, 10 pigs of 30 kg were exposed to a full body irradiation (^{60}Co) by a dose of 0.5 Gy with a dose rate of $0.98 \text{ Gy} \cdot \text{h}^{-1}$. Another 10 pigs of the same size represented control group. Pigs were slaughtered and samples from both, *musculus longissimus thoracis et lumborum* and *semimembranosus* muscle were collected for analysis.

Muscle (meat) pH and color were measured at 45 minutes and after 24 hours postmortem. pH was measured using the Orion 250 A+ digital pH meter equipped with an Orion puncture electrode. Meat color was determined in the CIELAB system using the portable Color-guide sphere spex spectrophotometer (Byk Gardner).

Determination of drip loss was carried out by a standard method in the interval from 24 to 48 hours *post mortem*, and also by a modified method using a container in the interval from 0 to 24 hours *post mortem* (Dvorak and Mikulík, 1990; Honikel, 1998).

Measurement of the lactic acid concentration was performed 24 hours *post mortem* according to Korenekova et al. (2014). Determination of the water content and fat content was performed according AOAC 2003.

A two-sample t-test with unequal variances was used for average difference significance testing in both, experimental and control groups. A pair t-test was used for comparing average values at different locations of *longissimus thoracis et lumborum* muscle each other or against *semimembranosus* muscle. The pair t-test was also applied for testing the differences of the values 45 minutes and 24 hour post mortem. A significance level of $\alpha=0.05$ was determined for all hypotheses in the planned experiments. Statistical values were calculated by means of MS Excel.

RESULTS

Results are summarized in Table. No statistical differences in monitored meat quality parameters were found between the experimental and the control groups.

Table: Meat quality parameters of longissimus thoracis et lumborum muscle at last rib and semimembranosus muscles in the control and experimental groups after total body irradiation by 0.5 Gy.

	<i>m. longissimus thoracis et lumborum</i>	<i>m. longissimus thoracis et lumborum</i>	<i>m. semimembranosus</i>	<i>m. semimembranosus</i>
Parameter	Irradiated group mean \pm sem	Control group mean \pm sem	Irradiated group mean \pm sem	Control group mean \pm sem
pH ₄₅	6.01 \pm 0.083	5.93 \pm 0.115	6.01 \pm 0.082	6.11 \pm 0.100
pH ₂₄	5.53 \pm 0.015	5.53 \pm 0.057	5.56 \pm 0.028	5.61 \pm 0.013
L* ₄₅	43.39 \pm 1.163	45.92 \pm 1.274	42.36 \pm 1.254	42.44 \pm 1.938
L* ₂₄	55.48 \pm 0.523	54.84 \pm 1.041	50.93 \pm 0.626	49.26 \pm 1.598
a* ₄₅	-1.01 \pm 0.309	-0.87 \pm 0.460	1.72 \pm 0.886	3.03 \pm 0.777
a* ₂₄	0.08 \pm 0.300	-0.73 \pm 0.320	2.52 \pm 0.497	4.05 \pm 1.201
b* ₄₅	4.47 \pm 0.315	4.77 \pm 0.426	6.26 \pm 0.302	6.14 \pm 0.680
b* ₂₄	6.95 \pm 0.300	6.32 \pm 0.410	7.31 \pm 0.400	7.01 \pm 0.360
ΔE^* ₄₅	37.58 \pm 1.142	36.48 \pm 1.656	39.26 \pm 1.426	38.58 \pm 1.887
ΔE^* ₂₄	26.78 \pm 0.470	27.04 \pm 1.025	31.85 \pm 0.640	34.10 \pm 1.913
C* ₄₅	4.67 \pm 0.323	5.04 \pm 0.421	6.94 \pm 0.440	7.17 \pm 0.754
C* ₂₄	7.01 \pm 0.298	6.46 \pm 0.382	7.86 \pm 0.425	8.62 \pm 0.776
Water (%)	74.81 \pm 0.271	76.42 \pm 0.291	75.30 \pm 0.247	74.62 \pm 0.475
Fat (%)	3.63 \pm 0.292	3.23 \pm 0.196	4.56 \pm 0.207	5.04 \pm 0.556
Drip losses 0 – 24h [%]	2.03 \pm 0.288	1.97 \pm 0.238	1.44 \pm 0.195	1.47 \pm 0.194
Drip losses 24 – 48h [%]	2.00 \pm 0.232	2.06 \pm 0.263	1.46 \pm 0.197	1.50 \pm 0.191
Lactic acid [g.kg ⁻¹]	14.13 \pm 0.692	13.84 \pm 0.439	14.09 \pm 0.503	13.91 \pm 1.286

mean - arithmetical mean

sem - standard error of mean

** statistical significance ($\alpha = 0.05$)*

L, a*, b*, ΔE^* , C*, hab - meat color parameters (CIELAB)*

Index 45 - 45 minutes post mortem

Index 24 - 24 hours post mortem

DISCUSSION

Low doses of ionizing radiation up to 0.5 Gy represent an approximate boundary between the stochastic and deterministic effects of ionizing radiation in both humans and pigs. Stochastic effect in particular cause a higher incidence of oncological diseases, which in the case of slaughter animals has no practical impact. However, these doses may already represent an oxidative stress. In general, any stress (transport, pre-slaughter activities, etc.) represents a key factor of the PSE meat defects (Van de Perre et al., 2010). It can be assumed that during an accident of a nuclear power plant, animals (pigs) from the farms located in the power plant protected zone, will not be exposed to whole body irradiation exceeding a dose of 0.5 Gy. Thanks to modern technologies in pig farming, it is possible for a certain time period to significantly reduce internal contamination of slaughter pigs by radionuclides. Additionally, even in internally contaminated pigs, there is a possibility to reduce internal contamination by the use of common meat processing technologies (Dvořák et al., 2008). In conclusion, total body exposure of pigs to irradiation by a single whole-body dose of 0.5 Gy does not affect the quality of pork meat. In a case of a nuclear accident, unless there is no internal contamination, and the effective dose does not exceed 0.5 Gy, pigs from the exposed area may be used for meat production.

CONCLUSION

Our data show, that if during the nuclear accident there is no internal contamination, and the effective radiation dose does not exceed 0.5 Gy, pigs may be used for slaughter purposes and meat production.

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TICKS IN THE URBAN ENVIRONMENT IN KOŠICE (EASTERN SLOVAKIA) AS AN IMPACT OF GLOBAL CHANGE

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ABSTRACT

Hard ticks are important vectors of pathogens. Abundance of hard tick in the Košice city was studied during March and April 2022. Adult ticks and nymph were collected from 13 center and pericenter area in Košice city. A total of 756 ticks were collected by flagging from ground vegetation and were identified to species level. We identified 3 species *Ixodes ricinus*, *Dermacentor reticulatus* and *Haemaphysalis concinna*. Of all the total ticks we found 709 *I. ricinus* (289 males, 283 females, 137 nymphs), 45 *D. reticulatus* (14 males, 31 females) and 2 females *H. concinna*. Their high density poses a serious risk to visitors in urban areas.

INTRODUCTION

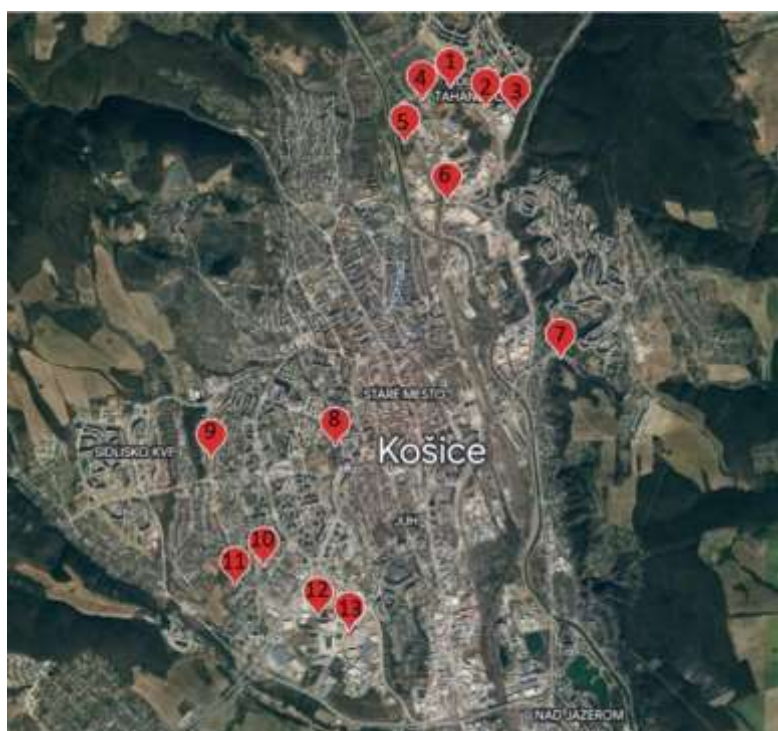
Ticks are an important group of ectoparasites, parasitic on various spectrum of hosts from reptiles to birds and mammals, and can vector a wide range of pathogens protozoal, bacterial, rickettsial and viral diseases of significance to both human and wildlife (Medlock et al., 2013a; Siuda, 1993). Urban landscapes are supported by green infrastructure, which provides a wide range of ecosystem services, from improving health and well-being to storing carbon and reducing heat (Office for National Statistics, 2018). Urban green infrastructure includes urban forests, open green spaces and green corridors (e.g. wildflower strips and hedgerows), which can form a network that supports diverse plant species and wildlife movement from surrounding rural habitats. This network can provide temporary or stable habitats for urban biodiversity and facilitate wildlife movement, particularly if nearby home ranges for wildlife exist (Evans et al., 2010; Hansford et al., 2023). However, this urban green is a tick habitat. Especially neglected, unmown areas in cities represent a risk to the public. Abandoned industrial sites and overgrown paths between different parts of the city are frequented by people walking their pets. These areas are risk of tick infestation. The presence of *I. ricinus* in urban green space is not necessarily a new phenomenon (Hansford et al., 2022; Medlock et al., 2013a; Uspensky, 2014), but tick encounters may have increased over time, particularly in recent years following initiatives to increase urban green space and its use by members of the public (Office for

National Statistics, 2018). The main goal of this work was to monitor ticks' abundance and species composition in central and pericentral as popular dog walking and sport localities which are often neglected in Košice, Eastern Slovakia, as potential risk areas for tick infestation.

MATERIAL AND METHODS

Questing ticks were collected by flagging with a white cotton blanket (1m^2) from 8 centre (C) and 5 pericentre (PC) localities in Košice, Eastern Slovakia, during the months of March and April 2022 (Fig. 1). The PC as in large urban developments on the periphery of the city, but with close contact to the open undeveloped area in the city and the C part as (green) completely isolated from peripheral urban green by extensive buildings and roads, of the city of Košice were chosen as the locations. These areas are used as recreational sites but are also neglected and the unmaintained vegetation provides an ideal environment for ticks and their hosts. The tick's ratio was estimated on the base of the number of active ticks collected by one person per one hour of flagging. To avoid affords during the study only the same two persons collected the ticks.

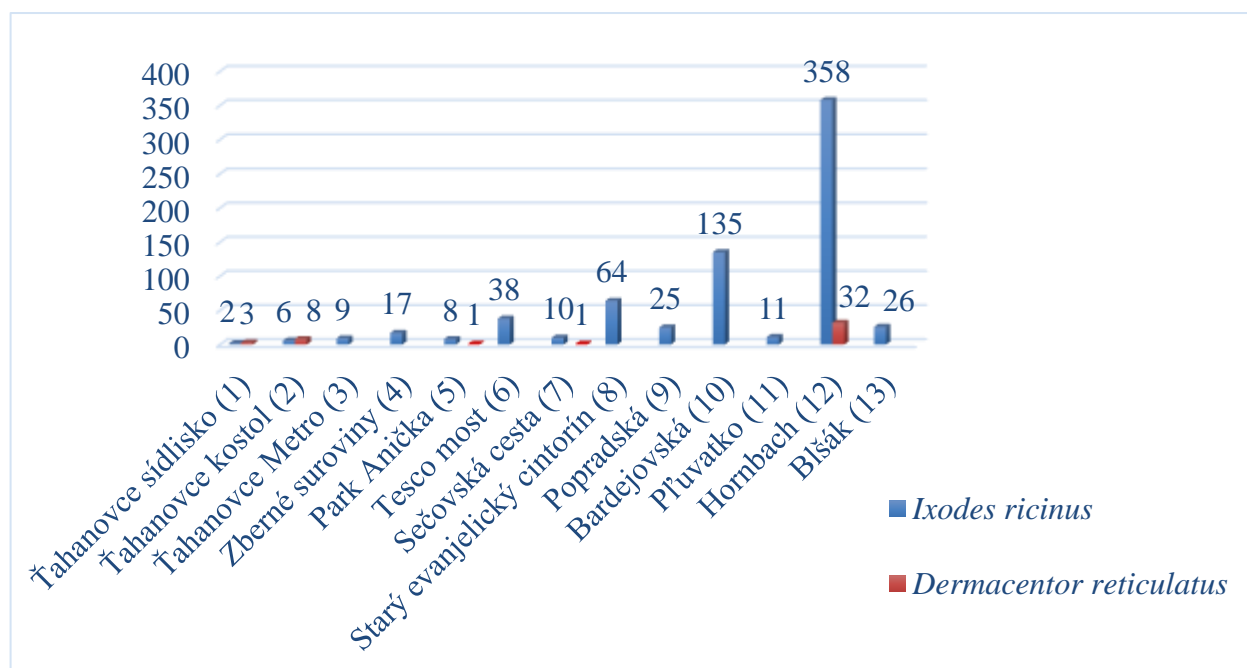
Figure 6, Selected localities in Košice (1 – Ťahanovce sídlisko (C), 2 – Ťahanovce kostol (C), 3 – Ťahanovce Metro (PC), 4 – Zberné suroviny (PC), 5 – Park Anička (PC), 6 – Tesco most (C), 7 – Sečovská cesta (PC), 8 – Starý evanjelický cintorín (C), 9 – Popradská (C), 10 – Bardejovská (C), 11 – Pľuvatko (PC), 12 – Hornbach (C), 13 – Blšák (C))



RESULTS

A total of 754 ticks we collected from 13 different locations in Košice in March and April 2022 (707 *I. ricinus*, 45 *D. reticulatus* and 2 *H. concinna*) (Fig. 2). No sampling was performed after July due to the particularly hot and dry weather conditions. Predominant species was *I. ricinus*. We found 289 males, 283 females and 137 nymphs *I. ricinus*, in all selected PC and C localities. In all C localities we found 651 *I. ricinus* (257 females, 268 males, 126 nymphs), more rarely collected ticks were *D. reticulatus*. We found 14 males and 31 females only in 3 C localities no. 1 - Ľahanovce sídlisko, 2 - Ľahanovce kostol, and 12 – Hornbach. The tick *D. reticulatus* was found for the first time in the central part of the city of Košice. On all PC sites we found 55 *I. ricinus* (24 females, 21 males, 10 nymphs) and 2 females *H. concinna* was found in Park Anička and Sečovská cesta.

Figure 2, Species representation of ticks on localities in Košice



DISCUSSION

The aim of this study was to evaluate the populations of hard ticks (Ixodidae) in the C and PC parts of the city Košice in recreational areas that are neglected almost all year round. The tick *Ixodes ricinus* is the most widespread and epidemiologically important tick species in Europe. It is mainly distributed in deciduous and mixed forests, forest edges and meadows. Its occurrence is influenced by host range, as well as by climatic conditions and the habitat (Lindgren et al., 2000; Vacek et al., 2023). A very detailed review of the occurrence of ticks in Slovakia towns was described by Stanko et al., 2021. The authors compiled a list of locations where *D. reticulatus* ticks occur in the surrounding area of Košice (Stanko et al., 2021). We

were the first to show a stable population of *D. reticulatus* by collecting directly in the centre localities in Košice. The increased risk of tick bites in urban green areas has been studied in many European countries (Medlock et al., 2013; Pet'ko et al., 1997; Uspensky, 2014). A number of studies show that ticks now find favorable conditions in urban green spaces in addition to pastures, meadows and forests. Stable tick populations have been reported from various urban parks in Europe, and many pathogens responsible for human and animal diseases have been detected in ticks collected from these urban areas (Kazimírová et al., 2016; Špitalská et al., 2014; Uspensky, 2014, Stanko et al., 2021). Parks and gardens are visited and enjoyed by many people every year during the spring and summer seasons, so it is important to fully assess the risk of tick bites in order to prevent this major public health threat. The occurrence and abundance of a certain tick species in a given habitat are mainly determined by abiotic factors, habitat characteristics and availability of hosts (MacLeod, 1935). In this study, we have shown the species representation of hard ticks in the city of Košice, in centre and pericentre areas, places popular with residents for walking dogs or playing sports, effective measures need to be implemented to reduce the tick abundance in urban areas.

CONCLUSION

In the centre and pericentre areas of Košice we found the presence of hard ticks of the *species* *I. ricinus*, *D. reticulatus* and *H. concinna*. The presence of *D. reticulatus* in the central part of the Košice was detected for the first time. These results indicate stable tick population in the city, regardless of the type of area surveyed, there is a high risk of tick bites and, consequently, of contracting tick-borne diseases.

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INFLUENCE OF FUNGICIDES TEBUCONAZOLE, EPOXICONAZOLE, AND PROTHIOCONAZOLE ON CONFORMATION OF HUMAN SERUM ALBUMIN

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ABSTRACT

Fungicides are widely used in agriculture as crop protection products. Knowledge about the effects and consequences of fungicide formulations' side - effects is necessary to prevent the use of inappropriate products or to limit the extent of their use. Studies of interactions between fungicides and target mammalian proteins are important steps toward an understanding a pesticide's toxicity. Human serum albumin is a mammalian transport plasma protein and in this study, it represents a target for impact of chosen fungicides on its macromolecule structure. Using biophysical methods of optical spectroscopy, a change of the protein conformation under fungicide influence was investigated.

INTRODUCTION

Binding of pesticides to plasma proteins has toxicological importance, and significantly affects the distribution and excretion of pesticides. Our study is expected to provide an important insight into the interactions of the transport protein human serum albumin (HSA) with fungicides tebuconazole (TB), epoxinazole (EPX), and prothioconazole (PTC), representatives of the conazoles group.

TB is the active ingredient used in Orius 25 EW, which protects a number of crops such as grapes, rice, fruits and vegetables because of its broad-spectrum antifungal activity. Its antifungal activity is due to its ability to inhibit the P450 enzyme, which blocks the conversion of lanosterol to ergosterol causing disruption of the fungal wall (Di Renzo et al., 2015). The US EPA has classified this fungicide as Group C-Possible Human Carcinogen (U.S. Environmental Protection Agency). EPX and PTC are the active ingredients used in Opal 7.5 EC (EPX) and Prosaro 250 EC (PTC), respectively. They actively stop the production of new fungi spores and

inhibit the biosynthesis of ergosterol. Inhibition of lanosterol-C14-demethylase (CYP51) leads to accumulation of methylated sterols in the fungal membrane, thereby impairing its function Goetz et al., 2006).

Serum albumins are the most abundant plasma proteins and contribute to a significant number of transport and regulatory processes. HSA has extraordinary binding properties. It can bind under the physiological conditions not only fatty acids, peptides, and proteins, but also low molecular weight endogenous and exogenous molecules (Wang et al., 2009; 2011). HSA is a monomeric protein composed of 585 amino acids with total molecular weight of 66 400 Da. They are organized in three main domains (I, II, III), where each can be divided into two subdomains (A and B). Domains II and III contain two primary drug's binding sites, known as Sudlow's site I and II [8]. An important part of Sudlow's site I is the only one Trp present in HSA (Trp214), as its indole ring and its rotation allow by a certain ligand nesting into the Sudlow's site and creating the stacking interactions. Molecules that specifically bind into the Sudlow's site I are bulky negatively charged heterocyclic molecules located in the middle of them.

The information about the possible impact of fungicide molecules on plasma proteins is still limited (Wang et al., 2011; Zhang et al., 2013). For this reason, we have studied an influence of three known fungicides TB, EPX, PTC on conformation state of HSA.

MATERIAL AND METHODS

TB, EPX, and PTC with 99% purity as well as HSA (fatty acid free, globulin free, purity no less than 99 %) were purchased from Sigma Aldrich, Darmstadt, Germany. The pesticides were dissolved in spectroscopic grade 100 % ethanol at concentration 10^{-3} mol/L. Stock solutions of HSA (concentration 5×10^{-4} mol/L) were prepared in Tris-Cl⁻ (0.05 mol/L Tris + 0.1 mol/L NaCl) buffer, pH 7.4 and conserved in the dark at 4 °C.

Three-dimensional fluorescence spectra were recorded on a SHIMADZU RF 5301 PC spectrofluorimeter (Shimadzu, Kyoto, Japan) in a 1 cm quartz cuvette at room temperature. Excitation wavelength was set at 220 nm with increments of 5 nm, and emission spectra were recorded in the range 250 nm to 500 nm. Fixed concentrations of HSA (2×10^{-6} mol/L, 2.5 mL) with 16 times higher concentration of TB, EPX and PTC were added to the sample cell. The samples were stabilized for 10 min.

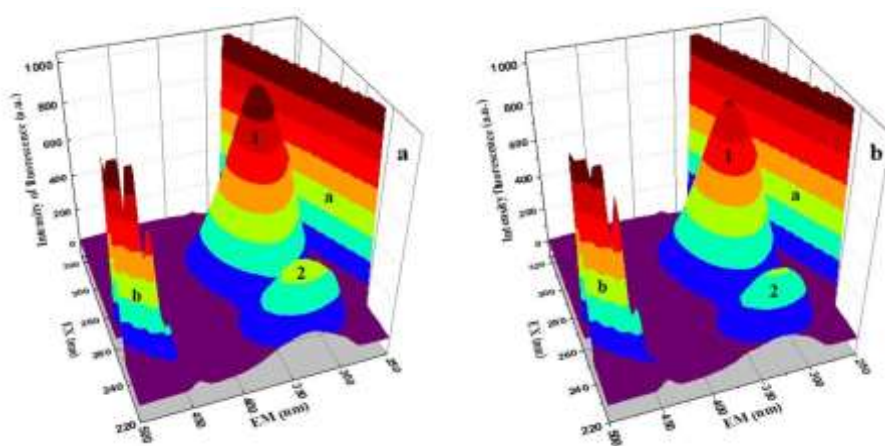
CD spectroscopy measurements were performed on a Jasco J-815 CD spectrometer (Jasco, Easton, MD, USA) in a 0.1 cm quartz cell at room temperature with constant nitrogen flush.

The CD spectra of HSA in the presence of TB, EPX, and PTC were recorded in the range 190 nm to 270 nm with scan rate 50 nm/min. Three scans were collected for each spectrum, taking the average as the final data. Complexes were prepared by means of titration of TB, EPX, and PTC into HSA (concentration 3×10^{-6} mol/L) to achieve final concentrations of TB, EPX, and PTC from 3×10^{-6} mol/L to 1.5×10^{-5} mol/L.

RESULTS AND DISCUSSION

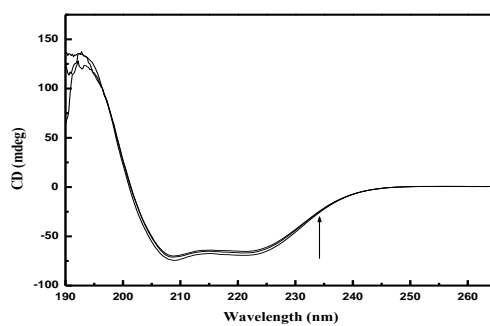
Three – dimensional fluorescence spectra (Fig. 1) and measurements of optical dichroism (CD spectroscopy) (Fig. 2) were carried out to investigate whether TB, EPX, and PTC affect HSA molecule conformation. In Figure 1b, it can be seen that the fluorescence intensity of peaks 1 and 2 decreased with the addition of EPX into HSA. Peak 1 represents the fluorescence contribution of Tyr and Trp and peak 2 represents the spectral properties of the polypeptide structural chain; its intensity correlated with the secondary structure of the protein [5]. This decrease indicates the binding of conazoles near the Tyr or Trp of the individual proteins. We can also observe a decrease in peak 2, which indicates changes in the peptide structure. Its declining trend after conazoles binding suggests that there is a slight destabilization of individual proteins and a slight unfolding of the polypeptide chain leading to conformational changes (Kalaierasi et al., 2018). This result correlates with absorption and CD spectroscopy measurements. No shifts in maximum emission wavelength were observed. This suggests that the conformation of HSA altered and TB, EPX, and PTC were located in the vicinity of Trp or Tyr residues on HSA.

Fig. 1. 3D fluorescence spectra of HSA (a) and EPX/HSA 16/1 complex (b)



Using the previous techniques, we found some conformation alteration in HSA cause by fungicides. To collect more information on the binding of pesticides to HSA we used the circular dichroism method. HSA presents two negative bands in the ultraviolet region at 208 nm and 222 nm, characteristic for the α -helical structure Lu et al., 1987; Kandagal et al., 2006). Two negative peaks contribute to $\pi \rightarrow \pi^*$ transfer (208 nm) and $n \rightarrow \pi^*$ transfer (222 nm) for the peptide bond in the α -helix (Greenfield, 2006). The CD measurements performed in the absence and presence of different concentrations of TB, EPX, and PTC revealed that the binding of pesticides to HSA caused a slight decrease in both bands (Fig. 2).

Fig. 2. CD spectra of HSA and HSA in the presence of EPX



According to mathematical equations (not shown), it is possible to determine α -helical structures content within the protein. Free HSA contains 55.75 % α -helical structures, and in the presence of fungicides, the content of α -helical structures gradually decreases to values approximately 2.5 % less. Therefore, we can state that fungicides TB, EPX, PTC cause changes in the secondary structure of HSA.

CONCLUSION

Better understanding of the interaction of pesticides with various possible molecular and/or cellular targets is essential for the determination of their function in biological systems. Moreover, this investigation contributes to the information about ecological impact on living organisms and nature.

The present paper focuses on results obtained in the study of chosen fungicides associations with serum albumins represented by HSA. The binding of fungicides to HSA leads to slight conformational changes in albumin macromolecule, as corroborated by our three-dimensional fluorescence and CD measurement outcomes.

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