

Experimental studies on virocidal and bactericidal properties of steam-water ozone-containing mixture

MYKHAILO KOSTYLEV¹, SVETLANA RYBALKO², NIKOLAY CHUKHRAIEV³,
ALEXANDER VLADIMIROV⁴, WALERY ZUKOW⁵, GEORGIY TEREHOV⁶, SVETLANA TITARENKO⁷,
DARYA STAROSYLA⁸, IRYNA SAVYTSKA⁹

^{1,6,7,9}State Institute «Shalimov National Institute of Surgery and Transplantation» to National Academy of
Medical Sciences of Ukraine, Kyiv, UKRAINE

^{2,8}State Institution "Institute of Epidemiology and Infectious Diseases named after L. Gromashevsky National
Academy of Medical Sciences of Ukraine", Kyiv, UKRAINE

³NMC MEDINTECH, Kyiv, UKRAINE

⁴National University of Health Protection of Ukraine named P. L. Shupyk, Kyiv, UKRAINE

⁵Nicolaus Copernicus University, Toruń, POLAND

Published online: February 28, 2022

(Accepted for publication February 15, 2022)

DOI:10.7752/jpes.2022.02037

Abstract

Introduction. The purpose of the work was to determine the antiviral and bactericidal action of the steam-water mixture containing ozone created by the POS-1 apparatus on models of the transmissible gastroenteritis virus of pigs of the coronavirus family and multi-resistant clinical strains of bacteria, as well as the selection of optimal parameters for the supply of ozone in the composition of the gas mixture, which does not cause pathological changes on the part of organs and body systems. **Materials and methods.** To create a gas mixture containing ozone, the POS-1 apparatus created by the team of NMC Medinteh LLC was used. Virological studies were conducted on the basis of the Institute of Epidemiology and Infectious Diseases named after L.V. Gromashevsky. As a working material, models of the vector-borne gastroenteritis virus of pigs of the coronavirus family were used. A series of bacteriological and experimental studies were performed on the basis of the State Institution of the National Institute of Surgery and Transplantation named after A.A. Shalimov of the National Academy of Medical Sciences of Ukraine. For bacteriological studies, polyresistant cultures at a concentration of 108 CCU/ml were used. To study the effects of an ozone-containing mixture of gases on living biological tissues, studies on white rats were performed in the experiment. **Results.** Virological studies have shown that at an exposure of 20 minutes in contact with an extracellular virus, the infectious titer decreased by 100,000 times, and at an exposure of 30 minutes, complete deactivation of the virus occurred. Bacteriological studies revealed a complete absence of culture growth after treatment with an ozone-containing mixture for 20 minutes. The results of swimming tests and behavioral reaction of white rats of the control and experimental groups did not differ from each other. Histological studies of the tissues of the respiratory system, as well as the spleen, thyroid gland, kidneys and adrenal glands did not reveal signs of pathological effects. **Conclusions.** 1. The parameters of ozone generation and supply in the composition of the steam-water mixture of gases were studied and optimally selected. 2. Studies of the effect of the ozone-containing mixture on the strain of the virus of the coronavirus family, as well as bacteriological studies on multi-resistant clinical bacterial cultures confirm the pronounced virocidal and bactericidal properties of the developed method. 3. The use of an ozone-containing mixture does not cause any pathological changes on the part of the organs and systems of a living organism. 4. The gas mixture can be used to prevent and treat infectious diseases of the respiratory system, both viral and bacterial genesis.

Key words: steam-water ozone-containing mixture, polyetiologiical respiratory diseases, vector-borne gastroenteritis virus of pigs, family of coronaviruses.

Introduction

In clinical practice, pneumonia is increasingly diagnosed, complicating the course of acute infectious diseases of the respiratory system and occupying one of the first places in the structure of child mortality (Bilichenko et al., 2016; Turchina et al., 2014). In 2017, pneumonia caused the deaths of 808,694 children under 5 years of age, accounting for 15.0% of all infant deaths worldwide (Chuchalin et al., 2014; Jakovlev, 2013). According to WHO, about 450 million people in the world annually fall ill with pneumonia, while approximately 7 million cases of the disease are fatal (Rukovodstvo ..., 2012).

According to the State Statistics Service, the number of deaths due to pneumonia in Ukraine in 2018 increased by 23.87% compared to 2017 and amounted to 6101 patients. According to the same agency, in 2018, 36 deaths from acute respiratory diseases of the respiratory system were registered in Ukraine, 13 from

influenza, 23 from viral pneumonia, 919 from bacterial pneumonia, as well as 5,095 from pneumonia, the causative agent of which was not established (Statistical Bulletin ..., 2018).

The vitrocidal and bactericidal properties of the steam-water ozone-containing mixture of gases were studied on models of the transmissible gastroenteritis virus of pigs of the coronavirus family and multi-resistant clinical strains of bacterial cultures. During the experiment, physiological tests and behavioral reaction of laboratory animals were analyzed, upon its completion, a series of histological studies of the organs of the respiratory system and the main organs of the stress response were performed, which showed that ozone-containing can be used to prevent and treat infectious diseases of the respiratory system organs, both viral and bacterial genesis.

The purpose of the study is to determine the antiviral and bactericidal effect of the steam-water mixture containing ozone created by the POS-1 apparatus on models of the transmissible gastroenteritis virus of pigs of the coronavirus family and multi-resistant clinical strains of bacteria, as well as the selection of optimal parameters for the supply of ozone in the composition of the gas mixture, which does not cause pathological changes on the part of organs and body systems.

Materials and methods

Procedure / Test protocol / Skill test trial / Measure / Instruments. For research, an apparatus for obtaining a steam-water ozone-containing mixture POS-1, created by the team of NMC Medintekh LLC, Kyiv, Ukraine, was used. The possibility of its application is confirmed by the "Conclusions of the state sanitary and epidemiological examination" No. 12.2-18-2 / 10782 of 15.05.2020 and No. 12.2-18-1 / 11376 of 22.05.2020.

The parameters of the developed device provide the possibility of formation of an ozone-containing mixture of gases with a capacity of 7 liters per minute at an ozone concentration of 1.2 mg per liter (Fig. 1).



Fig. 1. Apparatus for the preparation of steam-water ozone-containing mixture POS-1.

The developed model consists of an ozone generator, a Bobrov flask for humidifying ozone and an oxygen mask designed to additionally supply oxygen to the air in order to create a breathable mixture of gases.

The research program was developed by a team of authors and approved at a joint meeting.

Virological studies were carried out on the basis of the State Institution "Institute of Epidemiology and Infectious Diseases named after L.V. Gromashevsky" of the National Academy of Medical Sciences of Ukraine. As a working material, models of the transmissible gastroenteritis virus of pigs of the coronavirus family (TMGS), which is an etiological agent of transmissible gastroenteritis of pigs (TGS) and is a highly contagious intestinal disease, was used. The D52-5 (BRE79) virus strain has a high pathogenicity for pigs of all age groups at the level of 5 passage in a transcountable monolayer culture of ST piglet testicle cells. The strain is presented by Dr. Hubert Laude from the Laboratory of Molecular Virology and Immunology of the INRA Biotechnology Center in João-en-Josasi (France). He also proved the tropism of viruses of the gastrointestinal and respiratory tracts.

Titration of infectivity of viral materials on cell cultures was carried out by two methods: 1) the method of final dilutions according to GPA. The titer of infectivity was determined by the Kerber-Ashmarin method; 2) by the method of negative colonies (S-sign) under 1.35% agar coating (Difco-Bacto), a tissue cytopathogenic dose of TCD of 50 / ml was established. The titer of infectivity was determined in BOE / ml, the results were calculated after 120 hours of cultivation at a temperature of + 38 ° C.

A viral suspension at a dose of 1000 TCD 50 / ml was incubated with a lower concentration of CS50 at various exposures, taking samples to determine the infectious titer of the virus. As a control, a viral suspension was used, which was incubated under the same conditions without treatment by the POS-1 apparatus. A decrease in the infectious titer of the virus by 1.5-2.0 lgTCD50 compared with the control indicated a virocidal effect of the method of hyperbaric oxygenation with ozone.

To identify the direct destructive effect of the presented method on the extracellular virus, the following group of studies was conducted, in which a strain of the transmissible gastroenteritis virus of pigs with an infectious titer of 8.5 lg ID50 was used.

To determine the effect of the ozone-containing mixture (POS-1 apparatus) on the extracellular TGS coronavirus, transmissible cell cultures of SPEV (transplantable culture of pig embryo kidney cells) and Vero (transplantable culture of green monkey kidney cells) were used. The study was conducted as follows: a suspension of TGS coronavirus at a dose of 1000 TCD50 / ml was treated with an ozone-containing mixture, incubated at room temperature and samples of extracellular virus were taken after 5 min, 20 min and 30 min. Then the infectious titer of the TGS virus was determined in the SPEV and Vero cultures.

The isolation of RNA was performed using the "Ribosorb" kit according to the manufacturer's instructions (Ampli Sense, RF).

The reverse transcription reaction was performed using the kit "Revert Aid TM H Minus First Strandc DNA Synthesis Kit" according to the manufacturer's instructions (Thermo Scientific, Lithuania).

The viral neutralization reaction was carried out in 96 Well Plates "Costar" (USA) by the H. Laude method using the reference hyperimmune serum N6926 of the same author as a positive control.

The cell population was grown in dies on RPMI-1640 + 10% fetal serum at a temperature of +37.0 C in a thermostat with CO2 supply.

A series of bacteriological and experimental studies were performed on the basis of the State Institution of the National Institute of Surgery and Transplantology named after A.A. Shalimov of the National Academy of Medical Sciences of Ukraine.

In two sets of five Petri dishes, cultures were placed in isolation: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonis aeruginosa*, *Stafilococcus aureus*, *Candida albicans* in a concentration of 10⁸ CEU / ml.

Petri dishes with bacterial cultures were divided into a comparison group and a study group. The bacterial culture comparison group was left unspooned. The study group was placed in a laminar hermetic box of microbiological safety SHLV-02 with the POS-1 apparatus connected to it to create conditions for hyperbaric oxygenation (P = blood pressure + 0.2 mm Hg). The study group was treated with a steam-water ozone-containing mixture of gases for 20 minutes with a maximum ozone concentration (0.16±0.03) mg / m³ at a pressure of (1.2±1) atm. The volume of the mixture obtained was (7±0.4) l / min.

The experimental part of the work was carried out in compliance with measures of ethical and humane treatment of animals in accordance with the provisions of the Council of Europe Convention on Biomedicine and relevant laws of Ukraine, as well as agreed with the Commission on Medical Ethics of the National Institute of Surgery and Transplantology named after A.A. Shalimov of the National Academy of Medical Sciences of Ukraine.

Participants. To study the effects of an ozone-containing mixture of gases on living biological tissues in the experiment, we performed studies on white rats, the essence of which is given below.

Laboratory animals were divided into 3 groups of 10 individuals each. The first group was a comparison group, the second and third were experimental. Animals of the second group breathed an ozone-containing mixture for 10 minutes, animals of the third - 20 minutes once a day for 5 days while staying in an airtight container. After each procedure, the animals were given a load forced swimming test at the same time of day (10:00 a.m. to 11:00 a.m.) for 5 consecutive days, both in the comparison and experimental groups, immediately following the hyperbaric oxygenation procedure. After completing the swimming test, the "open field" test was performed (Gostjuhina et al., 2018). The behavioral response of animals (motor activity, orientation in space, hunger, reaction to external stimuli) was assessed subjectively during 10 minutes of observations. On day 6, the animals were removed from the experiment by an intrapleural overdose of a 5.0% solution of sodium thiopental.

Data collection and analysis / Statistical analysis. The digital material presented in the work was processed using the methods of variational statistics. Statistical assessment of the significance levels of differences in the obtained numerical indicators was carried out using the Student's T-criterion using Microsoft Excel and Microcal Origin programs. Differences at p < 0.05 were considered reliable.

Results

When performing virological studies, the detection of RNA of the transmissible gastroenteritis virus of pigs strain D52 was carried out by reverse polymerase chain reaction (RT-PCR). Cell cultures infected with the virus were treated with a steam-water ozone-containing mixture. The results for determining the virocidal effect of the gas mixture on the extracellular virus of transmissible gastroenteritis of pigs of the coronavirus family are presented in Fig. 2 and Fig. 3.

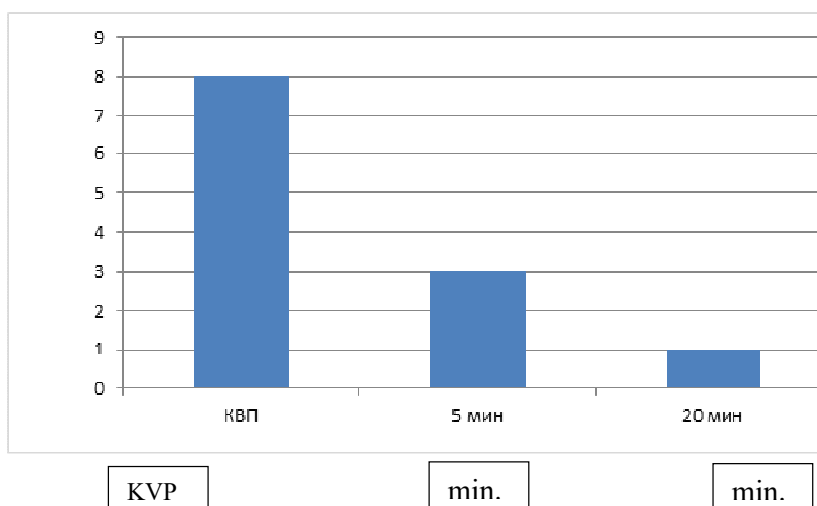


Fig. 2. Infectious titer of the TGS virus 100 ID50 in wells treated with ozone-containing mixture in SPEV cell culture.

KVP is a control seeding of the virus without treatment.

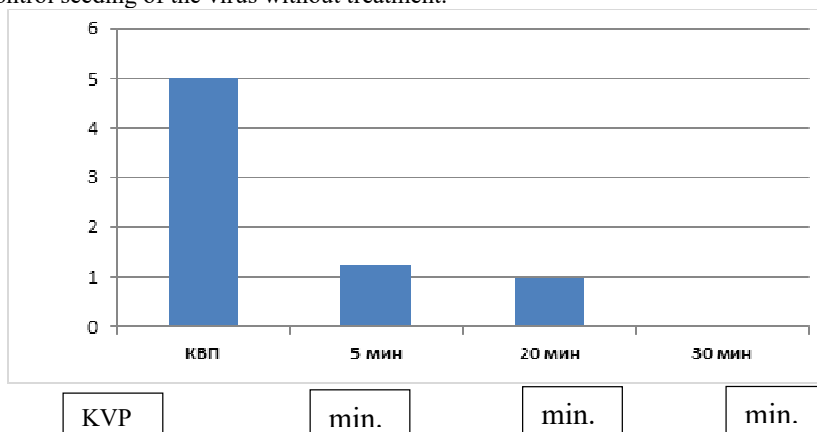


Fig. 3. Infectious titer of the TGS virus 100 ID50 in wells treated with ozone-containing mixture in Vero cell culture. KVP is a control seeding of the virus without treatment.

Infectious titer shown in the figures Fig. 2 and Fig. 3 is the degree of dilution of the virus, in which healthy cells die. Numerical values along the ordinate axis are logarithms of the dilution degree, each logarithm corresponds to the dilution of the virus by 10,000 times, ID50 is the semilethal concentration of the virus suspension, the exposure time in the ozone-containing vapor-water mixture of gases is represented along the abscissa axis.

According to the results of the study, it was found that the ozone containing the steam-water mixture produced by the POS-1 apparatus reduces the infectious titer of the TGS virus of the coronavirus family, depending on the exposure time, respectively, by 4.0; 5.0 and 7.0 lg ID50 in cell cultures SPEV and Vero, in other words - at exposure to 20 min in contact with extracellular virus, the infectious titer decreased by 100,000 times, and at an exposure of 30 minutes - complete deactivation of the virus occurred. The obtained results prove the presence of a pronounced virocidal effect of the use of a steam-water ozone-containing mixture of gases on the virus of transmissible gastroenteritis of pigs strain D52.

The results for determining the bactericidal capabilities of the proposed method are presented in Table 1.

Table 1. Concentration of polyresistent clinical strains of bacterial agents in the control group and the observation group

M/O	Control, (CCA/ml)	Observations, (CSA/ml)
Escherichia coli	10^6	no growth
Klebsiella pneumoniae	10^6	less than 10^3
Pseudomonas aeruginosa	10^8	no growth
Stafilococcus aureus	10^6	no growth
Candida albicans	10^6	less than 10^4

The performed bacteriological studies confirmed further culture growth in the control group and a complete absence of growth in the observation group after treatment with a steam-water ozone-containing mixture for 20 minutes in samples with *Escherichia Coli*, *Pseudomonis aeruginosa*, *Stafilococcus aureus*, as well as a significant decrease in the titer of *Klebsiella pneumoniae* (less than 103 CYU / ml) and *Candida albicans* (less than 104 CEU / ml), which is evidence of a pronounced bactericidal effect of the developed method.

The results of swimming tests and a comparison of the behavioral response of white rats of the control and experimental (after breathing with a gas mixture for 10-20 minutes) groups did not differ from each other.

Biological material for histological studies after the removal of animals from the experiment was represented by tissues of the larynx, trachea, extrapulmonary bronchi, lungs, liver, spleen, thyroid gland, kidneys and adrenal glands. No changes in the structure and parenchyma of the above organs of laboratory animals of all three groups were detected.

Discussion

Pneumonia, as a complication, is caused by a number of pathogens, including viruses, bacteria and fungal lesions of the respiratory system. The most frequent causative agents of acute respiratory diseases include various viruses, as well as bacteria, such as: *Streptococcus pneumoniae*, *Haemophilus influenzae*, β -hemolytic streptococci of group A, *Streptococcus pyogenes*, *Staphylococcus aureus* (Volchegorsky et al., 2000).

However, since 2020, due to the development of the SARS-COVID-19 pandemic, the main cause of pneumonia and mortality are various strains of this coronavirus, which have claimed hundreds of thousands of lives.

Given the expansion of the pandemic, the mutagenicity of the virus, as well as the likelihood of repeated waves, the authors developed a method for the prevention and treatment of viral and bacterial diseases of the respiratory system using hyperbaric oxygenation with a vapor-water ozone-containing mixture.

Due to its high reactogenic ability, ozone actively reacts with various biological objects, including viruses and bacteria. The main target of its oxidative action is the membrane structures of cells, in which double carbon bonds in polyunsaturated fatty acid molecules are destroyed to form organic peroxides and ozonides (Volkhovskaya & Kolesova, 2015; Fundiur, et al., 2021; Yaroshenko, 2019). With the introduction of minimal doses of ozone into the body, there is an activation of metabolic processes, which is accompanied by an increase in the content of dissolved oxygen in the blood (Kuril'chik et al. 2016).

Ozone improves blood circulation and oxygen delivery to damaged tissues, accelerates metabolism, improves overall well-being in patients, activates geroprotective systems, provides an increase in the effectiveness of therapy and a decrease in drug load (Bejnarovich et al., 2016). The results of the studies indicate that the use of low doses (0.6 μ g) of ozone is accompanied by a minimally initiating effect on lipid peroxidation in the blood with a predominant protective antioxidant effect both at the systemic level (blood) and at the cellular level (erythrocytes). The use of higher doses of ozone (2.0 and 8.0 μ g), also stimulating superoxide dismutase, is accompanied by a decrease in the effect of O₃ on redox processes in the body (Solov'eva et al., 2017).

It should be noted that a factor reducing the effectiveness of ozone treatment is its molecular instability, as well as a decrease in the clinically effective concentration in the solutions used for a short period of time (Leljanov & Listratenkov, 2013).

However, given the direct effect of ozone on the biological membranes of pathogenic agents, as well as its stimulating properties on organs and complexes of body tissues, ozone therapy is a promising method worthy of solving the problems of increasing the effectiveness of treatment of viral and bacterial diseases of the respiratory system, which is especially important in the context of the COVID-19 pandemic (Ruszel, & Pieciewicz-Szczęśna, 2020; Nebelyuk, 2021; Kozishkurt, 2019; Radziejowska, 2018; Divocha et al., 2021; Zhukov, & Zukow, 2020; Habchak, & Stasiv, 2021).

We have carried out studies on the use of steam-water ozone-containing mixture of gases as a prevention of contamination and further spread of infectious agents in the organs of the respiratory system. In addition, outdated ideas about the damaging effects of ozone in high concentrations on the human respiratory tract are one of the factors constraining the clinical use of ozone. The proposed intervention model (i.e., exercises using body weight and accessories, together with nutritional and psychological interventions online) effectively reduced body fat and improved health-related physical fitness parameters in overweight or obese females after 12 weeks (Araújo et al., 2021).

VD deficiency/insufficiency is widely spread both in elite wrestlers and the control individuals. Monitoring of VD level in elite athletes with subsequent correction is necessary. VD deficiency/insufficiency in athletes correlates with high morbidity with URTI and could be associated with OS (Kerimov et al., 2019). Females of the experimental group had significant positive changes according to the majority of indicators: economization of myocardium activity, oxygen transportation opportunities of an organism, aerobic opportunities of an organism, physical working capacity. It was also determined more significant positive changes: vital capacity, strength, flexibility, coordination. It is confirmed the expediency of systematic application of the breathing exercises with a full breath and interval hypoxia. It is expedient to perform breathing

exercises on the classes of the subject "Physical culture" with the students suffer from cardiological diseases (Kopeikina et al., 2018; Hashimoto et al., 2021; Campoi et al., 2019; Permadi, & Putra, 2018; Bala, et al., 2018).

Respiratory gymnastics for students with cardiological diseases. Research reported negative trend values in anthropometric variables and variables of cardiorespiratory fitness as predictors of cardiovascular risk both in schoolchildren of urban and rural sectors. Research should be focused on school populations, since values were found in the variables evaluated that suggest a predisposition for chronic Noncommunicable diseases (NCDs); metabolic syndrome and cardiovascular risk (Cresp et al., 2017; Popadynets et al. 2020; Babelyuk et al., 2017a; Babelyuk et al., 2017b; Ruzhylo et al., 2021).

Conclusions

1. Experimentally studied and optimally selected parameters of synthesis and supply of moistened ozone in the composition of the steam-water mixture of gases produced by the apparatus POS-1.

2. Studies of the effect of ozone-containing mixture on the strain of the gastroenteritis virus of pigs of the coronavirus family, as well as bacteriological studies on multi-resistant clinical bacterial cultures confirm the pronounced virocidal and bactericidal properties of the developed method.

3. The procedure of hyperbaric oxygenation with a vapor-water ozone-containing mixture does not cause any pathological changes on the part of the organs and systems of a living organism, as well as the development of stress reactions, which proves the safety of using the technique.

4. Steam-water mixture containing ozone can be used for the prevention and treatment of infectious diseases of the respiratory system, both viral and bacterial genesis, as well as disinfection of instruments, medical material, clothing and open premises.

Compliance with Ethical Standards

Conflict of Interest. The authors declare that there is no conflict of interest that could be perceived as interfering with publication of the article.

Competing Interests. The authors declare that they have no competing interests.

Ethical Approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent. Informed consent was obtained from all individual participants included in the study. All subjects of the institutional survey gave consent for anonymized data to be used for publication purposes.

Funding sources. This study has not received any financial support from any government, community or commercial organization.

References

- Araújo, C.G.A., Mariano, I.R., Valdes-Badilla, P., Sangirolamo, G.G., Santos, I.C., de Souza, H.B., & Branco, B.H.M. (2021). Effects of a hybrid multi-professional intervention on morphological and cardiorespiratory parameters in overweight or obese females. *Journal of Physical Education and Sport*, 21(5), 2641-2649.
- Babelyuk, V.Y., Dubkova, G.I., Korolyshyn, T.A., Holubinka, S.M., Dobrovol's'kyi, G.Y., Zukow, W., Gozhenko A.I., & Popovych, I.L. (2017a). Operator of Kyokushin Karate via Kates increases synaptic efficacy in the rat Hippocampus, decreases C3-θ-rhythm SPD and HRV Vagal markers, increases virtual Chakras Energy in healthy humans as well as luminosity of distilled water in vitro. Preliminary communication. *Journal of Physical Education and Sport*, 17(1), 383-393.
- Babelyuk, V.Y., Gozhenko, A.I., Dubkova, G.I., Korolyshyn, T.A., Kikhtan, V.V., Babelyuk, N.V., Zukow, W., & Popovych, I.L. (2017b). Causal relationships between the parameters of gas discharge visualization and principal neuroendocrine factors of adaptation. *Journal of Physical Education and Sport*, 17(2), 624-637.
- Bala, T., Krivoruchko, N., Masliak, I., Stasyuk, R., Aries'hyna, Y., & Kuzmenko, I. (2018). Influence of cheerleading classes on functional state of respiratory system of 10-16 year old teenagers. *Journal of Physical Education and Sport*, 18, 1850-1855.
- Bejnarovich, K.V., Kasum'jan, S.A., Leljanov, A.D., Kasum'jan, A.S., & Nesterov, A.A. (2016). Monooksid azota i ozon v kompleksnom lechenii rasprostranennogo peritonita. [Nitrogen monoxide and ozone in the complex treatment of advanced peritonitis]. *Bioradikaly i Antioksidanty*, 3(3), 195-198 [In Russian].
- Bilichenko, T.N., Bystrickaja, E.V., Chuchalin, A.G., Belevskij, A.S., & Batyn, S.Z. (2016). Smertnost' ot boleznej organov dyhanija v 2014–2015 gg. i puti ee snizhenija. [Mortality from respiratory diseases in 2014–2015 and ways to reduce it]. *Pul'monologija*, 26(4), 389-397. doi.org/10.18093/0869-0189-2016-26-4-389-397 [In Russian].

- Campoi, H.G., Campoi, E.G., Lopes, R.F., Alves, S.A., Regueiro, E.M., Regalo, S.C., Taube, O.L.S., da Silva, G.P., Verri, E.D. & Fabrin, S.C. (2019). Effects of physical activity on aerobic capacity, pulmonary function and respiratory muscle strength of football athletes and sedentary individuals. Is there a correlation between these variables? *Journal of Physical Education and Sport*, 19(4), 2466-2471.
- Chuchalin, A.G., Avdeev, S.N., Ajsanov, Z.R., Belevskij A.S., Leshhenko, I.V., Meshherjakova, N.N., Ovcharenko, S.I., & Shmelev, E.I. (2014). Federal'nye klinicheskie rekomendacii po diagnostike i lecheniju hronicheskoy obstruktivnoj bolezni legkih. [Federal Clinical Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease]. *Pul'monologija*. 3, 15-36. [In Russian].
- Cresp B.M., Quilaman, V.M., & Fernandes, F.J. (2017). Cardiorespiratory and nutritional status through anthropometric patterns of health in 12-14-year-old schoolchildren in urban and rural areas of the Araucanía Region, Chile. *Journal of Physical Education and Sport*, 17(1), 348-354.
- Divocha, V., Zukow, W., & Gozhenko, A. (2021). Incidence of COVID-19 after vaccination. Analysis of publicly available data. *Journal of Education, Health and Sport*, 11(7), 135-142.
- Fundiur, V.D., Grodetskyi, V.K., Yakobchuk, S.O., Khomko, O.Y., Kozlovskaya, I.M., Fundiur, Y.V., & Fundiur, O.V. (2021). Features of organ-saving foot amputation associated with ozone therapy, local application of autologous platelet-rich plasma and vacuum sanitization of postoperative wound in patients with ischemic-gangrenous form of diabetic foot syndrome. *Journal of Education, Health and Sport*, 11(5), 237-245. <https://doi.org/10.12775/JEHS.2021.11.05.025>
- Gostjuhina, A.A., Zamoshhina, T.A., Zajcev, K.V., Gutor, S.S., Zhukova, O.B., Svetlik, M.V., Abdulkina, N.G., & Zajcev, A.A. (2018). Adaptivnye reakcii kryz posle svetovykh desinhronozov i fizicheskogo pereutomleniya. [Adaptive rat responses after light desynchronization and physical fatigue]. *Bjulleten' sibirskoy mediciny*, 17(3), 22-34. [In Russian]. doi.org: 0.20538/1682-0363-2018-3-22-34.
- Habchak, A., & Stasiv, I. (2021). Use of ultrasonic diagnostics for investigation and detection of structural changes in the lungs in COVID-19. *Journal of Education, Health and Sport*, 11(12), 299-306.
- Hashimoto, H., Nakatani, E., Kida, N., & Nomura, T. (2021). A longitudinal survey of the effects of the Novel Coronavirus on exercise and sports among university students in Japan belonging to the Physical Education Faculty. *Journal of Physical Education and Sport*, 21, 2277-2287.
- Jakovlev, S.V. (2013). Kliniko-farmakologicheskoe obosnovanie vybora antibiotikov pri vnebol'nichnykh infekcijah dyhatel'nykh putej. [Clinical and pharmacological rationale for the choice of antibiotics for community-acquired respiratory tract infections]. *Consilium medicum*. Jekstravypusk: 4-5. [In Russian].
- Kerimov, F., Dumarov, J., Adyrkhaiev, S., Korobeynikov, G., Potop, V., Chiev, A., ... & Pavlenko, I. (2019). Possible associations of 25 (OH) vitamin D status with upper respiratory tract infections morbidity and overtraining syndrome among elite wrestlers. *Journal of Physical Education and Sport*, 19(Suppl. 6), 2177-2184.
- Kopeikina, E., Bogoeva, M., Kondakov, V., Iermakov, S., Gorelov, A., & Gruzdeva, N. (2018). Respiratory gymnastics for students with cardiological diseases. *Journal of Physical Education and Sport*, 18(Suppl. 4), 1801-1807.
- Kozishkurt, E.V. (2019). Prevalence of acute intestinal infections and their role in human pathology. *Journal of Education, Health and Sport*, 9(9):1308-1323.
- Kuril'chik, A.V., Smirnova, E.V., & Bul'da, V.I. (2016). Ozonoterapiya v sovremennoy klinicheskoy praktik. [Ozone Therapy in Contemporary Clinical Practice]. *Praktikuyuchiy likar*, 3(5), 92-94. [In Ukrainian].
- Leljanov, A.D. & Listratenkov, K.V. (2013). Antibakterial'nyj i ranozazhivljajushhij jeffekt ozona i interaktivnykh povjazok v lazerohirurgicheskom lechenii vrosshego nogtja. [Antibacterial and wound healing effect of ozone and interactive dressings in laser surgery for ingrown toenail]. *Vestnik Smolenskoj gosudarstvennoj medicinskoj akademii*, 3(12), 42-46. [In Russian].
- Nebelyuk, N. (2021). Effect of corvutin on changes in cytokin levels in the development of experimental bronchial asthma in combination with adrenaline miocardial damage. *Journal of Education, Health and Sport*, 11(2), 308-316.
- Permadi, A W., & Putra, I.M. (2018). Comparison of respiratory training methods for chest wall expansion in patients with chronic obstructive pulmonary disease. *Journal of Physical Education and Sport*, 18(4), 2235-2239.
- Popadynets, O., Gozhenko, A., Badyuk, N., Popovych, I., Skaliy, A., Hagner-Derengowska, M., Napierała, M., Muszkieta, R., Sokołowski, D., Zukow, W., Rybalko, L. (2020). Interpersonal differences caused by adaptogen changes in enropies of EEG, HRV, immunocytogram, and leukocytogram. *Journal of Physical Education and Sport*, 20(Suppl. 2), 982-999.
- Radziejowska, M. (2018). Use of physical education means in younger school-aged pupils after acute seasonal viral infections of the upper respiratory tract. *Journal of Education, Health and Sport*, 8(2), 407-421.

- Rukovodstvo po provedeniyu doklinicheskikh issledovaniy lekarstvennykh sredstv. (2012). Part I. Pod red. Mironova A.N. [Guidelines for preclinical drug research. Part I. Ed. Mironov A.N.]. M.: Grif i K; [In Russian].
- Ruszel, K., & Pieciewicz-Szczęśna, H. (2020). Are we still afraid of influenza during the Sars-Cov2 pandemic? - epidemiological analysis of influenza morbidity and vaccination in the 2019/2020 season. *Journal of Education, Health and Sport*, 10(8), 22–31. <https://doi.org/10.12775/JEHS.2020.10.08.003>
- Ruzhylo, S.V., Popovych, A.I., Zakalyak, N.R., Chopyk, R.V., Fihura, O.A., Bilas, V.R., Badiuk, N.S.; Gozhenko, A.I., Popovych, I.L., & Zukow, W. (2021). Bioactive water Naftussya and ozokerite have the same neuro-endocrine-immune effects in male rats caused by aryl hydrocarbons. *Pharmacologyonline*, 3, 213-226.
- Turchina, V.N., Dul'kin, L.A., & Tempel', N.A. (2014). Antibakterial'naja terapija pri lechenii infekcij dyhatel'nyh putej u detej na ambulatornom jetape. [Antibiotic therapy in the treatment of respiratory tract infections in children on an outpatient basis]. *Pediatricheskaja farmakologija*, 11(3), 66-69. <https://doi.org/10.15690/pf.v11i3.1011> [In Russian].
- Solov'eva, A.G., Peretjagin, P.V., Peretjagin, S.P., & Martusevich, A.K. (2017). Sostojanie sistemy lipoperoxidacii krovi krys pri dlitel'nom vozdejstvii ozona. [The state of the lipoperoxidation system of the blood of rats with prolonged exposure to ozone]. *Bioradikaly i Antioksidanty*, 4(1), 31-37. [In Russian].
- Statistical Bulletin in Ukraine. (2018). Kyiv.
- Volchegorsky, I.A., Dolgushin, I.I., Kolesnikov, O.L., & Tseylikman, V.E. (2000). Eksperimental'noe modelirovanie i laboratornaya otsenka adaptivnykh reaktsiy organizma. [Experimental modeling and laboratory examination of adaptive reaction of the organism]. Chelyabinsk: ChGPU; [In Russian].
- Volkhovskaya, N.B., & Kolesova, O.E. (2015). Eksperimental'nye issledovaniya vozdeystviya ozonirovannogo fiziologicheskogo rastvora na sostoyanie okislitel'no-vosstanovitel'nogo ravnovesiya. [Experimental Studies of the Effect of Ozonized Saline Solution on the State of Redox Equilibrium]. *Simvol nauki*, 10, 217–221. [In Russian].
- Yaroshenko, Z. (2019). The incidence of non-tumour diseases of the respiratory organs of offspring of the first generation of residents of radiation-contaminated territories of Ukraine under the age of 18 years. *Journal of Education, Health and Sport*, 9(12), 264–274. <https://doi.org/10.12775/JEHS.2019.09.12.027>
- Zhukov, A., & Zukow, W. (2020). Silver Against the Coronavirus Epidemic. *Journal of Education, Health and Sport*, 10(3), 83-97.