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Review Article

Campylobacter Species in Poultry Slaughterhouses: An Overview

Key words

Campylobacter species;
poultry;
slaughterhouses;
foodborne diseases;
food safety

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Abstract: *Campylobacter* is a constant concern in ensuring food safety, as it is one of the most common pathogens in food. The main source of *Campylobacter* species in food is poultry meat, and the primary production of this meat is a critical point where measures need to be taken to reduce its presence in the food chain. Therefore, poultry slaughterhouses are recognized as places where it is necessary to implement measures to control and reduce the number of *Campylobacter* spp. Food business operators are obliged to ensure greater hygiene on the slaughter line, through the inspection of equipment for each step of slaughter and the application of regular cleaning protocols. Continuous monitoring of the presence and abundance of *Campylobacter* spp. on the slaughter line provides data on the validity of hygiene control measures in the slaughterhouse, as well as the data needed to assess the microbiological risk in poultry meat. Monitoring the presence of *Campylobacter* spp. in poultry slaughterhouses is a basic activity that is necessary for taking measures to reduce contamination, improve microbiological safety in poultry processing and thus improve the food safety system as a whole. This review aims to highlight the importance of investigating the prevalence of *Campylobacter* spp. in poultry slaughterhouses, but also the importance of applying measures to prevent and control this pathogen both on farms and in slaughterhouses. These measures are necessary to minimize the presence and transmission of *Campylobacter* in poultry, thereby reducing the risk of foodborne diseases.

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Introduction

Campylobacter species is a commensal, enteric bacteria that causes campylobacteriosis, the most commonly reported foodborne zoonosis in the European Union (EU)/ European Economic Area (EEA) region since 2005 (1). As zoonotic infection that is the leading intestinal infection in humans worldwide (2), campylobacteriosis represents a serious public health problem. Poultry meat, especially raw, undercooked and recontaminated poultry meat or meat products, is the most common source of *Campylobacter* spp. in food (3). Although a microaerophilic bacte-rium, *Campylobacter* also manages to survive in aerobic conditions. (4). Surviving the presence of oxygen, as well as other stresses, such as osmotic stress and high temperature, Campylo-bacter spp. remains widespread throughout the poultry produc-tion chain, all the way to fresh meat at retail (5). Its presence in the human intestine is a consequence of consuming contaminated food, such as, undercooked poultry, but also unpasteurized dairy products and/ or contaminated water. Therefore, controlling the presence and elimination of *Campylobacter* species from agroecosystems is an important task of the food safety system (6).

Broilers are the dominant poultry for food production and most food safety research is conducted on them. Starting with farm animals, then through the entire agri-food system, this pathogen travels to the final consumer, mainly thanks to poor hygienic production practices. National competent authorities, respecting European regulations, seek to establish monitoring plans on the slaughter line, since the highest prevalence of *Campylobacter* spp. is found in poultry carcasses (7, 8). Although animals are usually infected with *Campylobacter* species at the farm level, the slaughter process is considered an important

factor in re- and cross-contamination (5). During 2008, a basic survey was conducted at the EU level (9), covering 26 member states, in broiler slaughterhouses to determine the prevalence of *Campylobacter* spp. in broiler carcasses. The prevalence of *Campylobacter* spp. on broiler carcasses in individual Member States ranged from 4.9% to 100% (9). In addition to whether *Campylobacter* spp. is present on broilers during slaughter, it is also important in what number it is present per gram of neck skin or per cm^2 of broiler carcass. Handling of contaminated poultry in the process of its processing and food production is the main route of *Campylobacter* infection (10). The high prevalence of these bacteria on broiler carcasses poses a risk to public health. Therefore, studies to identify *Campylobacter* spp. and determine its abundance on broiler carcasses, but also on the skin in the neck area, which has been identified as a critical point in *Campylobacter* contamination, are important for assessing the risk of infection for consumers (11). Numerous measures have been prescribed for the control and prevention of *Campylobacter* spp. in poultry, starting at the farm level, then in the slaughter-house and in the poultry processing sector. However, despite their use, *Campylobacter* remains a constant threat to food safe-ty.

Source and prevalence of *Campylobacter* spp. in poultry meat

Campylobacter spp. has been reported in both domestic and wild birds, especially commercial poultry (13). High population density is cited as one of the reasons for its widespread presence on poultry farms (14). In addition to this, there are various other factors that contribute to the widespread distribution of *Campylobacter* spp. in commercial poultry farms, such as the type of farming, housing systems and farm biosecurity standards (15). This bacterium is most commonly transmitted horizontally within the flock. Numerous epidemiological studies on the origin of *Campylobacter* in poultry have cited possible sources such as: insects (16), rodents (17), wild birds (18), other livestock farms and residual *Campylobacter* spp. populations from previous flocks (19). In addition, the environment of poultry farms, such as soil, water sources, dust, surfaces and air, is very often a source of this pathogen (20, 21). Poor biosecurity and hygiene standards in the poultry house are the most common reasons for the presence of *Campylobacter* species in poultry (22). Bailey et al. report a possible increased risk of contamination of poultry on the farm due to poor disinfection of drinking water and lack of adequate pest control treatments on poultry farms (23). Some other on-farm risk factors include waste reuse practices, time of year, general conditions on broiler farms, and age of broilers (24, 25). Due to the presence of numerous on-farm sources of *Campylobacter* spp. and the numerous risks of contamination, preharvest *Campylobacter* spp. control research has mainly focused on management practices (biosecurity and hygiene measures) and nutritional strategies that either prevent bacterial colonization or reduce their concentration in the gut of broilers (26). However, despite the implementation of such strategies, broilers from colonized flocks enter the slaughter process with high levels of *Campylobacter* bacteria on their feathers, most often due to contact with dirt and feces in the poultry house, as well as re-contamination during transport (27). There are numerous steps that each broiler carcass goes through in the slaughterhouse, and the most important processing steps for re-/cross-contamination of carcasses are plucking and evisceration (28). These steps are supplemented by scalding and plucking, practices known to increase the spread of foodborne pathogens (29). Scalding involves immersing the carcasses in hot water to open the feather follicles and allow for easy removal of the feathers during the harvesting process. The plucking phase occurs before the evisceration phase and involves the removal of the feathers and the upper layer of integument (30). Although it would be expected that *Campylobacter* spp. numbers would decrease after scalding, this process has not been proven to have any effect on reducing its abundance on broiler carcasses (31). Possible reasons for this include the fact that *Campylobacter* spp. are particularly well adapted to living on the skin of broilers, where they can form organic biofilms, and the fact that the temperature in the subcutaneous tissue is often 3°C to 4°C lower than the scalding temperature (32). Regarding the evisceration process, it is a step in which cross-contamination with *Campylobacter* spp. can significantly increase due to disruption of internal organs and stomach contents (33). Situations such as the spread of contamination from work equipment, from the environment, from the contents of injured internal organs are just some of the sources of contamination in this area of poultry carcass processing. Defecation also occurs in parallel with this step, which, if inadequately carried out, results in a high risk of contamination of broiler carcasses and slaughter equipment (34). Scientific evidence on the impact of these slaughterhouse processes on *Campylobacter* spp. abundance on carcasses is inconsistent. While some have observed an increase in the number of this pathogen during and after these steps (35), other authors have reported a decrease (19) or a complete absence of difference in the number of *Campylobacter* bacteria after evisceration (36, 37). In processing plants, different systems are used for cooling carcasses. One of the frequently used systems is cold water. Meat immersed in cold water has a significantly lower number of *Campylobacter* spp. than meat cooled in air (38). A study conducted in 20 US processing plants showed that the use of chlorine in the chilling tank significantly reduced the amount of *Campylobacter*, but did not completely eliminate the bacteria (39), making this carcass processing step a source of cross-contamination (40). It should be noted that chlorine-based rinses and similar antimicrobial agents are not approved for use on poultry carcasses in the European Union under Regulation (EC) No. 853/2004. Most other European countries, especially CEFTA countries, prohibit the use of these agents on poultry carcasses, while this is not the case in

certain non-EU countries, such as the United States. In facilities that use cold air to chill carcasses, it has been shown to have either no microbiological effects (40) or only minor effects (23) on *Campylobacter* levels. Close contact between carcasses and equipment also contributes to the spread of contamination, as it results in the accumulation of tissue fragments containing *Campylobacter* spp., which in turn contaminate subsequent carcasses (29). Furthermore, the risk of cross-contamination increases when carcasses are handled during processing and storage (41). Slaughterhouse workers and the equipment they use are a constant source of *Campylobacter* spp. contamination in slaughterhouses (42, 31). Aerosols and droplets generated by excessive washing during the hanging, scalding, and evisceration stages of slaughter can also contribute to the spread of the disease (43).

Prevention and control of *Campylobacter* spp. in poultry chain

The ability of *Campylobacter* spp. to colonize avian species (e.g., chickens, turkeys, starlings, quail, crows, and ducks) and domestic livestock (e.g., pigs, sheep, cattle, and goats) contributes to its spread among different animal species (44). Although domestic mammals and environmental contamination can be sources of infection, the main source of *Campylobacter* infection in humans is poultry products contaminated with this microbe (45). *Campylobacter jejuni* and *Campylobacter* coli are the most commonly isolated species in cases of human campylobacteriosis (46). Chicken has been identified as the main source of this food-borne pathogen, with the slaughter process as the step that contributes most to the contamination of chicken carcasses. In particular, the contamination of chicken carcasses with intestinal contents of *Campylobacter*-infected chickens during processing in slaughterhouses is the riskiest step in the entire process (47). Therefore, general disease control strategies have been introduced that include pre-harvest and post-harvest intervention control points. Pre-harvest control points include (I) implementation of biosecurity measures to reduce exposure to *Campylobacter* spp. from the environment, (II) vaccination of poultry, and (III) use of alternative antibiotic products to reduce or eliminate infection in chickens, such as: prebiotics, probiotics, essential oils, bacteriophages, etc. Post-harvest control points include (I) implementation of carcass sanitation methods in slaughterhouses, (II) the use of permitted methods for decontamination of carcasses, and (III) implementation of eggshell sanitation methods (47). Implementation of all these strategies and other control measures is necessary to reduce the burden of *Campylobacter* spp. on chicken carcasses, and thus the risk of its transmission from poultry products to humans.

On-farm biosecurity measures are part of pre-harvest measures and include, among others: (a) providing sufficient space for chickens on farms to reduce contact between them and thus possible horizontal transmission of *Campylobacter* spp.; (b) continuous sanitation of the environment on and around the farm to reduce the presence or elimination of *Campylobacter* spp. on farms; (c) strict control and application of biosecurity measures when it comes to human activities on the farm by eliminating or minimizing the transmission of *Campylobacter* spp. from the external environment via contaminated clothing, leather and boots of farm workers (46).

In addition to the application of biosecurity measures on farms, vaccination is one of the strategies applied to reduce the presence of *Campylobacter* spp. with poultry on farms. The presence of maternal antibodies in chicks up to 14 days old supports the application of this measure (48). But after this period of passive immunity, chickens become susceptible to infection, especially in cases where *Campylobacter* spp. is present in older flocks and the environment. Post-infection immune responses do not appear to limit *Campylobacter* spp. colonization until the age of broiler slaughter (46). Numerous techniques have been used in the development of effective vaccines, such as: whole cell or subunit vaccination, microorganism-vectored vaccines, and nanoparticle vaccines. However, despite attempts over the past decades to develop a successful vaccine against *Campylobacter* spp. in broilers, successful commercial vaccines are currently not available (49).

Another strategy implemented with the intention of reducing *Campylobacter* spp. colonization in broilers and ultimately reducing its presence on broiler carcasses is the use of feed additives. Given the efforts to minimize the use of antibiotics as growth promoters in poultry production, alternative strategies are being used to compensate for their effects. Therefore, prebiotics, probiotics, essential oils, bacteriophages and other alternative antibiotic products are added to poultry feed (46). Despite studies showing a small effect of prebiotics in reducing *Campylobacter* spp. in the gastrointestinal tract of poultry (50,51), their supplementation continues largely due to the knowledge that these indigestible fibers have a beneficial effect on the gut microbiota. The use of probiotics in poultry feed has been shown to protect farmed poultry from *Campylobacter* infection (52). These beneficial microorganisms are used as an antimicrobial alternative to antibiotics in poultry feed. They exert their antimicrobial effects by competing with microbial pathogens for adhesion and colonization sites and by modulating intestinal immune responses and microbiome composition (53). In addition, probiotic bacteria produce antimicrobial substances, such as bacteriocins, lactic acid, and hydrogen peroxide, which have direct bactericidal activity against enteric pathogens (53,54). Different Lactobacillus species have been shown to exert immunomodulatory and anti-campylobacter effects (52). The presence of different Lactobacillus species in the intestines of poultry results in inhibition of *Campylobacter* invasion in cultured intestinal epithelial cells and a reduction in the expression of *C. jejuni* virulence genes. In

addition, Lactobacillus species have shown the potential to enhance the phagocytic activity of chicken macrophages and modulate their immune responses (52). Although many studies support the role of probiotics in providing protection against *Campylobacter* infection, the results of these studies are largely heterogeneous. It is unclear whether the inconsistencies in probiotic efficacy are due to strain-specific effects and/or are related to differences in age and species of birds, dose and combinations of probiotics, route of administration, frequency and duration of application, and other environmental and management factors, including housing type and feeding regimen (55). In addition to prebiotics and probiotics, essential oils, organic acids, small molecule inhibitors, short-chain fatty acids, and bacteriophages are added to poultry feed. These additives have individual or symbiotic antibiotic effects in the poultry gut (46).

In addition to on-farm control measures, sanitation practices should be implemented in poultry processing facilities to further reduce *Campylobacter* levels along the food chain. Research on the control of *Campylobacter* spp. in broilers in primary production confirms that the proportion of broiler flocks infected with *Campylobacter* is directly related to the prevalence of *Campylobacter* spp. on broiler carcasses (4.9% to 100%) (56). *Campylobacter* spp. content in the caecum of chickens before slaughter reaches approximately 8 $\log_{10}$ CFU/g, and contamination of chicken feathers with faecal material during transport to the slaughterhouse can also be a significant external source of carcass contamination during the plucking/feathering process (57).

Given the poor tolerance of *Campylobacter* spp. to conditions outside the gastrointestinal tract, increasing the dwell time in cages between flocks and effective cleaning are suggested as options to reduce the risk of horizontal exposure (58). Effective carcass decontamination practices are another step in reducing the concentration of *Campylobacter* spp. in poultry meat. Contamination of meat products with intestinal contents is difficult to prevent during processing in slaughterhouses due to the high number of *Campylobacter* spp. in the intestines and the high percentage of infected broilers (46). Since chicken meat is recognized as the main cause of the most common foodborne zoonosis, studies on the presence of *Campylobacter* in fresh chicken meat at retail are frequent and numerous. They have shown that contamination is common, ranging from 59.9% of chicken meat samples from retail establishments (59). To reduce the burden of *Campylobacter* spp. on poultry carcasses, physical, chemical and biological control measures are taken on production lines.

European Food Safety Authority has recommended a series of control measures as part of the Process Hygiene Criteria (PHC) for *Campylobacter* spp. in accordance with Regulation (EU) No 2017/1495 (12). The purpose of the PHC is to ensure, through their mandatory and regular application, constant control of *Campylobacter* spp. on the slaughter line and thus ensure safe poultry meat (3). The treatment of carcasses at the end of the processing line is highlighted as significant, since in addition to the risk of carcass contamination during evisceration, cross-contamination from processing equipment, due to insufficient cleaning and disinfection, is considered another source of contamination during the slaughter process. Therefore, the use of previously proven safe physical and biological methods for carcass decontamination is considered potentially beneficial for food safety later at a later stage of the food chain. (46). In order to find safe and effective disinfectants on the slaughter line, the use of organic acids and quaternary ammonium compounds was experimentally tested. (60). Effective and safe agents for treating poultry carcasses to reduce *Campylobacter* infection are: agents with 2% lactic acid, then sodium chlorite (1200 mg/L) and trisodium phosphate (10-12%; pH 12) (61). The modest efficacy of these agents was not sufficient to justify their use in the treatment of poultry carcasses in the European Union countries. Therefore, the use of sodium chlorite and trisodium phosphate is not approved for use on poultry carcasses at all, and the use of lactic acid is only approved for the decontamination of bovine carcasses in accordance with Commission Regulation (EU) No. 101/2013. The slaughter phase is also the optimal point in the production cycle for decontamination using UV light and high temperatures, which have the potential for very high levels of efficiency if successfully implemented in slaughterhouses (62). However, the main disadvantage of these effective decontamination methods is their impact on the sensory properties of the meat. Freeze-thaw cycles, irradiation and pre-cooked meat are unfavorable and undesirable qualities for consumers in terms of sensory properties and overall food perception (63). In addition to chemical and physical control measures, biological treatments of broiler carcasses using essential oils, bacteriophages, bacteriocins and probiotics are also being applied. Data on the effectiveness of these methods in reducing *Campylobacter* spp. numbers after harvest are scarce, but based on their effects on other foodborne pathogens, their potential for controlling *Campylobacter* spp. in food is highlighted (64, 65).

Food safety and public health significance

Recognizing slaughterhouses as a risk point for contamination, many European countries have implemented a surveillance system for *Campylobacter* spp. in the broiler meat chain, which aims to reduce the contamination of broiler carcasses at the slaughter line. However, only a few European countries also take measures to reduce and control the spread of *Campylobacter* spp. in broiler flocks (66). According to the current legislation, each EU/EEA Member State is responsible for carrying out *Campylobacter* spp. surveillance, which includes the

collection, analysis and interpretation of data (67). In addition, surveillance includes control measures taken to mitigate the negative consequences of the pathogen in the food chain, which help to control and prevent the transmission of pathogens. The introduction of mandatory campylobacter surveillance (68, 12) was based on the scientific opinion of EFSA which estimated that broiler meat alone accounted for 20–30% of human campylobacteriosis cases. This scientific opinion highlights the fact that these cases of human campylobacteriosis could be reduced by >50% or even >90%, if the microbiological criteria in all slaughter batches tested on neck and breast skin were set at a critical limit of 1000 or 500 colony-forming units per gram (CFU/g) (3). Mandatory monitoring of *Campylobacter* spp. in poultry slaughterhouses is based on Process Hygiene Criteria (PHC) and includes quantification of *Campylobacter* spp. on neck skin samples. According to the established slaughter hygiene criteria, a limit threshold of acceptable carcass contamination (<1,000 CFU/g) was set (12). Subjects in the food sector, respecting the obligation to conduct surveillance tests weekly, assess the success of the slaughter process. The information obtained over time, helping to identify trends, variations and problems, provides the basis for decision-making and corrective action to maintain control within established limits. The ac-ceptance criteria, valid until January 1, 2025, stated that the hy-giene of the slaughter process was considered satisfactory if a maximum of 15 out of 50 samples (30%) did not exceed 1,000 CFU/g of neck skin for *Campylobacter* spp. As of January 1, 2025, this criterion has been tightened, so that a maximum of 10 out of 50 samples can exceed the limit value of 1000 CFU/g of Campyl-obacter (12). The regulations require interventions to effectively manage and reduce the microbial load on carcasses, ensuring food safety and regulatory compliance (68).

Tracking the source of the pathogen and its prevalence in poultry meat would facilitate better understanding and development of interventions from management practices and feeding regimens to further reducing the risk of contamination before entering the processing plant. The broiler sector has undergone dramatic changes in recent years, moving from conventional production to antibiotic-free programs, implementing biosecurity practices, changing broiler feeding strategies, and influencing their genetics (26). These and numerous other strategies (nutritional, vaccines, competitive inhibition, or biosecurity practices), at the pre- and post-slaughter level, are applied to reduce the presence of *Campylobacter* spp. in food and thus contribute to the protection of public health and the reduction of treatment costs. *Campylobacter*, along with *Salmonella*, causes the largest number of foodborne illnesses. Data from the United States show that their incidence contributes to approximately 70.7% of foodborne infections, 71.6% of hospitalizations, and 59% of deaths from foodborne illnesses (69). In the European Union, campylobacteriosis has been the most commonly reported food-borne gastrointestinal infection in humans since 2005. EFSA reports that in 2023 there were 148,181 confirmed cases of human campylobacteriosis in EU countries, corresponding to a European Union notification rate of 45.7 cases per 100,000 population (68). The same source states that the overall trend for *Campylobacter* human infections did not show a statistically significant increase or decrease overthe 2019–2023 period. In 5 member states, fresh broiler and turkey meat shows the highest percentages of contamination, 21.6% and 19.4%, respectively (68).

Campylobacteriosis is mostly caused by *C. jejuni*. Most people infected with campylobacteriosis either show no symptoms or only moderate symptoms, including diarrhea (which may or may not be bloody), nausea, fever, and abdominal pain (70). Some of the more serious complications that can occur due to *Campylobacter* infection include: pancreatitis, peritonitis, bacteremia, reactive arthritis, and Guillain-Barré syndrome (71). For these cases of complications with long-term consequences caused by *Campylobacter* infection, the precise mechanism is not clear. The estimated incidence of campylobacteriosis in the United States is 14 cases per 100,000 people (72). The actual incidence of campylobacteriosis is estimated at 9 million cases per year in Europe due to the asymptomatic nature of the disease (25). Campylobacteriosis cases are most common in July and August, coinciding with the peak season for *Campylobacter* isolation from chickens and other poultry in industrialized countries (73). There is no consensus on the infective dose, but it has been reported that a dose of 500 *C. jejuni* cells is sufficient to cause diarrhea and abdominal pain, and clinical symptoms can occur with exposure to a dose of 800 to 10^5 of *C. jejuni* cells (25). Control measures in the food production include numerous activities performed at different stages, and one of them is the education of producers, resulting in the improvement of biological security on farms and general purity of agricultural holdings and processing plants (74). The education of the population in safe practices for food management and preparation is also one of the measures in ensuring safe food and public health. Waganaar et al. have announced that after the application of the education strategy in Iceland, the number of cases of campilobacteriosis fell by 72% (75). Bearing in mind that cases of campilobacteriosis often occur when raw poultry is improperly handled in home cuisine (76), permanent population education is desirable.

One Health approach to prevention, treatment and control of campylobacteriosis

In the control and prevention of foodborne diseases, the concept of "One Health" seems to be effective, as there are joint efforts aimed at improving health between the human health, animal health and food safety sectors (77). Today, this concept is implemented in national action plans

for the control and prevention of foodborne diseases (78), where surveillance is based on risk and appropriate control actions. Surveillance and risk-based control actions can be set according to different levels of risk in different segments of the population of interest. Thus, for example, surveillance can be risk-based, with priority given to segments of the population that are at higher risk of: exposure, infection, impact, transmission of infection or other consequences (79). Similarly, control actions can be more effective in mitigating the risk of foodborne diseases in humans if they are targeted at individual levels of production, starting from livestock farming, agricultural operations and ending with the production and management of the food product (78).

In the context of public health, increasing attention has been paid to the ability of campylobacteriosis to cause relapses, as well as the spread of antimicrobial resistance (80). The increase in industrial poultry production, as well as the widespread use of antibiotics in animals during their fattening, but also in humans, has led to the emergence and spread of a new threat from antibiotic-resistant *Campylobacter* species. *Campylobacter* spp. can cause severe or systemic infections in immunocompromised or young/elderly patients. Treatment of such conditions often requires antibiotic therapy with first-line antibiotics, including fluoroquinolones and macrolides. Over time, *Campylobacter* spp. has acquired resistance to these clinically important antibiotics, compromising the effectiveness of antibiotic treatment. To address the problem of antimicrobial resistance, research and development of new and alternative measures for the control of antibiotic-resistant *Campylobacter* spp. in animal reservoirs and human hosts have been conducted. Special attention is being paid to the development of new alternative approaches to combat this pathogen (81). Therefore, several strategies have been evaluated to date for the control of *Campylobacter* infections in animal reservoirs and in humans, but none of the alternative approaches have yielded results that are as effective as antibiotic therapy (82, 83). For antibiotic therapy in humans, the use of alternative antibiotics is being considered, as well as the development and use of antibiotic adjuvants that can improve the usefulness of existing antibiotics. New clinical studies are needed for these approaches. Regarding vaccines, some of them have shown good protective effects in experimental animal models, but have not been able to produce protective immunity in human clinical trials (84). Such research results have led to the conclusion that host specificity plays an important role in immunization with *Campylobacter* vaccines and will certainly lead to different approaches for future vaccine development. In addition to activities within the framework of human health protection, a significant amount of research has been carried out in the animal health sector, all with the aim of mitigating *Campylobacter* spp. colonization in animal reservoirs. This is particularly true for poultry. In this segment, some approaches, such as the use of N-glycan-based vaccines, bacterial and phage therapies, have shown encouraging results (85), while others (e.g. prebiotics and probiotics) have achieved limited success because their effects are modest and highly variable (44, 86). In addition, when it comes to domestic livestock farming, it is necessary to take into account economic factors and the cost-effectiveness of using alternative methods (81). All these issues complicate the situation, and a combination of several approaches may be necessary to achieve optimal outcomes in the control of *Campylobacter* spp. in the food chain, which is why the "One Health" approach is considered potentially effective and efficient.

Conclusion

Campylobacter spp. is a persistent and widespread foodborne pathogen. Its prevalence in the food chain is most evident in fresh poultry meat and poultry products. Therefore, pre- and post-mortem measurements of poultry are a constant topic of attention from a food safety perspective. With continued research, an integrated approach of health, veterinary and food safety systems, through the "One Health" concept, seems to be a good solution for building a system for the control and monitoring of *Campylobacter* spp. along the entire food chain.

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Vrste kampilobaktrov v klavnicah za perutnino: pregled

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Izvleček: Bakterija kampilobakter je nenehna skrb pri zagotavljanju varnosti hrane, saj je eden najpogostejših patogenov v njej. Glavni vir kampilobaktra v hrani je perutninsko meso, pri čemer je primarna proizvodnja tega mesa kritična točka, kjer je treba sprejeti ukrepe za zmanjšanje njegove prisotnosti v prehranjevalni verigi. Zato so perutninske klavnice prepoznane kot kraji, kjer je treba izvajati ukrepe za nadzor in zmanjšanje števila bakterij Campylobacter spp. Izvajalci živilske dejavnosti so dolžni zagotoviti večjo higieno na klavni liniji, in sicer s pregledom opreme za vsak korak klanja in izvajanjem rednih protokolov čiščenja. Nenehno spremljanje prisotnosti in številčnosti bakterij Campylobacter spp. na klavni liniji zagotavlja podatke o ustreznosti ukrepov za nadzor higiene v klavnici, pa tudi podatke, potrebne za oceno mikrobiološkega tveganja v perutninskem mesu. Spremljanje prisotnosti bakterij Campylobacter spp. v klavnicah za perutnino je osnovna dejavnost, ki je neizogibna za sprejetje ukrepov za zmanjšanje onesnaženja, izboljšanje mikrobiološke varnosti pri predelavi perutnine in s tem izboljšanje sistema varnosti hrane kot celote. Namen tega pregleda je poudariti pomembnost preučevanja razširjenosti bakterij Campylobacter spp. v perutninskih klavnicah, pa tudi izvajanja ukrepov za preprečevanje in nadzor tega patogena tako na kmetijah kot v klavnicah. Ti ukrepi so nujni za zmanjšanje prisotnosti in prenašanja kampilobaktra pri perutnini, s čimer se zmanjša tudi tveganje za bolezni, ki se prenašajo s hrano.

Ključne besede: vrste kampilobaktrov; perutnina; klavnice; bolezni, ki se prenašajo s hrano; varna hrana

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Original Research Article

Determination of Oxidative Stress Responses Induced by the Combination of 4 Different Rare Earth Elements in *Dreissena polymorpha*

Key words

antioxidant enzymes;
Dreissena polymorpha;
oxidative stress;
rare earth element;
thiobarbituric acid reactive substances

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Abstract: Rare Earth Elements (REE), whose usage areas are increasing day by day, are increasing in the amount of mixing with the environment, causing changes in antioxidant enzyme activities by causing oxidative stress in living organisms. In this study, it was aimed to examine the oxidative stress responses induced by the mixture of 4 different REEs (terbium, gadolinium, Lanthanum, Praseodymium) in *Dreissena polymorpha*. For this purpose, sublethal concentration values were determined by literature review. Experimental application was carried out within 24 and 96 hours. In the analyzes performed to determine biomarker responses, samples taken from living organisms were weighed and homogenization processes were performed for the analysis of samples taken from the experimental groups, including the control group. After homogenization, samples were centrifuged at 4.000 rpm for 15 minutes. Supernatants were kept at -86 °C until measurements were made. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities, and glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) levels were determined using ELISA kits. Statistical analyses were performed using SPSS. One-way ANOVA (Duncan's multiple range test; $p < 0.05$) was used for comparison of measured parameters among groups. As a result of the application, a decrease in CAT activity and GSH level and an increase in TBARS levels were observed after 96 hours compared to the control group, while no statistically significant difference was detected in SOD and GPx activities.

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Introduction

The rare earth elements (REE) are a group of 17 elements in the periodic table, including scandium and yttrium with atomic numbers 21 and 39, and the lanthanides (Ln) with atomic numbers 57-71 (1). REEs are generally soft, pliable and easily workable. The reason why we call REEs that generally coexist in the earth's crust as "rare" is not because of their low amount in the ore, but because of the difficulty of processing these elements, separating them from each other selectively by enrichment methods and obtaining them in pure form (1). REEs occur together in minerals due to their chemical similarity. Some REEs have unique magnetic, phosphorescent, catalytic and electrical properties that make them highly valuable in industrial applications and some manufactured products. They are elements that have become extremely important for technology due to these features (2). These elements have an extremely important place in a wide range of technological fields, from mobile phones to televisions, from LED light bulbs to wind turbines. The estimated average REE concentration in the earth's crust is between about 130 mg/g^{-1} and 240 mg/g^{-1}, which is actually quite high compared to other commonly used elements (3).

Gadolinium (Gd) possesses remarkable metallurgical qualities, such that as little as 1% gadolinium can greatly improve iron, chromium, and related metals' machinability

and resistance to oxidation at high temperatures. Gd absorbs neutrons as a metal or salt and is thus occasionally used for shielding in radiography and nuclear reactors in neutron radiography. Terbium (Tb) is employed in solid-state electronics as calcium fluoride, calcium tungstate, and as a crystal stabilizer in high-temperature fuel cells. Terbium is utilized in actuators, maritime sonar systems, and sensors as a component of Terphenol-D (an alloy that expands and contracts more than any other alloy when subjected to magnetic fields). Catalysts, glass additives, carbon lamps for studio lights and projectors, ignition components in lighters and torches, electron cathodes, scintillators, and gas tungsten welding electrodes are just a few of the applications for lanthanum (La) compounds. Lanthanum carbonate is used as a phosphate binder in kidney failure patients who have excessive phosphate levels in their blood. Praseodymium (Pr), like other lanthanide trichlorides, can be anhydrous or hydrated. When exposed to moist air, it rapidly absorbs water and forms a light green heptahydrate (4).

Abiotic variations in the aquatic environment, such as emerging pollutants and those due to climate change, are stress factors that affect the resilience of organisms. These pollution factors consist of greenhouse gas emissions such as carbon dioxide (CO_2), methane (CH_4) and ozone (O_3), mixing with the atmosphere due to the burning of fossil fuels, industrial industrial wastes, household and agricultural wastes and wastes such as REE. The atmospheric layer generated by excessive concentrations of these gases absorbs and traps solar energy as heat, raising world average temperatures (5). The high amount of REEs in the environment and the increase in the concentration level taken into the living organism cause toxic effects in the living body. Depending on the organisms exposed to REE concentrations, toxic effects are known to produce growth-stimulating effects (6).

Oxidative stress occurs when reactive oxygen species (ROS) accumulate excessively. It regulates the physiological and molecular reactions that occur in response to almost all biotic and abiotic stimuli (7). A number of independent investigations in both plant and animal models have revealed the role of various REEs in the redox imbalance that leads to oxidative stress, and several REEs have been reported to produce oxidative stress (8, 9, 10). Aerobic organisms have a built-in antioxidant defense mechanism that includes both enzymatic and non-enzymatic compounds that can mitigate the damaging effects of ROS. Superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GSH) are among the antioxidant enzymes that have been widely used as effective biomarkers of environmental contamination in aquatic organisms and have been identified as effective protective barriers against ROS formation (11). Various studies have employed lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS), as a marker of oxidative stress in response to various environmental pollutants (12, 13, 14, 15). Depletion causes a redox imbalance and a decrease in the ability to cope with organic xenobiotics processed by glutathione S-transferase (GST) and glutathione peroxidases (GPx).

Mussels are particularly vulnerable to the elements present in the water as they are organisms with a sedentary filter feeding character. *Dreissena polymorpha* is vulnerable to environmental change and pollution because of its tendency to accumulate contaminants (16) and vast geographic range. *D. polymorpha* is commonly utilized in ecotoxicological tests due to its strong oxidative defense and relatively high tolerance to xenobiotics (17, 18).

The purpose of this study is to assess SOD, CAT and GPx activities, as well as TBARS and GSH levels, in order to determine the oxidative stress responses generated by a mixture of Tb, Gd, La, and Pr REEs on *D. polymorpha*.

Material methods

Supply of Living Organisms and Adaptation to the Laboratory Environment

D. polymorpha individuals were collected from the Euphrates (38º 48' 25" N, 38º 43' 51" E) (Figure 1). The organisms were quickly brought to the laboratory in plastic bottles. It was fed with plankton in a 12:12 hour light:dark cycle to design an environment similar to natural living conditions in a 20 L aerated aquarium in a room kept at 18 °C for 30 days before being used in the experiments. Organisms at similar developmental stages were selected for the study, were not fed during the experimental study and were checked every 24 hours.

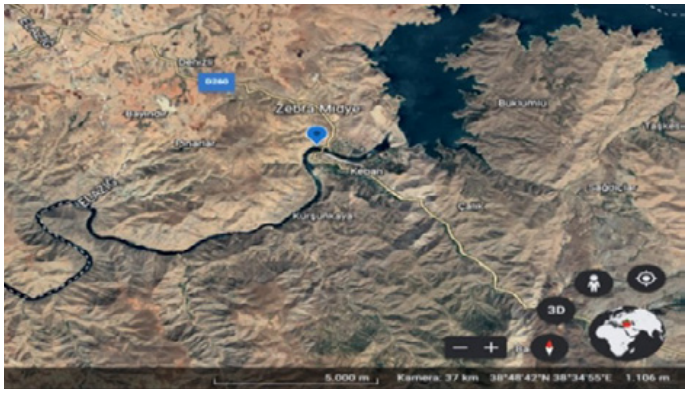

Figure 1: The area where the model organism *D. Polymorpha* was supplied

Chemical Substance Supply

Tb, Gd, Ln, Pr REEs used in the study were obtained from the commercial company Bostonchem with 99.99% purity.

Determination of Subletal Concentrations

As in all toxicological studies, the application concentrations determined in our Mix REE application study, taking into account the release rates to the environment, and after the literature review, the application concentrations were determined compared to the values in this range.

Experiment Design

Eight healthy models of similar size were placed in glass aquariums, each consisting of 2 liters. The O_2 need of living things was provided by air engines. Experimental study consisted of 4 groups, one of which was the control group. Two time slots (24 and 96 hours) were determined for the four groups.

Application concentration was created by mixing La, Gd, La, Pr REEs in equal proportions (1:1:1:1).

C1 (Control): The Mix was kept in water taken from the natural environment of the organisms, which was not exposed to any concentration of REE.

C2: Group exposed to 50 mg/L Mix REE concentration (1:1:1:1) at 24 and 96 hours

C3: Group exposed to 100 mg/L Mix REE concentration (1:1:1:1) at 24 and 96 hours

C4: Group exposed to 200 mg/L Mix REE concentration (1:1:1:1) at 24 and 96 hours

In the experimental research, all studies were carried out in triplicate.

Preparation and analysis of oxidative stress parameters in D. polymorpha soft tissue

For measurement of oxidative stress parameters, soft tissue samples were weighed and homogenized by adding 1/5 w/v PBS buffer (phosphate buffered salt solution) and using an iced homogenizer (DAIHAN brand). The homogenized samples were then centrifuged at 4.000 rpm for 15 minutes. Supernatants were maintained at -86 °C until measurements were taken. GSH and TBARS levels, as well as SOD, CAT, and GPx activities, were measured using corresponding ELISA kits. In the investigation, CAYMAN brand GSH (Catalog No 703002), SOD (Catalog No 706002), CAT Catalog No 707002), and GPx Catalog No 703102) were used.

Statistical Analysis (revised)

Statistical analyses were performed separately for each time point (24 h and 96 h) using One-Way ANOVA followed by Duncan's post-hoc test ($p<0.05$) to compare the four experimental groups (Control, 50 mg/L, 100 mg/L, 200 mg/L). Additionally, intra-group comparisons between 24 h and 96 h were conducted using an independent t-test, assuming normal distribution.

Results

TBARS Level

The TBARS assay results reveal a clear concentration- and time-dependent increase in lipid peroxidation in Dreissena polymorpha following exposure to mixed rare earth elements (REEs). At 24 hours, TBARS levels show a gradual elevation with increasing REE concentrations, while at 96 hours, this effect becomes markedly more pronounced particularly in the 200 mg/L group, which exhibits the highest TBARS value (0.137 µM), statistically distinct from all other groups (Figure 2).

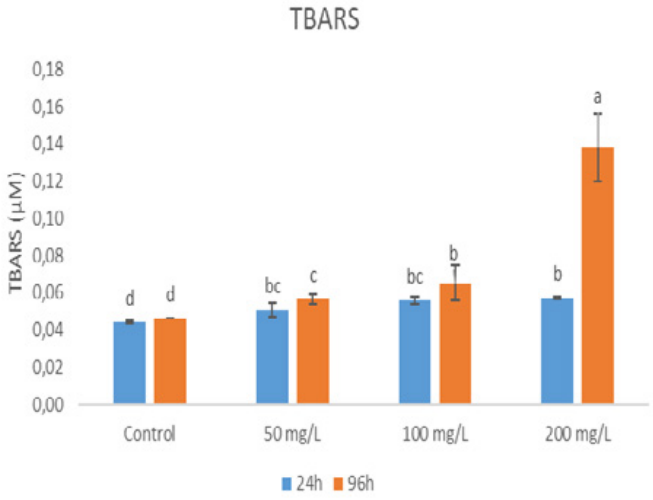

Figure 2: TBARS (µM tissue) levels of *D. polymorpha* exposed to Mix REE. Different letters indicate statistically significant differences ($p<0.05$)

GSH Level

The GSH assay results demonstrate a clear concentration- and time-dependent depletion of intracellular antioxidant capacity in Dreissena polymorpha following exposure to mixed rare earth elements (REEs). At both 24 h and 96 h, GSH levels were highest in the control group, with a progressive decline observed as REE concentration increased (Figure 3).

At 24 h, the control group exhibited a GSH concentration of approximately 53.3 µM, while the 200 mg/L group dropped sharply to 7.6 µM. This trend was similarly evident at 96 h, where the control group maintained relatively high GSH levels (49.3 µM), but the 200 mg/L group declined further to 6.5 µM. These reductions were statistically significant, as indicated by distinct lettering annotations (Figure 3) above the bars ($p < 0.05$).

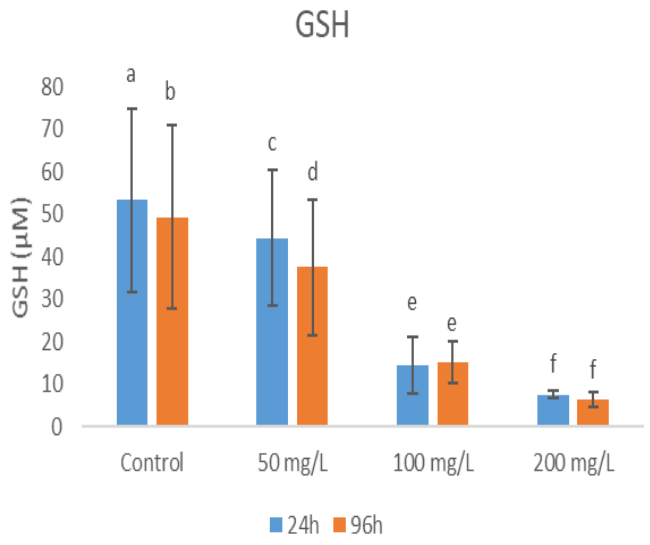

Figure 3: GSH (µM tissue) levels of *D. polymorpha* exposed to Mix REE. Different letters indicate statistically significant differences (p<0.05)

SOD Activity

SOD activities (U/mL tissue) in *D. polymorpha* exposed to different concentrations of Mix REE over time are given in Figure 4. There was no statistically significant difference in SOD activity in the 24 and 96 hour exposure groups compared to the control group (p>0.05).

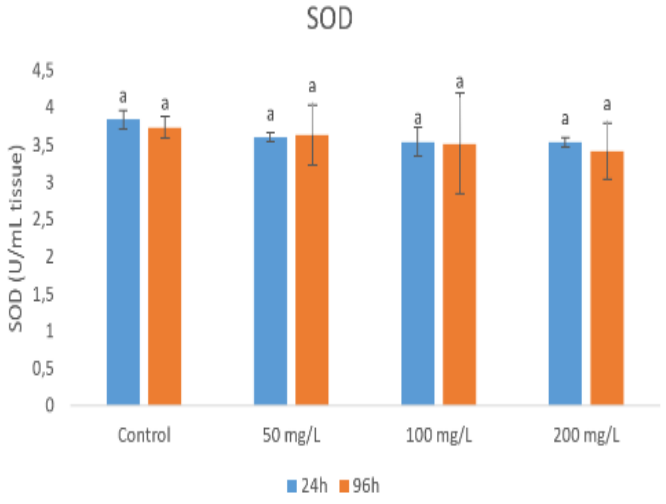

Figure 4: SOD (U/mL tissue) activities of *D. polymorpha* exposed to Mix REE. Different letters indicate statistically significant differences (p<0.05)

CAT Activity

Figure 5 presents CAT activity (nmol/min/mL tissue) in the control and experimental groups exposed to 50 mg/L, 100 mg/L, and 200 mg/L concentrations of the test substance at 24 h and 96 h exposure periods.

CAT activity was highest in the control group at both time points, with no statistically significant difference between 24 h and 96 h values (Figure 5). In the 50 mg/L group, CAT activity at 24 h remained comparable to the control (Figure 5), while 96 h values showed a significant reduction (p<0.05), compared to the control and 24 h values (Figure 5).

A marked and statistically significant decline in CAT activity was observed at 100 mg/L and 200 mg/L for both 24 h and 96 h exposures (p<0.05) compared to control.

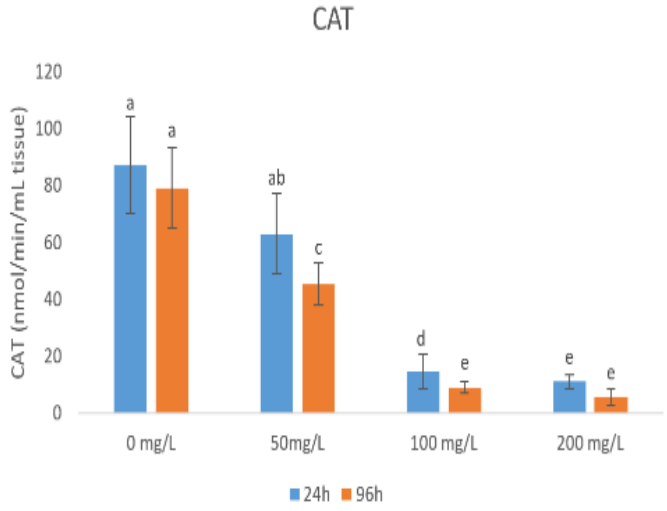

Figure 5: CAT (nmol/min/mL tissue) activities of *D. polymorpha* exposed to Mix REE. Different letters indicate statistically significant differences (p<0.05)

GPx Activity

The Figure 6 shows the GPx activity (nmol/min/mL tissue) in the experimental groups exposed to different concentrations of the tested substance (50 mg/L, 100 mg/L, and 200 mg/L) compared to the control, at two exposure durations: 24 hours and 96 hours.

In general, GPx activity remained relatively stable across groups, with no statistically significant reductions observed in the 50 mg/L and 100 mg/L treatments compared to the control at either time point. However, at 200 mg/L, a significant decrease in GPx activity was observed at 96 hours compared to 50 mg/L group (Figure 6).

Discussion

In the literature, there are many scientific studies investigating the effects of pollutants on aquatic organisms with various biomarkers. However, there are very few studies examining the oxidative stress responses by applying REEs to living organisms as a mixture. It is

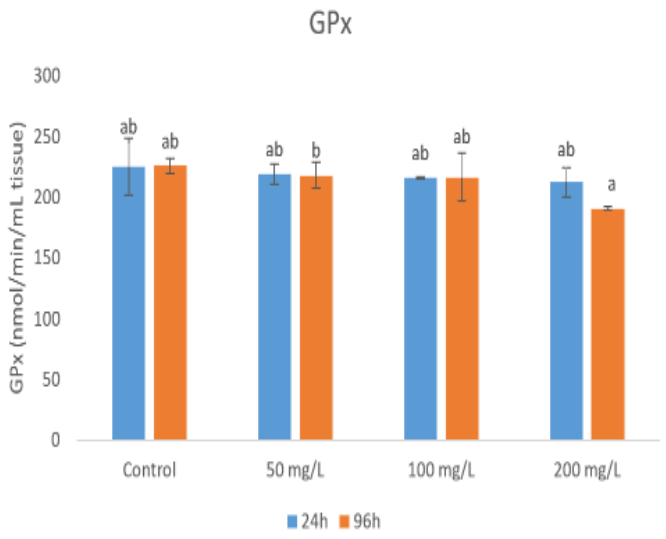

Figure 6: GPx (nmol/min/mL tissue) activities of *D. polymorpha* exposed to Mix REE. Different letters indicate statistically significant differences ($p<0.05$)

thought that this study will contribute to the literature by creating a source for the related field. Hanana et al., (2021), investigated the oxidative stress markers of terbium and praesodymium in *Rainbow trout* and stated that Tb is 2 times more toxic than Pr and plays a role in oxidative stress, calcium binding, hemoprotein activity and protein turnover Tb toxicity (19). Lompré et al. (2021), investigated the effect on the organism by exposing Tb and carbon nanotubes in native *Ruditapes decussatus* and *Ruditapes philippinarum* in their study, and as a result, they observed metabolic deterioration in oysters exposed only to Tb they stated that loss of redox balance and neurotoxicity were proven in this species (20). Freitas et al. (2020), evaluated the metabolic and oxidative stress responses of Dysprosium (Dy) of *Mytilus galloprovincialis* and found that Dy was responsible for the metabolic increase associated with glycogen expenditure of the mussel, activation of antioxidant and biotransformation defenses, and cellular damage with a clear loss of redox balance have emphasized (21). Hanana et al. (2021) evaluated the toxic effects of five mild REE mixtures in *Hydra vulgaris* in their study and stated that it caused a significant early toxic effect in hydra within the first 24 hours, and REE mixture affected hydra reproduction and head regeneration at the level of environmental concentration (22). Kang et al. (2022), investigated the toxic effects of lanthanum (La) and gadolinium (Gd) on zebrafish (*Danio rerio*) in their study and reported that they cause oxidative stress in living things (23).

Exposure to abiotic changes, such as the presence of pollutants, as well as reduced metabolic capacity of organisms can result in excessive production of ROS in bivalves. To prevent lipid membrane damage, organisms can differentiate their antioxidant defenses, namely the activity of SOD, CAT and GPx enzymes (24).

In the present study, decreased CAT activity was observed in *D. polymorpha* following exposure to Mix REEs. Our results suggest that CAT activity is significantly suppressed at higher concentrations of the substance, regardless of exposure duration. These findings indicate a clear concentration-dependent inhibition of CAT activity, particularly at 100 mg/L and 200 mg/L, pointing to potential oxidative stress and an impaired antioxidant defense system at elevated concentrations. Similarly, Dubé et al., investigated 7 different REE effects in young rainbow (*Oncorhynchus mykiss*) trout and reported reductions in CAT activity. Andrade et al. (2023), examined the oxidative stress results of yttrium (Y) in M. galloprovincialis and observed that CAT activity was inhibited. Figueiredo et al. (2018), evaluated the enzyme activities occurring in lanthanum exposure in Anguilla anguilla and observed reductions in CAT activity. Andrade et al. (2021), in their study, examined the response of *M. galloprovincialis* to lanthanum and reported that the decrease in CAT activity may be caused by pollution. Yang et al. (2016), examined the effects of yttrium on *Microcystis aeruginosa*, and determined reductions in CAT activities as a result of the examination. Figueiredo et al. (2022), In their study, they examined the oxidative stress responses in *Spisula solida*, which they exposed to La, and reported that there was a decrease in CAT activities as a result. Mixed REE exposure may parallel CAT activity reduction, which may reflect higher OH- production leading to CAT inhibition. In fact, the increase in intracellular ROS due to OH^- overproduction is associated with a decrease in CAT expression (30).

Activation of SOD under REE exposure reflects the organism's first enzymatic defense mechanism responsible for the dismutation of oxygen radicals to oxygen and hydrogen peroxide (31). No significant change was observed in SOD activities in *D. polymorpha* individuals exposed to mixed REE. Contrary to our results, Trapasso et al. (2021) who investigated the effect of Gd in *M. galloprovincialis* observed increases in SOD activity. Freitas et al. (2020), study, investigated the toxicological effects of neodymium on *M. galloprovincialis* and stated that SOD activities increased compared to the control. Andrade et al. (2021), their study, examined the response of *M. galloprovincialis* to La and observed decreases in SOD activity. Yang et al. (2016), examined the effects of yttrium on *M. aeruginosa* and found that there were decreases in SOD activities. Figueiredo et al. (2022), examined the oxidative stress responses in *S. solida* exposed to lanthanum in their study and stated that there was a decrease in SOD activity as a result.

Overall, GPx activity remained relatively stable across groups following exposure to Mix REEs. The only significant change was observed at 200 mg/L after 96 h, where GPx activity declined compared with the 50 mg/L group at the same time point. This finding suggests that prolonged exposure to the highest concentration tested may impair antioxidant defense, as reflected by reduced GPx activity.

Freitas et al. (2020), in their study, investigated the toxicological effects of neodymium on *M. galloprovincialis* and stated that no significant difference was observed in the control organisms in the case of GPx, at the highest exposure concentration. Alp et al. (2023), investigated the effects of Tb concentrations on *Lemna minor* and stated that there was an increase in GPx activities as a result. Yang et al. (2016), examined the oxidative stress responses in *S. solida* exposed to lanthanum in their study and observed decreases in GPx levels. Because GSH can be converted to GSSG in the presence of ROS, the GSH/GSSG ratio tends to drop when GSSG increases under stressful situations. Organisms that employ glutathione for redox homoeostasis can produce reduced glutathione, but they are also distinguished by their glutathione recycling capabilities. Glutathione reductase (GRed) is an enzyme that converts oxidized glutathione to its reduced form (34). As a result, the increased GSSG content seen in polluted mussels with decreased GSH/GSSG levels implies that GRed is unable to convert oxidized glutathione to its reduced form. In general, the GSH/GSSG ratio is used to quantify the oxidative stress of organisms exposed to contaminants (35, 36, 37). Freitas et al. (2020), study, they aimed to determine the oxidative damage of neodymium in *M. galloprovincialis* and as a result, they observed significant reductions in GSH levels. Trapasso et al. (2021), investigated the effects of Tb concentrations on *L. minor* and stated that there were decreases in GSH levels as a result. Pinto et al. (2010), study examined the effects of La in *M. galloprovincialis* and stated that there were significant decreases in GSH levels compared to the control. Ippolito et al. (2010), study, examined the oxidative stress responses of La, Ce, Pr, Nd, Gd REEs in *L. minor* and reported that GSH levels decreased depending on time and concentration.

ROS can cause membrane lipid peroxidation when they are overproduced and not adequately removed by antioxidant systems. Malondialdehyde (MDA) is one of the most extensively used oxidative stress markers among all the peroxidized compounds produced in the LPO process (24, 40). Our study demonstrated a significant increase in the lipid peroxidation marker TBARS in *D. polymorpha* exposed to Mix REEs compared to the control groups, which is likely attributable to elevated substance concentrations. These findings suggest that higher concentrations of the tested compound induce substantial lipid peroxidation, particularly during prolonged exposure. The observed increase in TBARS levels at 96 hours may reflect cumulative oxidative damage or a delayed response of the antioxidant defense system. Pagano et al. (2016), investigated the impacts of REEs such as Y(III), La(III), Ce(III), Nd(III), Sm(III), Eu(III), and Gd(III) on *Paracentrotus lividus*. They studied and discovered that Ce and Gd enhanced MDA levels, whereas Y(III), La(III), Sm(III), and Nd(III) did not. Trapasso et al. (2021), investigated the effects of Tb concentrations on *L. minor*, and as a result, they observed increases in TBARS levels. Pinto et al. (2019) study, examined the oxidative stress responses of La, Ce, Pr, Nd, Gd REEs in *L. minor* and stated that increases in MDA levels occurred. Yang et al. (2016), examined the effects of yttrium on *M. aeruginosa* and determined increases in MDA levels.

The results of oxidative stress responses in *D. polymorpha* exposed to Mix REE show parallelism with the studies in the literature. It is thought that the use of REEs in combination in experimental practice and the evaluation of their results will contribute to the literature.

Conclusion

According to the literature review and the study results, it is thought that the use of REEs alone or in combination causes environmental pollution and causes oxidative stress in the organism by penetrating living organisms, causing vital damages in the organism. It can be recommended to pay attention to the use of REE and not to release wastes to the environment.

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Determinacija odgovorov na oksidativni stres, ki ga povzroča kombinacija 4 različnih redkih zemljinskih elementov v *Dreissena polymorpha*

O. Serdar, A. N. Aydin

Izvleček: Redki zemeljski elementi (REE), katerih uporaba se iz dneva v dan povečuje, se vse bolj mešajo z okoljem in povzročajo spremembe v aktivnosti antioksidativnih encimov, saj v živih organizmih povzročajo oksidativni stres. V tej študiji smo želeli preučiti odzive na oksidativni stres, ki jih povzroča mešanica 4 različnih REE (Terbij, gadolinija, lantana, praseodima) v *Dreissena polymorpha*. V ta namen so bile na podlagi pregleda literature določene subletalne koncentracije. Eksperimentalna aplikacija je bila izvedena v 24 in 96 urah. V analizah, izvedenih za določitev odzivov biomarkerjev, so bili vzorci, odvzeti iz živih organizmov, stehtani in homogenizirani za analizo vzorcev, odvzetih iz eksperimentalnih skupin, vključno s kontrolno skupino. Po homogenizaciji so bili vzorci 15 minut centrifugirani pri 4000 rpm. Supernatanti so bili shranjeni pri temperaturi –86 °C do izvedbe meritev. Aktivnosti superoksid dismutaze (SOD), katalaze (CAT) in glutation peroksidaze (GPx) ter ravni glutationa (GSH) in s tiopropioninsko kislino reaktivnih snovi (TBARS) so bile določene z uporabo kompletov ELISA. Statistične analize so bile opravljene z uporabo SPSS. Za primerjavo izmerjenih parametrov med skupinami je bila uporabljena enosmerna ANOVA (Duncanov test večkratnega obsega; $p < 0,05$). Kot rezultat uporabe je bilo po 96 urah v primerjavi s kontrolno skupino opazno zmanjšanje aktivnosti CAT in ravni GSH ter povečanje ravni TBARS, medtem ko v aktivnostih SOD in GPx ni bila ugotovljena statistično značilna razlika.

Ključne besede: antioksidativni encimi; *Dreissena polymorpha*; oksidativni stres; redki zemeljski elementi; snovi, ki reagirajo s tiobarbiturno kislino

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Investigation of the Effects of Different Anesthesia Combinations on Cardiovascular Parameters in White New Zealand Rabbits

Key words

electrocardiography;
radiography;
rabbit;
VHS

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Abstract: The objective of this study is to assess the morphological and physiological alterations in the heart resulting from four distinct anesthesia combinations, with midazolam, medetomidine, and dexmedetomidine applied as preanesthetics in White New Zealand rabbits. For the study, a total of 32 white New Zealand rabbits were divided into four different groups. The vertebral heart score was measured in rabbits before (T_0) and at 5 (T_5), 10 (T_{10}), 30 (T_{30}), 50 (T_{50}) and 70 (T_{70}) minutes during the experiment. Concurrently, measurements were taken for the electrocardiographic parameters, all at consistent time intervals. Heart frequency, respiratory rate, rectal temperature, mean arterial pressure and peripheral blood oxygen saturation were measured for a total of 60 minutes with 5 minutes intervals before and during preanesthesia. The vertebral heart score changed in all groups except the Mid+Med group. In the electrocardiographic assessment, in the Mid+Med, Dex, and Mid+Dex groups, an extension in the duration of the QRS wave and QT interval was observed, while no significant change was detected in the durations of the PR interval and T wave. Conversely, in the Me group, a distinct prolongation was observed in the duration of the P wave. Peripheral blood oxygen saturation values increased, heart frequency, mean arterial pressure and rectal temperature parameters decreased in entire groups. Following a thorough analysis of all the data in this study,it was observed that the morphological and physiological effects on the heart induced by the Mid+Med group resulted in less pronounced changes compared to the other groups.

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Introduction

Among the various species, the New Zealand white rabbit is less aggressive than other species, has short life cycles and minimal health problems, and is easily accessible and affordable (1). Many experimental studies require sedation or anesthesia. Since the mortality rate due to anesthesia in rabbits is quite high (2), various anesthetic drugs and routes of administration have been investigated for protocols that minimize complication rates (3).

Pre-anesthesic drugs essentially, medetomidine and dexmedetomidine are highly selective, specific, and strong α2-adrenoreceptor agonists used mainly to provide sedation, analgesia, and antinociception. Adverse cardiovascular effects such as bradycardia, arrhythmia, hypertension or hypotension, and reduced cardiac output are also seen similarly to other α2-adrenoreceptor agonists. Medetomidine and dexmedetomidine cause a dose-dependent decrease in heart rate (HR), mean arterial pressure (MAP), and respiratory frequency (fR) in rabbits (4,5). Midazolam is a gamma-aminobutyric acid A receptor allosteric modulator that provides sedation without analgesia. In rabbits, midazolam administration induces sedation with excellent muscle relaxation, although depression of the cardiovascular and respiratory systems is minimal (6).

Radiography is the standard method for assessment of the respiratory tract anatomy as well as cardiac size and shape (7). Electrocardiography (ECG) is another non-invasive technique for assessing cardiac rhythm and electrical activity. These diagnostic methods are complementary in clinical practice (8).

Medetomidine, dexmedetomidine, and combinations of these sedatives with midazolam have been used for premedication in previous studies (6,9,10,11). However, studies comparing the effects of these drugs on the cardiovascular system are limited. This experiment was conducted to compare some cardiovascular effects of medetomidine (Med) and dexmedetomidine (Dex) alone and in combination with midazolam (Mid+Med, Mid+Dex) for premedication in White New Zealand rabbits. We hypothesized that the use of medetomidine and dexmedetomidine as preanesthetics would cause minimal cardiovascular effects, and in addition, the use of midazolam in combination with preanesthetics would further reduce the complications.

Material and methods

Animals

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by Ataturk University Local Board of Ethics Committee for Animal Experiments (no. 274/2021).

Thirty-two male New Zealand white rabbits (American Society of Anesthesiologists physical status I), aged 12–36 months and weighing 4.3 ± 0.4 kg, were obtained from the Medical Experimental Application and Research Centre of Ataturk University (Erzurum, Turkey). All rabbits were housed in individual stainless steel cages (60 × 50 × 60 cm high) without bedding material in a single room (temperature of 22 ± 2°C, humidity of 40%–60%, and illumination 12:12 hours light:dark cycle). The animals were fed a commercial pelleted diet and were provided water ad libitum.

Anesthesia Protocol

Rabbits that were not water-restricted before anesthesia but fasted for 4 hours were randomly divided into 4 groups. The groups were as follows: Medetomidine (0.3 mg/kg, Zoetis, New Zeland) (Group Med), Midazolam (1mg/kg, Zolamid 15 mg/3ml, Mefar Farma, Turkey) + Medetomidine (0.05 mg/kg, Zoetis, New Zeland) (Group Mid+Med), Dexmedetomidine (0.05 mg/kg, Sedadomid 200 µg/2ml, Kocak Farma, Turkey) (Group Dex), Midazolam (1 mg/kg) + Dexmedetomidine (0.025 mg/kg) (Group Dex). Preanesthetic agents were injected intramuscularly (IM) (5 ml/22 G cannula, Ayset, Tepefarma, Turkey) into the musculus quadriceps muscle group after T_0 measurement. At T_5, ketamine (30 mg/kg, ketasol 10%, Interhas, Turkey) was administered IM in entire groups to provide induction. For intubation, 10% local anesthetic spray (Vemcaine 10% pump oral spray, VEM, Tekirdag, Turkey) was used. After the rabbits were placed on the table in the dorsal position with the head and spine in a straight alignment, the intubation step was performed blindly using a laryngeal mask (No. R4, V-Gel Advanced Veterinary Airway Management System, Docsinnovet, London, UK), and the cuff of the laryngeal mask was inflated with 1 ml of room air through a syringe without using a manometer when sufficient depth of anesthesia was obtained (no swallowing reflex). Anesthesia maintenance isoflurane (Forane 99.9%, Aesica, Queenborough, UK) was administered in 100% oxygen at an oxygen flow rate of 2%/1L/min.

Radiographic Analysis

A radiographic evaluation of the VHS values of rabbits was performed in the right laterolateral position. For radiographic examinations, a 5 kilowatt (kW), 110 kilovolt (kV)/100 milliampere (mA) stationary X-ray machine (Mex-100, Oberhausen, Germany) was used. Radiographs were taken with a 35x43 x-ray cassette (DRx, Carestream, New York, United States of America) at 50 KV, 4.00 mAs, 100 mA dose, and at a distance of 80 cm from the tissue to be examined. The measurements were performed in the right lateral position before anesthesia (T_0), 5 (T_5), 10 (T_{10}), 30 (T_{30}), 50 (T_{50}), and 70 (T_{70}) minutes after anesthesia.

The vertebral heart score (VHS) was measured according to the protocol established by Ljubica (12). On the x-rays, the long heart axis (LA) was found by measuring from the farthest point in the ventral contour of the heart's x-ray image to the base of the heart, which is where the carina cranioventral border is. The short axis (SA) was measured at the widest cardiac image point on a line perpendicular to the long axis at the level of the clavicle vena cava. Both measurements (long and short axes) were compared with the distance from the cranial edge of the 4th thoracic vertebra (T4) to the cranial edge of the 5th thoracic vertebra. The VHS was calculated according to the formula given below:

$$VHS = (LA/T4) + (SA/T4)$$

Electrocardiographic Analysis

Biopac Systems, Inc Mp 150 400 kHz (Aero Camino, Goleta, United States of America) ECG device was used for electrocardiography measurement. The standard alligator clip electrodes, with the teeth curled outward, were applied just caudoproximal to each elbow and mid-caudal to the skin fold between the hocks and buttocks. The electrodes were placed in accordance with the manufacturer's instructions, similar to their placement in cats and dogs. Approximately 1 ml of alcohol was applied to each skin-electrode interface to enhance conductivity.

Electrocardiographic measurements were taken in the minutes specified in the VHS measurement. The evaluation of ECG analysis was performed with the Acqknowledge program (version 4.1.1.1 AcqKnowledge for MP Systems) (Intel® Core I5 CPU M460 2.53 Ghz, HP, California, United States of America). All measurements were taken for an average of 3 minutes, and the results were recorded. The amplitudes (mV) and durations (ms) of the P wave, QRS wave, and T wave were measured, while the durations (ms) of the PR interval and QT intervals were recorded by the same person at the same time.

Monitoring

In all animals, the HR and peripheral oxygen saturation (SpO_2) were monitored from the patient monitor (Comen C80-V, China) throughout the study by placing the tongue probe of the bedside patient on the tongue of the rabbits. Rectal temperature (T) was measured from the dorsal wall of the rectum with a digital thermometer throughout the study. *f*R was monitored on the patient monitor throughout the study by placing crocodile-tipped clips after shaving the skin on the musculus triceps on the forelimbs, the skin on the musculus quadriceps on the hindlimbs, and finally the skin dorsally (Heiniger Saphir, Switzerland). MAP was monitored on the patient monitor throughout the study using a size 2 cuff (4-6 cm) of the patient monitor after shaving the proximal tarsal joint of the left hind leg. These physiologic parameters of the rabbits were measured and recorded every 5 minutes for a total of 60 minutes with the aforementioned methods.

Statistical Analysis

Power analysis was performed to determine the minimum number of animals required in each group (PS-Power and Sample Size Calculation, Version 3.1.2, Vanderbilt University, TN, USA). Accordingly, in a calculation with a Type I error (α) of 0.05 and a Type II error (power, β) of 0.95, it was determined that 8 rabbits were required in each group for a difference of 0.2 (standard deviation ± 0.1) between the measured VHS values to be considered statistically significant between the groups. The difference in VHS values due to the administration of medetomidine in rabbits was investigated as a preliminary study before the main study. This preliminary study was utilized to generate the data for the current study.

All data used in the study were analyzed using SPSS software (IBM Company, SPSS Inc., IL, USA). The Shapiro-Wilk test was used before the analysis to determine the normal distribution of the data. Normally distributed data were analyzed using repeated analysis of variance (ANOVA). In cases where the assumption of sphericity was not met, the Greenhouse-Geisser or Huynh-Feldt value was used as the basis for the data obtained from the test results. Subsequently, differences between the groups were determined using the post-hoc Tukey test. For non-normally distributed data, the Kruskall-Wallis test followed by Dunn's multiple comparison test was used. All data were presented as mean ± standard deviation (SD), and p values <0.05 were considered statistically significant.

Table 1: VHS measurements before and during the experiment

	Time					
Groups	**T_0**	**T_5**	**T_{10}**	**T_{30}**	**T_{50}**	**T_{70}**
Med	7.75	7.8	7.8	7.8	7.85	7.95
	(6.7-7.8)[ab]	(6.8-8.5)*	(6.8-8.2)	(6.8-8.3)*	(6.9-8.4)*	(7-8.5)*[#]
Mid+Med	7.8	7.75	7.7	7.7	7.75	7.75
	(7.5-8.1)[a]	(7.5-8)	(7.6-8.2)	(7.3-7.9)	(7.3-7.9)	(7.4-7.9)
Dex	7.4	7.45	7.55	7.65	7.45	7.3
	(7-7.9)[b]	(7-8)	(7.2-8)*	(7.2-8)*	(7.1-7.9)	(6.9-7.8) [#]
Mid+Dex	7.7	7.7	7.9	7.65	7.65	7.5
	(7.5-7.9)[ab]	(7.5-8)	(7.5-8.1)*^	(7.3-7.9)[#]	(7.3-8)[#]	(7.3-7.8)*^[#]

Note: Measurements were recorded before drug administration (baseline) and every 5 minutes under anesthesia. Data are expressed as median (range). Different letters indicate differences in the same time period ($p<0.05$). Different letters indicate differences within the same time period ($p<0.05$). * indicates a within-group difference with T_0 ($p<0.05$). ^ indicates a within-group difference with T_5 ($p<0.05$). # indicates a within-group difference with T_{10} ($p<0.05$). The difference between groups for the same time period is indicated with [a and b].

Results

During the study, one of the rabbits in the Med group developed dyspnea at 55 minutes during anesthesia, and breathing became regular with pure oxygen support. Two rabbits in the Mid+Med group showed intermittent apnea for an average of 1 minute after ketamine administration. These rabbits were included in the study. At T_{15}, one rabbit in the Mid+Med group died. A new rabbit was used to replace the deceased rabbit, and the study was continued.

VHS measurement increased from T_0 to T_{70} following sedation in the Med group. The VHS value did not change in the Mid+Med group ($p<0.05$). In the Dex and Mid+Dex groups, an increase was observed until T_{10} and T_{30}, respectively, and then a decrease was observed. There was no statistically significant difference between the groups ($p>0.05$, Table 1).

P wave duration measurements showed a statistically significant increase at T_{50} in the Med group compared to T_5. In PR interval duration, QRS wave amplitude duration, and QT interval duration measurements, there was no statistically significant difference between groups. In T wave duration measurements, an inter-group difference was found at T_{70} ($p<0.05$, Table 2).

There was a gradually decrease in HR and T measurements in all groups until T_{60}. In addition, there was a difference between the groups in HR measurements from T_5 to T_{50} and in T measurements at T_{35} ($p<0.05$). In *f*R measurement, a statistically significant decrease was observed in the Med group at T_{40} and T_{60} compared to T_0 ($p<0.05$). In MAP value measurements, there was an increase in the Mid+Dex group at T_5 compared to T_0 and a gradual decrease after T_5 until T_{60} ($p<0.05$). There was a difference between the groups in MAP between T_{20} and T_{60}. Hypotension (MAP below 60 mmHg) was observed only in the Mid+Dex group. While no change was observed in the Med group in SpO_2 measurements, an increase was observed in the Mid+Med, Dex and Mid+Dex groups until T_{60}. There was also a statistically significant difference between the groups between T_5 and T_{20} (Table 3).

Discussion

The midazolam-medetomidine (Group Mid+Med) anesthesia combination was found to cause smaller changes in morphological and physiological effects on the heart compared to the other groups. An increase was observed in the VHS parameter in the Med group. While no change was observed in the Mid+Med group, an increase and then a decrease were observed in the Dex and Mid+Dex groups. The study revealed that P wave duration increased solely in the Med group. Prolongation in QRS wave and QT interval durations was observed, with no change in PR interval or T wave duration. Although not reaching statistical significance, the Mid+Med, Dex, and Mid+Dex groups exhibited a trend towards prolonged QRS wave and QT interval durations. Heart rate (HR), temperature (T), respiratory rate (*f*R), and mean arterial pressure (MAP) values decreased in all groups, while oxygen saturation (SpO_2) increased in all groups except the Med group.

During the study, the occurrence of dyspnea in one rabbit in the Med group and apneic respiration in two rabbits in the Mid+Med group was attributed to respiratory system depression following ketamine administration. This association was previously noted in a scientific study conducted on Wistar rats (13).

Moarabi et al. found an average VHS value of 7.8±0.33 in their radiographic assessment of New Zealand rabbits in the right lateral position. (14). Our study revealed that the Med, Mid+Med, and Mid+Dex groups exhibited a similar trend in T_0 measurement data compared to Moarabi et al.'s findings; however, the Dex group showed a comparatively lower VHS measurement. The underlying cause of this discrepancy is postulated to be the higher intrathoracic fat volume observed in rabbits as compared to other species. This results in a more cranial positioning of the rabbit's heart on lateral radiographs, leading to the superimposition of the fat layer with the cranial aspect of the heart and the consequent formation of artifacts. Fractional shortening (FS) is an index used to assess left ventricular systolic performance, and it has been reported to significantly decrease after dexmedetomidine administration in a study (14). In another study, it was reported that medetomidine administration led to a decrease in fractional shortening similar to that observed with dexmedetomidine administration (15). In parallel with these findings, it has been stated that the use of medetomidine and dexmedetomidine leads to an increase in E-point septal separation, resulting in left ventricular dilation and an observed enlargement in the cardiac silhouette. (15,16,17). Similarly, it was hypothesized that the increase in VHS measurement in the Med, Dex, and Mid+Dex groups in our study is related to cardiomegaly resulting from left ventricular dilation due to delayed atrioventricular conduction. The reason for the absence of any changes in the Mid+Me group is speculated to be the antiarrhythmic effect shaped by midazolam usage, as also suggested by Dupras et al., which may prevent slowing of cardiac conduction (18). The decrease in VHS at T_{50} and T_{70} time intervals in the Dex group, as well as at T_{30}, T_{50}, and T_{70} time intervals in the Mid+Dex group, is concluded to be potentially attributed to the shortened half-life of dexmedetomidine, as defined in previous studies (19), and the reduction in vascular resistance by ketamine, which may alleviate the atrioventricular delay caused by dexmedetomidine (20,21).

In the electrocardiographic assessment, the recording in Lead II indicated a positive P wave, QRS complex, and T wave. In mice administered with xylazine and ketamine, a decrease in the amplitude and an increase in the duration of the P wave have been reported (22). On the other hand,

Table 2: ECG parameter measurements before and during the experiment

		Time					
	Groups	**T_0**	**T_5**	**T_{10}**	**T_{30}**	**T_{50}**	**T_{70}**
P-Wave Amplitude	Med	0.05±0.03	0.05±0.02	0.04±0.01	0.03±0.01	0.04±0.02	0.03±0.01
	Mid+Med	0.08±0.02	0.07±0.02	0.06±0.02	0.06±0.02	0.06±0.05	0.04±0.02
	Dex	0.06±0.02	0.07±0.02	0.05±0.02	0.07±0.02	0.06±0.05	0.04±0.02
	Mid+Dex	0.06±0.01	0.05±0.01	0.06±0.01	0.04±0.02	0.06±0.02	0.15±0.28
P-Wave Duration	Med	0.03±0.01	0.03±0.01	0.04±0.02	0.04±0.01	0.04±0.01^	0.06±0.05
	Mid+Med	0.03±0.06	0.03±0.01	0.03±0.01	0.03±0.01	0.05±0.02	0.05±0.02
	Dex	0.03±0.01	0.03±0.01	0.03±0.01	0.05±0.01	0.05±0.03	0.04±0.01
	Mid+Dex	0.03±0.03	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
PR Interval Duration	Med	0.05±0.03	0.04±0.01	0.06±0.03	0.05±0.01	0.05±0.01	0.05±0.01
	Mid+Med	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.06±0.02	0.06±0.01
	Dex	0.04±0.01	0.04±0.01	0.05±0.01	0.06±0.02	0.06±0.02	0.05±0.01
	Mid+Dex	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.05±0.01
QRS Complex Amplitude	Med	0.12±0.07	0.12±0.04	0.10±0.06	0.10±0.05	0.07±0.02	0.07±0.02
	Mid+Med	0.14±0.04	0.12±0.04	0.13±0.04	0.11±0.04	0.09±0.05	0.08±0.04
	Dex	0.13±0.04	0.13±0.03	0.12±0.03	0.11±0.04	0.12±0.03	0.13±0.04
	Mid+Dex	0.11±0.02	0.11±0.02	0.11±0.02	0.11±0.01	0.12±0.03	0.22±0.31
QRS Complex Duration	Med	0.16±0.04	0.15±0.03	0.14±0.03	0.14±0.027	0.14±0.04	0.14±0.05
	Mid+Med	0.13±0.01	0.13±0.01	0.13±0.01	0.15±0.023	0.14±0.03	0.15±0.03
	Dex	0.14±0.02	0.15±0.01	0.14±0.02	0.15±0.028	0.16±0.03	0.17±0.03
	Mid+Dex	0.13±0.02	0.13±0.01	0.14±0.02	0.15±0.025	0.15±0.02	0.15±0.02
QT Interval Duration	Med	0.22±0.06	0.22±0.05	0.21±0.06	0.21±0.07	0.22±0.09	0.24±0.06
	Mid+Med	0.18±0.02	0.17±0.02	0.18±0.01	0.20±0.04	0.21±0.04	0.21±0.04
	Dex	0.18±0.02	0.20±0.02	0.19±0.03	0.20±0.06	0.21±0.04	0.23±0.04
	Mid+Dex	0.18±0.02	0.18±0.02	0.18±0.02	0.20±0.03	0.20±0.02	0.21±0.02
T-Wave Amplitude	Med	0.06±0.04	0.06±0.03	0.05±0.01	0.07±0.05	0.06±0.06	0.04±0.01
	Mid+Med	0.08±0.03	0.08±0.03	0.07±0.02	0.06±0.03	0.06±0.04	0.05±0.02
	Dex	0.07±0.02	0.07±0.03	0.06±0.02	0.08±0.05	0.07±0.03	0.07±0.02
	Mid+Dex	0.05±0.02	0.06±0.02	0.06±0.02	0.06±0.02	0.06±0.02	0.09±0.09
T-Wave Duration	Med	0.05±0.03	0.06±0.04	0.05±0.03	0.06±0.02	0.07±0.03^{a}	0.07±0.02^{a}
	Mid+Med	0.03±0.01	0.04±0.01	0.03±0.01	0.04±0.01	0.05±0.02^{ab}	0.06±0.02^{ab}
	Dex	0.03±0.01	0.03±0.01	0.04±0.01	0.05±0.04	0.04±0.01^{ab}	0.04±0.01^{b}
	Mid+Dex	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01^{b}	0.04±0.01^{b}

Note: Measurements were recorded before drug administration (baseline) and every 5 minutes under anesthesia. Data are expressed as mean ± standard deviation. Different letters indicate differences in the same time period ($p<0.05$). Different letters indicate differences a within the same time period ($p<0.05$). * indicates a within-group difference with T_0 ($p<0.05$). ^ indicates within-group difference with T_5 ($p<0.05$). # indicates a within-group difference with T_{10} ($p<0.05$). The difference between groups for the same time period is indicated with [a and b]

Table 3: Measurement of heart frequency (HR), rectal temperature (*T*), respiratory rate (*f*R), noninvasive mean arterial pressure (MAP) and peripheral arterial hemoglobin saturation (SpO_2) before and after administration of Medetomidine (Med), Midazolam+Medetomidine (Mid+Med), Dexmedetomidine (Dex) or Midazolam+Dexmedetomidine (Mid+Dex)

		Time												
	Groups	T_0	T_5	T_{10}	T_{15}	T_{20}	T_{25}	T_{30}	T_{35}	T_{40}	T_{45}	T_{50}	T_{55}	T_{60}
HR	Med	226 ±50	202±34^{a}	187 ±29^{a}	170±39^{a}	182±33^^{a}	178±32^{a}	170±25^{a}	171±25^{a}	163±37	166±35	162±31^{a}	166±31	167±37
	Mid+Med	244±36	236±37^{ab}	234±45^{ab}	217±46^{ab}	219 ±52^{ab}	213 ±52^{ab}	198±51$^{\#ab}$	185±54$^{\#ab}$	198±64	164±40	167±32^$^{\#ab}$	166±33^$^{\#}$	152 ±27*^
	Dex	268±15	262±25^{b}	255±28^{b}	214±79^{ab}	229±31$^{\#ab}$	231±24^{b}	220±25^{ab}	214±30^{ab}	206±32	197±35*^	187±34*^^{ab}	184±35*^	179±31*^$^{\#}$
	Mid+Dex	262±30	275±28^{b}	266±33^{b}	258±30^{b}	248±30^{b}	249±38^{b}	238±35^{b}	227±31^{b}	219±38	214±37	209±34^{b}	207±36	200±27*^
T	Med	39.2±0.5	38.8±0.8	38.4±0.8	38.2±0.6	38.2±0.7*	37.9±0.51*	37.7±0.5*	37.7±0.3*^{ab}	37.6±0.6	37.5±0.6	37.5±0.5*	37.4±0.3*	37.3±0.4*
	Mid+Med	38.7±1	38.5±0.9	38.3±0.9	38.1±1.2	37.9±1.1*	37.7±1*	37.6±0.9*^$^{\#}$	37.5±0.9*^^{a}	37.2±0.9*^$^{\#}$	37.2±0.9*^	36.9±0.9*^$^{\#}$	36.7±0.8*^$^{\#}$	36.6±0.8*^$^{\#}$
	Dex	39±0.44	38.8±0.4	38.7±0.4	38.6±0.4*	38.4±0.5*	38.1±0.4*^$^{\#}$	37.9±0.5*^	37.6±0.5*^$^{\#ab}$	37.4±0.5*^$^{\#}$	37.3±0.5*^$^{\#}$	37.1±0.5*^$^{\#}$	37.0±0.4*^$^{\#}$	36.7±0.3*^$^{\#}$
	Mid+Dex	39.3±0.2	39.2±0.2	39.0±0.2*	38.8±0.2*	38.7±0.3	38.6±0.2*^$^{\#}$	38.4±0.3*^$^{\#}$	38.3±0.3*^$^{\#b}$	38.1±0.4*^$^{\#}$	37.8±0.3*^$^{\#}$	37.6±0.4*^$^{\#}$	37.5±0.5*^$^{\#}$	37.4±0.5*^$^{\#}$
***f*R**	Med	55±19^{a}	45±20	33±13	31±12	34±13	32±16	31±14	37±17	31.9±15*	32±11	36±13	35±13	29±11*
	Mid+Med	40±25^{ab}	50±26	49±23	45±20	46±14	42±11	40±15	39±18	30±13	35±12	35±14	36±16	31±13
	Dex	31±14^{ab}	43±19	43±13	31±20	46±11	49±16	51±13	48±10	37±13	40±9	41±7	37±8	36±12
	Mid+Dex	30±6^{b}	46±17	41±19	55±29	46±29	39±22	38±18	36±6	29±10	32±5	32±6	27±10	28±8
MAP	Med	93±12^{a}	105±40	109±43	123±39	123±41^{a}	117±46^{a}	107±37	124±66^{a}	127±54^{a}	118±30^{a}	99±12^{a}	81±16	82±12^{a}
	Mid+Med	70±11^{b}	81±28	72±28	74±28	62±14^{b}	70±18^{b}	83±21	82±13^{ab}	79±14^{ab}	79±14^{ab}	82±20^{ab}	85±17	84±21^{a}
	Dex	74±12^{b}	77±12	85±19	104±47	106±34^{ab}	110±41^{ab}	108±61	109±62^{ab}	109±59^{ab}	109±63^{a}	98±53^{a}	87±40	74±33^{ab}
	Mid+Dex	77±12^{ab}	100±34*	96±34*^	97±57*$^{\#}$	95±41*^$^{\#ab}$	75±18^{ab}	64±22	56±27*^$^{\#b}$	56±31*^$^{\#b}$	56±34*^$^{\#b}$	50±25*^$^{\#b}$	57±44*^$^{\#}$	50±24*^$^{\#b}$
SpO_2	Med	91±6	95±3^{ab}	97 ±2^{a}	96±4	97±2^{ab}	96±4	98.5±1	96±5	96±5	97±4	97±4	97±3	96±6
	Mid+Med	95±1	97±1^{a}	97±1^{a}	98±1	98.5±1^{a}	97±1	98±1	98±1	98±1*	98±1	98±1*	98±1	99±1
	Dex	92±3	94±2^{b}	96±1^{ab}	97±1*	96±2^{b}	98±1*	97±1*^$^{\#}$	98±1	98±1	97±1	97±1	97±1	98±1*
	Mid+Dex	92±1	94±1*^{ab}	94±1*^{b}	96±1*^	96±1*^^{ab}	97±1*^$^{\#}$	98±1*^$^{\#}$	97±1*	97±1*	96±1*$^{\#}$	97±1*^$^{\#}$	97±1*^$^{\#}$	97±1*^$^{\#}$

Note: Measurements were recorded before drug administration (baseline) and every 5 minutes under anesthesia. Data are expressed as mean ± standard deviation. Different letters indicate differences in the same time period ($p<0.05$). Different letters indicate differences a within the same time period ($p<0.05$). * indicates a within-group difference with T_0 ($p<0.05$). ^ indicates within-group difference with T_5 ($p<0.05$). # indicates a within-group difference with T_{10} ($p<0.05$). The difference between groups for the same time period is indicated with [a and b]

administration of medetomidine in dogs has been reported to result in a decrease in both the duration and amplitude of the P wave (23). The prolongation of the P wave duration indicates left atrial dilation, while the increase in amplitude suggests right atrial dilation (24). In studies conducted, it has been reported that the amplitude and duration of the P wave in rabbits varied between 0.04 to 0.12 mV and 0.01 to 0.05 s, respectively (25,26). In our study, although the prolongation of P wave duration in the Med group was not statistically significant, the elongation at T_{70} time may be related to left atrial dilation and consequently, a slowdown in impulse conduction in the SA.

Although statistically significant differences were not observed in intra-group and inter-group comparisons of QRS complex durations, an elongation in QRS complex durations was identified in all groups in our study compared to the reported QRS complex durations in healthy rabbits (0.02-0.06 s) from a previous study (26). Cave et al. (27) investigated the effects of hypertonic fluid therapy following treatment with lipid emulsion on bupivacaine toxicity in New Zealand rabbits. In their study, they described that a wide QRS complex reflects left-sided intraventricular conduction delay, while a prolonged QRS complex reflects delayed depolarization of ventricular function. In light of the above information, in our study, it is contemplated that the prolongation of QRS complex durations following the administration of medetomidine and dexmedetomidine may be attributed to left-sided intraventricular delay. Our hypothesis is supported by the findings of Shekidef and colleagues, who reported an elongation in QRS complex durations in calves associated with the use of α2-adrenergic

receptor agonists. They attributed this phenomenon to delayed ventricular depolarization (28).

In a study assessing the risk factors for prolonged QT (0.15-0.17s) and cardiac arrhythmias in female rabbits due to drug administration, it was emphasized that the measurement of the QT interval is crucial in detecting cardiac repolarization abnormalities. The study highlighted that the prolongation of this interval is associated with the emergence of arrhythmias (29). In our study, the prolongation of the QT interval duration, compared to the data found in healthy rabbits by Lord et al. (26) (average 0.12s), was considered to be attributable to cardiomyopathy resulting from the delay between ventricular depolarization and repolarization, as also suggested by Yılmaz (24). Our hypothesis is supported by the findings of Drici et al., who described the prolonged QT syndrome in cats due to the use of antipsychotic drugs as being associated with drug-induced ventricular arrhythmias (30). Similarly, Kinjavdekar et al. reported that the subarachnoid administration of α2-adrenergic receptor agonists in goats could lead to the prolongation of the QT interval, suggesting that this prolongation may be attributed to delayed ventricular depolarization. (31).

In our study, it was observed that the average heart rate at T_0 is similar to the previously reported average heart rate for rabbits (32) (200-300 bpm), and it was found that the heart rate decreased over time in all groups. We hypothesized that the reason for this is attributed to the bradycardia induced by medetomidine and dexmedetomidine, as stated in the study conducted by Murrell and colleagues (33). Similarly, Yamashita et al. have reported a significant reduction in heart rate with the use of α2 adrenergic receptor agonists in horses. (34). In our study, the decrease in heart rate can be explained by sinus bradycardia induced by α2 adrenergic receptor agonists, which is thought to result from the reduction in sympathetic neurotransmitter release from the central nervous system. In addition, England et al. have reported a decrease in heart rate due to sinus bradycardia as a result of the use of α2-adrenergic receptor agonists (35). The heart rate in the Mid+Dex group was found to be higher than the heart rate in the Med group. It was hypothesized that the regulatory effect of midazolam, attributed to its antiarrhythmic properties, played a role in the occurrence of this difference in heart rates (36). Granholm et al., in their study investigating the reliability of medetomidine and dexmedetomidine in cats, reported an increase in heart rate in the group where dexmedetomidine was used compared to the group where medetomidine was used. They attributed this phenomenon to the reduction in sympathetic tone, shaped by inhibiting norepinephrine release in the central nervous system (37). Therefore, in our study, it was concluded that the higher heart rate in the Mid+Dex group could be attributed to the combined effects of both midazolam and dexmedetomidine administration.

Before the experiment, T was within the reference range (37.4-39.6°C) in all groups (38). It was observed that over time, all values gradually decreased but remained within the reference range. The gradual decrease in T observed over time after the administration of dexmedetomidine and medetomidine was considered to be associated with a reduction in muscle activation and the impact on thermoregulation following the administration of α2-adrenergic receptor agonists. These results were consistent with the findings of Ansah et al. and Selmi et al.(39,40). It has been reported that hypothermia develops in cats following the administration of α2-adrenergic receptor agonists, and this condition is attributed to a decrease in heat production due to reduced muscle activity during sedation. It is suggested that the direct effects of α2-adrenergic receptor agonists on thermoregulation may be responsible for this phenomenon. (41). Furthermore, in dogs, it has been reported that the administration of medetomidine and dexmedetomidine affects thermoregulation through central α2-adrenoreceptors, leading to a decrease in T. (42). However, in the study by Granholm and colleagues, no significant difference was found between the administrations of dexmedetomidine and medetomidine. In our study, a significant difference was observed at T_{35} between the Mid+Med and Mid+Dex groups, with the group receiving dexmedetomidine having a higher temperature than the group receiving medetomidine. We considered that this situation could be attributed to the difference in levels of muscle activity and the generation of heat through muscle activity. Our hypothesis is supported by the detection of intergroup differences in T following the administration of medetomidine and dexmedetomidine in cats. This was reported to be attributed to the difference in levels of muscle activity (39).

In rabbits, the normal *f*R during rest is between 30 and 60 breaths per minute. (43). n our study, all *f*R measurements recorded, except for the Med group, were within the normal range. In the Med group, a significant decrease was observed at T_{40} and T_{60} compared to T_0. The decrease observed in our study was considered to be related to a reduction in sympathetic tone, possibly due to the effects of medetomidine, as also indicated by Granholm et al. (37). A similar observation was reported in a study conducted in sheep, where bradypnea was noted between the T_5 and T_{60} minutes following the administration of medetomidine, attributed to a decrease in sympathetic tone. (44).

The report has documented that, for the identification of anesthesia-induced hypotension in rabbits, the non-invasive MAP value should be above 60 mmHg. (45). In our study, no statistically significant change was observed in MAP values evaluated with the non-invasive method over time in the Med, Mid+Med, and Dex groups. In the Mid+Dex group, an increase in MAP values was observed between T_5 and T_{20} minutes, followed by a gradual decrease after T_{35}. The initial increase in MAP was attributed to an elevation in vascular resistance and contraction of vascular smooth muscles, as indicated by Brunton et al. (46). The subsequent decline was considered to be influenced by dexmedetomidine activating presynaptic α2-adrenoreceptors, thereby

reducing plasma norepinephrine absorption (45). Additionally, the hypotensive effect of isoflurane, used for maintaining anesthesia, was presumed to contribute to the decrease in arterial blood pressure (33). Similarly, a study conducted in dogs (39) reported the hypotensive effects of dexmedetomidine while noting that medetomidine did not have a significant impact on this hemodynamic response.

Pulse oximetry is a non-invasive monitoring method that provides information about lung ventilation and can be utilized to assess a patient's oxygenation and perfusion (47). In rabbits, an SpO_2 reference value of 94% and above is considered acceptable, while measurements below 90% are interpreted as indicative of hypoxia. (48). In our study, all SpO_2 measurements in each group were within the reference range. In the T_{10} time interval, the lower measurement of the SpO_2 parameter in the Mid+Dex group compared to the Mid+Med groups may be attributed to the stronger sedative effect of dexmedetomidine. Indeed, supporting our hypothesis, a study conducted in dogs (49) reported that dexmedetomidine provided stronger and more predictable sedation and analgesia compared to an equivalent dose of medetomidine. Similarly, Yanmaz et al. (11) reported a gradual decrease in SpO_2 over time in their study using intranasal combinations of dexmedetomidine and midazolam in New Zealand rabbits. Despite the administration of oxygen, they observed that SpO_2 remained below 90%. On the other hand, Wei et al. (50) found a higher SpO_2 value after the intranasal administration of medetomidine compared to the SpO_2 measurement reported by Yanmaz and colleagues. As observed, these studies indicate the role of dexmedetomidine in the detection of low SpO_2 values. Additionally, a study conducted in rabbits reported that midazolam had less respiratory depression compared to dexmedetomidine. (45). In light of these findings, the SpO_2 values of animals in our study being within reference ranges and the highest SpO_2 values being observed in the Mid+Med group, can be interpreted as the combination of midazolam and medetomidine used in our study causing less respiratory depression.

The primary limitation of our study stems from the method of selecting two rabbits from each group and studying them on the same day, as opposed to examining all animals within a single group on a unified day. This approach could have minimized pre-experimental differences arising from the capture and handling method, allowing for greater control over potential variations between the groups.

Conclusion

Upon comprehensive examination of all the data in the study, it was observed that the anesthesia combination of midazolam and medetomidine (Group Mid+Med) induced comparatively fewer morphological and physiological alterations in the heart when contrasted with the other groups..

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Conflicts of interest. The authors declare no competing interests.

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Raziskava učinkov različnih kombinacij anestezije na kardiovaskularne parametre pri belih novozelandskih kuncih

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Izvleček: Cilj te študije je bil ovrednotiti morfološke in fiziološke spremembe srca, nastale zaradi štirih različnih kombinacij anestezije, pri čemer so bili kot sedativi uporabljeni midazolam, medetomidin in dexmedetomidin pri belih novozelandskih kuncih. V študiji smo 32 živali razdelili v štiri različne skupine. Vrednost vertebralnega srčnega indeksa je bila merjena pri zajcih pred poskusom (T0) in po 5 (T5), 10 (T10), 30 (T30), 50 (T50) in 70 (T70) minutah med poskusom. Hkrati so bile v enakih časovnih intervalih opravljene meritve elektrokardiografskih parametrov. Srčno frekvenco, frekvenco dihanja, rektalno temperaturo, srednji arterijski tlak in nasičenost periferne krvi s kisikom smo merili skupaj 60 minut, s 5-minutnimi presledki pred in med sedacijo. Vrednost vertebralnega srčnega indeksa se je spremenila v vseh skupinah, razen v skupini Mid+Med. Pri elektrokardiografski oceni smo v skupinah Mid+Med, Dex in Mid+Dex opazili podaljšanje trajanja vala QRS in intervala QT, medtem ko pri trajanju intervala PR in vala T nismo zaznali bistvenih sprememb. Nasprotno smo v skupini Me opazili izrazito podaljšanje trajanja vala P. Vrednosti nasičenosti periferne krvi s kisikom so se povečale, srčna frekvenca, srednji arterijski tlak in parametri rektalne temperature so se v vseh skupinah znižali. Po temeljiti analizi vseh podatkov v tej študiji je bilo ugotovljeno, da so morfološki in fiziološki učinki na srce, zaznani v skupini Mid+Med, povzročili manj izrazite spremembe v primerjavi z drugimi skupinami.

Ključne besede: elektrokardiografija; radiografija; kunec; VHS

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Original Research Article

Changes in Biochemical Markers in Broiler Chickens Exposed to Gadolinium and Lanthanum Orthovanadate Nanoparticles

Key words

gadolinium orthovanadate nanoparticles;
lanthanum orthovanadate nanoparticles;
biochemical markers;
broiler chickens;
blood serum

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Abstract: In our research, we were interested in the presence of changes in the biochemical profile of the blood serum of broiler chickens under the influence of nanoparticles of gadolinium orthovanadate (NP $GdVO_4:Eu^{3+}$), lanthanum orthovanadate (NP $LaVO_4:Eu^{3+}$) and their mixture in therapeutic doses, which were established by us in previous studies on white rats. Day-old broiler chickens of the *Cobb 500* cross (n=150) were used as the object of study. Chickens of the experimental group I received NP $GdVO_4:Eu^{3+}$ for 10 days at a dose of 0.2 mg/L of drinking water, experimental group II – NP $LaVO_4:Eu^{3+}$ at a dose of 0.2 mg/L of drinking water, experimental group III – NP $GdVO_4:Eu^{3+}$ and NP $LaVO_4:Eu^{3+}$ at a dose of 0.2 mg/L of drinking water (on average, chickens received 0.09 (0.13-0.05) mg/kg body weight of NP) and chickens of the experimental group IV received with water the veterinary vitamin drug Devivit Complex to compare the antioxidant effect at a dose of 0.3 ml/L of drinking water, chickens of the control group received drinking water without additives. After 10 days, NP administration was stopped and the chickens were observed for another 5 days. The administration of these nanoparticles to broiler chickens for 10 days was found to lead to a decrease in lipid metabolism (total cholesterol and triglycerides), protein metabolism (uric acid) and lipid peroxidation (diene conjugates and malondialdehyde) against the background of activation of carbohydrate metabolism (increased glucose concentration) and activity of hepatospecific enzymes (alanine and aspartate aminotransferases) with a prolonged effect after discontinuation of administration. The data obtained show that rare earth element orthovanadates nanoparticles have antioxidant properties. These nanoparticles are promising candidates for use in feed additives and veterinary drugs with an adaptogenic effect.

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Introduction

TIn the current state of veterinary medicine, clinical and pathological parameters alone may not provide sufficient diagnostic information for many pathological conditions. Therefore, it is necessary to conduct in-depth studies on the biochemical processes that sustain living matter, as well as the changes that occur during pathology (1, 2).

Determining the quantitative content of various components in biological fluids and tissues of healthy animals, as well as their changes in diseases, allows laboratory tests to perform timely diagnostics (even in the absence of clinical manifestations of the disease), study the pathogenesis, and test the effectiveness of therapeutic measures and drugs. In addition, biochemical studies can be used to monitor the health status of animals and the adequacy of their feeding (3, 4, 5).

Scientific progress has led to the development of many new substances for various industries, including those that use nanotechnology. However, studying the direct effects of these substances on living organisms and assessing their safety and efficacy requires biochemical studies (6, 7, 8). Special attention is given to creating new antioxidant drugs for livestock (poultry) farming in order to meet the demands of intensive agricultural production under stressful conditions (9, 10).

Some nanomaterials have been proven to have antioxidant activity. The most studied ones are nanocerium (11), silica nanoparticles (12), polydopamine nanoparticles (13), and nanoantioxidants based on nanocomposites of polysaccharides and proteins (14, 15, 16). Nanoantioxidants have many advantages over traditional antioxidants. They have increased bioavailability, controlled release, and can be delivered directly to the site of action (17).

Nanoparticles of rare earth orthovanadates, specifically gadolinium and lanthanum, are potential antioxidant substances. In a subchronic toxicological experiment conducted on white rats under conditions of feeding stress, these nanoparticles demonstrated an adaptogenic effect. When administered in doses of approximately 0.03-0.15 mg/kg body weight, the concentration of primary and secondary lipid peroxidation products decreased, while the activity of hepatospecific enzymes normalized (18, 19). Nanoparticles were found to have a positive effect on the intestinal mucosa by activating its mechanical and immunological barrier (20). This discovery led to further research on the effect of these nanoparticles on poultry.

The study aimed to analyze the changes in biochemical markers in broiler chickens' bodies when exposed to gadolinium and lanthanum orthovanadate nanoparticles.

Materials and methods

The place of the experiment

The experiment was conducted on the basis of the vivarium of the State Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise in Kyiv, Ukraine (experimental chickens of all five groups were kept in identical conditions) and in the laboratory of toxicological monitoring of the National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine» in Kharkiv, Ukraine.

Experimental bird, poultry keeping conditions and ration

Day-old broiler chickens of the *Cobb 500* cross (n=150) were used as the object of study. The birds were kept under optimal conditions: at a room temperature of (28 ± 4) °C with a relative humidity of (60-70) %; the day-night lighting cycle during the experiment was (15-9) h, and the vivarium room had 18 air volume changes per hour.

For feeding the chickens, we used the compound feed «PK 5 1-2 weeks Start Broiler», having previously determined the content of nutrients in it: the determination of crude protein content was carried out by the Kjeldahl method according to DSTU ISO 5983:2003, crude fiber - according to DSTU ISO 6865:2004, crude fat – according to DSTU ISO 6492:2003; vitamin content – according to DSTU 4687:2006, trace elements – according to DSTU EN 14082:2019. The research results are summarized in Table 1.

Table 1: Qualitative composition of the diet of broiler chickens (compound feed «PK 5 1-2 weeks Start Broiler»)

Indicator	Actually determined	Norm (21, 22)	± to the norm
Carbohydrates, g/100 g	57,18	Not standardized	–
Energy value, kcal	376,09	290,00	+ 86,09
Mass fraction of fat, %	6,69	Not standardized	–
Mass fraction of crude protein, %	21,79	21,00-22,00	Norm
Mass fraction of crude fiber, %	2,80	No more than 3.0	Norm
Vitamin B2, mg/kg	8,28	9,00	– 0,72
Vitamin A, IU/kg	7920,00	10000,00-13000,00	– 2080
Vitamin E, mg/kg	212,50	80,00	+ 132,50
Selenium, mg/kg	0,172	0,35	– 0,178
Copper, mg/kg	38,67	15,00	+ 23,67
Zinc, mg/kg	144,99	100,00	+ 44,99

The research program was reviewed and approved by the Bioethics Commission of the National Scientific Centre, Institute of Experimental and Clinical Veterinary Medicine in the current order. Animal experiments are in compliance

with the current legislation of EU (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 22 September 2010).

Experimental nanoparticles and comparator drug

In this work, we used experimental samples of gadolinium orthovanadate nanoparticles (NP $GdVO_4$:Eu^{3+}) (spindle-shaped geometry, size 8 × 25 nm) and lanthanum orthovanadate (NP $LaVO_4$:Eu^{3+}) (rod-shaped geometry, size 8 × 80 nm), with an initial concentration of 1.0 g/L (23, 24). The synthesis of gadolinium and lanthanum orthovanadate nanoparticles was carried out according to the method described in the scientific work of Klochkov et al. (23). The experimental samples of nanoparticles were synthesized and standardized according to their stability and size at the Department of Nanostructural Materials named after Yu. Malyukin of the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (Fig. 1).

Was used as a comparison drug veterinary vitamin preparation Devivit Complex (manufacturer LLC «DEVIE», Ukraine): one milliliter of the preparation contains active substances: vitamin A – 15000 IU, vitamin D_3 – 1000 IU, vitamin E – 20 mg, vitamins B_1 – 10 mg, B_2 – 0.5 mg, B_3 – 25 mg, B_5 – 35 mg, B_6 – 3.0 mg, B_{12} – 30 mg.

Experimental design

On the analog principle, 4 experimental and one control group of day-old broiler chickens (n=30) were formed: chickens of the experimental group I received NP $GdVO_4$:Eu^{3+} solution for 10 days at a dose of 0.2 mg/L of drinking water, experimental group II – NP $LaVO_4$:Eu^{3+} solution at a dose of 0.2 mg/L of drinking water, experimental group III – NP $GdVO_4$:Eu^{3+} and NP LaVO4: Eu3+ at a dose of 0.2 mg/L of drinking water (on average, chickens received 0.09 (0.13-0.05) mg/kg body weight of NP). The chickens of the experimental group IV received with water the veterinary vitamin drug Devivit Complex to compare the antioxidant effect at a dose of 0.3 ml/L of drinking water. The chickens of the control group received drinking water without additives. After 10 days, NP administration was stopped and the chickens were observed for another 5 days. The total duration of the experiment was 15 days.

During CO_2 anesthesia, 10 chickens from each group were euthanized 5 and 10 days after the start of administration and 5 days after the end of administration. Blood samples were taken from the chickens for further determination of biochemical parameters.

Research methods

The serum of experimental chickens was used to determine the content of total cholesterol (TC), total lipids (TL), triglycerides (TGL), total proteins, glucose, uric acid, and the activity level of indicator enzymes aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) – by conventional biochemical methods (27) using reagent kits produced by CORMAY (Poland) and SPE «Filisit-Diagnostics» (Ukraine), the intensity of lipid peroxidation (LPO) in blood serum was determined by the level of formation of its products: primary – diene conjugates (DC) and final – malondialdehyde (MDA) under conditions of extraction in a mixture of heptane-isopropanol (1:1) at wavelengths of 233 and 247 nm (28). The study was performed using a spectrophotometer (SHIMADZU UV-1800, Japan).

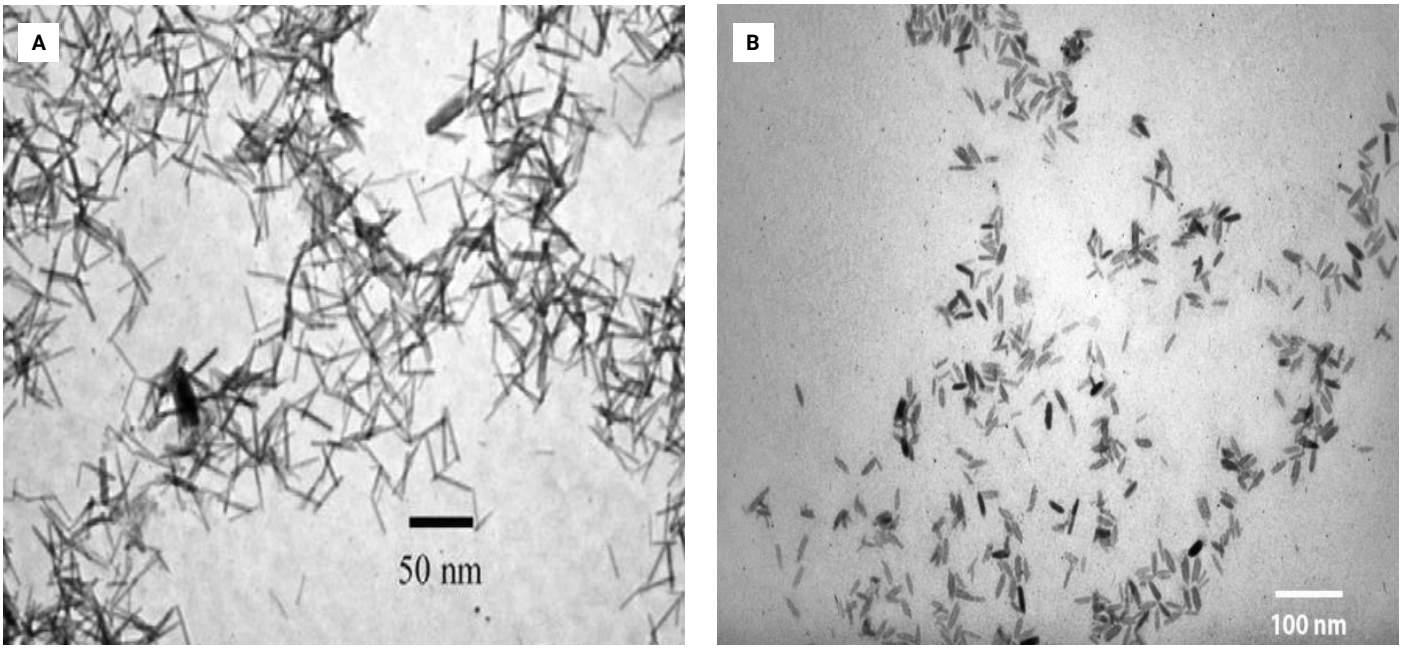

Figure 1: Photograph (transmission electron microscopy, TEM-125K, Selmi, Ukraine) of nanoparticles: A) $GdVO_4$:Eu^{3+}; B) $LaVO_4$:Eu^{3+} (25, 26).

Statistical analysis

The obtained results were processed by methods of variation statistics using the analysis of variance (ANOVA) software package StatPlus 7.6.5.0 (AnalystSoft Inc., USA). The reliability of the results was assessed by the Tukey's test (HSD difference of means) at a reliability level of 95.0% ($P < 0.05$)..

Results

Biochemical studies of blood of broiler chickens revealed certain changes in the lipid profile, LPO indicators and enzymatic activity. Thus, the concentration of TC in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$, 0.2 mg/L of drinking water) on the 5th day of administration exceeded the control value ($P < 0.05$) by 10.2%, on the 10th day – decreased by 19.7% ($P < 0.05$) and remained 21.4% lower than the control 5 days after the end of administration. With the introduction of NP $LaVO_4:Eu^{3+}$, 0.2 mg/L of drinking water (experimental group II), the concentration of TC was lower than the control at all study periods ($P < 0.05$), which was 7.8% on the 5th day, 23.4% on the 10th day, and 31.4% 5 days after the end of the administration. During the period of administration (10 days) of both types of nanoparticles (experimental group III), the concentration of TC in the blood serum of chickens tended to decrease, and 5 days after the end of administration it significantly decreased compared to the control by 26.5% ($P < 0.05$). Similar was the dynamics of TC in the blood serum of chickens after administration of the vitamin preparation Devivit complex: a downward trend during the administration of the drug and a significant decrease compared to the control by 23.8% ($P < 0.05$) 5 days after the end of administration (Table 2).

The concentration of TL in the blood serum of chickens of experimental group I (NP $GdVO_4:Eu^{3+}$) on the 5th day of administration exceeded the control value ($P < 0.05$) by 6.0%, on the 10th day – decreased by 10.9% ($P < 0.05$), while 5 days after stopping the administration it again exceeded the control by 22.1% ($P < 0.05$). When NP $LaVO_4:Eu^{3+}$ was administered (experimental group II), the concentration of TL was higher than the control at all study periods ($P < 0.05$), which was 24.0% on the 5th day, 21.1% on the 10th day, and 12.7% 5 days after stopping the administration. After 5 days of administration of both types of nanoparticles (experimental group III, the concentration of TL in the blood serum of chickens significantly increased compared to the control by 18.0% ($P < 0.05$), after 10 days – it had only a tendency to increase and after 5 days after the end of administration it increased again compared to the control by 34.0% ($P < 0.05$). With the introduction of the vitamin drug Devivit complex, a significant decrease ($P < 0.05$) in the concentration of TL was observed only after 10 days of experiment by 8.9%, while at the first and last terms of the experiment no significant changes were observed (Table 2).

The concentration of TGL in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) significantly decreased compared to the control ($P < 0.05$): after 5 and 10 days of administration by 68.1 and 46.9%, respectively, and 5 days after stopping the administration – by 32.8%. A similar dynamics of TGL concentration was observed in the blood serum of chickens of the second experimental group (NP $LaVO_4:Eu^{3+}$): after 5 and 10 days of administration, the decrease ($P < 0.05$) was 31.9 and 62.5%, respectively, and 5 days after stopping the administration – 13.4% ($P < 0.05$). After 5 days of administration of both types of nanoparticles (experimental group III), the concentration of TGL in the blood serum of chickens significantly increased compared to the control by 13.0% ($P < 0.05$), after 10 days – decreased by 25.0% ($P < 0.05$) and 5 days after stopping the administration remained significantly lower than the control by 13.4% ($P < 0.05$). With the introduction of the vitamin preparation Devivit complex (experimental group IV), the concentration of TGL in the blood serum of chickens was lower than the control at all periods of the study ($P < 0.05$), which was 43.5% on the 5th day, 37.5% on the 10th day and 10.4% 5 days after the stopping of administration (Table 2).

A significant decrease ($P < 0.05$) in the concentration of diene conjugates was observed in the blood serum of chickens of all experimental groups throughout the study period. Thus, the concentration of DCs in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was lower than the control by 29.6, 59.1, and 56.7%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 34.8, 44.1, and 55.4% after 5 and 10 days of administration and 5 days after cessation of administration, respectively. In the III experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of DCs was 25.1% lower than the control after 5 days, 37.1% after 10 days, and 51.0% after 5 days after stopping the administration.

With the administration of the vitamin preparation Devivit complex (experimental group IV), the concentration of DCs in the blood serum of chickens was lower than the control by 44.5, 71.3 and 45.8%, respectively, after 5 and 10 days of administration and 5 days after cessation of administration (Table 3).

Similarly to DCs, a decrease in the concentration of malondialdehyde in the blood serum of chickens of all experimental groups was found throughout the study period. Thus, the concentration of MDA in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was lower than the control by 6.4, 47.7 and 34.6%, respectively ($P < 0.05$). In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 8.0,

Table 2: Dynamics of lipid metabolism in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Total cholesterol (TC), mMol/L			
Control	5,28±0,08	5,17±0,09	5,13±0,08
Experimental group I (NP $GdVO_4:Eu^{3+}$)	5,82±0,10*	4,15±0,08*	4,03±0,08*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	4,87±0,10*	3,96±0,07*	3,52±0,09*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	4,98±0,11	4,82±0,09	3,77±0,08*
Experimental group IV, Devivit Complex	5,17±0,09	5,01±0,11	3,91±0,09*
Total lipids (TL), g/L			
Control	3,17±0,03	3,03±0,04	2,44±0,02
Experimental group I (NP $GdVO_4:Eu^{3+}$)	3,36±0,03*	2,70±0,04*	2,98±0,03*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	3,93±0,03*	3,67±0,02*	2,75±0,04*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	3,74±0,02*	3,17±0,03	3,27±0,07*
Experimental group IV, Devivit Complex	3,21±0,03	2,76±0,04*	2,45±0,03
Triglycerides (TGL), mMol/L			
Control	0,69±0,02	0,64±0,02	0,67±0,01
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,22±0,01*	0,34±0,01*	0,45±0,01*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,47±0,02*	0,24±0,01*	0,50±0,01*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,78±0,02*	0,48±0,01*	0,58±0,01*
Experimental group IV, Devivit Complex	0,39±0,01*	0,40±0,01*	0,60±0,01*

Note * p<0.05 – compared to the control group

17.8 and 34.6% after 5 and 10 days of administration and 5 days after stopping the administration, respectively ($P < 0.05$). In the third experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of MDA tended to decrease after 5 days, after 10 days it significantly decreased by 22.5% ($P < 0.05$) and 5 days after stopping the administration – by 29.2% ($P < 0.05$). In the case of the vitamin preparation Devivit complex (experimental group IV), the concentration of MDA in the blood serum of chickens was lower than the control ($P < 0.05$) by 32.0, 47.3 and 28.0%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 3).

The ALT activity in the blood serum of chickens of the experimental group I (NP $GdVO_4:Eu^{3+}$) had no significant deviations from the control group during the entire study period. In the second experimental group (NP $LaVO_4:Eu^{3+}$), after 5 days of administration, an increase in enzyme activity by 12.7% ($P < 0.05$) was observed, after 10 days no deviations from the control were found, and 5 days after the administration was stopped, an increase in ALT by 25.9% ($P < 0.05$) was again detected. After 5 days of administration of both types of nanoparticles (experimental group III), no significant changes in ALT activity were observed, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity exceeded the control values by 12.3 and 17.2% ($P < 0.05$). With the administration

Table 3: Dynamics of lipid peroxidation indicators and aminotransferase activity in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Diene conjugates DC, μMol/L			
Control	12,37±0,29	13,50±0,28	15,86±0,27
Experimental group I (NP $GdVO_4:Eu^{3+}$)	8,71±0,18*	5,52±0,14*	6,86±0,16*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	8,06±0,22*	7,55±0,21*	7,08±0,18*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	9,26±0,19*	8,49±0,32*	7,77±0,21*
Experimental group IV, Devivit Complex	6,86±0,21*	3,88±0,18*	8,60±0,17*
Malondialdehyde (MDA), ΔD/mL			
Control	2,50±0,04	2,58±0,03	3,18±0,06
Experimental group I (NP $GdVO_4:Eu^{3+}$)	2,34±0,03*	1,35±0,03*	2,08±0,05*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	2,30±0,03*	2,12±0,03*	2,08±0,05*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	2,38±0,03	2,00±0,03*	2,25±0,03*
Experimental group IV, Devivit Complex	1,70±0,04*	1,36±0,03*	2,29±0,03*
Alanine aminotransferase activity, mmol/h×L			
Control	0,55±0,01	0,57±0,01	0,58±0,01
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,55±0,01	0,55±0,01	0,60±0,01
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,62±0,01*	0,58±0,01	0,73±0,01*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,57±0,01	0,64±0,01*	0,68±0,01*
Experimental group IV, Devivit Complex	0,58±0,01	0,59±0,01	0,59±0,01
Aspartate aminotransferase activity, mmol/h×L			
Control	5,70±0,15	6,10±0,09	6,30±0,12
Experimental group I (NP $GdVO_4:Eu^{3+}$)	5,80±0,11	5,60±0,10*	5,80±0,11*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	5,80±0,12	5,80±0,11	6,40±0,10
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	5,90±0,11	5,80±0,08	5,90±0,12
Experimental group IV, Devivit Complex	6,00±0,14	6,80±0,17*	6,80±0,11*
De Ritis ratio, AST/ALT			
Control	10,4	10,7	10,9
Experimental group I (NP $GdVO_4:Eu^{3+}$)	10,5	10,2	9,7
Experimental group II (NP $LaVO_4:Eu^{3+}$)	9,4	10,0	8,80
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	10,4	9,1	8,70
Experimental group IV, Devivit Complex	10,3	11,5	11,5

Note * $p<0.05$ – compared to the control group

Table 4: Dynamics of key indicators of protein and carbohydrate metabolism in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Total proteins, g/L			
Control	43,68±0,63	43,91±0,44	42,65±0,34
Experimental group I (NP $GdVO_4:Eu^{3+}$)	43,56±0,48	43,34±0,42	42,94±0,40
Experimental group II (NP $LaVO_4:Eu^{3+}$)	42,88±0,47	44,36±0,28	43,28±0,32
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	43,79±0,37	43,68±0,41	43,68±0,35
Experimental group IV, Devivit Complex	43,00±0,51	45,27±0,24	43,28±0,26
Glucose, mmol/L			
Control	11,54±0,17	11,18±0,22	11,18±0,28
Experimental group I (NP $GdVO_4:Eu^{3+}$)	12,99±0,19*	13,28±0,28*	12,77±0,27*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	12,93±0,25*	13,43±0,24*	12,25±0,21*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	12,60±0,22*	13,75±0,25*	11,96±0,20
Experimental group IV, Devivit Complex	12,35±0,12*	13,16±0,28*	12,43±0,28*
Uric acid, mmol/L			
Control	0,53±0,016	0,66±0,014	0,73±0,011
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,52±0,014	0,61±0,015*	0,46±0,01*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,47±0,015*	0,60±0,013*	0,51±0,009*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,44±0,012*	0,53±0,01*	0,49±0,01*
Experimental group IV, Devivit Complex	0,47±0,014*	0,63±0,01*	0,61±0,009*

Note * p<0.05 – compared to the control group

of the vitamin preparation Devivit complex (experimental group IV), ALT activity had no significant deviations from the control group during the entire study period (Table 3).

The activity of AST in the blood serum of chickens of experimental group I (NP $GdVO_4:Eu^{3+}$) after 5 days of administration did not have a significant deviation from the control, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity was lower than the control by 8.2 and 7.9% ($P < 0.05$). With the administration of NP $LaVO_4:Eu^{3+}$ (experimental group II) and NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$ (experimental group III), AST activity did not have significant deviations from the control group during the entire study period. And in the case of vitamin preparation Devivit complex (experimental group IV), AST activity in the blood serum of chickens after 5 days of administration had no significant deviation from the control, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity exceeded the control values by 11.5 and 7.9% ($P < 0.05$) (Table 3).

The concentration of total proteins did not have significant deviations in all groups during the experiment, but during the period of administration in the first experimental group (NP $GdVO_4:Eu^{3+}$) a downward trend was recorded, in experimental groups II and IV (NP $LaVO_4:Eu^{3+}$ and Devivit complex) level of TP tended first to decrease, and then to increase, in experimental group III (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$) the level of TP was close to the control. It should

be noted that 5 days after the stopping the administration, the levels of TP in the blood serum of chickens of all experimental groups tended to increase (Table 4).

A significant increase ($P < 0.05$) in glucose concentration was observed in the blood serum of chickens of all experimental groups during the entire period of the study. Thus, the concentration of glucose in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was higher than the control by 12.6, 18.8 and 14.2%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the excess was 12.0, 20.1, and 9.6% after 5 and 10 days of administration and 5 days after stopping the administration, respectively. In the third experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the glucose concentration was 9.2% higher than the control after 5 days, 23.0% higher after 10 days, and 7.0% higher 5 days after stopping the administration. When administering the vitamin preparation Devivit complex (experimental group IV), the concentration of glucose in the blood serum of chickens was higher than the control by 7.0, 17.7 and 11.2%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 4).

The concentration of uric acid in the blood serum of chickens of all experimental groups during the entire period of research was lower ($P < 0.05$) than the control values. Thus, the concentration of uric acid in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 days of administration tended to decrease, after 10 days of administration and 5 days after stopping the administration was lower than the control by 7.6 and 37.0%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 11.3, 9.1, and 30.1% after 5 and 10 days of administration and 5 days after stopping the administration, respectively. In the III experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of uric acid was 17.0% lower than the control in 5 days, 19.7% lower in 10 days, and 32.9% lower in 5 days after the administration was stopped. When administering the vitamin preparation Devivit complex (experimental group IV), the concentration of uric acid in the blood serum of chickens was lower than the control by 11.3, 4.5 and 16.4%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 4).

Discussion

Today, broiler chickens reach slaughter weight twice as fast as 60 years ago. Such changes are associated with hyperphagia and excessive fat deposition, mainly in the body of broilers, which require restricted feed and feeding regimens. Obesity in broilers disrupts reproductive functions and sexual activity and, as a result, changes their productivity: it reduces the yield and quality of meat, which together leads to a loss of profit (29). At the same time, changes in adipocyte differentiation, lipid synthesis, lipolysis, β-oxidation of fatty acids and lipid content were detected in the adipose tissue of chicken embryos from the 12th to the 9th day before hatching, with the number of mitochondrial copies and β-oxidation of fatty acids increasing after hatching, indicating an important role of subcutaneous adipose tissue in providing energy to the poultry (30).

That is why a lot of scientific literature is devoted to lipid metabolism in broiler chickens, especially its control by biochemical markers of blood serum. Thus, the concentration of certain components in the blood serum (plasma) of broiler chickens: TC (31) and TGL (32) mainly decreased in the dynamics of the use of vitamin and mineral preparations, and in the case of the use of cerium dioxide nanoparticles (33), the concentration of TL increased, which is consistent with the results of our studies.

Lipid metabolism is closely related to the antioxidant defense system of broiler chickens and lipid peroxidation (34). Diene conjugates are the primary products of lipid peroxidation, which are toxic metabolites that can damage lipoproteins, proteins, nucleic acids, and enzymes. During the free radical oxidation of arachidonic acid, hydrogen is detached in the alpha position in relation to the double bond, which leads to its movement with the formation of diene conjugates, which are subsequently metabolized into secondary malondialdehyde. The body's antioxidant defense system blocks these compounds, interrupting the chain reaction (35, 36).

The administration of gadolinium and lanthanum orthovanadates nanoparticles led to a decrease in the concentration of primary and secondary lipid peroxidation products (DC and MDA) in the blood serum of experimental chickens, as well as with the administration of a vitamin preparation, indicating their antioxidant effect, with a prolonged effect (a decrease was also observed 5 days after the stopping the administration of nanoparticles).

The liver plays a significant role in neutralizing toxicants, including LPO products. The degree of liver damage is characterized by alanine and asparagine transaminases. However, in poultry, the activity of AST is ten times higher than that of ALT. This can be explained by the fact that the reactions of transamination with alanine, catalyzed by ALT, play a less important role in the metabolism of amino acids in chickens than those with AST. AST is a central component of metabolism, providing substrates for the tricarboxylic acid cycle. Therefore, it participates in the regulation of energy production in oxidative phosphorylation. In poultry metabolism, AST serves as a marker of the intensity of the catabolic vector (3, 4).

Our study found that chickens that received orthovanadate nanoparticles had a decrease in serum AST and De Ritis ratio (AST to ALT ratio) compared to the control group and

the vitamin drug group, where both indicators increased during the experiment. The change in aminotransferase activity is attributed to the hepatoprotective effect of nanoparticles, as established in previous studies (37, 38).

The liver synthesizes various plasma proteins and lipoproteins, as well as low molecular weight biochemically active substances such as creatine, 25-oxycholecalciferol, and heme. Additionally, it produces cholesterol and the end product of nitrogen metabolism, urea (uric acid in poultry) (39, 40). In our study, the concentration of total protein remained unchanged throughout the experiment. However, the concentration of uric acid in the blood serum of chickens decreased ($P < 0.05$) after administration of gadolinium and lanthanum orthovanadates nanoparticles, their mixture, and a vitamin drug. This result is consistent with the findings of Tsekhmistrenko et al., 2020 (38) in the case of the use of nanoparticles of another rare earth element (cerium dioxide) and indicates the activation of protein metabolism. And the concentration of uric acid in the blood serum of poultry decreased under the influence of antioxidant drugs (41, 42, 43).

Rare earth elements have similar biochemical properties to calcium and can replace it in reactions (44). This can affect phosphorus metabolism and, consequently, energy (carbohydrate) metabolism. For instance, when broiler chickens were fed a diet with mineral supplements replaced by 40%, 60%, 80%, and 100% with dicalcium phosphate nanoparticles, glucose concentration increased at 60% replacement but decreased at 40%, 80%, and 100% replacement (45). This partially aligns with our findings on the administration of gadolinium and lanthanum orthovanadate nanoparticles, which resulted in increased serum glucose concentrations in all experimental groups at all study periods.

Conclusions

Nanoparticles of orthovanadates of rare earth elements (gadolinium and lanthanum) show promise as candidates for inclusion in feed additives and veterinary drugs with adaptogenic action, since their administration to broiler chickens for 10 days at a dose of 0.09 mg/kg of body weight (0.2 mg/L of drinking water) leads to a decrease in certain biochemical markers of lipid metabolism (TC and TGL), protein metabolism (uric acid) and lipid peroxidation against the background of activation of carbohydrate metabolism and activity of hepatospecific enzymes. The introduction of nanoparticles (both in mono-solutions and in a mixture) along with a vitamin preparation-comparison confirms their adaptogenic effect on the body of experimental chickens and indicates a prolonged effect (the above trends persisted 5 days after the cessation of administration), but gadolinium orthovanadate nanoparticles showed the best results.

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Spremembe biokemičnih označevalcev pri pitovnih piščancih, izpostavljenih nanodelcem gadolinijevega in lantanovega ortovanadata

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Izvleček: V raziskavi nas je zanimala prisotnost sprememb v biokemičnem profilu krvnega seruma piščancev brojlerjev pod vplivom nanodelcev gadolinijevega ortovanadata (NP $GdVO_4:Eu^{3+}$), lantanovega ortovanadata (NP $LaVO_4:Eu^{3+}$) in njihove mešanice v terapevtskih odmerkih, ki smo jih ugotovili v prejšnjih študijah na belih podganah. Raziskava je bila izvedena na enodnevnih brojlerjih *Cobb 500* cross (n = 150). Piščanci eksperimentalne skupine I so 10 dni prejemali NP $GdVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode, piščanci eksperimentalne skupine II – NP $LaVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode, piščanci eksperimentalne skupine III pa NP $GdVO_4:Eu^{3+}$ in NP $LaVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode (v povprečju so piščanci prejemali 0,09 (0,13–0,05) mg/kg telesne mase NP). Piščanci poskusne skupine IV so z vodo prejemali veterinarski vitaminski pripravek Devivit Complex v odmerku 0,3 ml/l pitne vode za primerjavo antioksidativnega učinka, piščanci kontrolne skupine pa pitno vodo brez dodatkov. Po 10 dneh smo prenehali dajati NP in piščance opazovali še 5 dni. Ugotovili smo, da je 10-dnevno dajanje omenjenih nanodelcev piščancem brojlerjem povzročilo zmanjšanje presnove lipidov (skupnega holesterola in trigliceridov), presnove beljakovin (sečne kisline) in peroksidacije lipidov (dienskih konjugatov in malondialdehida) ob aktivaciji presnove ogljikovih hidratov (povečani koncentraciji glukoze) in aktivnosti hepatospecifičnih encimov (alanina in aspartat aminotransferaze) s podaljšanim učinkom po prekinitvi dajanja. Pridobljeni podatki kažejo, da imajo nanodelci ortovanadatov redkih zemeljskih elementov antioksidativne lastnosti, zato so obetavni kandidati za uporabo v krmnih dodatkih in veterinarskih zdravilih z adaptogenim vplivom.

Ključne besede: nanodelci gadolinijevega ortovanadata; nanodelci lantanovega ortovanadata; biokemični označevalci; piščanci brojlerji; krvni serum

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Original Research Article

Effect of LED Light Colour and Stocking Density on Some Hematological and Oxidative Stress Parameters in Japanese Quails

Key words

quail;
LED light;
heterophile/lymphocyte ratio;
oxidative stress;
stocking density

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Abstract: The study evaluated the effects of light-emitting diode (LED) light colour and stocking density on the hematological parameters, oxidative metabolism, and organ weights of quails. Several key management factors that influence the welfare of broilers include light color and stocking density. For this reason, this study aimed to reveal the effect of different light colors and stocking densities on the hematological and oxidative stress parameters in Japanese quails. For this purpose, levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) were measured in quails subjected to different light colors and stocking densities, using commercially available ELISA kits. In this study, 720 1-day-old mixed-sex Japanese quails (*Coturnix coturnix japonica*) were randomly assigned to one of six treatments, each having four duplicates of 30 birds. The experiment was designed as a 3 x 2 factorial arrangement of treatments, with three LED light colours (white, blue, and green) and two stocking densities (low = 200 cm^2/bird and high = 100 cm^2/bird). At 42 days of age, 20 quails from each treatment group were randomly selected for some hematological parameter analysis. According to the results obtained, the heterophile/lymphocyte (H/L) ratio was significantly higher in the white LED light colour treatments. However, the effects of stocking density on the H/L ratio were not significant. The oxidative stress indicator MDA was unaffected by the light colour, however, the high stocking density drastically reduced liver CAT and GSH activities. The heart weight was lower in the quail subjected to blue LED light. The heart and liver organ weights were not affected by stocking density. In conclusion, whereas white LED light increases the H/L ratio and the stress situation, it does not affect the oxidative stress indicators. These findings highlighted the need to identify optimum LED light colours for quails in commercial production settings in order to increase flock welfare. More research is needed to understand the effects and find the best colour of LED light for quails at varied stocking densities.

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Introduction

Light intensity, source, and spectrum are vital in modern poultry management (1). The perception of light colour in chickens has a substantial impact on their physiological reactions and stress levels. Exposure to mixed green-blue, blue, or green light resulted in lower heterophile/lymphocyte (H/L) ratios, indicating less stress in broilers (2). Pure blue light was also found to lower the H/L ratio, potentially lowering anxiety and oxidative stress by increasing melatonin release via the pineal gland (3). Green light at 560 nm, on the other hand, encouraged muscle growth and reduced oxidative stress during the early period, while blue light at 480 nm was more effective later (4).

The number of birds in a given space, or stocking density, is an important aspect of poultry management, particularly for quails. Stress, resource competition, hostility, and limited

movement can all result from overcrowding. High stocking density raises hematological stress hormones, the H/L ratio, and oxidative stress, while decreasing immunological response (5, 6).

Previous research has looked at how light colour affects stress indicators in chickens (4, 7). However, no prior research has been conducted on the effects of hematological parameters and oxidative stress in quails subjected to high stocking density. As a result, this study was conducted to investigate how white, blue, and green LED light colours, as well as stocking densities of 100 and 200 cm^2 per bird, influence various hematological parameters and oxidative metabolism in Japanese quails from day 1 to day 42 of their growth period.

Material and methods

Experimental Design: All the experimental procedures involved in this study were performed after ethical approval was taken from the Animal Care and Use Committee of Aydin Adnan Menderes University (64583101/2022/96).

This study took place at the Poultry Research Unit of Aydin Adnan Menderes University, Turkey, over six weeks. LED light bulbs (9W power, CT-4277 CATA, Turkey) were placed inside cages. Quails were randomly assigned to one of three LED light color treatments: white (400-770 nm), blue (480 nm), or green (560 nm). The light/dark cycle was set to 24 hours of light and 0 hours of darkness. Each LED colour treatment was evaluated at two different stocking densities: low (200 cm^2/bird) and high (100 cm^2/bird).

A total of 720 1-day-old mixed-sex Japanese quails (*Coturnix coturnix japonica*) chicks were initially weighted individually so that the cages had a similar beginning weight distribution. These chicks were divided into six groups, with different LED light colors (white, blue, and green) and two stocking densities (100 and 200 cm^2/quail). Each group had 20 quails in four replicates, making a total of 80 quails in the study. The quail chicks were raised in cages measuring 25 x 44 x 30 cm and were provided with the same number of heaters, feeders, and drinkers throughout the experiment. The temperature for the first three days was 33 ºC, gradually decreasing by 3 ºC per week until it reached 23 ºC. The relative humidity was maintained at 50-60% throughout the experiment. All quails were fed with balanced diets (0-14 d; 2910 kcal metabolic energy (ME)/kg, 24% crude protein (CP), and 15-42 d; 2900 kcal ME/kg, 22% CP) (8). Feed and water were ensured *ad libitum* throughout the study.

Hematological Parameters: At the end of the experiment, five quails from each replicate cage (20 birds/group; a total of 120 quails) were randomly selected and slaughtered by decapitation. In EDTA-coated tubes, were collected 1 mL of blood from each quail. It was stained with a blood smear on glass slides using May-Grünwald and Giemsa stain to analyze peripheral blood leukocyte populations. The cells were then enumerated and identified as heterophils, eosinophils, basophils, lymphocytes, and monocytes. The proportional proportions of each cell type were estimated based on the total number of leukocyte cells collected, and the H/L ratio was calculated using the Gross and Siegel method established in 1983 (9).

A total of 48 quails, two quails per replicate, were slaughtered by decapitation at the end of the experiment. The heart and liver were individually weighed, and the weights were noted. Tissue samples from the heart and liver were collected to assess oxidative metabolism, including measurements of MDA, superoxide dismutase (SOD), catalase (CAT), and GSH. To do this, the samples were first homogenized in cold 150 mM PBS (pH 7.4) at 2,000 rpm for 2 min by a tissue homogenizer (IKA WERKE Yellowline OST Basic S2 Analog Overhead Stirrer, Athy, Ireland). Obtained tissue homogenate supernatants were stored at -80 °C (NU 9668E, Nuaire, Japan) until spectrophotometric analyzes. MDA assay was performed as described by Ohkawa et al. (1979) (10). SOD activity was determined as described earlier by Sun et al. (1988) (11). The determination of CAT activity was done according to a modified method by Luck (1965) (12). GSH activity was also measured as described by Tietze (1969) (13). The assays were performed with the use of a spectrophotometer (Shimadzu UV-1601, Duisburg, Germany).

Statistical Analysis: The data were analyzed using the SPSS 22.0 (Statistical Package for the Social Sciences for Windows, IBM Corp., Armonk, NY, US). Data were tested for normality using Shapiro-Wilk's test. Using Levene's test, the assumption of homogeneity of variances was verified. Analysis of variance was performed with the GLM (Univariate General Linear Model) procedure to reveal the effects of some hematological parameters and the oxidative metabolism data (MDA, SOD, CAT, and GSH activity). The assumption of homogeneity of variances was evaluated using Levene's test. Hematological and oxidative stress parameter measurements were subjected to analyzes using a general linear model procedure, and means were compared using the least square difference (LSD) method. The experimental model for the design was defined as follows:

$$Yijk= \mu+\alpha i+\beta j+(\alpha\beta)ij+ Eijk$$

Where Yijk= the observed value, μ=the overall mean, αi=the effect of light colour (white, blue, and green), βj= the effect of stocking density (100 and 200 cm2/quail), (αβ)ij= the interaction between light colour and stocking density, and Eijk= the test error per observation. The partial effects of LED light color and stocking density for each factor were analyzed with the Least Squares Means Test and multiple comparisons were performed with a Duncan Test, with $P < 0.05$ indicating statistical significance. The correlations

between hematological parameters were calculated using Person's correlation coefficients.

Results

Table 1 displays the hematological parameters of quails in various light colour and stocking density treatments. The white LED light group had the greatest heterophile, eosinophil, monocyte counts, and H/L ratio (%28.30, %10.63, %9.78, and 0.88, respectively), with significant differences ($P < 0.01$, $P < 0.001$, $P < 0.05$, and $P < 0.05$, respectively). Hematological values did not differ significantly between stocking density groups. The combination of white LED light and a stocking density of 100 cm^2/bird resulted in the greatest heterophile percentage (30.95), which was statistically significant. On the lymphocyte percentage, there was also a significant interaction between LED light colour and stocking density.

Table 1: Influences of LED light color and stocking density on some hematological parameters of quails[1]

Treatment main effects	Hematological Parameters						
	n	Heterophile (%)	Lymphocyte (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)	H/L
Expected mean (μ)	120	25.38	55.13	7.77	3.52	8.20	0.62
LED light treatment							
White	40	28.30^{a}	48.70^{b}	10.63^{a}	2.60^{b}	9.78^{a}	0.88^{a}
Green	40	20.33^{b}	61.10^{a}	6.75^{b}	3.73^{a,b}	8.10^{a,b}	0.41^{b}
Blue	40	27.53^{a}	55.60^{a}	5.95^{b}	4.23^{a}	6.73^{b}	0.57^{a,b}
Stocking density							
200 cm^2/bird	60	23.90	57.07	7.25	3.82	7.98	0.63
100 cm^2/bird	60	26.87	53.20	8.30	3.22	8.42	0.61
Pooled SEM[2]		1.12	1.22	0.44	0.23	0.40	0.07
LED color x stocking density							
White - 200 cm^2/bird	20	25.65^{a}	52.65^{b,c}	9.75	2.15	9.80	0.94
White - 100 cm^2/bird	20	30.95^{a}	44.75^{c}	11.50	3.05	9.75	0.81
Green - 200 cm^2/bird	20	15.15^{b}	66.50^{a}	6.25	4.15	7.95	0.26
Green - 100 cm^2/bird	20	25.50^{a}	55.70^{b}	7.25	3.30	8.25	0.56
Blue - 200 cm^2/bird	20	30.90^{a}	52.05^{b,c}	5.75	5.15	6.20	0.68
Blue - 100 cm^2/bird	20	24.15^{a}	59.15^{a,b}	6.15	3.30	7.25	0.47
Pooled SEM[3]		2.74	3.00	1.09	0.57	0.99	0.18
Significance of main effects				***P* value**			
LED light color		0.007	< 0.001	< 0.001	0.017	0.010	0.034
Stocking density (SD)		0.188	0.117	0.239	0.203	0.592	0.941
LED light color x SD		0.007	0.007	0.824	0.057	0.850	0.319

n: The total number of quails in the group. H/L: Heterophile/ Lymphocyte ratio. [a, b, c]: Means with different superscript letters in the same column differ ($P < 0.05$). [1]: Data presented as the least square means, [2]: Pooled SEM for main effects, [3]: Pooled SEM for interaction effect.

Table 2: Effect of LED light color and stocking density on oxidative stress parameters and weights of the heart and liver organs of quails

Treatment main effects		MDA (nmol/mg protein)		SOD (U/mg protein)		CAT (k/mg protein)		GSH (mg/g protein)		Organ weights (g)	
	n	Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
Expected mean (μ)	48	21.48	2.99	1.09	0.29	0.10	2.74	29.65	22.37	1.80	4.62
LED light treatment											
White	16	23.28	2.93	1.07	0.30	0.09	2.63	29.43	21.93	1.94[a]	4.70
Green	16	20.06	2.92	1.07	0.28	0.11	2.83	31.48	22.79	1.77[a,b]	4.33
Blue	16	21.09	3.12	1.12	0.27	0.10	2.75	28.04	22.40	1.71[b]	4.81
Stocking density											
200 cm^2/bird	24	23.56	2.98	1.11	0.29	0.09	3.08	29.57	24.29	1.86	4.68
100 cm^2/bird	24	19.40	3.00	1.07	0.28	0.11	2.39	29.73	20.46	1.75	4.55
Pooled SEM[1]		1.32	0.16	0.06	0.02	0.01	0.12	1.10	0.80	0.04	0.25
Significance of main effects						***P* value**					
LED light color		0.598	0.851	0.907	0.741	0.253	0.807	0.447	0.907	0.042	0.719
Stocking density (SD)		0.122	0.953	0.727	0.626	0.026	0.008	0.942	0.021	0.126	0.799
LED light color x SD		0.333	0.977	0.989	0.240	0.207	0.568	0.135	0.233	0.554	0.478

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione. n: The number of pens. [a,b]: Means with different superscript letters in the same column differ ($P<0.05$). [1]: Pooled SEM for main effects.

Table 3: Pearson correlation coefficients and correlation significance among hematological parameters of quails

	Heterophile	Lymphocyte	Eosinophil	Basophil	Monocyte	H/L
Heterophile	—					
Lymphocyte	-0.879**	—				
Eosinophil	0.058	-0.391**	—			
Basophil	-0.051	-0.012	-0.236**	—		
Monocyte	-0.074	-0.248**	0.091	-0.133	—	
H/L	0.784**	-0.727**	0.087	-0.087	0.045	—

H/L: Heterophile/ Lymphocyte ratio; **: $P<0.01$.

The colour of the LED light did not influence MDA (nmol/mg protein) (23.28 for heart and 2.93 for liver in white light; 20.06 for heart and 2.92 for liver in green light; 21.09 for heart and 3.12 for liver in blue light), SOD (U/mg protein) (1.07 for heart and 0.30 for liver in white light; 1.07 for heart and 0.28 for liver in green light; 1.17 for heart and 0.27 for liver in blue light), CAT (k/mg protein) (0.09 for heart and 2.63 for liver in white light; 0.11 for heart and 2.83 for liver in green light; 0.10 for heart and 2.75 for liver in blue light), or GSH (mg/g protein) (29.43 for heart and 21.93 for liver in white light; 31.48 for heart and 22.79 for liver in green light; 28.04 for heart and 22.40 for liver in blue light) activities

(Table 2). However, higher stocking density increased CAT activity in cardiac tissue ($P < 0.05$). Furthermore, the 200 cm^2/bird group had significantly higher CAT and GSH activities in liver tissue than the 100 cm^2/bird group ($P < 0.01$ and $P < 0.05$, respectively). In this investigation, there was no relationship between light colour and stocking density for MDA level, SOD, CAT, and GSH activities in quails.

The correlations among hematological parameter values within quails are presented in Table 3. Lymphocytes had a significant negative correlation with heterophile, eosinophil, monocyte, and H/L values (-0.879, -0.391, -0.248, and -0.727, respectively). A negative correlation of r= -0.236 (P<0.01) was observed between eosinophil and basophil groups.

Discussion

The LED light hue had a strong influence on the heterophile, lymphocyte, eosinophil, basophil, and monocyte count in Japanese quails in our current study. Blue and green LED lights, in particular, increased lymphocyte percentage when compared to the effects of white light. Furthermore, green LED lights had a lower heterophile proportion than both blue and white LED lights. Notably, both blue and green light sources reduced the H/L ratio substantially. Similar results were obtained in other studies on other poultry species (1, 3, 7, 14). Because of the medium wavelength (about 560 nm) of the LED light, the lowered H/L ratio seen with green LED light in our study implies its beneficial impact on stress reduction and subsequent increases in welfare. It could be, that green light likely reduces stress in quails by promoting a more balanced immune response, improving circadian rhythms, and inducing a calming effect. This results in a lower H/L ratio, suggesting that green light may have a positive impact on quail welfare, particularly in terms of stress reduction. Similar to the effects reported on lymphocyte percentages and the H/L ratio, the use of blue and green LED lights resulted in lower eosinophil percentages when compared to white light exposure. While the precise role of eosinophils in avian biology is unknown, they are thought to function similarly to mammalian counterparts and are linked to glucocorticoid levels. However, the relationship between higher eosinophil counts (eosinophilia) or decreased eosinophil granulocytes (eosinopenia) and increasing glucocorticoid levels remains unclear (15). Eosinophil numbers often have an inverse connection with stress levels. They may, however, increase as a result of parasite infection or inflammation, making them potentially useful for distinguishing between infection and chronic stress (16). Furthermore, when compared to white light, blue light was found to decrease the number of monocytes while increasing the percentage of basophils. This finding contradicts the expected increase in basophil numbers and decreases in monocyte counts, which is especially noticeable in broiler chickens suffering from acute heat stress. In summary, the observed disparity between expected stress changes and the effects of blue light shows that the influence of light on immune cells may include distinct mechanisms that are not purely stress-driven. It alludes to the complexities of biological responses to various stimuli, as well as the necessity for further research to completely know the specific mechanisms underlying these cellular changes under various environmental situations.

Assessing stress and the balance between antioxidants (SOD, CAT, GSH) and oxidants (MDA-lipid peroxidation) is crucial for biological balance. Stress can increase oxidant production, resulting in a decrease in antioxidants (17). The colour of the LED light did not affect MDA levels, SOD, CAT, or GSH activities in the heart and liver of quails in our investigation. This is the first study to look at the effects of LED light colour on quails reared at densities of 200 and 100 cm^2/bird. Studies have shown that different light colours have different effects on oxidative metabolism in serum and different tissues, especially in broiler chickens (18, 19). Aside from light exposure, a variety of factors influence oxidative stress and antioxidant activity, including food, environmental circumstances, stocking density, and genetic factors. These variables may have interacted differently in our study than in other studies, resulting in different outcomes. Furthermore, various tissues may react differently to light exposure. While our study concentrated on the heart and liver, future studies exploring different tissues or organs may reveal differences in reactivity to LED light colour.

In the present study, it was determined that high stocking density had no significant effect on the H/L ratio in quails. The lack of a significant difference in H/L ratios between the low and high stocking density groups could be due to a combination of factors such as the birds' adaptation to stocking density, mitigating effects of other environmental variables (such as green light), individual variations in stress response, and the experimental conditions under which the measurements were taken.

Stocking density also did not show a significant effect on oxidative stress in the heart and liver, similar to Cengiz et al. (2015) (20). The high density of 100 cm^2/bird in our study lowered liver CAT and GSH activities, which is similar to Koç Yildirim et al. (2023) (21). However, Sevim et al. (2021) found no variations in SOD and GSH activity in broilers with different stocking densities (22). These diverse consequences illustrate the intricate link that exists between an organism's responses and external circumstances. Understanding this complexity is critical for animal well-being, yet varying research methodologies, housing, and management practices may contribute to inconsistencies in stress research in animals.

Conclusion

Finally, the study discovered that the colour of LED lights impacts numerous hematological parameters and welfare indicators in Japanese quails. Quails exposed to green LED light showed a decreased stress ratio, which could help their well-being. Stocking density had no discernible effect on hematological values. Different LED colours did not affect oxidative stress markers in the heart and liver. Studying how LED color and stocking density interact is crucial for understanding quail stress. More research is needed to improve bird welfare practices.

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Conflict of Interest. The datasets used and/or analyzed during the current study are available from the corresponding author (Evrim DERELİ FİDAN) upon reasonable request. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Author Contributions. The study's inception and design were contributed to by all authors. Evrim DERELI FIDAN and Ece KOC YILDIRIM prepared the materials, collected data, and analyzed the results. Evrim DERELI FIDAN wrote the first draft of the manuscript, and all contributors provided feedback on prior draughts. The final manuscript was read and approved by all writers.

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Vpliv barve led-svetlobe in gostote živali na nekatere hematološke parametre in oksidativni stres pri japonskih prepelicah

E. D. Fidan, E. K. Yildirim

Izvleček: Študija je ocenjevala učinke barve svetlobe svetlečih diod (LED) in gostote naselitve na hematološke parametre, oksidativno presnovo in težo organov prepelic. Več ključnih dejavnikov upravljanja, ki vplivajo na dobro počutje brojlerjev, vključuje barvo svetlobe in gostoto naselitve. Zato je bil namen te študije ugotoviti učinek različnih barv svetlobe in gostote naselitve na hematološke parametre in parametre oksidativnega stresa pri japonskih prepelicah. V ta namen so bile pri prepelicah, ki so bile izpostavljene različnim barvam svetlobe in gostoti naselitve, izmerjene ravni malondialdehida (MDA), superoksid dismutaze (SOD), katalaze (CAT) in glutationa (GSH) z uporabo komercialno dostopnih kompletov ELISA. V študiji je bilo 720 enodnevnih japonskih prepelic (*Coturnix coturnix japonica*) naključno razvrščenih v eno od šestih obravnav, vsaka s štirimi dvojniki po 30 ptic. Poskus je bil zasnovan kot faktorska razporeditev obdelav 3 x 2 s tremi barvami LED-svetlobe (bela, modra in zelena) in dvema gostotama naselitve (nizka = 200 cm^2/ptico; visoka = 100 cm^2/ptico). Pri starosti 42 dni je bilo naključno izbranih 20 prepelic iz vsake skupine za analizo nekaterih hematoloških parametrov. Glede na dobljene rezultate je bilo razmerje med heterofilci in limfociti (H/L) znatno višje pri prepelicah, izpostavljenih beli LED-svetlobi, vendar vpliv gostote naselitve na razmerje H/L ni bil značilen. Na indikator oksidativnega stresa MDA barva svetlobe ni vplivala, je pa visoka gostota naselitve drastično zmanjšala aktivnosti CAT in GSH v jetrih. Teža srca je bila manjša pri prepelicah, ki so bile izpostavljene modri LED-svetlobi. Gostota naselitve ni vplivala na maso srca in jeter. Zaključimo lahko, da bela LED-svetloba sicer poveča razmerje H/L in stresne razmere, vendar ne vpliva na kazalnike oksidativnega stresa. Te ugotovitve so poudarile potrebo po določitvi optimalnih barv LED-svetlobe za prepelice v komercialnih proizvodnih okoljih, da bi povečali dobrobit jate. Za razumevanje učinkov in iskanje najboljše barve LED-svetlobe za prepelice pri različnih gostotah naselitve je potrebnih več raziskav.

Ključne besede: prepelice; LED-svetloba; razmerje med heterofilci in limfociti; oksidativni stres; gostota naselitve

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Case Report

Ovarian Cancer Diagnosis and Treatment in a Geriatric Bitch: Challenges in Treating an Elder pet Aggravated by COVID-19 Pandemic Restrictions

Key words

canine ovarian cancer;
COVID-19 pandemic lockdown;
metastatic adenocarcinoma;
ovariohysterectomy;
pulmonary metastasis

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Abstract: Ovarian tumors are considered rare in dogs but pose a diagnostic challenge, requiring oncological expertise to establish diagnosis and accurate treatment. Diagnostic imaging is crucial to cancer diagnosis and treatment follow-up but, as in Medicine, this service was impacted by quarantine imposed by COVID-19 pandemic. This report aimed to describe a senior canine patient diagnosed with ovarian adenocarcinoma in a Brazilian country town during COVID-19 lockdown, evaluating the decision-making process in the absence of a veterinary oncology specialist, complicated by pandemic restrictions. A 15-year-old, nonspecific breed, intact bitch presented apathy and emesis. Abdominal palpation revealed increased epigastric volume. Ultrasonography suggested tumoral mass of unknown origin. Exploratory laparotomy was performed and a unilateral ovarian mass was removed. Patient recovered well from surgery. Histopathological evaluation revealed ovarian adenocarcinoma with lymphatic vascular invasion. No further treatment was indicated. Semesterly revisions were scheduled but metastasis developed in less than a year, after first revision. By this time, thoracic radiography was unavailable in owner's town, impairing metastasis screening and prognosis. Chemotherapy was offered only after metastasis detection but was declined by the owner. Patient was lost in follow-up. This present case illustrates the importance of a prompt diagnosis and suitable treatment for ovarian cancer-bearing patients based on veterinary oncology expertise. It also emphasizes how pandemic lockdown prejudicated imaging exam availability, especially in country towns, similar to what was observed in human patients, reflecting an undeniable One Health issue.

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Introduction

TOvarian tumors are considered rare in dogs and represent 0,5 to 1,2% of all neoplasms, reaching up to 6% in intact bitches (1). They are divided according to their original ovarian tissue in epithelial (tubular and papillary adenocarcinomas, carcinoma, adenoma and cystadenoma), germ cells (dysgerminoma and teratoma), sex cord stromal (granulosa

cell tumor, thecoma and luteoma) and mesenchymal tumors (e.g.: hemangiosarcoma, leiomyoma), this last ones with sparse cases in literature (1,2).

Epithelial ovarian tumors are the most frequent, approximately 50% of reported ovarian tumor cases in bitches, and half of the malignant types (adenocarcinoma and carcinoma) will metasta-size to lymph nodes and abdominal organs via tumor cell im-plantation, a process known as carcinomatosis (1,2). Carcinomas can begin insidiously and grow up to palpable abdominal mass (1,3). Abdominal ultrasound is capable of identifying such mass and effusion associated with carcinomatosis, and thoracic radi-ography is indicated for metastatic disease identification (1,4). Ovariohysterectomy is the treatment of choice although adju-vant chemotherapy can be adopted in an attempt to prevent tumor progression (1,3). Definitive diagnosis is determined by histopathological evaluation of excised ovarian mass (1,2,3,4).

Routine early-age neutering adopted in many countries prevents ovarian tumor development in bitches (1,5,6). Even nowadays, many owners are insecure about preventive neutering, which aims to avoid reproductive system cancer in addition to birth control (5,6).

Companion animal oncology has evolved rapidly in recent dec-ades and new therapies have emerged for treating dogs and cats with cancer. Nevertheless, veterinary oncology specializa-tion is required to adequately approach a cancer-bearing pet, considering not only the patient's health but also the owner's own experience with cancer and the animal-human bond (7). The vast majority of veterinary specialists remain in the urban centers, leading to a shortage in rural and remote areas (8,9). In addition, corporations focusing in companion animals practice are often established in urban areas (9). Therefore, facilities such as veterinary laboratories and diagnostic imaging centers for pets are not often found in the countryside (9), what can compromise diagnostic investigation in dogs and cats living in these areas.

This countryside panorama was worsened during COVID-19 pandemic, when treating oncological patients, whether humans or pets, has become a crossroads. Worldwide decreed lockdown led to oncological treatment delays and postponed routine and screening tests for early-stage cancer and metastasis detection in human patients (10,11). This situation yielded health and economic impacts: an increase in late-stage cancer diagnosis, leading to poorer prognosis, and health service demand, with a huge expense increment (10,11).

This report aims to describe the case of a female dog diagnosed with ovarian cancer, at the beginning of COVID-19 pandemic, in a countryside town. Clinical course, imaging diagnosis, surgery and treatment options in the absence of a veterinary oncology spe-cialist are described in addition to oncological geriatric patient approach complications as well as all limitations imposed by COVID-19 pandemic lockdown. The importance of a timely-manner treatment decision in oncological patient is depicted under One Health aspects.

Case Presentation

A 15-year-old intact female dog of no specific breed (NSB), weighing 14.5 kg was presented to private clinical attendance. Two days before, the bitch had an emesis episode, was apathic and hyporectic. On the next dawn, many emesis episodes fol-lowed and by morning the dog presented hematochezia.

On clinical examination, grade 2 dehydration (12) and swollen popliteal and submandibular lymph nodes were noticed. Ab-dominal palpation revealed an increased epigastric volume, suggesting tumoral mass. Clinical suspicion was pyometra or neoplasia.

Abdominal ultrasonography, complete blood count (CBC) and serum biochemistry were requested. Ultrasound revealed a heterogeneous tumoral mass, 7,39 cm wide

Table 1: Complete blood count (CBC) results; dog, female, NSB, 14y, with ovarian adenocarcinoma: CBC parameters revealed mild neutro-philia (a) and marked lymphopenia (b) on relative values (asterisk)

Variable	Value	Reference Value*
RBC	6.81 x 10^{12} /mL	5.5–8.5 x 10^{12} /mL
HGB	14.00 g/dl	12-18 g/dl
HCT	42.80 %	37-55%
MCV	62.84 fl	60-77 fl
MCHC	32.71%	32-36%
WBC	12460/µl	6000 – 17000/µl
Bands	2% (249)	0-2%
Neutrophils	90% (11214)[a]	58-87%
Lymphocytes	5% (623)[b]	12-30%
Monocytes	3% (374)	3-10%
Basophils	0	Rare
Eosinophils	0	2-10%
Platelets	282000/µl	175 – 500000/µl

RBC: total red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; WBC: total white blood cells. *Reference values provided by the private laboratory

Table 2: Serum biochemistry results; dog, female, NSB, 14y, with ovarian adenocarcinoma: parameters within normal range, except for alanine aminotransferase (ALT) (a) and alkaline phosphatase (ALP) (b), and reference values

Variable	Value	Reference Value*
Urea	36.0 mg/dL	20-50 mg/dL
Creatinin	0.64 mg/dL	0.5-1.6 mg/dL
AST	87.0 mg/dL	10-88/ul
ALT	307.8 mg/dL[a]	10-88/ul
ALP	222.0 mg/dL[b]	20-156/ul
GGT	6.0 mg/dL	≤ 10 mg/dL

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase. *Reference values provided by the private laboratory

(Fig. 1). No specific origin was mentioned. CBC values revealed mild neutrophilia and marked lymphopenia on relative values (Table 1). Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) showed increased values (Table 2). CBC and serum biochemistry anal-yses were performed by automated hematological equipment. Abdominal ultrasound was performed by SonoScape® equip-ment.

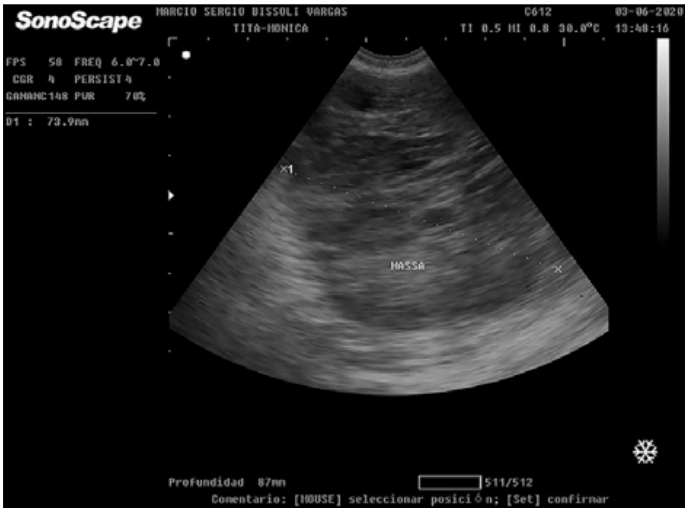

Figure 1: Dog, female, NSB, 14y, abdominal ultrasound image: well-defined, heterogeneous mass (massa) on the epigastric region, meas-uring 7,39 cm long

Patient was hospitalized for three days, under venous fluid ther-apy (Lactate Ringer's solution, intravenously) and antiemetic medication (ondasentron, 0,5 mg/kg, BID, IV). An exploratory laparotomy was scheduled. Surgical approach was performed by pre-retro-umbilical incision. Abdominal access revealed a tumoral mass adhered to right ovary (Fig. 2). Ovariohysterecto-my (OHE) was performed (13)

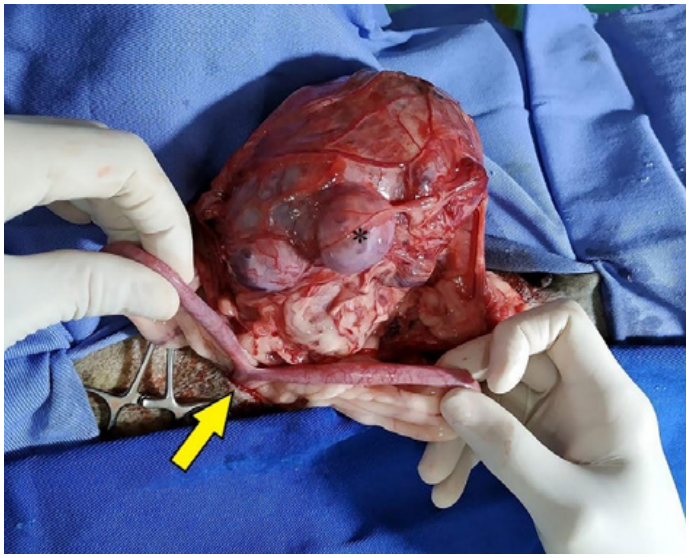

Figure 2: Dog, female, NSB, 14y, abdominal exploratory laparotomy: tumoral mass adhered to the right ovary site (asterisk). Uterine horn bifurcation is exposed (arrow)

with owner consent. Surgery was concluded without further intercurrence. Postoperative prescrip-tions included antibiotics (enrofloxacin, 5 mg/kg, BID, PO, 10 days), anti-inflammatory (meloxicam, 0,1 mg/kg, SID, PO, 3 days) and antiemetic medication (ondansetron, 0,5 mg/kg, BID, PO, 5 days) and wound management (allantoin-based moisturiz-ing ointment, topic, SID, 7 days). Patient recovered well with medical discharge 10 days after surgery.

Excised piece was sent to histopathological evaluation. On mac-roscopic analyses, ovarian mass was 12 cm wide, 13,5 cm long and 6,9 cm depth, with well-defined margins, red color and well vascularized. Left ovary and uterine horns were macroscopically normal (Fig. 3). The cut surface of the mass showed pale, yellow and red colored multinodular

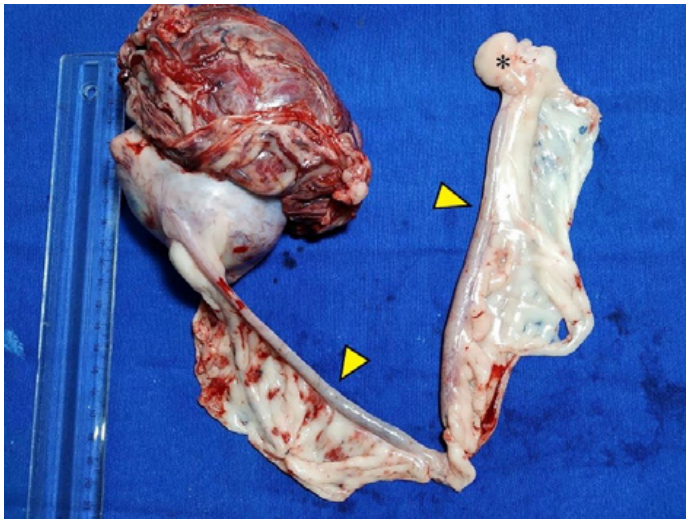

Figure 3 Dog, female, NSB, 14y, surgically excised piece: macroscopi-cally normal uterine horns (arrow heads) with left ovary pouch (aster-isk) and right ovarian mass (papillary adenocarcinoma), red and well vascularized

tissues with hemorrhage, necrosis and vascular spaces (Fig. 4). Samples were fixed in 10% formalin and submitted to routine histological process in alcohol and xy-lene passages until paraffin embedding and microtomy. Histo-pathological evaluation of hematoxylin-eosin-stained sections revealed high-density cellular epithelial neoplastic cells, with solid areas accompanied by papillae formation. Neoplastic cells presented scarce basophilic cytoplasm and prominent nucleolus. Moderate to intense pleomorphism was noticed. Up to six mito-sis figures were observed under high power field (HPF). Lym-phatic vascular invasion was noticed. An ovarian papillary ade-nocarcinoma diagnosis was established (2).

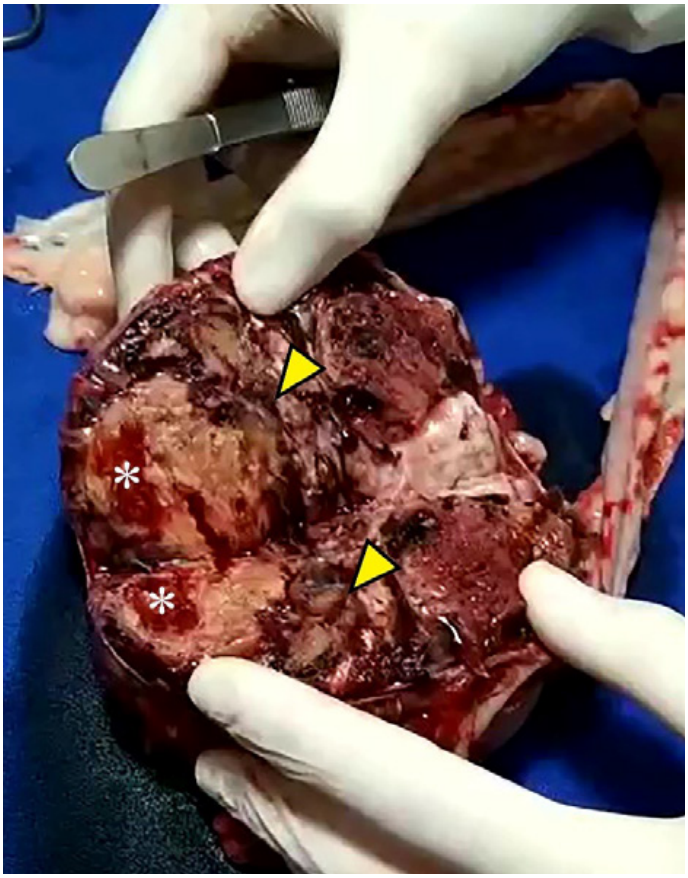

Figure 4: Dog, female, NSB, 14y, surgical excised piece: tumoral mass cut surface presenting different areas of multinodular tumoral tissue formation (pale, yellow and reddish tissue), with hemorrhage (aster-isk), necrotic areas (arrow heads) and some vascular spaces, features commonly associated with malignant neoplasms

Fifteen days after surgery, patient was well, normophagic and normodipsic, going for daily walks, without any apathy signs. Veterinary clinical recommendation was for clinical follow-up every six months for thoracic radiography, abdominal ultraso-nography for metastasis investigation, besides CBC and serum biochemistry.

On the first revision, six months later, aforementioned exams showed no alterations. Before the second revision, the owner noticed subcutaneous nodules in the thoracic region. Thoracic radiography was requested, but it was unavailable in the owner's rural town due to the COVID-19 pandemic lockdown. Therefore, the examination was delayed. On clinical return, a few weeks later, follow-up exams were performed: CBC showed no altera-tions and thoracic radiography revealed multiple radiopaque pulmonary nodules. On this occasion, palliative chemotherapy was indicated to restrain metastasis evolution and improve pa-tient overall survival. The owner declined chemotherapy due to dog's advanced age and patient was lost in follow-up.

Discussion

This reported patient was an intact, senior bitch, conditions that predispose canine ovarian tumor development (1, 2, 14). No adenocarcinoma was found in ovaries from clinically healthy bitches submitted to elective OHE in a previous report (5). Mean age of these bitches was 5,8 years-old and the oldest record was 8,0 years-old (5). Another study specifically focused on malig-nant ovarian tumors: mean age of the included dogs was 12 years, ranging from 7 to 15 years (3). More recently, a retro-spective study found five adenocarcinomas out of 35 ovarian tumors (benign and malignant), in bitches with mean age of 9,6 years (4). Nonspecific clinical signs such as hyporexia and apa-thy are commonly described, followed by weight loss and ascites (1, 3); the latter did not occur in the patient presented. This pa-tient presented with emesis, accompanied by ALT and AP alter-ations, indicating carcinomatosis of the abdominal organs. Ovar-ian tumors are initially insidious but might grow to become a palpable abdominal mass (1, 4, 5, 15), as presented by this pa-tient.

Abdominal ultrasonography (US) is adamant to elucidate ovarian masses (1, 4, 16), although in the present case, mass origin was not identified by the evaluator. Ovarian adenocarcinoma does not usually alter blood or serum parameters, as it is not hor-mone-producing (1, 2). This patient's increased ALT and ALP may be related to anorexia, gastrointestinal symptoms, or carci-nomatosis.

OHE is the treatment of choice for ovarian cancers in dogs (1, 4, 5). Despite being senior, patient evolved well after surgery, putting down the myth of old dogs being inoperable patients.

Surgery revealed a unilateral ovarian mass, with no macroscopic sign of carcinomatosis or ascites. Unilateral ovarian adenocarci-noma is considered uncommon (2, 3, 8). Ovarian carcinomas can produce abdominal carcinomatosis and ascites (2, 14). Neither processes were identified on US nor OHE. Likewise, pyometra and/or cystic endometrial hyperplasia usually accompany ovari-an tumors (1, 5, 16). Although not histologically evaluated, uter-us was macroscopically normal, even for a geriatric intact dog.

Tumor macroscopy evaluation indicated many malignant fea-tures, with US correspondence. Large ovarian tumors are more likely to be malignant (2, 4). Median diameters of 3,5 cm (17) and 6,9 cm (3), with maximum measures of 8,0 cm and 19,0 cm, respectively, have been reported for malignant ovarian tumors. Ovarian mass described here was within this range. Solid ovarian masses are often related to malignancy on US and macroscopy examination, as in the present case, while cystic lesions tend to be benign (4, 16). Haemorrhage and necrosis are common mac-roscopic features of malignant neoplasms and contribute to heterogeneous echotexture, along with multinodular tumour tissue formation, on ultrasound (1, 3, 4, 6, 22), as seen in this case. Unfortunately, these malignant features were overlooked by the general practitioner at the time of surgery.

Tumor etiology is defined by histopathological evaluation and carcinoma is the most frequent canine ovarian epithelial tumor (1, 2, 4, 16). An extremely important information was overlooked in the histopathological report: lymphatic vascular invasion by neoplastic cells was observed. This is a morphological feature of metastatic process in progress (2, 3). Hence, according to the World Health Organization (WHO) TNM staging scheme for canine ovarian tumors (3), although regional lymph nodes were not evaluated, this finding was enough to categorize this patient as N1 (positive for lymph node metastasis), with poor prognosis. As a unilateral tumor, T category was classified as T1 and since no distant metastasis was observed, M category was M0, at the time of diagnosis.

In this present case, oncological indication was for monthly or quarterly clinical revision (1), reinforced by lymphatic cellular invasion evidence, instead of semesterly. Due to COVID-19 pan-demic and lockdown, human oncological treatment protocols were revised, to balance patient exposure risk to SARS-CoV-2 and adequate metastasis screening intervals (10, 18). Delaying revisions were not discussed with the dog's owner and it was not possible to determine if semesterly revisions were chosen based on pandemic restrictions or lack of oncological knowledge.

Regional and distant metastases are frequent on adenocarcino-mas, especially to regional lymph nodes (1, 2 ,3). Carcinomatosis is more common in ovarian bilateral tumors (1, 2, 14, 17) and was not observed in this case. Peritoneal and pleural neoplastic implants are considered distant metastases in ovarian cancer (3, 14) but might be difficult to diagnose even under computed to-mography (CT) (14, 15), increasing the importance of a thorough macroscopy evaluation during surgery in search for these le-sions (15).

Despite radiography low sensibility, it is still the most commonly used imaging modality for pulmonary evaluation in oncological companion animals (16, 19) since lungs are among the most cited sites for malignant neoplasm distant metastasis (1, 2). However, in canine (1, 3, 15, 17) and human (20) ovarian cancer, pulmonary metastasis is rarely described. CT is more sensitive for pulmonary nodules but it has a higher cost and lower availa-bility when compared to radiography (14, 15, 19, 21), especially in Brazil.

No further oncological treatment besides surgery was offered to presented patient and metastasis developed quickly, in less than a year. This phenomenon is identified in many cancers as meta-static progression suppression by primary site or concomitant tumor resistance (22, 23). In presented case, adjuvant chemo-therapy (CTx) was recommended because of lymphatic vascular invasion (3). CTx was indicated as complimentary treatment only after subcutaneous nodule detection, which worsened patient's prognosis (1, 2, 3). Since no further US evaluation or nodule cy-tology was performed, it is not possible to affirmatively correlate these nodules with ovarian cancer in this patient. Although very rare, cutaneous metastasis is described in women with ovarian carcinoma (24), but no such canine case was found.

Chemotherapy was declined by the owner, concerned with dog's quality of life. Such preoccupation is frequent in veterinary geri-atric patients with cancer, mirroring human disease and its ad-verse reactions (7). However, new treatment modalities aimed at metastasis control, such as metronomic chemotherapy, are already available for companion animals, preserving quality of life and increasing overall survival time (25, 26, 27).

Currently, many veterinary clinicians feel unable to proceed with oncological treatments, especially chemotherapy (8). Another decision-making complication in this case is oncology specialist availability on the country side of a continental country such as Brazil (8). In addition, most of the time, companion animal onco-logical treatment is not affordable to the vast majority of popula-tion, even more so during COVID-19 pandemic which has led to an increase in unemployment rates worldwide (28). The same scenario regarding oncological treatment expense concerns during pandemic was described in Medicine (10, 28, 29).

Another difficulty observed in this case was diagnostic imaging realization. This veterinary service may again be deficient on the country side (9). COVID-19 pandemic lockdown contributed to diagnosis delays and treatment interruptions in thousands of cancer cases worldwide (10, 11, 29). During quarantine, routine medical appointments and exams, which often identify pre-tumoral or initial malignant lesions, were left aside (10, 11, 29) although they were still available as essential services by Brazili-an national law (30). Such an unprecedent situation was ex-pected to impair health systems, increasing the number of newly diagnosed advanced-stage cancer patients and compromising an already debilitated health budget (10, 29). The same impact was expected and is by now observed in Veterinary Medicine routine, reflecting One Health issues.

Conclusions

The present case illustrates the importance of a timely diagnosis and adequate treatment for oncological companion animals, regardless of age. Histopathological analysis is adamant for ovar-ian tumor identification, such as adenocarcinoma and can also inform metastatic progression such as lymphatic vascular inva-sion. Adjuvant chemotherapy is indicated in such cases to prevent rapid metastasis dissemination and improve overall survival time. Unfortunately, lack of veterinary oncology specialists is a reality, mostly in the countryside of Brazil. Follow-up imaging exams are important for staging bitches with ovarian adenocarcinoma but this service was dramatically impacted by COVID-19 pandemic lockdown, similar to what occurred in oncological human patients, under a One Health system view.

Acknowledgements

Ethics Approval and Consent to Participate: An Ethics Committee on the Use of Animals approval was waived since the presented case does not include animal experimentation. Informed consent obtained from owner.

Consent for publication: Written informed consent was obtained from the participant (dog owner) for publication of this case re-port and accompanying images.

Competing Interests: The authors declare that they have no competing interest.

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List of Abbreviation

COVID-19 – coronavirus disease (2019), OHE - ovariohysterecto-my, NSB – no specific breed, CBC – complete blood count, ALT - alanine aminotransferase, ALP - alkaline phosphatase, HPF – high power field, US - ultrasonography, WHO – World Health Organi-zation, TNM – tumor node metastasis, SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2, CTx - chemotherapy, CT – computed tomography

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Case Report

Urethral Obstruction Secondary to Hyperplasia of Seminal Vesicle Glands in an Intact Male African Pygmy Hedgehog (*Atelerix albiventris*)

Key words

accessory sex glands;
small mammals;
reproductive tract

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Abstract: A 4-year-old, male, intact African pygmy hedgehog was presented at the clinic with a 2-day history of weakness. Clinical examination revealed a large fluid-like mass, suspected to be the urinary bladder. The surveying ultrasound confirmed an enlarged urinary bladder with anechoic content and a small amount of floating echoic material. The patient was not able to urinate nor was it possible to place an urinary catheter. After three days he continued to be non-responding to any medical approach. The patient was prepared for explorative surgery, during which he collapsed and was declared deceased. The following autopsy revealed an extramural urethral obstruction caused by the enlarged seminal vesicle glands. The histology examination confirmed hyperplasia of the glandular epithelial cells, with focal squamous metaplasia. A focal mild perivascular inflammatory infiltrate was also present, formed mainly by lymphocytes and plasma cells with isolated macrophages and admixtures of eosinophilic granulocytes.

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Introduction

A 4-year-old, male, intact African pygmy hedgehog was presented at the clinic with a 2-day history of weakness of hind legs, lethargy, and visible abdominal distension. Clinical examination revealed a body conditional score of 2/5 with the weight being 420g, ongoing periodontitis, and a massive *Caparinia tripilis* infestation. Palpation of the abdomen was limited due to a fluid-like mass, suspected to be the urinary bladder. The owner could not state whether the animal was urinating and defecating normally. The patient was kept as a solitary pet in an aquarium with paper bedding. He was adopted from his previous owners at 2 years old. The current owner was not able to change his dietary preference, therefor he was eating mostly mealworms and occasionally kibble for kittens.

Case presentation

Surveying ultrasound (linear probe LA4 – 18B, frequency 4 – 18 MHz, Samsung RS85 prestige, Samsung Medison Co., Ltd., Seoul, South Korea) was performed under inhalation anaesthesia using a 3 % isoflurane (Aerrane 100% 250 ml, Baxter S. A. Bd., Belgium) concentration with a 2 l/min O2 flow. The ultrasound confirmed an enlarged urinary bladder with anechoic content and a small amount of floating echoic material (Fig. 1). The bladder wall was not thickened. Cystocentesis was not performed due to the risk of rupture of the urinary bladder. It was possible to examine other organs like testicles (Fig. 2) and a part of the accessory sex glands (Fig. 3) with a visible heteroechoic structure (sized 0,77 x 1,77 cm). It was not possible to visualize a large part of the abdominal cavity. This was due to the overexpanded urinary bladder.

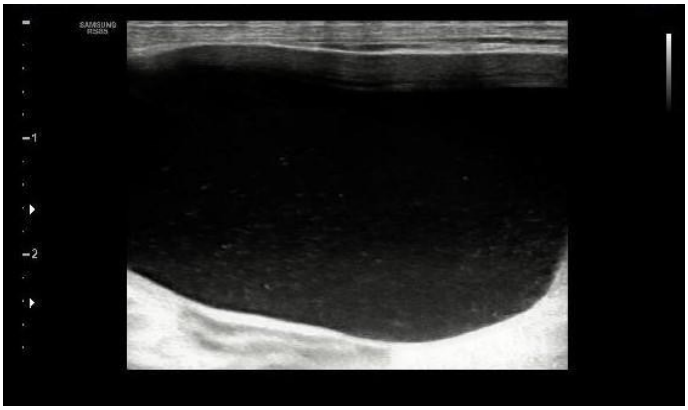

Figure 1: Ultrasonography of the abdominal cavity of the male African pygmy hedgehog. Distended urinary bladder with anechoic fluid and a small amount of echoic material, gravity-dependent

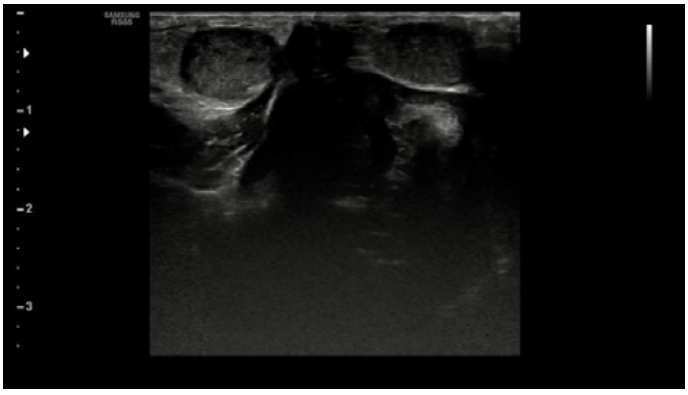

Figure 2: Ultrasonography of the African pygmy hedgehog. Visualization of both testicles (1) inside of the abdominal cavity and penis (2)

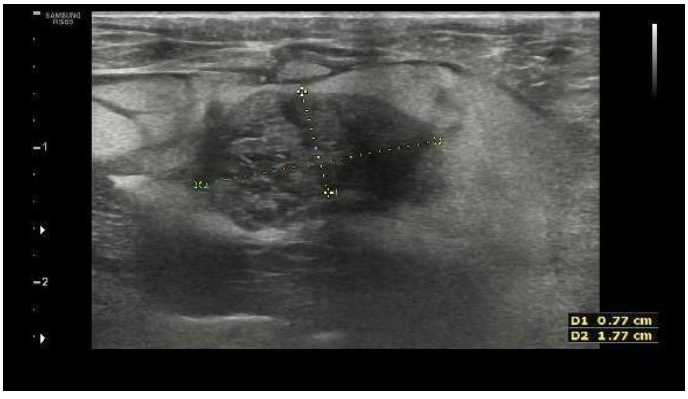

Figure 3: Ultrasonography of the abdominal cavity of the male African pygmy hedgehog. Enlarged seminal vesicle glands with hyperechoic surrounding fat and a small amount of free fluid

The blood collection was performed from *v. cava cranialis* later that day, under inhalation anaesthesia using a 3 % isoflurane concentration with a 2 l/min O2 flow. Biochemistry of the plasma revealed elevated urea, with normal creatinine levels, and altered liver enzymes (elevated ALT and ALP) (Tab. 1). Haematological examination revealed mild leukocytosis with eosinophilia and monocytosis (Tab. 2). Remaining values were within normal range.

Table 1: Plasma biochemistry profile of the male African pygmy hedgehog

Parameters	SI units	Value	RI[1]
Protein	g/l	65.6	58 ± 7
Albumin	g/l	29.1	29 ± 4
Bilirubin	µmol/l	<0.9	5.13 ± 5.13
Creatine	µmol/l	31.8	35.37 ± 17.68
Urea	mmol/l	23.2	10.68 ± 3.21
Glucose	mmol/l	5.6	4.94 ± 1.67
ALP	µkat/l	1.35	0.85 ± 0.35
ALT	µkat/l	2.50	0.88 ± 0.4
AST	µkat/l	0.96	0.56 ± 0.37

Table 2: Haematology of the male African pygmy hedgehog

Parameters	SI units	Value	RI[1]
Haemoglobin	g/l	122	120 ± 28
Haematocrit	%	40.5	36 ± 7
Erythrocytes	10^{12}/l	6.0	6 ± 2
Leukocytes	10^{9}/l	17.9	11 ± 6
Thrombocytes	10^{9}/l	109	226 ± 108
Lymphocytes	10^{9}/l	5.12	4.0 ± 2.2
Monocyte	10^{9}/l	1.16	0.3 ± 0.3
Neutrophils	10^{9}/l	6.85	5.1 ± 5.2
Basophils	10^{9}/l	0.21	0.4 ± 0.3
Eosinophils	10^{9}/l	4.63	1.2 ± 0.9

The owners were offered the option of an explorative laparotomy, however, they decided on conservative therapy. The patient was therefore admitted for stabilization and further observation.

Supportive care included subcutaneous administration of fluids with amino acids and vitamins – Hartmann solution (B Braun, Germany) + Duphalyte (Zoetis, Spain) in the ratio of 5:1 (4 ml pro toto SC, q12h).

Analgesia consisted of meloxicam (0.2 mg/kg SC, q12h; Melovem 5 mg/ml; Dopharma research B. B., Netherlands).

Other medications included an antiemetic drug maropitant (1 mg/kg SC, q24h; Cerenia 10 mg/ml; Zoetis Belgium SA, Belgium), and H2 blockers famotidine (0.4 mg/kg IM, q12h; Quamatel 20 mg/5 ml; Gedeon Richter Plc. Hungary). To relieve the spasm of the urinary bladder the patient received butylscopolaminium-bromid (10 mg/kg IM, q12h; Buscopan 20 mg/ml; IPSEN Consumer HealthCare, France). As for antibiotic therapy, due to leukocytosis, the patient received amoxicillin-clavulanate (25 mg/kg PO, q12h; Noroclav 50 mg/tbl; Norbrook, Ireland) in his meal, which was shortly eaten. At one time, when the patient refused to eat, the antibiotics were administered directly *per os* with a syringe.

On the third day of hospitalization, less than 48 hours after admission and the start of conservative treatment, there were no changes in the abdominal distension, nor was the patient able to urinate. The following attempt to place an urinary catheter to release some pressure was unsuccessful. The case was consulted with the owners who this time agreed to perform an explorative laparotomy, due to the high risk of rupture of the urinary bladder and kidney damage.

The patient was prepared for anaesthesia according to the standard protocol used at the clinic: premedication consisted of medetomidine (0.02 mg IM pro toto; Cepetor 1 mg/ml; CP – Pharma Handelfsges. mbH, Germany), ketamine (1 mg IM pro toto; Narkamon 50 mg/ml; Bioveta a.s., Czech Republic) and midazolam (0.05 mg IM pro toto; Midazolam Accord 5 mg/ml; Accord Healthcare Limited, Great Britain). Anaesthesia was induced with a mask using a 5% concentration of isoflurane and an oxygen flow at the rate of 2 O2l/min. After induction, the concentration was lowered to 2.5 % of isoflurane. The patient received an IV catheter to the cephalic vein. After the sterile surgical field was prepared, the patient collapsed. The anaesthesia was turned off, maintaining the patient on a high O2 flow at 4l/min. Cardiac massage via chest compressions was performed to maintain blood flow at the highest possible rate, approximately 120 – 150 beats per minute. The breathing was secured by mouth-to-mouth-to-nose breathing as it wasn't possible to perform the intubation. As neither of these attempts was successful a bolus of epinephrine (0.1 mg/kg IV; Adrenalin Léčiva 1 mg/ml; Zentiva, k.s., Czech Republic) diluted in 1 ml of Hartmann solution was administered directly in the IV catheter. The resuscitation was unsuccessful and the patient was declared deceased.

The autopsy revealed an enlarged urinary bladder as a result of an extramural urethral obstruction (Fig. 4). This obstruction was caused by applied pressure and partial strangulation from one of the enlarged vesicular seminal glands. A cytology impression smear and a sample for microbiological and histological examination were obtained from one of these glands.

Cytology showed the presence of multiple leucocytes, with neutrophils, eosinophils, and monocytes equally present together with clusters of cuboidal epithelial cells, with granular basophilic cytoplasm and an oval nucleus located at the base of the cells (Fig. 5). Cultivation of the samples presented two colonies of bacteria: *Staphylococcus simulans* and *Enterococcus faecalis*.

In the obtained biopsy of the altered gland, a mild chronic interstitial inflammation with cystic dilatation of acini and ducts of the gland was observed. The cells of the

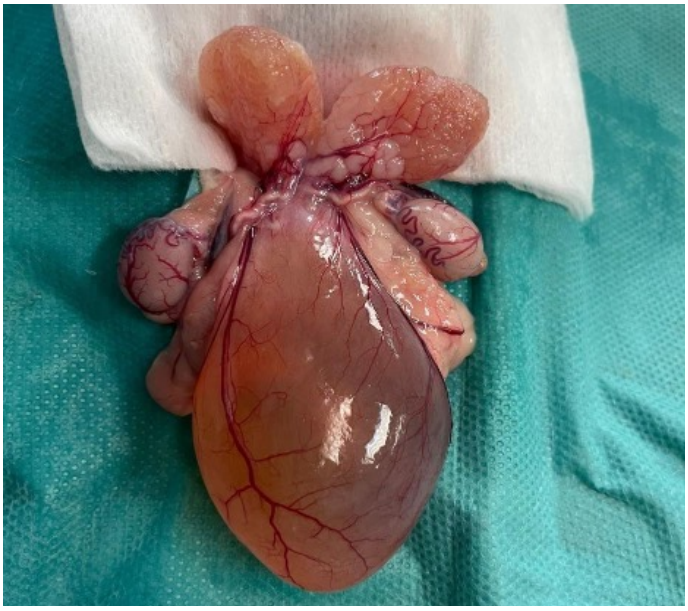

Figure 4: Obstructed and distended urinary bladder (1). Visualization of the reproductive tract: testicles (2), enlarged seminal vesicle glands (3) obstructing the urethra

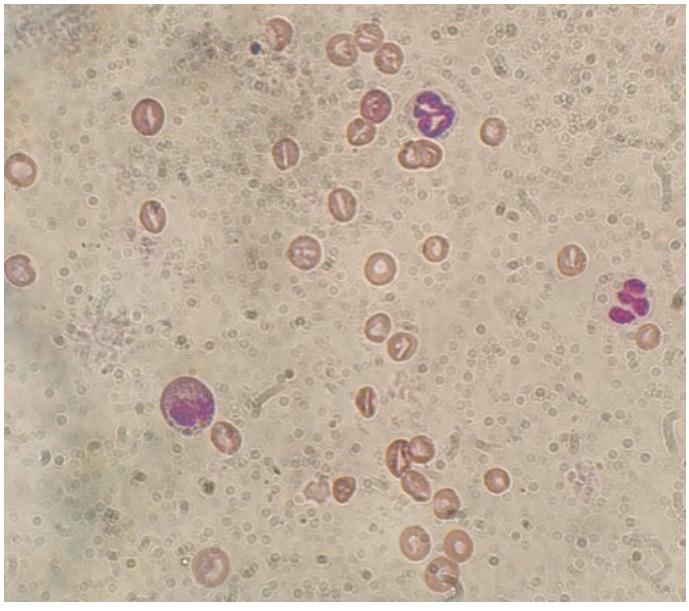

Figure 5: Impression smear from the enlarged seminal vesicle glands. Cytology revealed erythrocytes (1) and leucocytes, with a different number of neutrophils (2), eosinophils (3), monocytes, and lymphocytes

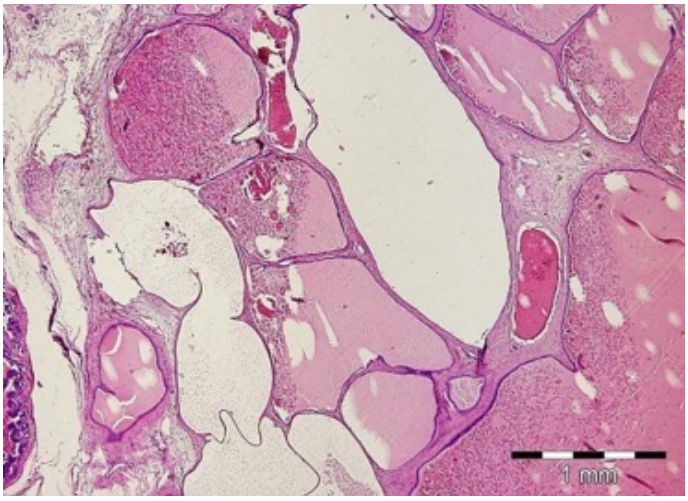

Figure 6: Histology examination from the biopsy of seminal vesicle gland of African pygmy hedgehogs. Hyperplasia of the glands with cystic structures. Dilatation of acines with a small amount of condensated secret. HE, magnification 40x

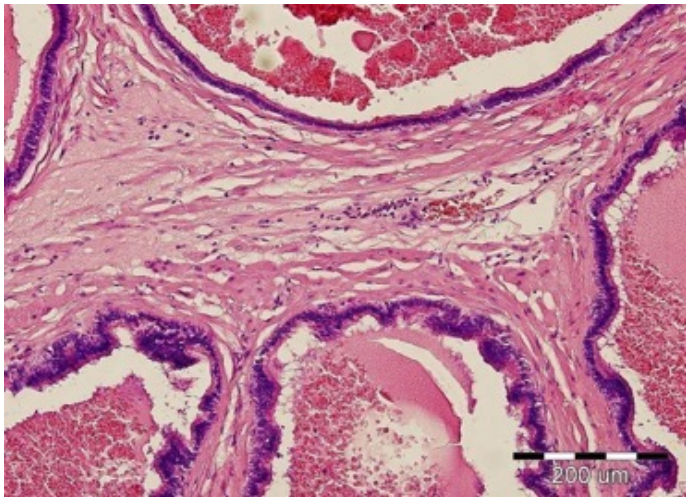

Figure 7: Histological examination from the biopsy of seminal vesicle gland of African pygmy hedgehog. In the interstitial connective tissue of the seminal vesicles, focally mild perivascular inflammatory infiltrate consisting mainly of lymphocytes and plasma cells with isolated macrophages and admixture of eosinophilic granulocytes. Focal oedema of the interstitium and mild fibrosis, hyperemia of blood vessels, and isolated hemorrhages. HE, magnification 200x

glandular epithelium presented signs of hyperplasia with focal squamous metaplasia. A focal mild perivascular inflammatory infiltrate was also present, formed mainly by lymphocytes and plasma cells with isolated macrophages and admixtures of eosinophilic granulocytes (Fig. 6, Fig. 7).

Discussion

To date, there is limited information available about the reproductive tract of the African pygmy hedgehog, especially the males.

The presented case was a male, older than 4 years of age. African pygmy hedgehogs are considered reproductively old from 2 – 2.5 years old, with their life span being 4 – 6 years old (1, 2). Pathologies of the reproductive tract in intact individuals (both males and females) are a common problem in geriatric patients. The majority of cases are described in females, with the most common one being a neoplasia of the uterus (3,4).

A recent study has focused on the ultrasonographic description of both male and female reproductive systems, however, the gross anatomy of the seminal vesicle glands was not described (5). A retrospective study mentioned hyperplasia of seminal vesicles in an intact male, without any further detailed description (6). Another recent study describes the first case of hyperplasia of the seminal vesicles that obstructed the gastrointestinal system (7).

The authors of the article mentioned that seminal vesicle glands in African pygmy hedgehogs may behave similarly to the prostate of intact dogs (7). Benign prostatic hyperplasia is a common disease documented mainly in old non-sterilised canine males. This condition develops as the exposure of the prostate to the hormone dihydrotestosterone (8). Untreated prostatic hyperplasia may develop into a prostatic abscess, which can cause further complications (8).

There is a discussion about whether a similar process can occur in other small mammals as well, such as African pygmy hedgehogs (7). A recent study described a case of vesicular gland infection and prostatitis in an intact Guinea pig (9). The patient was presented with ongoing anuria and lethargy. After not succeeding with conservative treatment the patient was admitted for surgery. This consisted of abdominal castration and extirpation of the vesicular glands, as well as cleaning multiple prostate abscesses (9). A similar surgical approach could be considered in the case of inflammation and hyperplasia of the seminal vesicle glands in African pygmy hedgehogs, where castration would be also recommended.

In this case, the infection could be a result of either an ascended infection from the urinary bladder or a haematogenous infection from a different primary source. An infection of the accessory sex glands may occur if the patient is suffering from untreated cystitis. In this presented case the cultivation showed the presence of *Staphylococcus simulans* and *Enterococcus faecalis*. A retrospective study on Guinea pigs showed that 55.5 % of presented cases were diagnosed with bacterial cystitis and/or other UTIs (10). Cultivation from this study confirmed the presence of more than one bacterial species, presenting *Pseudomonas* spp., *Staphylococcus* spp., and *Escherichia coli* (10).

Conclusion

This case study supports the theory that more attention should be given to preventive sterilization of the males. Hyperplasia of seminal vesicle glands in African pygmy hedgehogs might be more frequent than previously thought, similar to prostate hyperplasia in dogs. However, due to their behaviour and problematic handling, male hedgehogs might show less noticeable symptoms than dogs. If specific signs are seen, then the disease associated with accessory sex glands is usually more advanced with further complications. Therefor castration of African pygmy hedgehog males should be offered to owners as a way to prevent possible complications such as obstruction of different organs inside of the abdominal cavity.

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Obstrukcija sečnice zaradi hiperplazije mehurnice pri nekastriranem samcu afriškega beloprsega ježa (*Atelerix albiventris*)

L. Kasalova, H. Černochová, R. Dvořáková, A. Angelová, Z. Knotek

Izvleček: Štiriletnega nekastriranega samca afriškega beloprsega ježa so pripeljali na kliniko zaradi znakov oslabelosti, ki so trajali dva dni. Klinični pregled je pokazal veliko tekočini podobno maso, za katero se je domnevalo, da gre za sečni mehur. Ultrazvočni pregled je potrdil povečan sečni mehur z anehogeno vsebino in majhno količino plavajočega ehogenega materiala. Jež ni mogel urinirati, prav tako ni bilo mogoče namestiti urinskega katetra. Po treh dneh se še naprej ni odzival na noben način zdravljenja. Izveden je bil eksplorativni kirurški poseg, med katerim je poginil. Pri obdukciji je bila ugotovljena ekstramuralna obstrukcija sečnice, ki jo je povzročila povečana mehurnica. Histološka preiskava je potrdila hiperplazijo žleznih epitelijskih celic z žariščno skvamozno metaplazijo. Prisoten je bil tudi blag žariščni perivaskularni vnetni infiltrat, ki so ga sestavljali pretežno limfociti in plazmatke s posameznimi makrofagi ter primesmi eozinofilnih granulocitov.

Ključne besede: pomožne spolne žleze; mali sesalci; reproduktivni trakt

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Urethral Obstruction Secondary to Hyperplasia of Seminal Vesicle Glands in an Intact Male African Pygmy Hedgehog (*Atelerix albiventris*)

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