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# *A great valuable start!*

**Maureen Ellis, Executive Director,**
**International Federation of Biosafety Associations**
**Ontario, Canada**

*Population health is the most valuable asset of a state, and maintaining public health is and should be a priority for any country. Improving the health status of the population is the main objective of reforms and improvement of health systems. National and international strategies for health highlight the increasing role of promoting population and education health.*

*The One Health concept recognizes that the health of people is connected to the health of animals and of the environment. In order to disseminate the valuable results of scientific research on the local, regional, national, and global levels – having as goal to achieve optimal health outcomes by recognizing the interconnection between people, animals, plants and their shared environment, in 2019 the Moldavian Biosafety and Biosecurity Association (MDBBA) founded the „One Health & Risk Management" (OH&RM) Journal.*

*On this occasion, The International Federation of Biosafety Associations (IFBA) is pleased to congratulate the members of the MDBBA for successfully launching the OH&RM Journal. This scientific journal provides a platform for exchanging the best practices and an increased awareness of global risks in the handling of dangerous biological materials. For the past two years MDBBA has been an active member of IFBA and a biosafety champion in Moldova. The Association brings together a diverse community of individuals who share their passion for biosafety issues.*

*Good luck!*

mellis

OH&RM ONE HEALTH & RISK MANAGEMENT

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ARTICOLE DE CERCETARE – НАУЧНЫЕ СТАТЬИ – RESEARCH ARTICLES – ARTICLES DE RECHERCHE



# THE EFFICACY OF DIRECT-ACTING ANTIVIRAL THERAPY IN PATIENTS WITH CHRONIC HEPATITIS C

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***Key words:*** *chronic HCV, treatment, sofosbuvir, ledipasvir, daclatasvir.*

***Introduction****. Viral hepatitis C (HCV) is a significant global health problem. The risk of developing chronic HCV is up to 80% of patients, of whom 10-20% can develop liver cirrhosis or hepatocellular carcinoma which can lead to death. Treatment with direct-acting antiviral agents (DAAs) contributes to a sustained virological response (SVR) in 97-99% of cases.*
***Material and methods.*** *The study was conducted on 206 patients with chronic HCV who underwent two generic antiviral agent therapies: group I (103) – Sofosbuvir 400 mg + Ledipasvir 80 mg, whereas group II (103) – Sofosbuvir 400 mg + Daclatasvir 60 mg orally, once a day, for 12 weeks. The assessment of hepatitis C virus RNA and genotype, as well as the degree of hepatic fibrosis by Fibroscan, biochemical and complete blood count (CBC) indices were carried out.*
***Results.*** *The study results showed high efficacy of the generic DAAs treatment in patients with chronic HCV over 12 weeks. The SVR rate made up 90.3% in Sofosbuvir + Ledipasvir therapy and 86.4% – in Sofosbuvir + Daclatasvir. Similar treatment response was recorded in naive patients and those who previously underwent unsuccessful treatment with Pegylated Interferon and Ribavirin. The DAAs treatment showed the following minor adverse reactions: asthenia, headache, sleep* disorder*, and nausea, which did not require treatment discontinuation.*
***Conclusions.*** *The 12-week course of DAAs therapy exhibited high SVR rate in both chronic HCV naive patients and those previously treated with Pegylated Interferon and Ribavirin.*

***Cuvinte cheie:*** *HVC cronică, tratament, sofosbuvir, ledipasvir, daclatasvir.*

**EFICACITATEA TRATAMENTULUI CU PREPARATE ANTIVIRALE CU ACȚIUNE DIRECTĂ LA PACIENȚII CU HEPATITĂ VIRALĂ C CRONICĂ**

***Introducere.*** *Hepatita virală C (HVC) este o maladie cu un impact semnificativ la nivel mondial. Riscul de cronicizare a HVC este de până la 80% dintre pacienții diagnosticați cu această maladie iar 10-20% dintre aceștea dezvoltă ciroza hepatică, carcinomul hepatocelular sau poate surveni decesul. Tratamentul cu preparatele antivirale, cu acțiune directă (PAAD) contribuie la obținerea răspunsului virusologic susținut (RVS) în 97-99% de cazuri morbide.*
***Material și metode.*** *Studiul științific a inclus 206 de pacienți cu HVC cronică, care au primit două scheme de preparate antivirale generice: I lot (103) – Sofosbuvir, 400 mg + Ledipasvir, 80 mg, însă cei din lotul II (103) – Sofosbuvir, 400 mg + Daclatasvir, 60 mg per os, o dată în zi, timp de 12 săptămâni. Au fost evaluate ARN-VHC, genotipul virusului C, gradul de fibroză hepatică prin Fibroscan, indicii biochimici și hemoleucograma.*
***Rezultate.*** *Rezultatele studiului au demonstrat randamentul ridicat al tratamentului cu PAAD, efectuat pe parcursul a 12 săptămâni pacienților cu HVC cronică. Rata RVS a fost de 90,3% – în cazul tratamentului cu Sofosbuvir + Ledipasvir și 86,4% – cu Sofosbuvir + Daclatasvir. Răspunsul la tratament a fost similar la pacienții naivi și la cei tratați anterior cu Interferon pegilat și Ribavirină* – fără succes. Tratamentul antiviral cu PAAD a înregistrat reacții adverse minore*: astenie, cefalee, dereglarea somnului, grețuri, care nu au pretins întreruperea tratamentului.*
***Concluzii.*** *Tratamentul cu PAAD, administrat timp de 12 săptămâni, a atins RVS ridicat atât la pacienții cu HVC cronică naivi, cât și cei tratați anterior cu PEG-INF și Ribavirină.*

## INTRODUCTION

Viral hepatitis C is a significant global health problem. According to the World Health Organization data, 71 million people with hepatitis C virus are estimated worldwide, accounting for 1.2% of the world population. Approximately 399 000 people die annually from HCV-related complications, which include cirrhosis, hepatocellular carcinoma and liver failure (1). Annually, 3-4 million new cases of HCV occur, including 1300-1400 cases in the Republic of Moldova. The prevalence of HCV infection considerably ranges from a very low level (0.05-0.1%) in the UK and Scandinavian countries, to the highest level in Asia and Egypt (>5-10%) (2, 3). The HCV incidence in the Republic of Moldova was estimated at 4.5-5.0% in the overall population with the prevalence of genotype 1b, accounting for 98% (4, 5). People aged between 30-49 are predominantly affected, men being more common than women. The risk of chronic HCV infection is very high. Untreated acute hepatitis C can develop into a chronic form in up to 80% of people, of whom 10-20% develop liver cirrhosis over 20 years, followed by decompensated liver disease, hepatocellular carcinoma and even death (6). Once the onset of chronic infection occurs, the rate of spontaneous recovery is significantly reduced. Chronic HCV in most patients develops asymptomatically or nonspecifically, as long as no cirrhosis is present (7, 8, 9). As a result, HVC is often diagnosed accidentally or remains undiagnosed. It has been estimated that only 30-50% of HCV people are aware of their disease. Therefore, the lack of an effective vaccine, that would contribute to a decrease in HCV morbidity rate and subsequent consequences, requires the development of early diagnostic measures and treatment in order to eradicate the infection. Once HCV is identified, the assessment of the therapeutic success is possible by dosing the amount of RNA-HCV and the sustained virologic response. Over time, the SVR rate increased from 5-20% in interferon monotherapy, to 40-50% in combined IFN + Ribavirin treatment. Currently, the SVR rate accounts for 95-100% in cases treated with DAAs (10). The major advantage in the treatment of chronic HVC is the opportunity to administer DAAs – NS3/4A protease inhibitors, NS5B polymerases and NS5A replication complex.

These drugs require oral short-term administration, having a high SVR rate and minimal side effects. The purpose of antiviral treatment is to definitely eliminate the virus and get negative RNA-HCV at a 6-month interval after treatment completion by obtaining a SVR. The literature data confirm that over 99% of patients who have obtained a SVR remain HCV-negative over 4-5 years after the treatment was discontinued, with no signs of hepatitis. The long-term SVR showed a 75% reduction of hepatocellular carcinoma cases, decompensated and compensated cirrhosis and death over the next 15 years (11). It has recently been shown that patients with SVR have a similar quality-of-life status as the overall population (7), whereas those with compensated and decompensated cirrhosis do not require liver transplant (9, 12).

## MATERIAL AND METHODS

*The purpose of the research*: to study the effectiveness of Interferon-free treatment in patients with chronic HCV infection.

*The research objectives:* to assess the efficacy of the DAAs therapy: Sofosbuvir + Ledipasvir and Sofosbuvir + Daclatasvir in patients with chronic HCV infection. To study the evolution of clinical, biochemical, virological and paraclinical indices in patients with chronic HCV infection both at the beginning and end of treatment, as well as over 24 weeks after treatment. To analyze the adverse reactions and complications that can occur after the DAAs treatment.

*The research hypothesis:* the emergence of new therapeutic opportunities allows each patient diagnosed with chronic HCV infection to receive antiviral treatment. DAAs have shown a higher efficacy and tolerability, as well as shorter treatment duration compared to interferon therapy. A combination of at least two of the three major classes of drugs results in SVR≥95% in just 8-12 weeks of treatment. Patients who were treated for HCV infection exhibited a better quality of life, as well as a reduced risk of developing liver cirrhosis, hepatocellular carcinoma and death related to liver and extrahepatic diseases.

The study was conducted on 206 adult patients with chronic HCV who initiated DAAs treatment within the PHMI *Toma Ciorba* Clinical Hospital of Infectious Diseases during 2017-2018.

The inclusion criteria were as follows: patients ≥18 years of age, with chronic HCV confirmed by anti-HCV, anti-HCV IgM, RNA-HCV>25 IU/mL, with F0-F3 fibrosis of all genotypes, naive patients

or patients subjected to a previous unsuccessful antiviral treatment.

The exclusion criteria were as follows: pregnant and nursing patients, HIV-HBV-HVD co-infection, liver cirrhosis, F4 fibrosis, hepatocellular carcinoma or other malignancies.

The patients included in the study were randomly divided into two groups of 103 patients each. The study groups were comparable in terms of age and gender. The degree of liver fibrosis was assessed via Fibroscan. Patients with F0, F1, F2 and F3 fibrosis were selected. Chronic HCV patients were initially diagnosed by detecting anti-HCV via the immuno-enzymatic assay and confirmed by ARN-HCV testing. Serum levels of ARN-HCV were determined by real-time polymerase chain reaction (PCR) with a low detection limit <25 IU/mL. Prior to treatment initiation, the HCV genotype was assessed (1a, 1b, 2, 3 and 4). Anamnestic, epidemiological, clinical, biochemical, serological and molecular biology data were collected in all patients at the beginning, over 4 and 12 weeks of treatment, as well as over 24 weeks after the antiviral treatment completion.

The 1st group included 103 patients treated with Twinvir (Sofosbuvir 400 mg/Ledipasvir 90 mg), (manufacturer: Incepta Pharmaceuticals, Bangladesh), one pill orally, once daily, for 12 weeks.

The 2nd group included 103 patients, undergoing treatment with Nucleobuvir (Sofosbuvir 400 mg) – one pill, and Daclavirdin (Daclatasvir 60 mg) – one pill orally (manufacturer: EVAPHARMA, Egypt), for 12 weeks.

All patients signed an informed consent. The study protocol was positively endorsed by the Research Ethics Committee of *Nicolae Testemitanu* SUMPh (meeting no. 75 of 26.04.2017).

## RESULTS

Of the 206 patients with chronic HCV included in the study, there were 104 (50.5%) men and 102 (49.5%) women. There were patients aged between 20-79 years old, mean age 50.13±1.28 years. Patients aged 41-50 years – 55 (26.7%) were the most affected, as well as patients aged 51-60 years – 63 (30.6%). There were 19 (9.2%) patients aged 20-30, 33 (16%) patients aged 31-40 years, 30 (14.5%) patients aged 61-70 years, and 6 (2.9%) patients aged 71-80 years. More than half of the patients had concomitant digestive diseases or extrahepatic manifestations. The results were similar in both research groups. Most patients were naive, whereas 14 (13.6%) patients from group I and 11 (10.7%) patients from group II previously administered antivirals with Interferon, Ribavirin, Boceprevir or Telaprevir, but unsuccessfully. Of the total number of patients, 174 (84.4%) were infected with genotype 1. Of them, 170 cases were detected with 1b GT, 2 patients with GT 1a and mixed GT (1a+1b) each, 2 (1%) patients – genotype 2, 10 (4.9%) patients – genotype 3, of whom 6 – genotype 3a and 2 patients had mixed genotypes (3a+ 3b) and (3a+1b) each, one patient (0.5%) – genotype 4+1b. The only diagnostic tests available in the Republic of Moldova were for genotypes 1-4. Genotypes 5, 6 and 7 have remained unidentified. Therefore, 19 (9.2%) patients with detectable quantitative HCV RNA have not been assessed for genotype (tab. 1).

FibroScan elastometry assessment or elastography detected minor F0-F1 fibrosis in 70 (34%) patients, moderate fibrosis (F2) in 55 (26.7%) cases and advanced fibrosis (F3) in 81 (39.3%) cases. Patients of group I more frequently exhibited minor fibrosis – in 52 (50.5%) cases, and group II had predominantly advanced fibrosis in 58 (56.3%) cases. At the beginning of antiviral treatment, the level of HCV viremia ranged from 2 168 to 64 402 936 copies/mL (mean value – 6 413 266 ± 776 462 copies/mL) and did not differ significantly in both groups (tab. 1).

At least one gastrointestinal disease, such as chronic pancreatitis, chronic cholecystitis or chronic gastroduodenopathy was found in 64 (62.1%) patients from group I and in 60 (58.2%) patients from group II.

Concomitant chronic hepatitis C virus infection with diabetes were found in 62 (30.1%) patients, with hypertensive disease – in 63 (30.6%), ischemic heart disease – in 27 (13.1%), autoimmune thyroiditis – 18 (8.7%), and vasculitis – 20 (9.7%), which could be extrahepatic manifestations of HCV, due to unknown disease onset (tab. 2).

Patients with extrahepatic manifestations were older (55.86±0.97 years) than those without manifestations (45.08±1.07 years) ($p<0.001$). Patients without extrahepatic manifestations exhibited a short-term disease from the time of detection (9.52±0.58 years), compared to those who had extrahepatic manifestations (11.54±

0.63 years), (p<0.05). There were no contraindications for DAAs, compared to Interferon treatment, in 9 (8.73%) patients from group I and in 6 (5.82%) patients from group II, who exhibited extrahepatic manifestations, as well as cancer remission.

Table 1. General characteristics of chronic HCV patient groups at the initiation of antiviral treatment.

| Indices | Lot SOF+LDV (n=103) | Lot SOF+DCV (n=103) | OR 95% CI, P |
|---|---|---|---|
| **Age, years** | 50.03±1.29 | 50.24±1.28 | >0.05 |
| **Males, n/(%)** | 51 (49.5) | 53 (51.4) | 0.93 (0.54-1.60), >0.05 |
| **Females, n/(%)** | 52 (50.5) | 50 (48.6) | 0.78 (0.63-1.87), >0.05 |
| **Gastrointestinal comorbidities, n/(%)** | 64 (62.1) | 60 (58.2) | 1.18 (0.67-2.06), >0.05 |
| **Extrahepatic manifestations, n/(%)** | 56 (54.3) | 60 (58.21) | 0.85 (0.49-1.48), >0.05 |
| **Previous antiviral treatment, n/(%)** | 14 (13.6) | 11 (10.7) | 1.32(0.57-3.05), >0.05 |
| **Genotype, n/(%)** | | | |
| **1b** | 98 (95.1) | 72 (69.9) | 8.43 (3.13-22.76), <0.001 |
| **2** | 1 (0.97) | 1 (0.97) | |
| **3** | 0 | 10 (9.7) | |
| **4** | 0 | 1 (0.97) | |
| **Mixed** | 0 | 4 (3.88) | |
| **Unidentified** | 4 (3.88) | 15 (14.5) | 0.24 (0.08-0.74), <0.05 |
| **Fibrosis staging, n/(%)** | | | |
| **F0-F1** | 52 (50.5) | 18 (17.5) | 4.81 (2.54-9.12), <0.001 |
| **F2** | 28 (27.2) | 27 (26.2) | 1.05 (0.57-1.94), >0.05 |
| **F3** | 23 (22.3) | 58 (56.3) | 0.22 (0.12-0.41), <0.001 |
| **ARN-VHC, copies /mL, mean value** | 6.231.148± 745.259 | 6.595.385± 1.450.617 | >0.05 |

Table 2. Comorbidities of patients with chronic HCV at the initiation of antiviral treatment.

| Comorbidities | Lot SOF+LDV (n=103) | Lot SOF+DCV (n=103) | OR 95% CI, P |
|---|---|---|---|
| **Chronic pancreatitis, n/(%)** | 23 (22.33) | 26 (25.24) | 1.17 (0.61-2.23), >0.05 |
| **Chronic cholecystitis, n/(%)** | 31 (30.09) | 26 (25.24) | 0.78 (0.42-1.44), >0.05 |
| **Chronic gastroduodenopathy, n/(%)** | 26 (25.24) | 25 (24.27) | 0.95 (0.50-1.78), >0.05 |
| **Diabetes mellitus, n/(%)** | 32 (31.06) | 30 (29.12) | 0.91 (0.50-1.65), >0.05 |
| **Hypertensive disease, n/(%)** | 22 (21.35) | 41 (39.8) | 2.43 (1.32-4.50), <0.01 |
| **Cardiomyopathy, n/(%)** | 17 (16.5) | 10 (9.7) | 0.54 (0.24-1.25), >0.05 |
| **Tumors, n/(%)** | 9 (8.73) | 6 (5.82) | 0.65 (0.22-1.88), >0.05 |
| **Obesity, n/(%)** | 11 (10.68) | 13 (12.62) | 1.21 (0.51-2.83), >0.05 |
| **Autoimmune thyroiditis, n/(%)** | 9 (8.73) | 9 (8.73) | 1.0 (0.38-2.63), >0.05 |
| **Vasculitis, n/(%)** | 9 (8.73) | 11 (10.68) | 1.25 (0.49-3.15), >0.05 |

The DAAs treatment showed a good biochemical response even from the first month of treatment. There was a significant ALAT decrease in both groups, thus over 4 weeks of treatment, the mean ALAT values showed decreasing rate of 3.47 times in group I and 2.81 times in group II, which reached the normal indices and maintained during the treatment and after its completion (p<0.001) (fig. 1).

Virologic response over 12 weeks after the treatment was recorded in 95 (92.2%) patients treated with SOF+LDV; in 90 (87.4%) patients with SOF+DCV, RNA-HCV it was undetectable. Virologic failure at the end of treatment was recorded in 8 (7.8%) patients from group I and in 13 (12.6%) from group II.

Over 24 weeks after antiviral treatment completion, undetectable RNA-HCV was maintained in 93 (90.3%) patients in group I and 89 (86.4%) in group II. Although the $SVR_{24}$ rate in patients who administered SOF+LDV treatment was higher than in those with SOF+DCV, there was not any statistically significant difference OR=0.68, 95% CI (0.20-1.61), p=0.39 (fig. 2).

Table 3. Mean values of biochemical indices at the initiation of antiviral treatment.

| Biochemical parameters | Lot SOF+LDV (n=103) | Lot SOF+DCV (n=103) | P |
|---|---|---|---|
| Thymol test, U | 4.78±0.28 | 6.0±0.31 | <0.01 |
| Total bilirubin, µmol/L | 16.54±0.93 | 15.98±0.73 | >0.05 |
| ALAT, IU/L | 108.23±12.2 | 118.8±14.78 | >0.05 |
| ASAT, IU/L | 81.8±8.44 | 90.38±7.49 | >0.05 |
| Alkaline phosphatase, IU/L | 206.17±9.18 | 183.94±8.68 | >0.05 |
| GGT, UI/L | 67.03±5.72 | 76.63±8.24 | >0.05 |
| Glucose, mmol/L | 5.96±0.18 | 6.05±0.19 | >0.05 |
| Amylase, IU/L | 89.44±4.83 | 99.14±5.25 | >0.05 |
| Prothrombin time index, % | 88.56±0.65 | 86.27±0.65 | <0.05 |

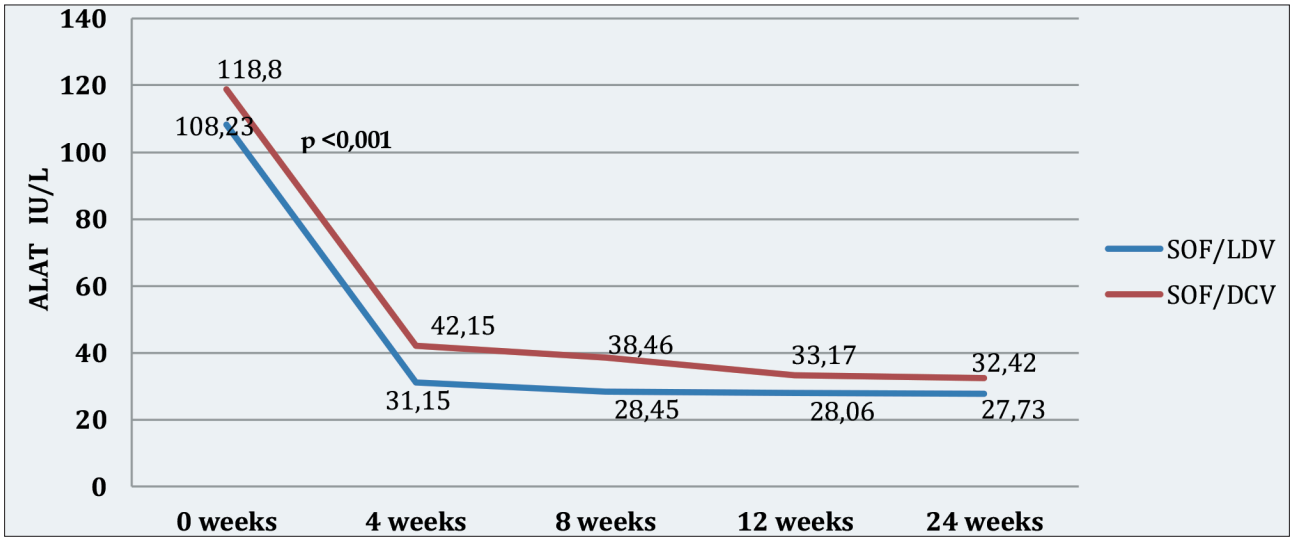


Figure 1. Dynamics of ALAT activity in patients with chronic HCV treated with DAAs.

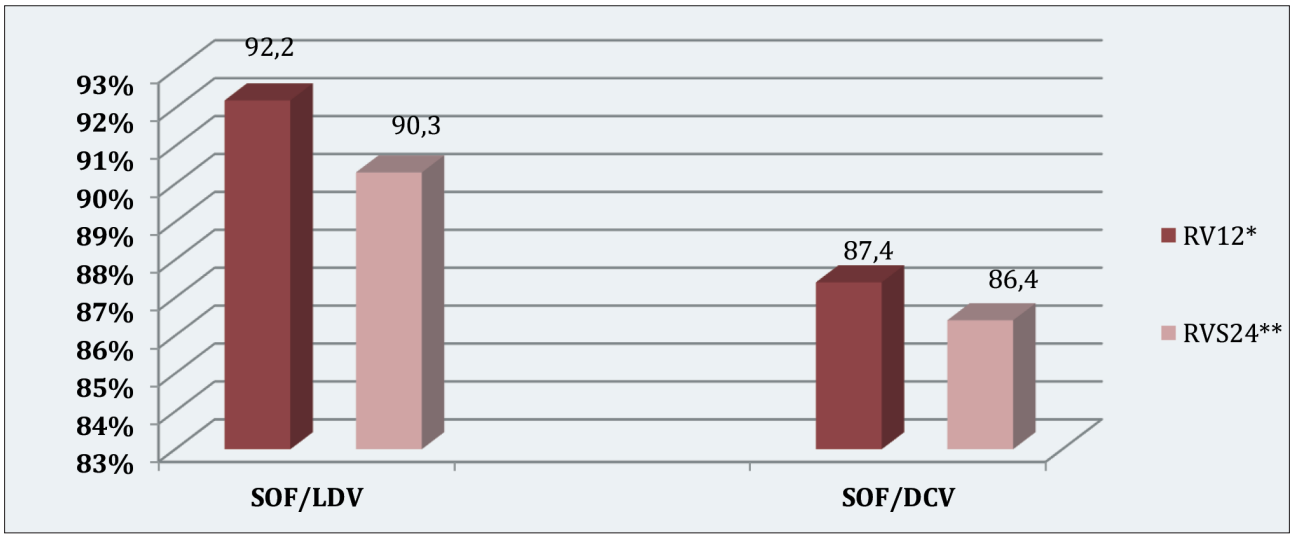


Figure 2. Virologic response in chronic Hepatitis C patients.

* VR12 – virologic response at the end of treatment

** $SVR_{24}$ – sustained virologic response over 24 weeks after treatment

The analysis of the SVR-related predictive factors in DAAs treatment (tab. 4, tab. 5), revealed that SVR was mostly found in patients with high cytolytic activity compared to those who had serum aminotransferase level within normal range. Young patients showed a more frequent SVR than those over 60, although the differences were not statistically significant. At the same time, patients with SVR showed a higher initial level of ALAT (117.75±10.69 IU/L), compared to those in whom virologic failure was recorded (79.68±11.88 IU/L), ($p<0.05$). The obtained data help to conclude that age, gender, level of viremia, liver fibrosis stage and genotype are not predictive factors of SVR in DAAs treatment.

Table 4. SVR-related predictive factors for Sofosbuvir+Ledipasvir treatment.

| Factors | SVR n=93 | Total n=103 | % | 95% CI | P |
|---|---|---|---|---|---|
| Age | | | | | |
| ≤40 years | 22 | 24 | 91.6 | 1.0 | |
| 41-60 years | 51 | 56 | 91.1 | 0.93 (0.17-5.15) | 0.93 |
| ≥60 years | 20 | 23 | 86.9 | 0.61 (0.09-4.01) | 0.60 |
| Gender | | | | | |
| Males | 48 | 51 | 94.1 | 1.0 | |
| Females | 45 | 52 | 86.5 | 0.40 (0.10-1.65) | 0.21 |
| ALAT | | | | | |
| Normal range | 18 | 22 | 81.8 | 1.0 | |
| > normal range | 75 | 81 | 92.6 | 2.78 (0.71-10.89) | 0.14 |
| ARN-VHC | | | | | |
| < 1 mln copies/mL | 13 | 13 | 100 | 1.0 | |
| 1-5 mln copies/mL | 43 | 50 | 86.0 | 0.21 (0.01-4.01) | 0.30 |
| >5 mln copies/ mL | 37 | 40 | 92.5 | 0.40 (0.02-8.20) | 0.55 |
| Fibrosis stage | | | | | |
| 0-1 | 49 | 52 | 94.2 | 1.0 | |
| 2-3 | 44 | 51 | 86.3 | 0.38 (0.09-1.58) | 0.19 |
| Genotype | | | | | |
| 1 | 88 | 98 | 89.8 | 1.0 | |
| 2,3,4 | 1 | 1 | 100 | 0.36 (0.01-9.31) | 0.53 |
| Unidentified | 4 | 4 | 100 | 1.06 (0.05-21.25) | 0.97 |

Table 5. SVR-related predictive factors for Sofosbuvir+Daclatasvir treatment.

| Factors | SVR n=89 | Total n=103 | % | 95% CI | P |
|---|---|---|---|---|---|
| Age | | | | | |
| ≤40 years | 24 | 27 | 88.9 | 1.0 | |
| 41-60 years | 47 | 53 | 88.7 | 0.98 (0.23-4.26) | 0.98 |
| ≥60 years | 18 | 23 | 78.3 | 0.45 (0.09-2.13) | 0.31 |
| Gender | | | | | |
| Males | 46 | 53 | 86.8 | 1.0 | |
| Females | 43 | 50 | 86 | 0.93 (0.30-2.88) | 0.91 |
| ALAT | | | | | |
| Normal range | 14 | 18 | 77.8 | 1.0 | |
| > normal range | 75 | 85 | 88.2 | 2.14 (0.59-7.8) | 0.25 |
| ARN-VHC | | | | | |
| < 1 mln copies/mL | 22 | 24 | 91.7 | 1.0 | |
| 1-5 mln copies/mL | 33 | 39 | 84.6 | 0.50 (0.09-2.70) | 0.42 |
| >5 mln copies/ mL | 34 | 40 | 85 | 0.52 (0.10-2.79) | 0.44 |
| Fibrosis Stage | | | | | |
| 0-1 | 15 | 18 | 83.3 | 1.0 | |
| 2-3 | 74 | 85 | 87 | 1.35 (0.33-5.41) | 0.68 |
| Genotype | | | | | |
| 1 | 60 | 71 | 84.5 | 1,0 | |
| 2, 3, 4 | 17 | 17 | 100 | 6.65 (0.37-118.64) | 0.20 |
| Unidentified | 2 | 15 | 80 | 0.73 (0.17-3.03) | 0.66 |

Treatment failure was established in 24 (11.6%) patients. Of them, there were 14 women (58.3%) and 10 men (41.7%). There were 19 (79.2%) patients over 40 years and those with an average (F2) and advanced (F3) fibrosis level 18 (75%) were more likely to fail the treatment. The ARN-HCV viremia level was similar in patients who had SVR and those with virologic failure. The SVR rate was similar in naive patients and those previously treated with antivirals, that was 160 (88.4%) and 22 (88%) cases, respectively.

The DAAs therapy was well tolerated by patients from both groups, whereas 27 (13.1%) patients experienced minor adverse reactions like asthenia, headache, nausea, insomnia, hypertensive crisis, which were quickly compensated without suspending the treatment (tab. 6).

Table 6. Adverse reactions to antiviral treatment in patients with chronic HCV.

| Indices | SOF/LDV | SOF/DCV | OR 95% CI, P |
|---|---|---|---|
| | I | II | |
| | N=103 | N=103 | |
| Asthenia, n (%) | 4 (3.9) | 5 (4.9) | 0.79 (0.21-3.04), p= 0.73 |
| Headache, n (%) | 4 (3.9) | 3 (2.9) | 1.35 (0.29-6.17), p= 0.70 |
| Nausea, n (%) | 2 (1.9) | 4 (3.9) | 0.49 (0.09-2.73), p=0.42 |
| Insomnia, n (%) | 3 (2.9) | 2 (1.9) | 1.51 (0.24-9.26), p=0.65 |
| Hypertensive seizures, n (%) | 0 | 1 (0.97) | 0.33 (0.01-8.20), p=0.50 |

## DISCUSSIONS

The implementation of the National Program of combating viral hepatitis B, C and D over 2017 and 2021 has as an overall purpose to further reduce the morbidity rate of acute and chronic hepatitis B, C and D viral infections and cirrhosis, as well as minimize their socioeconomic consequences. Access to modern DAAs schemes leads to the disappearance of HCV RNA over four weeks after treatment initiation, as well as to virus elimination in about 90% of cases. Reactivation of HCV over 24 weeks after DAAs treatment completion is rare, although reinfection should not be excluded. About 99% of patients who exhibited SVR had a negative ARN-HCV after treatment, thus the progression of liver cirrhosis and hepatocellular carcinoma being stopped (8, 9). Our study results have proved a high efficacy of generic antiviral agents administered over 12 weeks in patients with chronic HCV, thus a sustained virologic response was recorded over 24 weeks after treatment in 90.3% of patients undergoing Sofosbuvir + Ledipasvir and 86.4% patients receiving Sofosbuvir+Daclatasvir. The treatment was effective in both naïve patients and those who underwent a previous unsuccessful treatment with PEG-INF, RBV, Boceprevir or Telaprevir.

## CONCLUSIONS

1. The generic direct-acting antiviral agents showed a high efficacy, having a sustained virologic response in approximately 90% of patients treated with both treatment schemes.

2. The sustained virologic response was similar in both naïve patients and in those who underwent a previous unsuccessful antiviral treatment.

3. Generic antiviral agents have been well tolerated by most patients, whereas minor side effects did not require treatment discontinuation.

## CONFLICT OF INTERESTS

The authors do not declare any conflict of interest.

## REFERENCES

1. World Health Organization. Global Hepatitis Report 2017. Available from: http://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf?sequence=1 [Accessed 10th January 2019].

2. Pântea V. *Hepatitele virale acute și cronice (etiologie, epidemiologie, patogenie, tablou clinic, diagnostic și profilaxie).* Chișinău: 2014.
3. World Health Organization. Global Hepatitis Report 2016. Available from: https://www.who.int/hiv/pub/arv/ global-AIDS -update-2016_en.pdf [Accessed 27th December 2018].
4. Agenția Națională pentru Sănătate Publică. *Notă informativă cu privire la realizarea Programului Național de combatere a hepatitelor virale B, C și D pentru anii 2017-2021*. 2017. p.5-6. Available from: https://msmps.gov.md/ro/content/nota-informativa-cu-privire-la-realizarea-programului-national-de-combatere-hepatitelor [Accessed 10th January 2019].
5. European Association for the Study of the Liver. *Recommendations on Treatment of Hepatitis C 2018*. Available from: http://www.easl.eu/medias/cpg/HEPC-2018/Full-report.pdf [Accessed 10th January 2019].
6. Lok AS, Seeff LB, Morgan TR, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology*. 2009; 136:138-148.
7. American Association for the Study of Liver Diseases and the Infectious Diseases Society of America. *Recommendations for Testing, Managing, and Treating Hepatitis C*. 2016. Available from: https://www.hcvguidelines.org/ [Accessed 10th January 2019].
8. Chhatwal J, Wang X, Ayer T, et al. Hepatitis C disease burden in the United States in the era of oral direct-acting antivirals. *Hepatology.* 2016; 64:1442-1450.
9. Asselah T, Boyer N, Saadoun D, et all. Direct-acting antivirals for the treatment of hepatitis C virus infection: optimizing current IFN-free treatment and future perspectives. *Liver Int.* 2016; 36:47-57.
10. Ahmed OA, Kaisar HH, Badawi R, et al. Efficacy and safety of sofosbuvir-ledipasvir for treatment of a cohort of Egyptian patients with chronic hepatitis C genotype 4 infection. *Infect Drug Resist*. 2018; 11:295-298.
11. Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H. *Hepatology – A clinical textbook.* 7th Edition 2016.
12. Săndulescu O, Streinu-Cercel A, Stoica A, et al. Regression of liver fibrosis following sustained virological response in patients with chronic HCV infection and cirrhosis. *BMC Infectious Diseases.* 2016; 16(602):46-47.

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# СОВРЕМЕННАЯ ЭПИДЕМИОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА ВЕТРЯНОЙ ОСПЫ В РОССИИ

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***Key words:*** *chicken-pox, vaccination, prevention.*

**CURRENT EPIDEMIOLOGICAL CHARACTERISTIC OF CHICKENPOX IN RUSSIA**

***Introduction.*** *The varicella vaccine introduction in the National immunization schedule is planned in Russia by 2020. Since 2013, vaccination against chicken-pox has been carried out according to epidemiological indications and as part of regional immunization program in some regions.*

***Material and methods.*** *A descriptive epidemiological study has been carried out to assess the current epidemiological features of chicken-pox in Russia. A retrospective analysis of the incidence distribution in the time, age and territorial aspects was carried out, as well as taking into consideration the number of doses administered to certain population groups. Materials for a research include the official data on incidence of chicken-pox and quantity of varicella vaccine doses administered in 2006-2018 in the country in general, and in two megalopolises – Moscow and St. Petersburg.*

***Results.*** *It is established that long-term and seasonal recurrence, the age structure and the epidemic nature of chicken-pox incidence did not change in comparison with the prevaccinal period due to low vaccination coverage of the children in country scales. On the example of the immunization program in Moscow it is shown that vaccination before attendance at childcare allowed to reduce incidence of children at the age of 3-6 years. However, a long-term epidemiological effect was not achieved due to incomplete vaccination coverage of the entire cohort of children.*

***Conclusions.*** *When introducing vaccination, it is necessary to strengthen the epidemiological surveillance of chicken-pox and its vaccine prophylaxis in order to timely respond to unfavorable trends of shift in the age of chicken-pox occurrence.*

***Cuvinte cheie:*** *varicela, vaccinare, prevenire.*

**CARACTERISTICA EPIDEMIOLOGICĂ ACTUALĂ A VARICELEI ÎN RUSIA**

***Introducere.*** *În Rusia introducerea vaccinului împotriva varicelei este planificată în conformitate cu Programul național de imunizare până în 2020. Din anul 2013, vaccinarea contra varicelei se efectuează corespunzător indicațiilor epidemiologice, iar în unele regiuni – în baza prevederilor Programului regional de imunizare.*

***Material și metode.*** *A fost realizat un studiu descriptiv epidemiologic pentru a evalua particularitățile epidemiologice actuale ale varicelei în Rusia. A fost efectuată o analiză retrospectivă a distribuției incidenței în timp, după vârstă și aspecte teritoriale, luând în considerație și factorii sociali, precum și după numărul de doze administrate anumitor grupuri de populație. Materialele pentru cercetare includ datele oficiale privind incidența varicelei și cantitatea de doze de vaccin administrate în perioada 2006-2018 în țară, în general, și în două orașe mari – Moscova și Sankt Petersburg.*

***Rezultate.*** *S-a stabilit că recurența pe termen lung și ciclicitatea sezonieră, structura de vârstă și caracterul epidemic al incidenței varicelei nu s-au modificat în comparație cu perioada prevaccinală, din cauza nivelului scăzut de vaccinare a copiilor pe întreg teritoriul țării. Luând ca exemplu Programul de imunizare din orașul Moscova observăm că vaccinarea înainte de încadrarea copilului în instituțiile preșcolare a permis reducerea incidenței bolii la copiii de 3-6 ani. Însă, nu a fost obținut un efect epidemiologic pe termen lung din cauza acoperirii incomplete a vaccinării tuturor copiilor.*

***Concluzii.*** *La inițierea vaccinării este necesar să se consolideze supravegherea epidemiologică a varicelei și a profilaxiei acesteia pentru a răspunde în timp util tendințelor defavorabile de schimbare a vârstei apariției varicelei.*

## ВВЕДЕНИЕ

Успехи вакцинопрофилактики инфекций с аэрозольным механизмом передачи возбудителя привели к изменению социально-экономической значимости массовых инфекционных болезней и смене приоритетов в противоэпидемической и профилактической работе. Например, в течение последних лет в России по уровню экономического ущерба (без учета ВИЧ-инфекции и туберкулеза) третье место после ОРВИ и кишечных инфекций неустановленной этиологии занимает ветряная оспа (1). Несмотря на сравнительно легкое клиническое течение и относительно редкие случаи осложнений и летальных исходов бремя ветряной оспы велико из-за ее широкой распространенности и высокой заболеваемости. Необходимость снижения экономических потерь обусловило актуальность внедрения вакцинации против ветряной оспы (2, 3).

В России лицензированы две живые моновакцины против ветряной оспы зарубежного производства. В соответствии с Приказом Минздрава России от 21.03.2014 г. №125н прививки против ветряной оспы внесены в Календарь профилактических прививок по эпидемическим показаниям. Вакцинация проводится бесплатно для населения за счет бюджета здравоохранения субъектов федерации. Прививкам подлежат дети и взрослые из групп риска, ранее не привитые и не болевшие ветряной оспой (4). Иммунизация возможна и на коммерческой основе за счет средств населения. В некоторых субъектах федерации за счет средств бюджета региона реализуются региональные программы иммунизации. Так в Москве вакцинация против ветряной оспы введена в Региональный календарь профилактических прививок, утвержденный Приказом Департамента здравоохранения г. Москвы от 04.07.2014 г. № 614, согласно которого вакцинация проводится детям в возрасте 12 месяцев и старше перед поступлением в дошкольные организации, детям домов ребенка, а также по эпидпоказаниям контактным лицам из очагов заболевания, не болевшим, не привитым и не имеющим сведений о профилактических прививках против ветряной оспы, призывникам и детям, выезжающим на отдых в летние оздоровительные лагеря (5).

В связи с тем, что в Российской Федерации к 2020 году вакцину против ветряной оспы планируется внедрить в Национальный календарь профилактических прививок, возникла необходимость дать современную эпидемиологическую характеристику этой инфекции и определить, в какой степени на нее повлияла различная тактика проведения вакцинопрофилактики.

## МАТЕРИАЛЫ И МЕТОДЫ

Материалами для исследования послужили данные Федерального государственного статистического наблюдения о заболеваемости ветряной оспой (формы 2 и 23-17) и количестве проведенных прививок против этой инфекции (формы 5 и 6) в 2006-2018 годах как в Российской Федерации в целом, так и в двух мегаполисах – Москве и Санкт-Петербурге. Задачами исследования являлось выявление групп населения, наиболее подверженных риску заболевания ветряной оспой, периодов риска подъема заболеваемости, а также оценка влияния на заболеваемость вакцинации. В рамках описательного эпидемиологического исследования выполнен ретроспективный анализ распределения заболеваемости во временном, возрастном и территориальном аспектах, а также с учетом охвата вакцинацией отдельных групп населения.

Для проведения статистического анализа связи между величинами (показатели заболе-ваемости, число привитых) был использован метод корреляции с расчетом коэффициента линейной корреляции Пирсона (r) и оценкой его достоверности.

## РЕЗУЛЬТАТЫ

На фоне снижения инфекционной заболеваемости в России значимость ветряной оспы остается высокой, поскольку удельный вес случаев ветряной оспы от общего числа зарегистрированных случаев инфекционных заболеваний (без учета гриппа и ОРВИ) за период наблюдения ежегодно составлял от 21,3 до 30,5%.

В течение 2006-2018 годов каждый год в Российской Федерации регистрировали от 674 797 до 936 917 случаев ветряной оспы. Минимальный показатель заболеваемости за период наблюдения составил 475,5 (в 2010 году), максимальный – 642,4 на 100 тыс. населения (в 2014 году). В многолетней динамике заболеваемости (рис. 1) ветряной оспой населения России в 2006-2018 гг. отмечалась слабовыраженная тенденция роста показателей.

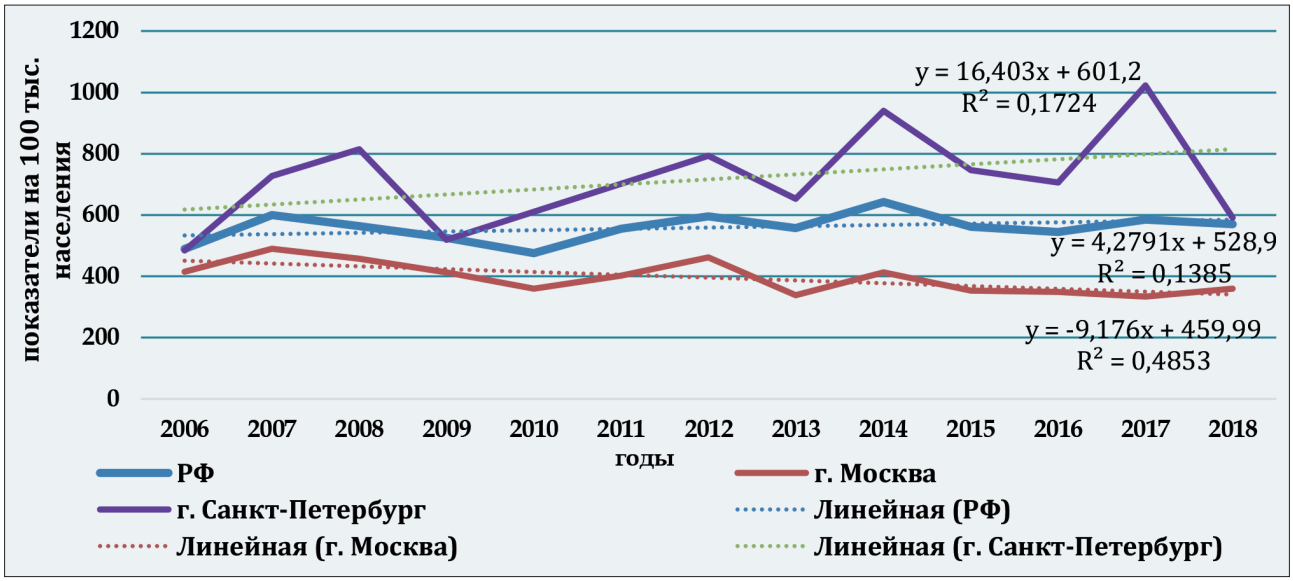


Рисунок 1. Заболеваемость ветряной оспой населения Российской Федерации, Москвы и Санкт-Петербурга в 2006-2018 годах (показатели на 100 000 населения).

Для ветряной оспы характерна цикличность эпидемического процесса: за период наблюдения отмечено 4 многолетних эпидемических цикла продолжительностью от 2-х до 4-х лет с «пиками» заболеваемости в 2008, 2012, 2014 и 2017 годах.

Ветряная оспа по-прежнему оставалась «детской» инфекцией: дети в возрасте до 18 лет составляли 94,0-95,2% от общего числа заболевших (рис. 2).

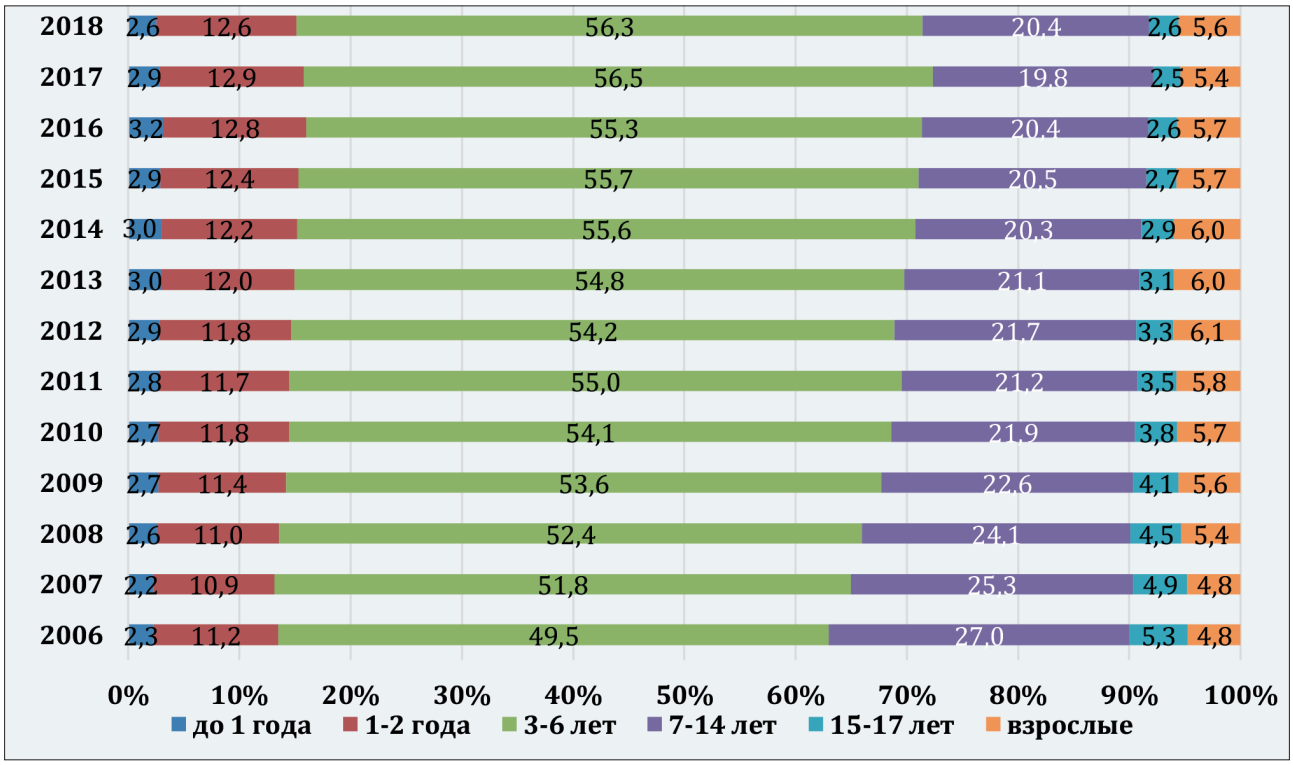


Рисунок 2. Возрастная структура заболевших ветряной оспой в России в 2006-2018 годах (%).

Установлено неравномерное распределение заболеваемости по возрастным группам населения (рис. 3). Наибольшая заболеваемость отмечена среди детей в возрасте 3-6 лет, максимальный показатель среди них был зарегистрирован в 2007 году и составил 8400,5 на 100 тысяч детей данного возраста, наименьший показатель зафиксирован в 2016 году – 6265,9 на 100 тысяч детей данного возраста.

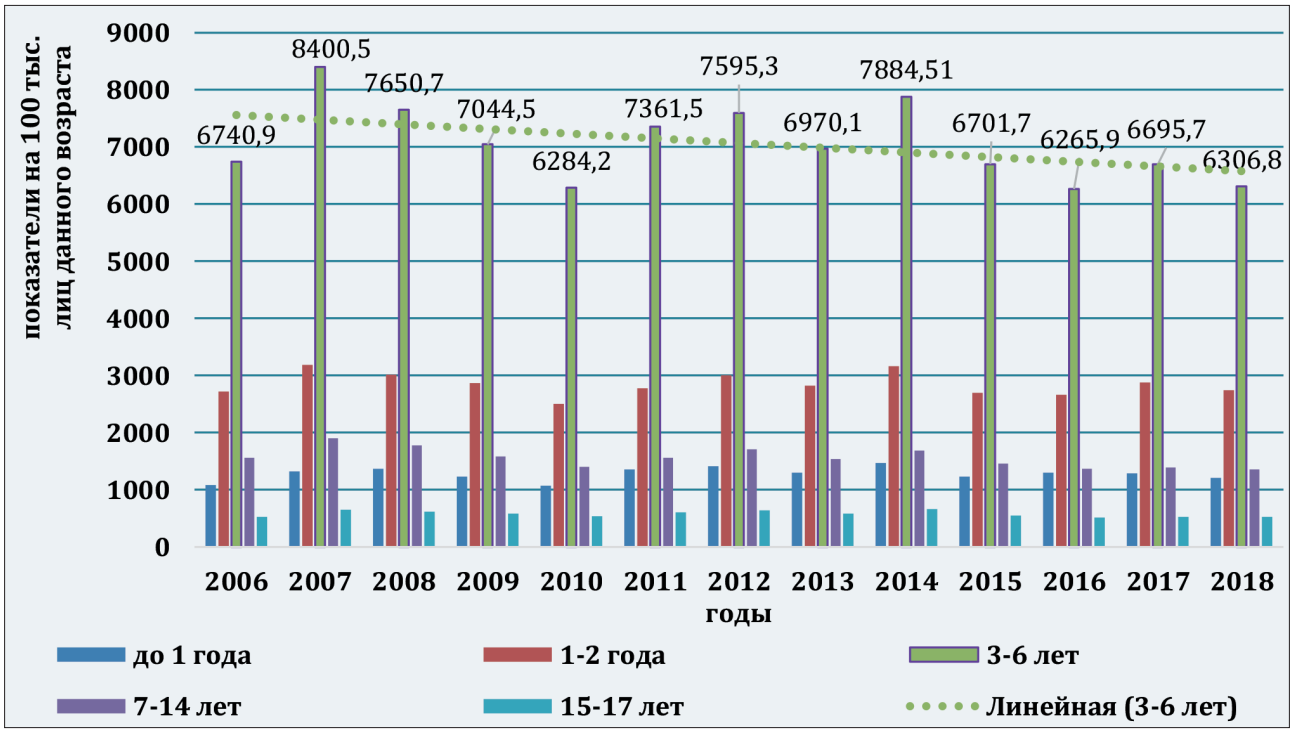


Рисунок 3. Заболеваемость ветряной оспой детей различных возрастных групп в России в 2006-2018 годах (показатели на 100 000 детей данного возраста).

Высокие показатели заболеваемости детей дошкольного (2-6 лет) и школьного (7-14 лет) возраста обусловлены, в том числе, и вспышечной заболеваемостью, поскольку в детских образовательных учреждениях и школах создаются условия, когда группа детей находится в одном помещении, при этом легко реализуется аэрозольный механизм передачи возбудителя ветряной оспы, возникают и развиваются эпидемические вспышки с большим количеством заболевших. Например, в 2017 и 2018 годах, соответственно, 6,35% и 5,07% от общего числа случаев заболевания за год зарегистрировано в крупных эпидемических очагах ветряной оспы с числом больных 10 и более.

По данным статистического наблюдения в 2017 году в России имела место 3271 вспышка ветряной оспы с общим числом заболевших 54 513 человек (54 399 детей и 114 взрослых), в 2018 году – 2 851 вспышка с 42 521 заболевшим (42 474 ребенка и 47 взрослых). Структура учреждений, в которых были зарегистрированы вспышки, представлена в Таблице 1. Приведенные данные подтверждают, что дети, посещающие дошкольные образовательные учреждения и учащиеся школ представляют собой социальные группы риска заболевания ветряной оспой.

Внутригодовое распределение случаев заболевания ветряной оспой также обусловлено заболеваемостью детей дошкольного и школьного возраста. Сезонный подъем заболеваемости начинался в сентябре, что совпадает с началом учебного года в образовательных учреждениях, а с марта наблюдалось снижение заболеваемости, которая достигала минимума в июле. Таким образом, сезонное распределение показателей заболеваемости непосредственно связано с формированием детских коллективов. Выявлена прямая сильная корреляционная связь между показателя-

ми помесячной заболеваемости всего населения и детей 3-6 лет, посещающих дошкольные учреждения (r=0,83; p<0,05), а также школьников (r=0,83; p<0,05).

Таблица 1. Удельный вес учреждений разного типа, в которых в 2017-2018 гг. были зареги-стрированы эпидемические вспышки ветряной оспы с числом случаев заболевания 10 и более.

| Тип учреждения | Зарегистрировано эпидемических вспышек | | | |
|---|---|---|---|---|
| | 2017 год | | 2018 год | |
| | **абс.ч.** | **%** | **абс.ч.** | **%** |
| Детские дошкольные учреждения | 2436 | 74,47 | 2158 | 75,69 |
| Школы | 811 | 24,79 | 662 | 23,22 |
| Колледжи, ВУЗы | 4 | 0,12 | 1 | 0,04 |
| Лечебные учреждения | 6 | 0,18 | 10 | 0,35 |
| Социальные учреждения для детей | 11 | 0,34 | 16 | 0,56 |
| Прочие | 3 | 0,10 | 4 | 0,14 |
| ВСЕГО | **3271** | **100** | **2851** | **100** |

Несмотря на то, что удельный вес взрослых среди заболевших ветряной оспой оставался небольшим (5-7%), за период наблюдения ежегодно в России регистрировали от 33 293 до 56 114 случаев ветряной оспы у лиц в возрасте 18 лет и старше. Показатели заболеваемости взрослых находились на уровне 29,0 – 47,4 на 100 тыс. взрослого населения (рис. 4)

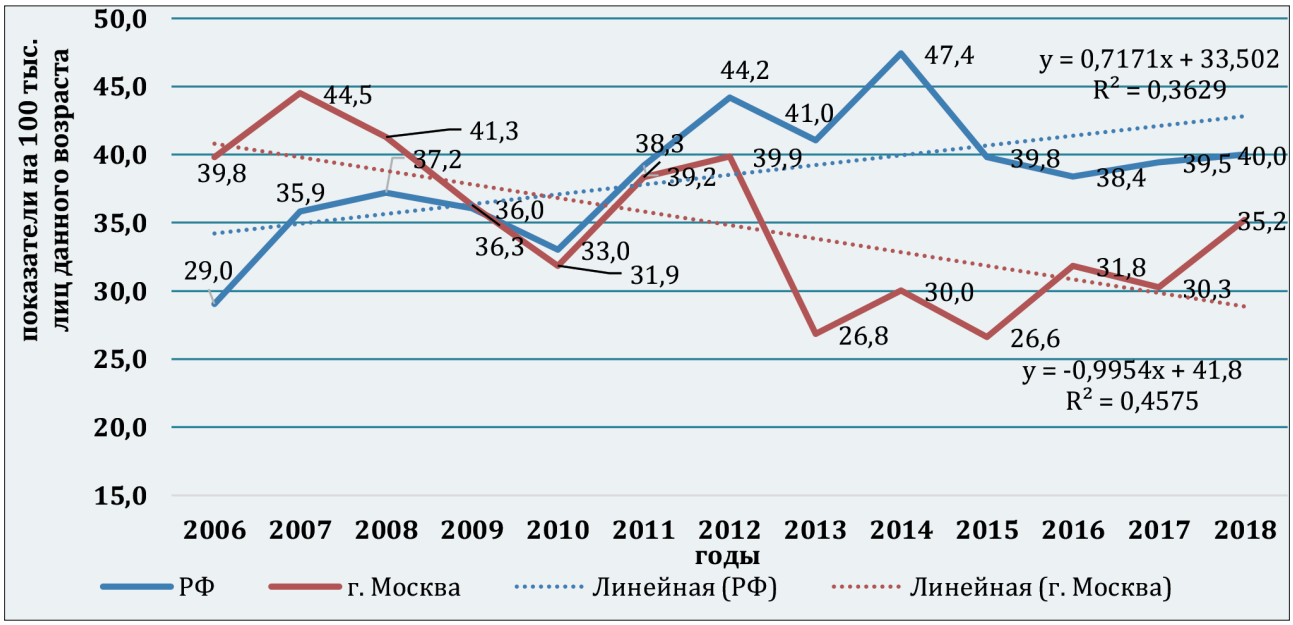


Рисунок 4. Заболеваемость ветряной оспой взрослого населения в Российской Федерации и в г. Москве в 2006-2018 годах (показатели на 100 000 населения в возрасте 18 лет и старше).

При этом в целом по стране наметилась тенденция повышения показателя заболеваемости взрослого населения. Однако для подтверждения данного вывода необходимы дальнейшие наблюдения.

Одной из эпидемиологических особенностей ветряной оспы является внутрибольничная заболеваемость: удельный вес ветряной оспы в структуре госпитальных инфекций (без учета гнойно-септических и внутриутробных инфекций) в Москве за период наблюдения ежегодно составлял 10-18%, что в абсолютных числах составило от 22 (в 2017 г.) до 101 (в 2011 году) случая в год. За анализируемый период зарегистрировано 5 случаев заражения ветряной оспой медицинских работников по месту работы. В многолетней динамике отмечалась выраженная тенденция к снижению

числа внутрибольничных случаев ветряной оспы в городе.

В рамках проведенного исследования была дана оценка влиянию вакцинации на уровень заболеваемости ветряной оспой в Москве, Санкт-Петербурге и Российской Федерации в целом.

С 2013 г. по 2018 г. в России было проведено 368 973 прививок против ветряной оспы, ежегодно вакцинировали от 32 112 до 85 517 детей. В 2016-2018 годах объемы вакцинации уменьшились с 78 833 до 57 128 прививок в год, что было обусловлено отсутствием отечественной вакцины и трудностями организации закупок импортного препарата. На этом фоне заболеваемость ветряной оспой в стране не имела тенденции к снижению (рис. 1).

По регионам России проведенные прививки распределялись неравномерно: значительная часть из них (около 40%) были осуществлены в столице – г. Москве (табл. 2). Ежегодно в Москве были вакцинированы более 20 000 детей. В результате в динамике заболеваемости ветряной оспой в Москве, в отличие от страны в целом, наблюдалась тенденция к снижению (рис. 1).

В другом мегаполисе, Санкт-Петербурге, за тот же период времени ежегодно прививали от 876 до 3 567 детей, что составило от 2,1 до 4,8% от общего числа введенных доз вакцины против ветряной оспы в России. В этих условиях заболеваемость ветряной оспой в Санкт-Петербурге не уменьшилась и даже приобрела тенденцию к росту (рис. 1).

На Рисунке 5 отражена динамика заболева-емости ветряной оспой населения г. Москвы и г. Санкт-Петербурга в сопоставлении с количеством проведенных детям прививок против этой инфекции.

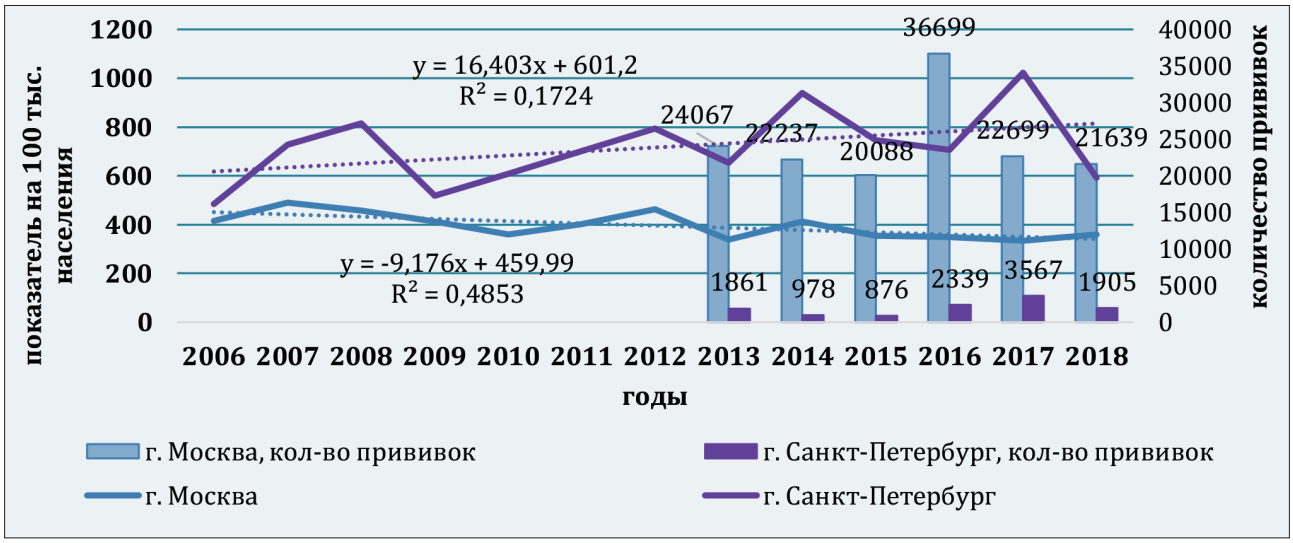


Рисунок 5. Заболеваемость ветряной оспой и количество проведенных прививок против ветряной оспы в г. Москве и в г. Санкт-Петербурге в 2006-2018 гг.

С введением вакцинации заболеваемость детей 3-6 лет в Российской Федерации снизилась на 28%: с 7057,9 до 5105,5 на 100 тысяч детей данного возраста. В то же время уменьшилась и заболеваемость детей в возрасте 7-14 лет, причем в Москве, по сравнению с Россией в целом, тенденция снижения заболеваемости детей дошкольного и школьного возраста была более выражена (рис. 6, 7).

Для оценки зависимости показателей забо-ле-ваемости ветряной оспой от объемов иммунизации против этой инфекции был проведен расчет коэффициента корреляции Пирсона для попарно связанных параметров (табл. 2). Результаты количественного анализа не выявили достоверной сильной степени корреляционной связи заболеваемости от числа прививок против ветряной оспы. На примере населения страны в целом прослеживалась отрицательная слабой степени зависимость показателей заболеваемости детей, подростков и взрослых от числа проведенных прививок. По данным, полученным в Москве, установлена лишь слабой степени отрицательная корреляционная связь показателей заболеваемости детей 3-6 лет и количества прививок. Более того, выявлена слабая прямая зависимость заболеваемости взрослых и детей в возрасте до 1 года от числа сделанных прививок.

В условиях начального этапа вакцинопрофилактики ветряной оспы заболеваемость взрослого населения Российской Федерации демонстрировала тенденцию к росту, однако в Москве наблюдалось снижение показателей заболеваемости взрослых (рис. 4).

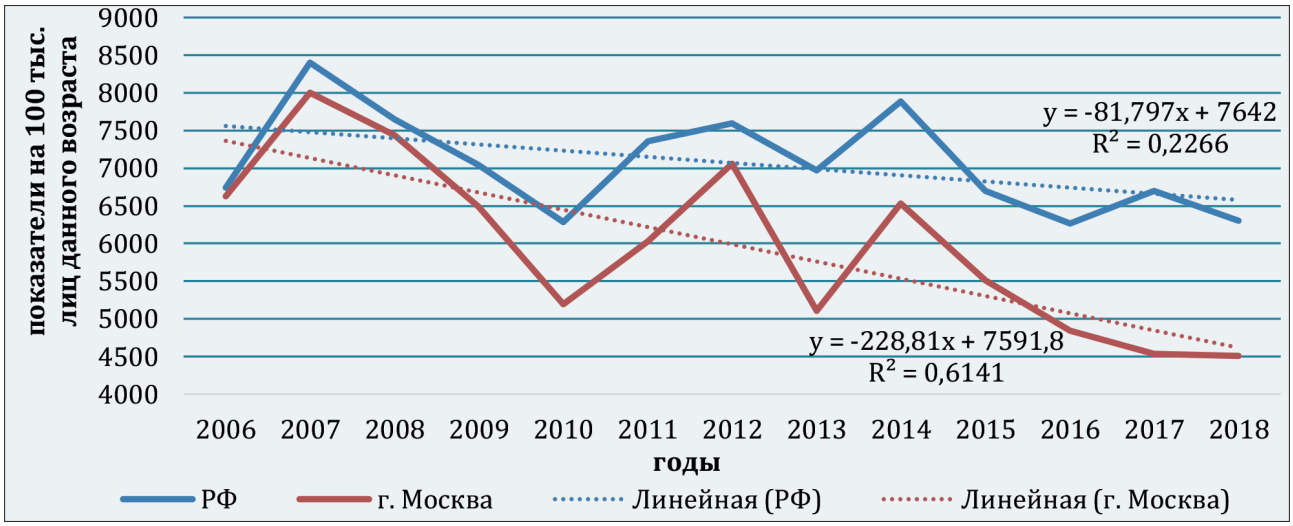


Рисунок 6. Заболеваемость ветряной оспой детей 3-6 лет в Российской Федерации и в г. Москве (2006-2018 годы).

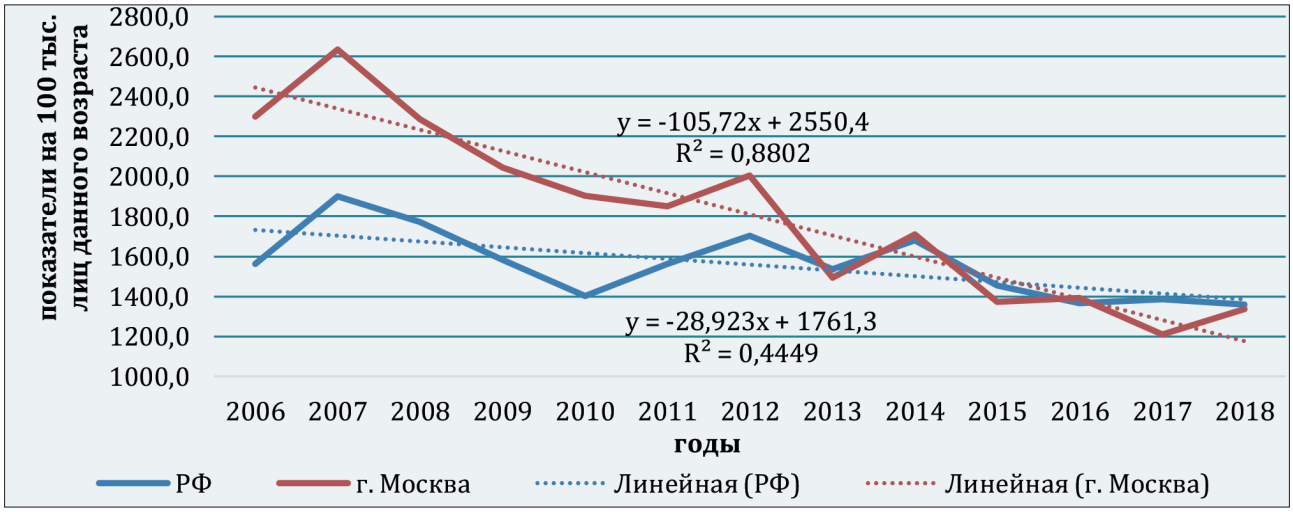


Рисунок 7. Заболеваемость ветряной оспой детей 7-14 лет в Российской Федерации и в г. Москве (2006-2018 годы).

Таблица 2. Зависимость между уровнями заболеваемости ветряной оспой (ВО) разных возрастных групп населения и числом привитых против ветряной оспы лиц (Российская Федерация, г. Москва, 2006-2018 годы).

| Связанные параметры | г. Москва | | РФ | |
|---|---|---|---|---|
| | коэфф. коррел. Пирсона r | Характеристика корреляционной связи | коэфф. коррел. Пирсона r | Характеристика корреляционной связи |
| Уровень заболеваемости ВО детей 3-6 лет/ число привитых против ВО | -0,24 | отрицательная, слабая | -0,36 | отрицательная, слабая |
| Уровень заболеваемости ВО детей 7-14 лет/ число привитых против ВО | -0,04 | отсутствует | -0,33 | отрицательная, слабая |
| Уровень заболеваемости ВО подростков/ число привитых против ВО | -0,04 | отсутствует | -0,39 | отрицательная, слабая |
| Уровень заболеваемости ВО взрослых/ число привитых против ВО | +0,23 | положительна, слабая | -0,41 | отрицательная, слабая |
| Уровень заболеваемости ВО детей до 1 года/ число привитых против ВО | +0,20 | положительна, слабая | -0,11 | отрицательная, очень слабая |

## ДИСКУССИИ

Результаты исследования показали, что тенденция, многолетняя и внутригодовая цикличность, возрастная структура и вспышечный характер заболеваемости ветряной оспой в России не изменились по сравнению с довакцинальным периодом (6, 7). Современные проявления эпидемического процесса ветряной оспы в России аналогичны тем, которые наблюдались в США и странах Европы до начала вакцинации против этой инфекции (8-12).

Практически все авторы отмечали, что ветряная оспа относится к «детским» инфекциям вследствие легкости реализации аэрозольного механизма передачи возбудителя, при котором большинство детей инфицируются уже в первые годы жизни.

В 1998 г. Всемирная Организация Здраво-охранения на основании опыта Японии и США рекомендовала плановую («универсаль-ную») иммунизацию детей в странах, где ветряная оспа является значительной проблемой здравоохранения, экономики и общества в целом, где вакцина является доступной и где достижим и может поддерживаться высокий (85-90%) охват вакцинацией (13).

В Российской Федерации вакцинопрофи-лактика ветряной оспы началась с исполь-зованием двух тактик иммунизации: первая- проведение прививок по эпидемическим показаниям, что, по сути, является вариантом выборочной вакцинации, вторая – плановая (когортная) вакцинация в некоторых регионах с охватом вакцинацией ниже 90% вследствие ограниченного финансирования закупок импортной вакцины из средств бюджета здравоохранения субъекта федерации.

Как было показано, применение данных тактик не позволила существенно улучшить ситуацию с ветряной оспой в целом по стране. При этом результаты вакцинации против ветряной оспы в Москве продемонстрировали преимущество эффекта плановой вакцинации по сравнению с выборочной.

К аналогичному заключению в свое время пришли исследователи из Канады, которые показали, что плановая иммунизация эффективнее выборочной вакцинации, проводимой на коммерческой основе частными врачами. Вакцина для профилактики ветряной оспы была лицензирована в Канаде в 1998 г., а плановая иммунизация началась в 2004 г. в Онтарио. Уровень госпитализаций, обращений за неотложной помощью и количество амбулаторных визитов снизились, в среднем, на 53%, 43% и 45%, соответственно, по сравнению с 9%, 23% и 29% по тем же показателям при внедрении вакцинации только в частном секторе (14).

Эксперты ВОЗ предупреждали, что при недостаточном охвате прививками детей может наблюдаться «повзросление» инфекции и рост числа тяжелых случаев заболевания среди подростков и взрослых (13). Подобное наблюдалось, например, в США. До 1995 г. почти 73% случаев ветряной оспы регистрировали у детей в возрасте до 6 лет с «пиком» заболеваемости в группе детей 3-6 лет. А уже в 2004 г. дети в возрасте до 6 лет включительно составили только 30% заболевших этой инфекцией. Максимальные показатели заболеваемости среди вакцинированных регистрировали у детей в возрасте 6-9 лет, а среди непривитых – у детей 9-12 лет. При этом абсолютное число заболеваний среди детей старшего возраста не увеличилось по сравнению с довакцинальным периодом (15).

В условиях начального этапа вакцинопрофи-лактики ветряной оспы заболеваемость взрослого населения России демонстрирова-ла тенденцию к росту, однако в Москве наблюдалось снижение показателей заболе-ваемости населения в возрасте 18 лет и старше (рис. 4). Выявленная в 2018 году слабая положительная корреляция заболева-емости взрослых в Москве с количеством сделанных прививок, возможно, является следствием «повзросления» ветряной оспы, обусловленной недостаточным уровнем охвата детей плановой вакцинацией.

Следует подчеркнуть, что причиной относительного «сдвига» заболеваемости на старшие возрастные группы может являться также использование однодозной схемы вакцинации против ветряной оспы, поскольку после однократной прививки остается значительное число неиммунных лиц (16-18).

Чтобы предупредить это последствие вакцинации, необходимо предусмотреть повторную прививку для детей, получивших первую дозу вакцины. К подобному заключению приводит опыт профилактики кори, краснухи и эпиде-

мического паротита. Развитие вакцинопрофилактики этих трех инфекций в стране проходило через период однократной вакцинации. При всех трех инфекциях для профилактики эпидемических вспышек в школах, снижения риска возникновения врожденных и тяжелых клинических форм заболевания проводят повторную вакцинацию детей перед поступлением в школу, а также дополнительную иммунизацию взрослых. Общие эпидемиологические закономерности указанных инфекций и ветряной оспы позволяют использовать единые подходы к управлению эпидпроцессом при всех четырех нозологиях (19). Поэтому перспективы вакцинопрофилактики ветряной оспы в России связаны с внедрением двукратной прививки и использованием комбинированных вакцин против кори, краснухи и эпидемического паротита и ветряной оспы.

В рекомендациях ВОЗ (20) подчеркивалось, что до того, как страны примут решение о внедрении вакцины против ветряной оспы в программы плановой иммунизации детей, они должны создать адекватный эпиднадзор за болезнью для оценки бремени ветряной оспы с проведением постоянного эпиднад-зора после внедрения вакцинации. В связи с этим на этапе расширения вакцинации в России продолжается совершенствование системы эпидемиологического надзора за ветряной оспой и ее вакцинопрофилактикой.

## ВЫВОДЫ

1. В России на начальном этапе вакцинопрофилактики эпидемиологическая характеристика ветряной оспы не изменилась по сравнению с довакцинальным периодом, что обусловлено недостаточным уровнем охвата иммунизацией детского населения.

2. На примере организации иммунизации против ветряной оспы в городе Москве продемонстрировано, что однократная вакцинация детей перед поступлением в дошкольные образовательные учреждения позволила снизить заболеваемость детей в возрасте 3-6 лет, однако вследствие неполного охвата прививками всей когорты детей данного возраста стойкого эпидемиологического эффекта достичь не удалось.

3. При внедрении вакцинации необходимо усиление эпидемиологического надзора за ветряной оспой и ее вакцинопрофилактикой для своевременного реагирования на неблагоприятные тенденции в виде «повзросления» инфекции.

## КОНФЛИКТ ИНТЕРЕСОВ

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## ЛИТЕРАТУРА

1. *О состоянии санитарно-эпидемиологического благополучия населения в Российской Федерации в 2018 году: Государственный доклад.* – Москва: Федеральная служба по надзору в сфере защиты прав потребителей и благополучия человека; 2019.
2. Воронин Е.М, Михеева И.В. К вопросу об оценке экономического ущерба от заболеваний, вызываемых вирусом ветряной оспы. *Профилактическая медицина – практическому здравоохранению: Сб. научных статей МПФ ППО ММА им. И.М.Сеченова*. *Вып. 3*. Москва: ФЦГЭ Роспотребнадзора; 2007. С. 259-264.
3. Шаханина И.Л, Горелов А.В, Лыткина И.Л, Толкушин А.Г. Экономическая оценка вакцинопрофилактики ветряной оспы на примере Москвы. *Эпидемиология и инфекционные болезни*. 2009; 3:49-57.
4. Приказ Минздрава России от 21.03.2014 г. №125н *«Об утверждении национального календаря профилактических прививок и календаря прививок по эпидемическим показаниям»*.
5. Приказ Департамента здравоохранения г. Москвы от 04.07.2014 г. №614 *«Об утверждении регионального календаря профилактических прививок и календаря прививок по эпидемическим показаниям»*.
6. Воронин Е.М, Ермоленко М.В, Чернова А.М, Лыткина И.Н, Михеева И.В. Современные особенности эпидемического процесса ветряной оспы. *Эпидемиология и вакцинопрофилактика.* 2010; 6(55):17-23.
7. Ясинский А.А. Ветряная оспа в Российской Федерации. *Бюллетень «Вакцинация».* 2009; 1:9-11.
8. Prevention of Varicella: recommendations of the Advisory Committee on Immunization Practices/ Centers for Disease Control and Prevention. *MMWR*. 1996; 45(11):43.
9. Meyer PA, Seward JF, Jumaan AO. et al. Varicella mortality: trends before vaccine licensure in the

United States, 1970-1994.*The Journal of Infectious diseases*. 2000; 182(2):383-390.

10. Socan M, Blaško M. Surveillance of varicella and herpes zoster in Slovenia, 1996 – 2005. Euro Surveill. 2007; 12(2):pii=687. Available from: https://doi.org/10.2807/esm.12.02.00687-en
11. Heininger U, Seward JF. Varicella. *Lancet.* 2006; 368:1365-76.
12. Чистенко ГН, Гузовская ТС, Шиманович В.П. Закономерности эпидемического процесса ветряной оспы на территории Республики Беларусь. *Журн. Гродн. гос. мед. университета*. 2008; 2:68-71.
13. Varicella vaccines. WHO position paper. *MMWR*.1998; 73:241-248.
14. Kwong J.C, Tanuseputro P, Zagorski B, et al. Impact of varicella vaccination on health care outcomes in Ontario, Canada: Effect of a publicly funded program? *Vaccine*.2008; 26:6006-6012.
15. Chaves S.S, Gargiullo P, Zhang JX. et al. Loss of vaccine-induced immunity to varicella over time. *N Engl J Med.* 2007; 356:1121-1129.
16. Kuter B, Matthews H, Shinefield H, et al. Ten year follow-up of healthy children who received one or two injections of varicella vaccine. *Pediatr Infect Dis J.* 2004; 23:132–137.
17. Sosa LE, Hadler JL. Epidemiology of varicella in Connecticut 2001–2005. *J Infect Dis.* 2008; 197(Suppl 2):S90- S93.
18. Seward J, Marin M., Vazquez M. Varicella vaccine effectiveness in the US vaccination program: a review. *J Infect Dis.* 2008; 197:S82- S87.
19. Лыткина ИН, Михеева И.В. Унификация системы управления эпидемическим процессом кори, эпидемического паротита и краснухи. Эпидемиология и вакцинопрофилактика. 2011; 1(56):8-14.
20. Meeting of the Strategic Advisory Group of Experts on immunization, April 2014 – conclusions and recommendations. *Weekly epidemiological record.* 2014, 2(89):221-236 Available from: http://www.who.int/we.

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# STUDY OF ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA* SPP. ISOLATED FROM FOOD AND BIOLOGICAL MATERIAL

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***Key words:*** *Salmonella spp., biological properties, antibiotics, resistance, susceptibility.*

***Introduction.*** *Antimicrobial resistance is a global public health problem that the world is facing today.*

***Material and methods.*** *Isolation and identification of Salmonella spp. were performed according to DSTU EN 12824: 2004; DSTU ISO 18593: 2006 standards; Nutrient media were manufactured in accordance with DSTU EN ISO 11133: 2014 standard. The antibiotic susceptibility of cultures was determined by the disk diffusion method. The research findings were both studied and interpreted in accordance with EUCAST recommendations.*

***Results.*** *Out of 10 samples (material was obtained from animals, humans and foods), 10 cultures of Salmonella spp. were isolated and analyzed. The cultures were mainly susceptible to semisynthetic and inhibitor-protected penicillins, cephalosporins, carbapenems and more often to tetracyclines, macrolides, lincosamides. Most Salmonella spp. strains were intermediate susceptibility to aminoglycosides, 30% of Salmonella spp. strains were susceptible to pefloxacin (II) and only 10% were susceptible to ciprofloxacin (II).*

***Conclusions.*** *The obtained data indicate the screening feasibility of antibiotic susceptibility of Salmonella spp. including a wide range of tested drugs, which may be important in determining the antibiotic therapy.*

***Cuvinte cheie:*** *Salmonella spp., proprietăți biologice, antibiotice, rezistență, sensibilitate.*

**STUDIEREA SENSIBILITĂŢII LA ANTIBIOTICE A SPECIILOR DE *SALMONELLA*, IZOLATE DIN MATERIALE BIOLOGICE ŞI PRODUSE ALIMENTARE**

***Introducere.*** *Actualmente, rezistența antimicrobiană este o problemă globală de sănătate publică.*

***Material şi metode.*** *Izolarea și identificarea Salmonella spp a fost efectuată conform standardelor DSTU EN 12824:2004; DSTU ISO 18593:2006; mediile nutritive fiind preparate în conformitate cu standardul DSTU EN ISO 11133:2014. Sensibilitatea tulpinilor la antibiotice a fost determinată prin metoda disc-difuzimetrică, iar studiul și interpretarea rezultatelor au fost realizate în conformitate cu recomandările EUCAST.*

***Rezultate.*** *Din 10 probe (material prelevat de la animale, oameni și din alimente) au fost izolate și studiate 10 tulpini de Salmonella spp. Preponderent, tulpinile sunt sensibile la penicilinele de semisintetice și cele protejate de inhibitori, cefalosporine, carbapeneme; mai frecvent prezentând rezistență la tetracicline, macrolide și lincosamide. Pentru aminoglicozide însă majoritatea tulpinilor Salmonella spp. au prezentat sensibilitate intermediară, astfel, din 30% tulpini Salmonella spp. sensibile la pefloxacină (II), doar 10% au fost sensibile la ciprofloxacină (II).*

***Concluzii.*** *Datele obținute indică fezabilitatea screening-ului sensibilității Salmonella spp. la antibioticele din cea mai largă listă posibilă de preparate testate, impunându-se drept acțiune oportună în determinarea antibioticoterapiei.*

## INTRODUCTION

Over the XXI century, infectious diseases are still the main causes of death worldwide (1, 2). Recently, the spread of antibiotic-resistant microorganisms has become a public concern. Antimicrobials have been used in the production of livestock products for therapeutic and prophylactic purposes, which contributed to the development of adaptive mechanisms to the applied antibiotics. This, in turn, served as an impetus for the production of new antimicrobial agents.

The Ukrainian agriculture sector shows a considerable sample size among global production volumes (3, 4). An increase in both the demand and production volume of agricultural products is accompanied by a free section of the continental boundaries of semi-finished or finished agri-food products (5). Migration of people, birds and terrestrial wild animals also has contributed to the microbial long-distance transfer (5, 6, 7, 8). All these factors have differently promoted the spread of multi-resistant microbial strains to the existing antibiotics both due to their transportation by living organisms over long distances, and due to the production of the agro-industrial complex.

*The purpose of this work* is to study the biological properties and assess the antibiotic resistance among various groups *Salmonella* spp. cultures, isolated from biological samples taken from Kiev, Volyn region.

## MATERIAL AND METHODS

*Salmonella* spp. strains were isolated and identified from the research materials according to: DSTU EN 12824:2004 Microbiology of food products and animal nutrition. Horizontal method for detecting *Salmonella* spp.; DSTU 4769:2007 Bacteriological study of pathological material from animals. Methods of detecting salmonella; MU 4.2.2723-10 "Laboratory diagnostics of salmonella, detection of *Salmonella* in food products and environmental objects", 2010; DSTU ISO 18593:2006 "Microbiology of food products and animal nutrition. The study of microbial fingerprinting and washings from the surface". Antibiotic susceptibility testing by disc diffusion method, using HiMedia's discs. The research findings were studied and interpreted in accordance with EUCAST guidelines (9).

## RESULTS

The microbial cultures were isolated during the microbiological assessment of materials taken from Kiev, Volyn region.

The study confirmed 6 samples of biomaterial, obtained from birds and 6 *Salmonella* spp. strains were identified: *S.* Typhimurium (from geese), *S.* Virchow (from ducks), *S.*Virchow (from chickens), *S.* Gallinarum, S. Pullorum (from chickens), *S.* Enteritidis (from chickens) *S. Dublin* (from calf); two cultures – from food samples (*S. Enteritidis*, which are rare *Salmonella* species (F-67+) and two cultures (*S.* Enteritidis, *S.* Typhimurium) found in people with symptoms of food poisoning).

The bacterial cultures formed a uniform turbidity and a small amount of white amorphous sediment in the Pepted Meat Broth, which was easily broken-down while being shaken. The cultures of *S.* Dublin, *S.* Gallinarum, *S.* Pullorum formed growth rings. The bacterial cultures formed transparent, tender, greyish and 3-4 mm S-colonies in Meat Infusion Agar.

The laboratory animals (white mice weighing 16-18 g) which were subcutaneously administered doses of $0.5\times10^9$ CFU/cm$^3$ died in 100% of experimental animals within 8 hours.

The research findings showed that the studied cultures in 100% of cases were susceptible to ampicillin and ampicillin/sulbactam, whereas were resistant to benzylpenicillin and methicillin; 90% of strains were resistant to oxacillin; 50% of cultures were susceptible to ticarcillin and 10% – resistant to ticarcillin/clavulanic acid; 30% of strains were susceptible and 60% were resistant to amoxicillin; 30% of cultures were susceptible 30% – resistant to piperacillin; 20% of strains were susceptible and 80% – resistant to carbenicillin.

The structural similarity between cephalosporins and penicillins causes the same mechanism of antimicrobial action and cross-allergies in some patients (10, 11). The studied *Salmonella* spp. strains showed susceptibility to most of cephalosporins; no direct dependence of levels of antimicrobial susceptibility to various generation of drugs was found: 80% of the studied strains showed susceptibility and 10% showed resistance to cefazolin (I); 60% of cultures showed susceptibility and 30% showed resistance to cephalexin (I); 90% of the studied bacterial strains were susceptible and 10% of cultures were resistant to cefaclor (II).

20% of the studied cultures were susceptible and 60% are resistant to cefuroxime (II); 90% of the studied cultures were susceptible to cefamandole (II), no resistant cultures were detected; 80% of the studied cultures were susceptible and 20% are resistant to cefixim (III); 30% of the studied cultures were susceptible and 30% were resistant to cefoperazone (III); 80% of the cultures of the studied cultures showed susceptibility to cefotaxim (III), no resistant cultures were detected; 50% of the studied bacterial strains showed susceptibility and 20% were resistant to ceftazidime (III); 20% of the studied strains were susceptible and 20% were resistant to ceftriaxone (III); 100% of the studied strains showed resistance to cefepim (IV).

Due to the natural activity of carbapenems (imipenem and meropenem) against enterobacteria, the studied cultures were found susceptible to imipenem (70%) and meropenem (80%).

No *Salmonella* spp. resistant to carbapenems was detected.

The most studied cultures exhibited a moderate resistance to aminoglycosides: 20% of cultures were susceptible and 30% were resistant to streptomycin (I); 60% – susceptible and 10% – resistant to gentamicin (I); 20% – susceptible and 20% – resistant to kanamycin (I); 20% of cultures are susceptible and 80% – intermediate susceptible to neomycin (I); 40% –and 10% – resistant to tobramycin (II); except for netilin (II), to which 100% of the cultures were susceptible, and amikacin (III), which showed an inhibitory effect on 20% of the studied *Salmonella* spp. strains

*Salmonella* spp. have natural resistance to macrolides (12, 13). Our studies also proved that *Salmonella* spp. is resistant to macrolides. However, cultures of *S.* Gallinarum, *S.* Pullorum, *S.* Dublin, *S.* Virchow (Q) were susceptible to azithromycin (15); *S.* Virchow (Q) strain was susceptible to clarithromycin.

Most of the *Salmonella* spp. showed natural resistance to tetracyclines. The culture of *S.* Gallinarum Pullorum was susceptible to tetracycline, doxycycline; *S.* Typhimurium *(F)* showed sensitivity to Tetracycline.

High concentrations of linquamides may also exhibit bactericidal effects to relatively high susceptible microorganisms (13, 14). The studies conducted on microbial resistance to lincosamides showed susceptibility to lincomycin and clindamycin, except for *S.* Virchow (Q) strain.

Depending on the mechanism of action, quinolones differ completely from other AMP drugs. 50% of *Salmonella* spp. were susceptible to nalidixic acid (I), norfloxacin (II), levofloxacin (III), and gatifloxacin (IV). The remaining drugs showed a susceptibility of 30% (pefloxacin (II) to 10% (ciprofloxacin (II), ofloxacin (II), lomefloxacin (II)) of the studied cultures of *Salmonella* spp.

Pathogenic microorganisms rarely develop antibiotic resistance to nitrofurans. (19, 20, 21). The study results showed that 40% of the studied cultures were susceptibile to furazolidone.

All the studied *Salmonella* spp. strains were susceptibile to chloramphenicol

## DISCUSSIONS

Ten *Salmonella* spp. strains were isolated from 10 samples of food products and biological material of various origin. Among the isolated cultures, 2 isolates belong to *S.* Enteritidis, 2- to *S.* Typhimurium, 2- to *S.* Virchow, 1- to *S.* Dublin, *S.* Gallinarum, *S.* Pullorum, *S.* Muenchen, *Salmonella* F-67+. Cultural-morphological, enzymatic and antigenic properties of the selected cultures correspond to species characteristics; all bacterial cultures proved to be pathogenic in white mice.

The cultures were predominantly susceptible to semisynthetic and inhibitor-protected penicillins, cephalosporins and carbapenems; cultures showed resistance to tetracyclines, macrolides, and lincosamides in most cases. As regarding the aminoglycosides, most of *Salmonella* spp. strains showed intermediate resistance; up to 50% of studied *Salmonella* spp. strains were susceptible to some quinolones of different generations, however, cultures showed resistance to an overwhelming number of cases; 30% of the selected *Salmonella* spp. cultures were susceptible to amoxicillin, whereas the cultures were non-susceptible to ampicillin, a screening recommended by EUCAST (version 8) on the enterobacteria sensitivity to amoxicillin. From 30% of *Salmonella* spp. strains susceptible to pefloxacin (II) (EUCAST screening recommendations on susceptibility of *Salmonella* spp. to ciprofloxacin), 10% cultures showed susceptibility to ciprofloxacin (II).

The study analysis found that the studied cultures isolated from food products, sick and dead animals, and biomaterial obtained from people were characterized by natural susceptibility to

antibiotics; no cases of acquired resistance was found within this study. This may indicate that, the current rational use of antibiotic therapy may be effective.

## CONCLUSIONS

1. The data obtained may indicate the need for screening studies on the susceptibility of *Salmonella* spp. strains to antibacterial drugs, while the list of studied drugs should be expanded as much as possible. It might be important when choosing an appropriate antibiotic therapy.

## CONFLICT OF INTERESTS

All authors declare no competing interests.

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## REFERENCES

1. Patel R, Kandefer D, Walsh M et al. Causes and timing of death in extremely premature infants from 2000 through 2011. *New england journal of medicine*. 2015;372(4):331-340.
2. Crump J, Medalla F, Joyce K et al. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: national antimicrobial resistance monitoring system, 1996 to 2007. *Antimicrob agents chemother*. 2011;55(3):1148-54.
3. Панорама аграрного сектора України/Міністерство аграрної політики та продовольства, ДУ «Інститут економіки та прогнозування НААН України, ГО «Центр аграрних реформ»; 2017.
4. State statistics service of Ukraine. Statistical collection: livestock of Ukraine, 2016. 2017 Available from: http://www.ukrstat.gov.ua/ [Accessed 10th Septemer 2019].
5. Feshhenko Y, Gumenyuk M, Denisov O. Antibiotic resistance of microorganisms. The state of the problem and the ways to solve it. *Ukrainian chemotherapy journal.* 2010;23(1):4-10.
6. Pruden A, Larsson D, Amezquita A, et al. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environmental health perspectives*. 2013;121(8):878.
7. Mc Nulty K, Soon J, Wallace C. et al. Antimicrobial resistance monitoring and surveillance in the meat chain: a report from five countries in the european union and european economic area. *Trends in food science & technology*. 2016; 58:1-13.
8. Moroz E, Bliznyuk M, Roshchin G. Risk-oriented approach in the structure of indicative planning in the system of medical protection of the population of Ukraine during military emergencies. Personnel policy in the field of health care in the face of threats to the national security of Ukraine. *Proceedings of the Annual All-Ukrainian Scientific and Practical Conference on International Participation*. Kyiv; 2017.
9. EUCAST. The european committee on antimicrobial susceptibility testing. 2018 Available from: http://www.eucast.org/ [Accessed 10th September 2019].
10. Threlfall E, Fisher I, Berghold C, et al. Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of salmonellosis in humans in Europe in 2000: results of international multi-centre surveillance. *Euro surveill*. 2003;8(2):41-5.
11. Parry C. *Management of multiple drug-resistant salmonella infections. Management of multiple drug-resistance infections*. Totowa: Humana press Inc; 2004.
12. Zuckerman J. Macrolides and ketolides: azithromycin, clarithromycin, telithromycin. *Infectious disease clinics of north America*. 2004;18(3):621-649.
13. Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin b reveals the nascent peptide exit path in the ribosome. *Journal of molecular biology*. 2003; 330(5):1005-1014.
14. Morar M, Bhullar K, Hughes D, et al. Structure and mechanism of the lincosamide antibiotic adenylyltransferase linb. *Structure*. 2009; 17(12):1649-1659.
15. Emmerson A, Jones A. The quinolones: Decades of development and use. *J. Antimicrob. Chemother*. 2003; 51(1):13-20.
16. Mitscher L. Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents. *Chem rev*. 2005; 105(2):559-92.
17. Linder J, Huang E, Steinman M, et al. Fluoroquinolone prescribing in the United States: 1995 to 2002. *American journal of medicine*. 2005; 118(3):259-268.
18. Cramer D. The Mode of Action of Nitrofuran Compounds: II. Application of Physicochemical Methods to the Study of Action against *Staphylococcus aureus*. *J Bacteriol.* 1947; 54(2):119-125.
19. Brodie A, Gots J. Nitrofurans as electron acceptors for certain respiratory enzymes. *Arch Biochem Biophys.* 1952; 39(1):165-173.
20. Röschenthaler R, Kindler P, Herrlich P, et al. The action of nitrofurantoin: inhibition of growth of Esche-

richia coli K 12 and of IPTG-induced beta-galaotosidase synthesis. *Zentralbl Bakteriol Orig.* 1970; 215(2):203-211.

21. Anderle C, Stieger M, Burrell M, et al. Biological activities of novel gyrase inhibitors of the aminocoumarin class. *Antimicrob. Agents Chemother.* 2008; 52(6):1982-1990.

22. Allsop A. New antibiotic discovery, novel screens, novel targets and impact of microbial genomics. *Curr. Opin. Microbiol*. 1998; 1(5):530-534.

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# К ВОПРОСУ ВАКЦИНОПРОФИЛАКТИКИ ПНЕВМОКОККОВОЙ ИНФЕКЦИИ СРЕДИ НЕДОНОШЕННЫХ ДЕТЕЙ С БРОНХОЛЕГОЧНОЙ ДИСПЛАЗИЕЙ

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***Key words:*** *PCV13 vaccine, pneumococcal infection, bronchopulmonary dysplasia.*

**VACCINE PROPHYLAXIS OF PNEUMOCOCCAL INFECTION AMONG PREMATURE INFANTS WITH BRONCHOPULMONARY DYSPLASIA**

***Introduction.*** *Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease among children of the first year of life, especially children born prematurely with extremely low and very low body weight.*

***Material and methods.*** *To estimate the number of children born alive in Perm Territory between 2015 and 2017, official statistics data were used. Experimental epidemiological studies were used to assess safety, reactogenicity, immunogenic activity and preventive efficacy of the Prevnar 13 vaccine when immunizing preterm infants with bronchopulmonary dysplasia under prospective controlled randomized clinical observation.*

***Results.*** *There were 29 premature infants with bronchopulmonary dysplasia under observation, the control group comprised 29 unvaccinated premature infants with BPD and 30 full-term infants. The PCV 13 vaccination of preterm infants with BPD established good tolerance, poor reactogenicity (17.2±0.57%) and vaccine tolerance like full-term infants (16.5±0.55%), high safety profile, high immunogenic properties (seroconversion – 93.1%, seroconversion factor – 5.5). Evaluation of immunogenic activity among children with BPD vaccinated with the PCV 13 vaccine revealed high immunological efficacy compared to unvaccinated healthy children.*

***Conclusion.*** *The need for vaccination of premature babies with BDL against pneumococcal infection has been scientifically substantiated. The study has confirmed safety, poor reactogenicity, high immunogenic activity and preventive efficacy of the Prevenar 13 vaccine among premature babies with BDL under the conditions of the catamnesis department of the perinatal center within the follow-up monitoring of vaccinated infants over three years.*

***Cuvinte cheie:*** *vaccin VPC13, infecție pneumococică, displazie bronhopulmonară.*

**VACCINOPROFILAXIA INFECȚIEI PNEUMOCOCICE LA SUGARII NĂSCUȚI PREMATUR, DIAGNOSTICAȚI CU DISPLAZIE BRONHOPULMONARĂ**

***Introducere.*** *Cea mai frecventă boală pulmonară cronică la copii în primul an de viață, în special la sugarii născuți prematur, cu o greutate corporală foarte mică, este displazia bronhopulmonară (DBP).*

***Material și metode.*** *Pentru a estima numărul copiilor născuți vii în perioada 2015-2017, în regiunea Permi, Rusia au fost utilizate datele statistice oficiale. Au fost proiectate studii epidemiologice experimentale pentru evaluarea eficacității profilactice și a reactivității, demonstrarea siguranței și activității imunogene a vaccinului pneumococic conjugat (VPC 13) la imunizarea sugarilor născuți prematur cu DBP.*

***Rezultate.*** *Au fost investigați 29 de copii născuți prematur, diagnosticați cu DBP și pentru comparație – 29 de copii născuți prematur, nevaccinați cu DBP și, respectiv, 30 de copii născuți la termen. Vaccinul VPC 13 administrat la sugarii născuți prematur care suferă de DBP a stabilit o toleranță bună, reactivitate scăzută (17,2±0,57%) și toleranță similară la vaccin ca și la copiii născuți la termen (16,5±0,55%), siguranță înaltă, proprietăți imunogene crescute (seroconversia – 93,1%, factorul seroconversiei – 5,5). Evaluarea activității imunogene la copiii cu DBP, vaccinați cu VPC 13 a evidențiat o eficacitate imunologică ridicată la copiii născuți prematur cu DBP, în comparație cu copii sănătoși vaccinați.*

***Concluzii****. Astfel, a fost dovedită științific necesitatea vaccinării copiilor născuți prematur cu DBP împotriva infecției pneumococice și a fost confirmată siguranța vaccinului, reactivitatea scăzută, activitatea imunogenă ridicată și eficacitatea profilactică.*

## ВВЕДЕНИЕ

В настоящее время достаточно велико глобальное бремя пневмококковой инфекции среди детей до 5 лет. Наиболее частой хронической патологией легких среди детей первого года жизни, особенно среди детей, родившихся недоношенными с экстремально низкой массой тела и очень низкой массой тела, является бронхолегочная дисплазия (БЛД)(1).

БЛД – это полиэтиологическое хроническое заболевание морфологически незрелых легких, развивающееся у новорожденных, главным образом глубоко недоношенных детей, в результате интенсивной терапии респираторного дистресс-синдрома и (или) пневмонии (1). Бронхолегочная дисплазия выявляется практически у всех новорожденных, родившихся со сроком гестации менее 28 недель, у 40% – с гестационным возрастом 28-30 недель, у 4% – более 30 недель (1). Доказано, что обострение БЛД связано с присоединением вирусных и вирусно-бактериальных ассоциаций. Наличие бронхолегочной дисплазии обуславливает наиболее высокую восприимчивость к пневмококковой инфекции (2, 3). Центральное место в патогенезе БЛД занимает незрелость легких недоношенного ребенка, что предрасполагает к баротравме и токсическому действию кислорода при искусственной вентиляции легких (ИВЛ), приводящие к системной воспалительной реакции легких, нарушая процесс формирования альвеол. Наличие БЛД у ребенка нередко приводит к ремоделированию стенки бронхов и развитию легочной гипертензии (4, 5). Среди детей с БЛД вне обострения заболевания при исследовании назофарингеальных мазков с помощью полимеразной цепной реакции инфицирование пневмококком достигает 12,6% (6). При инфицировании пневмококком дети с БЛД развивают тяжелые обострений основного заболевания (7).

## МАТЕРИАЛЫ И МЕТОДЫ

Целью настоящего исследования определить распространенность бронхолегочной патологии среди недоношенных детей и установить влияние вакцинации недоношенных детей, страдающих бронхолегочной дисплазией, против пневмококковой инфекции на состояние их здоровья.

Оценка количества детей, родившихся живыми, на территории Пермского края в 2015-2017 гг., проведена по официальным данным оказания медицинской помощи беременным, роженицам и родильницам (форма №32) и данных годовых отчетных форм отделения катамнеза краевого перинатального центра ГБУЗ ПК «Ордена «Знак Почета» Пермская краевая клиническая больница». Экспериментальные эпидемиологические исследования применяли для оценки безопасности, реактогенности, иммуногенной активности и профилактической эффективности вакцины ПКВ 13 при иммунизации недоношенных детей с бронхолегочной дисплазией в условиях проспективного контролируемого рандомизированного клинического наблюдения, проведенного в 2015-2017 гг. Для иммунизации недоношенных детей с бронхолегочной дисплазией использовалась вакцина ПКВ 13 с 2-х месячного возраста по схеме «3+1». Критериями включения детей явились: гестационный возраст менее 32 недель; отсутствие противопоказаний к введению вакцины Превенар 13, содержащей 13 серотипов пневмококка – 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F и 23F, по схеме «3+1», предусмотренных инструкцией по применению; наличие письменного информированного согласия родителей на профилактическую прививку. Вакцину ПКВ 13 в дозе 0,5 мл вводили внутримышечно в область переднебоковой поверхности бедра в соответствии с инструкцией по применению препарата.

Безопасность вакцины оценивалась врачом иммунологом-аллергологом в ходе клиниче-ского наблюдения за недоношенными детьми с БЛД, привитыми и не привитыми против пневмококковой инфекции, на основании данных лабораторных исследований привитых и не привитых в динамике: до и после иммунизации (на 28-й день) с оценкой общего анализа крови (общего гемоглобина, содержания эритроцитов, лейкоцитов, лейкоцитарная формула), уровня общего содержания IgE в сыворотке крови, а также результатов биохимического анализа сыворотки крови с определением общего билирубина, аспартатаминотрансферазы (АСТ), аланинаминотрансферазы (АЛТ), общего белка и креатинина, общего анализа мочи (реакция мочи, плотность, число плоского эпителия и лейкоцитов), выполненных стандартными унифицированными методами.

Реактогенность вакцины оценивали по наличию системных и местных поствакцинальных реакций, степени их выраженности и продолжительности на основе активного клинического наблюдения за привитыми детьми по специальной программе – через 30 минут и на 28 сутки после иммунизации, а также по результатам ежедневных записей родителей в дневниках самонаблюдения. Системные реакции оценивали по степени повышения температуры тела (температурные реакции были дифференцированы на слабые – 37,1-37,5°С, средние – 37,6-38,5°С и сильные – 38,6°С и выше) и местные – по величине гиперемии и отека (в мм) в месте введения препарата. Результаты наблюдения фиксировались в индивидуальных «Дневниках наблюдения за привитым» и сертификатах профилактических прививок Ф.157/у-93.

Иммуногенную активность вакцины оценивали на основании определения в парных сыворотках крови (до и через 28 дней после вакцинации) общего уровня специфических IgG – антител (IgG-АТ) к смеси полисахаридов (СП) *Streptococcus pneumoniae*, входящих в состав вакцины методом иммуноферментного анализа (ИФА) на твердофазном носителе с расчетом средней геометрической титров (СГТ) специфических антител, уровня сероконверсии (процент лиц, у которых наблюдался 4-кратный прирост специфических антител после введения вакцины) и фактора сероконверсии (кратность прироста специфических антител после вакцинации).

Профилактическую эффективность вакцины оценивали в группе привитых и не привитых лиц по количеству случаев заболеваний острыми респираторными инфекциями, внебольничной пневмонии, синуситов, бронхитов на 1000 детей за 12 месяцев (6 месяцев до и 6 месяцев после вакцинации) и при проспективном наблюдении через 3 года после проведенной вакцинации с расчетом индекса эффективности.

Статистический анализ результатов проведен с использованием методов параметрической и непараметрической статистики с определением средней арифметической (M), стандартной ошибки (m) и среднего стандартного отклонения (δ). Достоверность различий между явлениями оценивали с помощью t-критерия Стьюдента. Разность результатов считали статистически значимой при $p<0,05$. Для иммунизации недоношенных детей с БЛД использовалась вакцина ПКВ13.

## РЕЗУЛЬТАТЫ

Методом случайной выборки (единица выборки – один ребенок) были сформированы 2 группы наблюдения. Дети основной группы (n=29) имели БЛД и привиты вакциной против пневмококковой инфекции (первая группа). Группу сравнения (n=29) составили непривитые недоношенные дети с БЛД (вторая группа), находящиеся на диспансерном наблюдении в краевом перинатальном центре ГБУЗ ПК «Ордена «Знак Почета» Пермская краевая клиническая больница» с 2-х месячного возраста. Группы наблюдения были равноценны и однородны по возрасту, половому признаку, срокам иммунизации и состоянию здоровья. Для сравнительной оценки переносимости вакцинации недоношенными детьми с БЛД с 2-х месячного возраста была сформирована вторая группа сравнения – здоровые доношенные дети (n=30).

Первые результаты данного исследования были опубликованы авторами в журнале «Эпидемиология и Вакцинопрофилактика» в 2018, №17/2 (8).

Количество детей, родившихся живыми, на изучаемой территории в 2015-2017 гг. по официальным данным оказания медицинской помощи беременным, роженицам и родильницам (форма №32) колеблется от 31 372 до 37 972 детей. Доля недоношенных детей составляла 6,0±0,3%, удельный вес детей с экстремально низкой массой тела (ЭНМТ), родившихся с массой от 500 до 999 гр., и очень низкой массой тела (ОНМТ), родившихся с массой от 1 000 до 1 499 гр., составлял 6,7±2,0 и 34,1±1,7 %% соответственно (табл. 1).

В структуре первичной заболеваемости детей с ЭНМТ уровень бронхолегочной дисплазии среди недоношенных детей с ЭНМТ (рожденные с массой до 1 000 гр.) был наибольшим (53,6±2,0%): среднемноголетний показатель составил 767,0±3,9 на 1000 при общем показателе первичной заболеваемости 1 429,3±4,1; показатели заболеваемости ретинопатией (462,3±3,0 на 1 000), поражения ЦНС тяжелой степени (158,5±2,6 на 1 000), нейросенсорной тугоухости (32,2±2,3 на 1 000) – наименьшими ($p<0,05$) (табл. 2).

Таблица 1. Данные о численности новорожденных детей, родившихся живыми и количество недоношенных детей в Пермском крае, в 2015-2017 гг. (абс. число, %, M±m).

| Годы наблюдения | Количество новорожденных детей, родившихся живыми, абс. | Количество недоношенных детей | | Количество детей с ЭНМТ | | Количество детей с ОНМТ | |
|---|---|---|---|---|---|---|---|
| | | абс. ч. | % | абс. ч. | % | абс. ч. | % |
| **2015** | 37 972 | 2 351 | 6,2 | 161 | 6,8 | 783 | 33,3 |
| **2016** | 36 654 | 2 165 | 5,9 | 156 | 7,2 | 765 | 35,3 |
| **2017** | 31 372 | 1 916 | 6,1 | 116 | 6,1 | 646 | 33,7 |
| **M±m** | 35 333 | 2 144 | 6,0±0,3 | 144 | 6,7±2,0 | 731 | 34,1±1,7 |

Таблица 2. Структура первичной заболеваемости недоношенных детей, родившихся живыми с ЭНМТ, в г. Перми в 2015-2017 гг. (абс. число, показатель на 1000 детей, M±m, %).

| Нозологические формы | 2015 | | | 2016 | | | 2017 | | | M±m |
|---|---|---|---|---|---|---|---|---|---|---|
| | абс. ч. | на 1000 | % | абс. ч. | на 1000 | % | абс. ч. | на 1000 | % | |
| **БЛД** | 67 | 752,8 | 52,3 | 67 | 779,1 | 53,2 | 30 | 769,2 | 55,6 | 767,0±3,9 |
| **Ретинопатия** | 34 | 382,0 | 26,5 | 44 | 511,6 | 34,9 | 19 | 487,2 | 35,2 | 462,3±3,0 |
| **Поражение ЦНС тяжелой степени** | 21 | 235,9 | 16,4 | 14 | 162,8 | 11,1 | 3 | 76,9 | 5,6 | 158,5±2,6 |
| **Тугоухость** | 3 | 33,7 | 4,8 | 1 | 11,6 | 0,7 | 2 | 51,3 | 3,6 | 32,2±2,3 |
| **Итого:** | 128 | 1438,2 | 100 | 126 | 1465,1 | 100 | 54 | 1384,6 | 100 | 1429,3±4,1 |

В структуре первичной заболеваемости детей с ОНМТ уровень выявленной бронхолегочной дисплазии среди недоношенных детей с ОНМТ (дети, рожденные с массой от 1001 до 1500 г) аналогично был наибольшим (41,1±1,8%): среднемноголетний показатель составил 157,3±2,3 при общем показателе первичной заболеваемости 382,4±3,2 на 1000. Показатель заболеваемости ретинопатией (94,6±1,9 на 1000), поражения ЦНС тяжелой степени (75,9±1,8 на 1000) и нейросенсорной тугоухостью – аналогично наименьшим – 21,1±1,6 на 1000 ($p<0,05$) (табл. 3).

Таблица 3. Структура первичной заболеваемости детей, родившихся живыми с ОНМТ, в г. Перми в 2015-2017 гг. (абс. число, показатель на 1000 детей, M±m, %).

| Нозологические формы | 2015 | | | 2016 | | | 2017 | | | M±m |
|---|---|---|---|---|---|---|---|---|---|---|
| | абс. ч. | на 1000 | % | абс. ч. | на 1000 | % | абс. ч. | на 1000 | % | |
| **БЛД** | 30 | 180,7 | 41,6 | 25 | 174,8 | 54,3 | 21 | 216,5 | 55,3 | 157,3±2,3 |
| **Ретинопатия** | 15 | 90,4 | 20,8 | 10 | 69,9 | 21,7 | 12 | 123,7 | 31,5 | 94,6±1,9 |
| **Поражение ЦНС тяжелой степени** | 24 | 144,6 | 33,3 | 6 | 41,9 | 13,0 | 4 | 41,2 | 10,5 | 75,9±1,8 |
| **Тугоухость** | 3 | 18,1 | 4,3 | 5 | 34,9 | 11,0 | 1 | 10,3 | 2,7 | 21,1±1,6 |
| **Итого:** | 72 | 433,7 | 100 | 46 | 321,7 | 100 | 38 | 391,8 | 100 | 382,4±3,2 |

Фоновая клиническая характеристика детей представлена в Таблице 4. Средний возраст детей на момент вакцинации против пневмококковой инфекции составил в основной группе 9,12±4,29 мес., в группе сравнения – 11,29±4,58 мес. Средний гестационный возраст – 28±1,46 и 31±1,59 недель соответственно. В основной группе 15 детей родились с экстремально низкой массой тела (менее 1000 граммов), 13 детей с очень низкой массой тела (от 1000 до 1500 граммов). В группе сравнения детей с очень низкой массой тела при рождении было 11, с экстремально низкой массой – 10 детей. В кислородной поддержке не нуждался ни один ребенок с БЛД. Дети в обеих группах имели последствия перинатального поражения центральной нервной системы.

Сопутствующее заболевание дыхательной системы (внутриутробные пневмонии в анамне-

зе) имели 21,0±1,02 детей основной группы и 10±0,9%% в группе сравнения.

Сравнительная оценка реактогенности конъюгированной пневмококковой вакцины ПКВ13 в группе привитых недоношенных детей с БЛД и в группе привитых доношенных детей в условиях проспективного контролируемого рандомизированного клинического наблюдения не выявила достоверных различий в частоте поствакцинальных реакций. Общее суммарное число поствакци нальных системных реакций в группе привитых недоношенных детей с БЛД в течение 28 дней после первой иммунизации составило 17,2±0,57 против 18,5±0,55% в группе привитых доношенных детей (p>0,05).

Таблица 4. Фоновая клиническая характеристика привитых и непривитых детей, участвующих в клиническом исследовании (абс. число, M±m, %).

| Показатели (единицы измерения) | Привитые дети, n=29 | Группа сравнения, n=29 |
|---|---|---|
| **Возраст детей, M±m (мес.)** | 9,12±1,09 | 11,29±1,01 |
| **Количество мальчиков, n (%)** | 8 (28±0,85) | 9 (31±0,82) |
| **Масса тела при рождении, г, Me (мин; макс)** | 1172,5 (690; 1780) | 1510 (860; 1910) |
| **Гестационный возраст при рождении, M±m, недель** | 28±1,46 | 31±1,59 |
| **Количество детей с экстремально низкой массой тела при рождении, абс. число** | 15 | 10 |
| **Количество детей с очень низкой массой тела при рождении, абс.** | 13 | 11 |
| **Количество детей, перенесших внутриутробную пневмонию, n (%)** | 6 (21±1,02) | 3 (10±0,9) |

В группе привитых недоношенных детей с БЛД выявлены легкие общие реакции у 4 детей (13,8±0,57%), которые купировались без назначения лекарственных средств. У одного ребенка (3,4±0,57%) отмечена сильная реакция в виде подъема температуры тела до 39,5°С, потребовавшее назначения жаропонижающих лекарственных средств в возрастной дозировке. Местных реакций зарегистрировано не было. В основном все поствакцинальные реакции были слабой и средней степени выраженности, быстропроходящими в течение 2 дней и не требовали медикаментозного лечения.

Наиболее часто первая иммунизация против пневмококковой инфекции совмещалась с введением вакцины против вирусного гепатита В (34,5±0,57%), реже с инактивированной полиомиелитной вакциной (3,5±0,57%) и тетраксимом (3,5%±0,57%). Поствакцинальных реакций и поствакцинальных осложнений у детей при симультанной иммунизации в условиях проспективного контролируемого рандомизированного клинического наблюдения не отмечалось.

В группе привитых здоровых доношенных детей по схеме «2+1» отмечены легкие общие реакции у 2 детей (6,6±0,55%), которые купировались самостоятельно без назначения лекарственных средств. У одного ребенка (3,3±0,55%) отмечена сильная реакция с подъемом температуры до 38,7°С, которому однократно назначено жаропонижающее средство в возрастной дозировке. Местная реакция (гиперемия в месте введения вакцины в размере до 2,5 см) наблюдалась у 2 детей (6,6±0,55%). Комбинированных реакций зарегистрировано не было. В структуре поствакцинальных реакций (16,5±0,55%) преобладали реакции слабой и средней степени интенсивности и все клинические проявления были быстро проходящими (в течение 3 дней) и не требовали лекарственного лечения.

Следовательно, сравнительная оценка реактогенности вакцины среди недоношенных детей, имеющих бронхолегочную дисплазию, и привитых доношенных детей выявила слабую реактогенность (17,2±0,57%), и схожую переносимость вакцины с доношенными детьми (16,5±0,55%). В группах привитых детей как основной, так и в группе сравнения изменений со стороны дыхательной системы (апное, десатурация, бронхообструктивный синдром) не наблюдались.

В ходе мониторинга за показателями общего анализа крови у привитых детей в динамике не отмечено существенных отклонений от нормы (p>0,05). Лейкоцитарная формула соответствовала возрастной норме. Результаты клинических исследований крови у привитых и не привитых детей в динамике представлены в Таблице 5.

Результаты исследований биохимического анализа крови у привитых и не привитых детей в динамике представлены в Таблице 6.

Таблица 5. Показатели общего анализа крови детей привитых и непривитых – до вакцинации и через 28 суток после вакцинации (M±m).

| Показатели (единицы измерения) | Привитые дети, n=29 | | Группа сравнения, n=29 | |
|---|---|---|---|---|
| | до вакцинации, (M±m) | 28 сутки после вакцинации, (M±m) | до вакцинации, (M±m) | 28 сутки после вакцинации, (M±m) |
| **Гемоглобин (г/л)** | 117,78±1,83 | 119±2,25 | 126±7,0 | 118±0,6 |
| **Эритроциты ($\times 10^{12}$/л)** | 4,13±0,37 | 4,05±0,21 | 4,46±0,28 | 4,25±0,11 |
| **Лейкоциты ($\times 10^{9}$/л)** | 6,53±1,03 | 9,5±1,22 | 8,11±1,1 | 7,13±0,48 |
| **Палочкоядерные (%)** | 2,75±1,02 | 2,33±1,01 | 1,0±0,48 | 1,0±0,51 |
| **Сегментоядерные (%)** | 31,5±3,37 | 38,5±3,5 | 32,0±2,00 | 32,0±2,33 |
| **Эозинофилы (%)** | 2,6 ±0,75 | 2,33±0,44 | 4,8±1,27 | 3,4±1,29 |
| **Лимфоциты (%)** | 57,63±3,37 | 51±9,5 | 53,16±3,18 | 62,6±2,44 |
| **Моноциты (%)** | 9,13±2,16 | 6,75±1,63 | 6,8±2,44 | 6,0±2,0 |
| **СОЭ (мм/ч)** | 4,86±2,41 | 5,33±3,11 | 4,2±1,04 | 4,0±1,41 |

Таблица 6. Показатели биохимического анализа крови у привитых и не привитых детей – до вакцинации и через 28 суток после вакцинации (M±m).

| Показатели (единицы измерения) | Привитые дети, n=29 | | Группа сравнения, n=29 | |
|---|---|---|---|---|
| | до вакцинации, (M±m) | 28 сутки после вакцинации, (M±m) | до вакцинации, (M±m) | 28 сутки после вакцинации, (M±m) |
| **Билирубин общий (мкмоль/л)** | 8,33±1,51 | 7,25±0,43 | 8,36±1,51 | 7,2±0,24 |
| **АЛТ (Ед/л)** | 27,03±1,63 | 25,73±2,45 | 27,2±1,63 | 31,5±5,16 |
| **АСТ (Ед/л)** | 42,13±2,95 | 44,4±2,34 | 40,8±2,95 | 45,33±4,0 |
| **Общий белок (г/л)** | 59,28±3,46 | 58,3±1,31 | 57,03±4,04 | 57,14±1,19 |
| **Креатинин (мкмоль/л)** | 45,89±1,9 | 38,4±3,52 | 41,3±1,9 | 35,5±2,75 |

Оценка показателей биохимического анализа сывороток крови привитых детей не выявил существенных изменений. Основные показатели биохимического анализа крови соответствовали норме в обеих группах, также не было обнаружено статистически достоверных различий между фоновыми показателями и показателями, полученными через 28 дней после вакцинации (p>0,05). Содержание общего белка в сыворотке крови до вакцинации (59,28±3,46%) и после (58,3±1,31%) свидетельствовало об отсутствии влияния вакцины на белоксинтезирующую функцию печени.

Динамика содержания общего иммуноглобулина класса Е в сыворотках крови привитых и не привитых детей представлена в Таблице 7.

Таблица 7. Содержание общего уровня IgE в сыворотках крови привитых и непривитых детей – до вакцинации и через 28 суток после вакцинации (M±m).

| Показатели (норма) | Привитые дети n=29 | | Группа сравнения n=29 | |
|---|---|---|---|---|
| | до вакцинации | 28 сутки после вакцинации, | до вакцинации | 28 сутки после вакцинации, |
| **Общий уровень IgE (до 130МЕ/мл)** | 37,46±3,12 | 33,65±2,27 | 15,55±3,72 | 14,48±3,59 |

В основной группе детей уровень общего IgE изначально был выше, чем у детей группы сравнения и на 28 сутки существенно не изменился (p>0,05) в обеих группах.

Результаты общего анализа мочи у привитых находились в пределах нормальных физиологических величин, что свидетельствовало об отсутствии токсического воздействия вакцины на мочевыделительные органы привитых детей.

Оценка содержания специфических антител к полисахаридам пневмококка в сыворотке крови на 28 сутки после иммунизации детей, страдающих бронхолегочной дисплазией, выявило увеличение суммарных IgG в 5,5 раза, в сравнении с 29 ребенком группы сравнения. Среднегеометрическое титров (СГТ) антител в основной группе детей до вакцинации составила 25,46 у.е. Через 28 дней после вакцинации уровень суммарных антител IgG возрос и составил 142 у.е. (p<0,05). Во второй группе сравнения у детей уровень IgG к полисахаридам пневмококка через 28 дней остался на исходном уровне и не изменился (35,24 у. е. и 35,39 у.е. соответственно, p>0,05) (рис. 1).

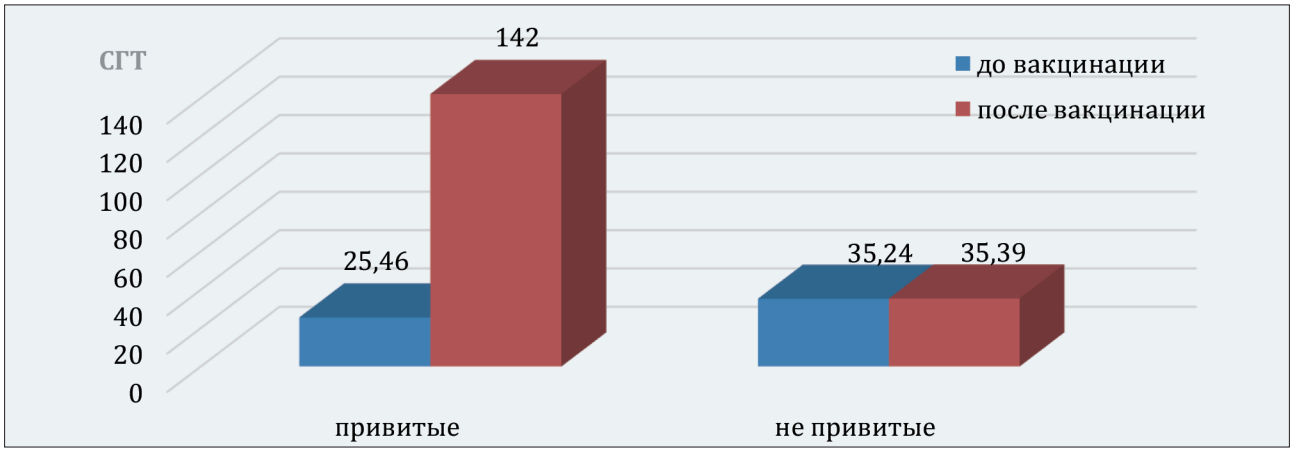


Рисунок 1. Уровень специфических антител (IgG) к полисахаридам пневмококка у привитых недоношенных детей с бронхолегочной дисплазией (n=29), и непривитых (n=29) в динамике (до вакцинации и через 28 дней после вакцинации) в у. е.

Проспективное наблюдение в течение 3 лет за привитыми недоношенными детьми с бронхолегочной дисплазией по схеме «3+1» и в группе сравнения позволило выявить определенный уровень заболеваемости внебольничными пневмониями и отитами среди привитых и не привитых (табл. 8).

Таблица 8. Уровень заболеваемости внебольничными пневмониями и отитами среди привитых и непривитых (показатели на 1000 детей).

| Нозологические формы | Привитые дети, n=29 | Группа сравнения, n=29 |
|---|---|---|
| **Заболеваемость внебольничными пневмониями** | 0 | 33,3 |
| **Заболеваемость отитами** | 66,7 | 241,4 |

В группе привитых недоношенных детей с БЛД показатель заболеваемости отитами (66,7 на 1000 детей) был ниже в 3,5 раза чем среди не привитых детей (241,4 на 1000 детей), а заболевших внебольничной пневмонией среди привитых не зарегистрировано (p <0,05). В группе сравнения у заболевшего ребенка с внебольничной пневмонией (33,3 на 1000) в посеве мокроты выделился *Streptococcus pneumoniae*.

Проспективное наблюдение за привитыми недоношенными детьми с бронхолегочной дисплазией в течение трех лет позволило установить 4 случая заболевания внебольничной пневмонией (показатель составил 137,9 на 1000 детей), вызванных респираторно-синцитиальным вирусом. Случаев внебольничной пневмонии среди привитых детей, вызванных *Streptococcus pneumoniae*, в ходе проспективного наблюдения в течение 3 лет не отмечено. В группе сравнения уровень заболеваемости внебольничной пневмонией не изменился до возраста 24 месяцев, в дальнейшем дети этой группы были привиты против пневмококковой инфекции путем проведения однократной вакцинации.

Полученные результаты высокого профиля безопасности, умеренной реактогенности, высокой иммуногенной активности и профилактической эффективности вакцины ПКВ 13 у детей, родившихся недоношенными, и имеющих бронхолегочную дисплазию, позволили внедрить в медицинскую деятельность отделения катамнеза краевого перинатального центра технологию вакцинации недоношенных детей с высоким риском (3-й уровень оказания неонатологической медицинской помощи). Охваты вакцинацией недоношенных детей, имеющих бронхолегочную дисплазию, в г. Перми в 2015-2017 гг. представлены в таблице 9.

Таблица 9. Охват вакцинацией недоношенных детей, имеющих бронхолегочную дисплазию, в г. Перми в 2015-2017 гг. (абс. число, %).*

| Годы | Количество недоношенных детей с БЛД, абс. | Количество привитых, абс. | % |
|---|---|---|---|
| 2015 | 107 | 80 | 74,7 |
| 2016 | 92 | 76 | 82,6 |
| 2017 | 51 | 49 | 96,0 |

*по данным реестра краевого перинатального центра ГБУЗ ПК «Ордена «Знак Почета» Пермская краевая клиническая больница».

## ДИСКУССИИ

Выявленная высокая частота (53,6±2,0%) распространения бронхолегочной дисплазии среди родившихся недоношенных детей с экстремально низкой массой тела – среднемноголетний уровень первичной заболеваемости составил 767,0±3,9 при общем показателе 1429,3±4,1 на 1000 и среди детей с очень низкой массой тела (41,1±1,8%) среднемноголетний уровень первичной заболеваемости – 157,3±2,3 при общем показателе 382,4±3,2 на 1000 на фоне неснижаемого количества недоношенных детей (6,0±0,3%) диктует необходимость своевременной вакцинопрофилактики пневмококковой инфекции среди наиболее восприимчивых детей, имеющих бронхолегочную патологию.

Введение вакцины ПКВ 13 недоношенным детям, страдающих бронхолегочной дисплазией, выявило хорошую переносимость (отсутствие клинических проявлений бронхообструктивного синдрома и негативного влияния на дыхательную систему – не наблюдалось апное и десатурации среди привитых), слабую реактогенность (17,2±0,57%) и схожую переносимость вакцины с доношенными детьми (16,5±0,55%), высокий профиль безопасности (отсутствие каких-либо патологических отклонений в показателях общего анализа крови, биохимического анализа крови, общего анализа мочи и содержания общего IgE в динамике), высокие иммуногенные свойства (сероконверсия – 93,1%, фактор сероконверсии – 5,5) и ее высокую профилактическую эффективность (наблюдалось существенное снижение уровня заболеваемости отитами и не регистрировались случаи внебольничной пневмонии среди привитых).

При совмещении иммунизации против пневмококковой инфекции недоношенным детям

с бронхолегочной патологией при первом введении вакцины Превенар 13 и вакцины против вирусного гепатита В (34,5±0,57%), с инактивированной полиомиелитной вакциной (3,5±0,57%) и тетраксимом (3,5%±0,57%) поствакцинальных реакций у привитых, в условиях проспективного контролируемого рандомизированного клинического наблюдения, ни в одном случае поствакцинальных реакций не отмечено.

## ВЫВОДЫ

1. Научно обоснована необходимость вакцинации недоношенных детей с бронхолегочной дисплазией против пневмококковой инфекции и подтвержден высокий профиль безопасности и высокая профилактическая эффективность применения вакцины ПКВ 13 позволяют внедрить во всех регионах Российской Федерации новую медицинскую технологию – вакцинацию недоношенных детей с бронхолегочной дисплазией против пневмококковой инфекции на третьем уровне оказания неонаталогической медицинской помощи детям в условиях отделения катамнеза перинатальных центров в рамках реализации Национального календаря профилактических прививок.

## КОНФЛИКТ ИНТЕРЕСОВ

Нет конфликта интересов.

## ЛИТЕРАТУРА

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015; 13(1).
2. Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES, Agarwal A. Critical appraisal of world health organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. *Urology*. 2012; 79(1):16-22.
3. Huyghe E, Izard V, Rigot JM, Pariente JL, Tostain J. Évaluation de l'homme infertile: recommandations AFU 2007. *Prog en Urol*. 2008; 18(2):95-101.
4. Gill K, Jakubik J, Rosiak-Gill A, Kups M, Lukaszuk M, Kurpisz M, et al. Utility and predictive value of human standard semen parameters and sperm dna dispersion for fertility potential. *Int J Environ Res Public Health.* 2019; 16(11).
5. World Health Organization. Department of Reproductive Health and Research. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010; 1:287.
6. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2009; 16(3):231-45.
7. Niang L, Ndoye M, Labou I, Jalloh M, Kane R, Diaw JJ, et al. Profil épidémiologique et clinique de l'infertilité masculine à l'hôpital général de Grand-Yoff, Sénégal: à propos de 492 cas. *Andrologie*. 2009; 19(2):103-7.
8. Owolabi AT, Fasubaa OB, Ogunniyi SO. Semen quality of male partners of infertile couples in Ile-Ife, Nigeria. *Niger J Clin Pract*. 2013; 16(1):37-40.
9. Basnet P, Hansen SA, Olaussen IK, Hentemann MA, Acharya G. Changes in the semen quality among 5739 men seeking infertility treatment in Northern Norway over past 20 years (1993–2012). *Journal of Reproductive Biotechnology and Fertility.* 2016; 5:1-7.
10. Geoffroy-Siraudin C, Dieudonné Loundou A, Romain F, Achard V, Courbière B, Perrard MH, et al. Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. *Asian J Androl*. 2012; 14(4):584-90.
11. Louis JF, Thoma ME, Sorensen DN, Mclain AC, King RB, Sundaram R, et al. The prevalence of couple infertility in the United States from a male perspective: Evidence from a nationally representative sample. *Andrology*. 2013; 1(5):741-8.
12. Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: Systematic review and meta-analyses. *Fertil Steril*. 2011; 95(1):116-23.
13. Gupta Sanjay, Swapnil S. Singhai. Management of oligoasthenozoospermia: an observational clinical study. *European Journal of Pharmaceutical and Medical Research*, 2016; 3(6):387-90.
14. Punab M, Poolamets O, Paju P, et al. Causes of male infertility: a 9 – year prospective monocentre study on 1737 patients with reduced total sperm counts. *Hum Reprod*. 2017; 32(1):18-31.
15. Lee JY, Dada R, Sabanegh E, Carpi A, Agarwal A. Role of genetics in azoospermia. *Urology*. 2011; 77(3):598-60.
16. Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. *Clinics.* 2013; 68 (SUPPL.1):39-60ф.

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# DEVELOPMENT OF SEMEN QUALITY IN MALE PARTNERS OF INFERTILE COUPLES IN THE REPUBLIC OF MOLDOVA

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***Key words:*** *human semen, male infertility, diagnosis, reference values.*

***Introduction.*** *It is estimated that over 15% of couples of reproductive age face infertility worldwide. In about half of these cases the male factor is involved. To assess the potential of male fertility the spermiogram analysis may not always be an optimal diagnostic tool, but it remains the basic clinical tool.*
***Material and methods.*** *The purpose of the study is to analyze the regional tendencies of the semen quality in male partners of couples facing infertility. A retrospective study of 4625 patients subject to semen analysis between 2012-2018 was conducted. All semen samples were collected after a recommended period of sexual abstinence of three to five days. The spermiogram analysis was performed by the computerized method according to WHO guidelines for Human Semen analysis, 2010.*
***Results.*** *Of the total number of 4625 men examined, 1861 (40.2%) presented normal values of semen – normozoospermia, and 2764 (59.8%) showed abnormal semen parameters. Asthenozoospermia was the most common abnormality profile recorded in 1394 (30.2%) men, followed by oligoasthenozoospermia diagnosed in 973 men (21.0%). Azoospermia was found in 200 men with an estimated prevalence of 4.3%. In 113 men examined, oligozoospermia was found in 2.4%. Oligoasthenoteratozoospermia was diagnosed in 1.5% and necrozoospermia in 0.3%.*
***Conclusion.*** *The study provides the first evidence that semen quality in men in the Republic of Moldova who are facing infertility in couples has deteriorated over the years.*

***Cuvinte cheie:*** *material seminal, infertilitate masculină, diagnostic, valori de referință.*

## EVOLUȚIA CALITĂȚII MATERIALULUI SEMINAL LA BĂRBAȚII PARTENERI DIN CUPLURILE INFERTILE ÎN REPUBLICA MOLDOVA

***Introducere.*** *La nivel mondial se estimează că peste 15% dintre cuplurile de vârstă reproductivă se confruntă cu infertilitatea, iar în aproximativ jumătate din aceste cazuri este implicat factorul masculin. Pentru evaluarea potențialului de fertilitate masculină spermograma poate să nu fie întotdeauna un instrument de diagnostic optim, cu toate acestea însă rămâne în continuare instrumentul clinic de bază.*
***Material și metode.*** *Scopul: analiza tendințelor regionale ale calității materialului seminal la bărbații din cadrul cuplurilor ce se confruntă cu infertilitatea. Studiu retrospectiv a fost efectuat în perioada 2012-2018 pe un eșantion de 4625 bărbați care au făcut analize ale materialului seminal. Recoltarea probelor a fost făcută după abstinență de la ejaculare timp de 3-5 zile în condiții de laborator. Spermograma s-a realizat prin metoda computerizată conform criteriilor și valorilor de referință stabilite de OMS în anul 2010.*
***Rezultate.*** *Din numărul total de 4625 de bărbați investigați, 1861 (40,2%) au prezentat valori normale ale materialului seminal normozoospermie și 2764 (59,8%) au prezentat tulburări de spermatogeneză. Cea mai frecventă anomalie a spermatogenezei a fost înregistrată astenozoospermia – la 1394 de bărbați cu o frecvență de 30,2%, urmată de oligoastenozoospermia – la 973 de bărbați în 21,0% din cazuri. La 200 de bărbați a fost înregistrată azoospermia, frecvența fiind de 4,3%, iar la 113 bărbați investigați a fost depistată oligozoospermia cu frecvența de 2,4%, oligoastenoteratozoospermia – în 1,5% și necrozoospermia – în 0,3%.*
***Concluzii.*** *Studiul confirmă, că calitatea materialului seminal a bărbaților din Republica Moldova, care se confruntă cu infertilitatea în cuplu, se deteriorează de-a lungul anilor.*

## INTRODUCTION

Infertility affects an estimated rate of 15% of couples of reproductive age worldwide, and in about half of these cases the male factor is involved (1).

The causes of infertility can be divided into four broad categories: 1) female factor; 2) male factor; 3) couple factor – due to cumulative female and male infertility; 4) idiopathic infertility, unexplained. The exact percentage for each of these categories is difficult to determine; however, it is generally reported that in about 40% of cases infertility is due to female cause, in 40% – male cause, and in 20% – anomalies detected in both partners (1, 2). Thus, the examination of the male partner is as important as the female one for the assessment of couple's fertility. Medical history and physical examination are standard assessments for all men, including semen analysis.

The spermiogram evaluation is relevant for the appreciation of the functional status of the seminiferous tubules, epididymis, and accessory sex glands. The prognostic value of semen characteristics, such as sperm concentration, percentage of motility, and morphology represents the first line of examination in the diagnosis of male infertility (3). Semen analysis may not always be an optimal diagnostic tool, but it still remains the basic clinical tool for the evaluation of male fertility potential (4).

Important treatment decisions in male infertility are largely based on spermiogram results. Therefore, it is essential that the human semen analysis be performed according to the updated requirements of the World Health Organization (WHO), 2010 (5). In recent years, the European Society for Human Reproduction and Embryo-logy (ESHRE), in collaboration with the WHO, have developed a program to improve laboratory standardization in terms of sperm sample diagnosis and assessment criteria (6).

## MATERIAL AND METHODS

*The purpose* of the study is to analyze the regional tendencies of semen quality in male partners of couples facing infertility.

The study presents a retrospective evaluation of 4 625 patients in the Republic of Moldova subject to semen analysis during 2012-2018. All semen samples were collected in laboratory conditions after a recommended period of sexual abstinence of three to five days. Each sample was incubated at 37°C and analyzed within an hour. The spermiogram analysis was performed by the computerized method on the automated analyzer SQA IIC-P (Medical Electronic Systems, USA). Semen analysis was performed according to the WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edition, 2010 (tab. 1). All patients are part of infertile couples who made appointments for doctor`s consultation in the Repromed Center.

Table 1. Semen parameters and reference values according to WHO (2010).

| Abstinence (days) | 3-7 days |
|---|---|
| Volume (mL) | ≥ 1.5 mL |
| Color | ≤2 cm |
| Liquefaction time | ≤60 min |
| Viscosity | 2 cm |
| pH | ≥7.2 |
| Leukocytes | ≤ 1 mln/mL |
| Sperm Concentration (mln/mL) | ≥ 1.5 mL |
| Total number of spermatozoa (mln/ejaculate) | ≥ 39 mln |
| Progressives sperm motility (%) | ≥ 32 % |
| Concentration of motile spermatozoa (mln/mL) | ≥ 10 mln/mL |
| Total number of motile spermatozoa (mln/ejaculate) | ≥ 15 mln |
| Concentration of functional spermatozoa (mln/mL) | ≥ 7 mln/mL |
| Total number of functional spermatozoa (mln/ejaculate) | ≥ 10.5 mln/mL |
| Motility index | ≥ 80 |
| Morphology (Normal forms) (%) | ≥4% |
| Vitality (9%) | ≥ 58 |

Interpreting the results, the spermiogram diagnosis was made according to the descriptive terminology of the same WHO guidelines as follows:

- normozoospermia: total number/percentage of sperm with progressive mobility and normal morphology, being of equal value or above the reference values;
- oligozoospermia: total number of sperm/sperm concentration below lower reference limit;
- asthenozoospermia: sperm motility below 40% or rapid progressive sperm motility <32%;
- teratozoospermia: percentage of normal sperm below 4%;
- oligoasthenozoospermia: low concentration and low percentage of progressively motile sperm;
- oligoteratozoospermia: low total number of sperm and low percentage of normal forms;
- asthenoteratozoospermia: percentage of motile sperm and normal sperm below low reference limit;
- oligoasthenoteratozoospermia: low total number of sperm/low percentage of motile sperm and normal forms;
- cryptozoospermia: very low spermatozoa concentration in ejaculate ≤1 million/mL;
- hypospermia: semen volume < 1,5 mL;
- hyperspermia: semen volume >1,5 mL;
- leukospermia/pyospermia: presence of leukocytes in ejaculate above reference limit;
- hematospermia: presence of blood in ejaculate;
- necrozoospermia: low percentage of live and high percentage of immotile sperm;
- aspermia: complete lack of semen with ejaculation;
- azoospermia: absence of spermatozoa in the sediment of a centrifuged semen sample.

## RESULTS

In the biology laboratory of the Repromed Center, Chisinau, Republic of Moldova, the spermiogram was performed in 4625 men, during 2012-2018. The Repromed Center is an Assisted Reproduction Center where the vast majority of infertility couples from all over the country present themselves. Spermiograms were performed over the following years: 2012 – 206 patients, 2013 – 702 patients, 2014 – 854 patients, 2015 – 800 patients, 2016 – 717 patients, 2017 – 703 patients, and 2018 – 643 patients. The number of semen analyses in the studied period is relatively constant. Except for 2012, because patients were registered since the middle part of the year since the Repromed Center started activity (tab. 2).

Table 2. Distribution of men according to their semen characteristics in the period 2012-2018 in the Republic of Moldova.

| Semen parameters | Absolute number | | | | | | | | % |
|---|---|---|---|---|---|---|---|---|---|
| | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | Total | |
| **Normozoospermia** | 103 | 358 | 348 | 340 | 260 | 235 | 217 | **1 861** | 40.2 |
| **Oligozoospermia** | 13 | 24 | 26 | 16 | 18 | 10 | 6 | **113** | 2.4 |
| **Oligoasthenozoospermia** | 27 | 90 | 166 | 173 | 165 | 170 | 182 | **973** | 21.0 |
| **Asthenozoospermia** | 44 | 179 | 245 | 231 | 235 | 250 | 210 | **1 394** | 30.2 |
| **Oligoasthenoteratozoospermia** | 5 | 13 | 11 | 12 | 11 | 13 | 3 | **68** | 1.5 |
| **Necrozoospermia** | | 6 | 6 | | 3 | | 1 | **16** | 0.3 |
| **Azoospermia** | 14 | 32 | 52 | 28 | 25 | 25 | 24 | **200** | 4.3 |
| **Total** | **206** | **702** | **854** | **800** | **717** | **703** | **643** | **4 625** | **100** |

Of the total number of 4 625 men examined, 1 861 (40.2%) presented normal values of semen – normozoospermia, 2 764 (59.8%) showed abnormal semen parameters. The most common profile of abnormality recorded in our study was asthenozoospermia in 1 394 men from 2012 to 2018 with a frequency of 30.2%, followed by oligoasthenozoospermia being diagnosed in 973 men – 21.0%. Azoospermia was recorded in 200 men with an estimated prevalence of 4.3%. In 113 men examined, the frequency of oligozoospermia accounted for 2.4%. In 113 men examined, oligozoospermia was detected in 2.4%. Oligoasthenoteratozoospermia was diagnosed in 68 men (1.5%) and necroszoospermia in 16 men (0.3%) (tab. 2, fig. 1).

Normozoospermia was considered according to the following WHO criteria: sperm concentration ≥1.5 mln/mL, total number of sperm cells ≥39 mln, progressive sperm motility ≥32% and morphology ≥4%. The results of normozoospermia analysis were recorded in 2012 and 2013 with a

frequency of 50.9%. In 2014, the normal values of spermiogram decreased by 10.2%, the frequency accounted for 40.7%. In 2015 the situation improved insignificantly compared to 2014 by 1.8%. Over the following years a decrease of normal values of spermatogenesis was also observed. In 2016 the frequency accounted for 36.3%, in 2017 – 33.4%, in 2018 – 33.7% (fig. 2).

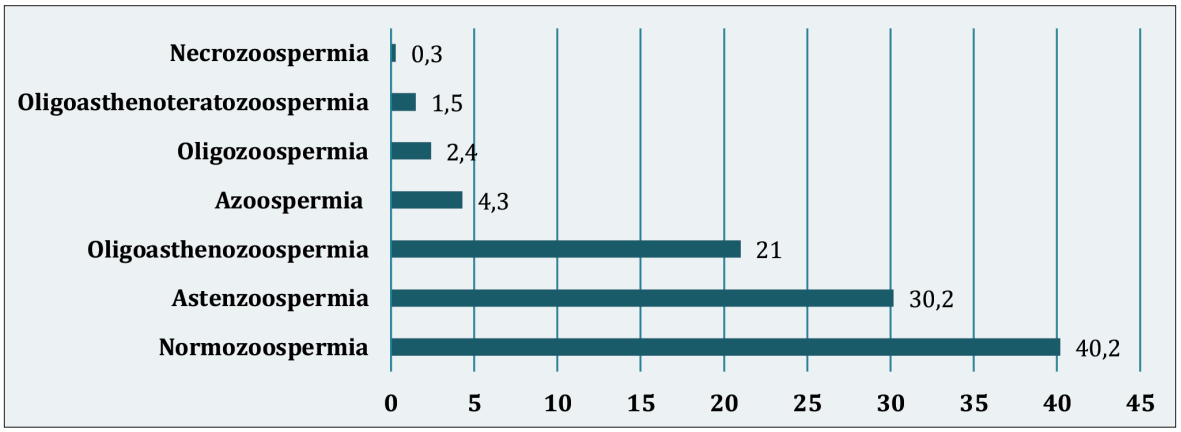


Figure 1. Distribution of patients according to semen analysis during the period 2012-2018.

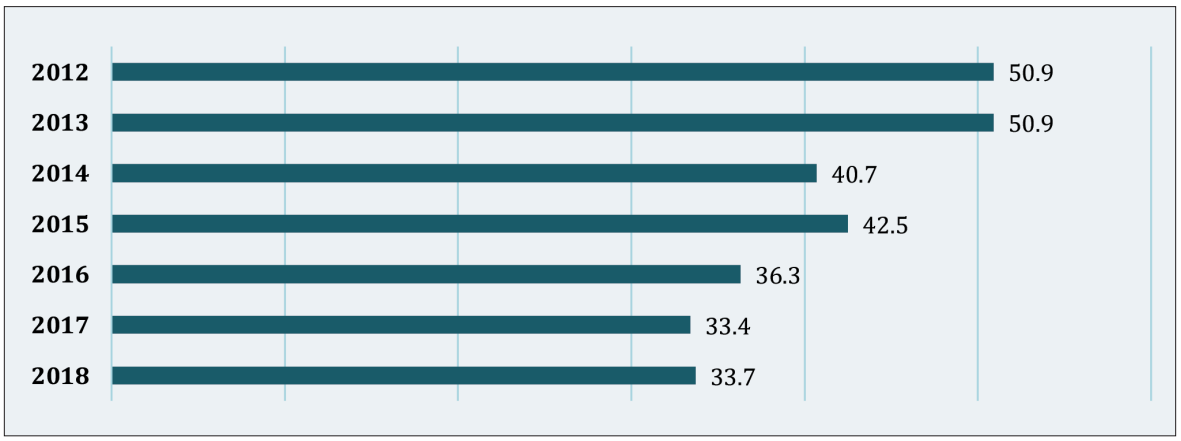


Figure 2. Frequency of normozoospermia in men during 2012-2018.

According to the results of asthenozoospermia analysis in 2012 the frequency was 21.7%. In 2013 the frequency increased by 3.7% compared to the previous year. Over the following years the values of asthenozoospermia also increased, in 2014 – 28.6%, in 2015 – 28.8%, in 2016 – 32.7%, in 2017 – 35.5%, and in 2018 – 32.6% (fig. 3).

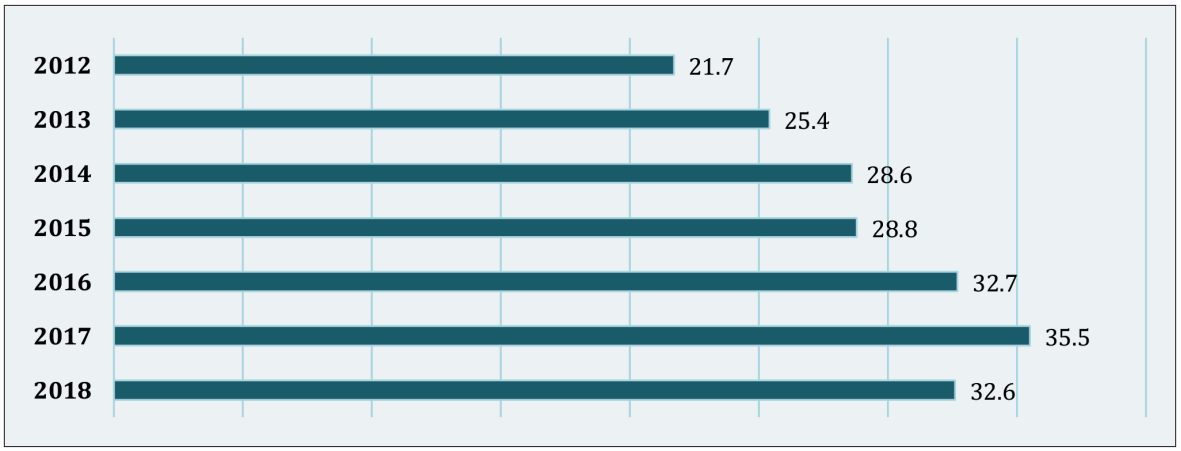


Figure 3. Frequency of men with asthenozoospermia during 2012-2018.

## DISCUSSIONS

The study shows that during 2012-2018, the abnormal semen quality was found in approximately 59.8% of male partners of couples facing infertility (tab. 2). A high incidence of spermatogenic disorders is also found in other studies (7, 8, 9) According to our data, this percentage increased from 50% in 2012 to 66.3% in 2018 (tab. 2).

This represents a significant increase in spermatogenesis abnormalities, although the WHO introduced lower baseline values in 2010. Thus, an alarming phenomenon of decreased male fertility can be observed in fertility clinics in the Republic of Moldova, which could be correlated with sperm decline described in the literature. This has been illustrated by several studies from multiple world regions that argue that men's reproductive health has been in rapid decline in the recent years (10, 11).

In the present study we observe that sperm concentration in the semen and healthy sperm count have decreased considerably. If in 2012 and 2013 the frequency of normozoospermia was 50.9%, over the following years a decrease in the normal values of spermatogenesis could be observed, accounting for 33.7% in 2018 (fig. 2).

The most common profile of abnormality in our study was asthenozoospermia recorded in 1394 men with a frequency of 30.2% (fig. 1). It is very noticeable that the abnormal frequency of sperm motility increased from 2012 to 2018. Thus, in 2012 the frequency of asthenozoospermia was 21.7%, in 2014 – 28.6% it increased, in 2015 – 28.8%, 2016 –32.7%, in 2017 – 35.5%. In 2018 there was a slight decrease of asthenozoospermia – 32.6%, most probably due to a lower number of appointments of couples with infertility compared to the previous year (fig. 3). According to literature data, this abnormality is found in 40% of men, affecting their fertility. The role of socio-psycho-behavioral factors in the development of this abnormality has been demonstrated. Psychological stress, smoking and alcohol are modifiable risk factors for the number of motile sperm (12). Also, sperm motility depends on its specific structures such as microtubules, outer dense fibers and mitochondria that provide energy for sperm movements (13).

The second cause of spermatogenesis disorder identified in our study was oligoastenozoospermia with a frequency of 21.0%. Oligoastenozoospermia is a combination of reduced sperm motility and low sperm count. According to bibliographic sources it is the most common cause of male infertility (14). The causes of this disorder are heterogeneous, such as cryptorhidia, varicocele, chronic infections, hormonal causes, psychoemotional causes, metabolic causes, etc. In 20% its etiology and pathogenesis are not fully elucidated and may be associated with specific gene abnormalities (13).

The frequency of azoospermia in the current study accounts for 4.3%. According to bibliographic sources azoospermia is found in 8% of infertile men and in 1% of the male population (15). In the case of lack of sperm or an extremely small number, a genetic cause may be identified in about 21-29% (16). In this context, cytogenetic tests for karyotype analysis as well as molecular genetic evaluation of Y-chromosome microdeletions analysis and mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene are fully justified and important to exclude a possible cause of genetic origin. Abnormal genotype may be present in up to 12% of azoospermic men and 4% of oligospermic men. Cystic fibrosis screening is recommended for azoospermia if it is due to congenital bilateral absence of the vas deferens (CBAVD). Optional Y-chromosome microdeletion screening can be carried out if sperm count is <5 million/mL.

## CONCLUSION

1. Our results clearly show that semen quality in the population of men in couples with infertility in the Republic of Moldova decreased from 2012 to 2018. As many authors suggest, we also believe that environmental and lifestyle factors have negatively affected the quality of semen development. The contribution of genetic factors cannot be excluded either.

2. Therefore, the analysis of the regional tendencies of semen quality is necessary and can be considered an indirect factor in assessing the tendencies in male infertility.

## CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

## REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol*. 2015;13(1).
2. Esteves SC, Zini A, Aziz N, Alvarez JG, Sabanegh ES, Agarwal A. Critical appraisal of world health organization's new reference values for human semen characteristics and effect on diagnosis and treatment of subfertile men. *Urology*. 2012; 79(1):16-22.
3. Huyghe E, Izard V, Rigot JM, Pariente JL, Tostain J. Évaluation de l'homme infertile: recommandations AFU 2007. *Prog en Urol*. 2008; 18(2):95-101.
4. Gill K, Jakubik J, Rosiak-Gill A, Kups M, Lukaszuk M, Kurpisz M, et al. Utility and predictive value of human standard semen parameters and sperm dna dispersion for fertility potential. *Int J Environ Res Public Health.* 2019; 16(11).
5. World Health Organization. Department of Reproductive Health and Research. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization; 2010; (1)-287.
6. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2009; 16(3):231-45.
7. Niang L, Ndoye M, Labou I, Jalloh M, Kane R, Diaw JJ, et al. Profil épidémiologique et clinique de l'infertilité masculine à l'hôpital général de Grand-Yoff, Sénégal: à propos de 492 cas. *Andrologie*. 2009; 19(2):103-7.
8. Owolabi AT, Fasubaa OB, Ogunniyi SO. Semen quality of male partners of infertile couples in Ile-Ife, Nigeria. *Niger J Clin Pract*. 2013; 16(1):37-40.
9. Basnet P, Hansen SA, Olaussen IK, Hentemann MA, Acharya G. Changes in the semen quality among 5739 men seeking infertility treatment in Northern Norway over past 20 years (1993–2012). *Journal of Reproductive Biotechnology and Fertility.* 2016; 5:1-7.
10. Geoffroy-Siraudin C, Dieudonné Loundou A, Romain F, Achard V, Courbière B, Perrard MH, et al. Decline of semen quality among 10 932 males consulting for couple infertility over a 20-year period in Marseille, France. *Asian J Androl*. 2012; 14(4):584-90.
11. Louis JF, Thoma ME, Sorensen DN, Mclain AC, King RB, Sundaram R, et al. The prevalence of couple infertility in the United States from a male perspective: Evidence from a nationally representative sample. *Andrology*. 2013; 1(5):741-8.
12. Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: Systematic review and meta-analyses. *Fertil Steril*. 2011; 95(1):116-23.
13. Gupta Sanjay, Swapnil S. Singhai. Management of oligoasthenozoospermia: an observational clinical study. *European Journal of Pharmaceutical and Medical Research*, 2016; 3(6):387-90.
14. Punab M, Poolamets O, Paju P, et al. Causes of male infertility: a 9 – year prospective monocentre study on 1737 patients with reduced total sperm counts. *Hum Reprod*. 2017; 32(1):18-31.
15. Lee JY, Dada R, Sabanegh E, Carpi A, Agarwal A. Role of genetics in azoospermia. *Urology*. 2011; 77(3):598-60.
16. Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. *Clinics.* 2013; 68 (SUPPL.1):39-60.

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# CONTROLUL ŞI EVALUAREA RISCULUI EXPUNERII POPULAŢIEI LA RADON ÎN REPUBLICA MOLDOVA

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***Key words:*** *radon, public health, risk.*

**CONTROL AND EVALUATION OF THE RISK OF POPULATION EXPOSURE TO RADON**

***Introduction.*** *A safe way to reduce the onset of oncological diseases is to protect the population from exposure to radon. In order to know the risk of radon influence on the health of the population, it is necessary to quantify the radon concentrations in the homes air and environment components.*

***Material and methods.*** *The aim of the study was to monitor radon concentrations in the air from different types of housing (n=2500), in rural and urban areas, on the territory of the Republic of Moldova, by using RADTRAK2-type detectors, with the assessment of the risk of population exposure to radon. The exhibition period was 90 days.*

***Results.*** *The results indicate on the radon problem existence on the country territory and the need for a strict solution of the problem. Thus, in 615 homes from the studied ones (25%) the radon concentration was higher than the national norms; in 662 homes (26%) radon concentration was higher than European norms. In 1277 homes (51%) radon concentration was higher than National/European norms. In connection with the above, the Government Decision draft was elaborated. A special role is given to the need to elaborate and make changes to the Building Code, with the need to monitor radon when commissioning residential buildings.*

***Conclusions.*** *The results denote increased variability of the radon concentrations according to the geographical area, the type of housing and other factors. Radon mapping identified the localities with high risk of exposure of the population to radon.*

***Cuvinte cheie:*** *radon, sănătate publică, risc*

***Introducere.*** *O cale sigură de diminuare a declanşării maladiilor oncologice o constituie protecţia expunerii populaţiei la radon. În vederea cunoaşterii riscului influenţei radonului asupra sănătăţii populaţiei este necesară cuantificarea concentraţiilor de radon în aerul din locuinţe şi din componentele mediului ambiant.*

***Material şi metode.*** *Scopul studiului a constat în monitorizarea concentraţiilor de radon în aerul din diferite tipuri de locuinţe (n=2500), din zonele rurale şi urbane, de pe teritoriul Republicii Moldova, prin utilizarea detectorilor de tip RADTRAK2, cu evaluarea riscului expunerii populaţiei la radon. Perioada de expoziţie a constituit 90 de zile.*

***Rezultate.*** *Rezultatele indică asupra existenţei problemei radonului în locuinţe, pe teritoriul ţării şi necesitatea soluţionării stringente a problemei. A fost constatat că, în 615 locuinţe studiate (25%) concentraţia radonului depăşea normele naţionale, iar în 662 de locuinţe (26%) a depăşit normele europene. Astfel, în 1277 de locuinţe (51%) analizate concentraţia radonului a fost mai mare decât normele naţionale/europene. În rezultatul celor expuse a fost elaborat proiectul Hotărârii de Guvern „Cu privire la aprobarea Strategiei Naţionale privind reducerea riscului asociat iradierii naturale, inclusiv a radonului". Un rol deosebit îl constituie elaborarea şi efectuarea modificărilor la Codul construcţiilor, cu necesitatea monitorizării radonului la darea în exploatare a clădirilor locative.*

***Concluzii.*** *A fost stabilită variabilitatea sporită a radonului în aerul din locuinţe în funcţie de zona geografică, tipul locuinţei, dar şi de alţi factori. Cartarea radonului pe teritoriul ţării a rezultat identificarea localităţilor cu risc sporit de expunere a populaţiei la radon.*

## INTRODUCERE

Expunerea populaţiei la sursele radioactive naturale se datorează în primul rând radonului ($^{222}$Rn), aceasta constituind peste 50% din expunerea totală (1). Radonul este un gaz radioactiv, care este produs în mod continuu de $^{226}$Ra, descendent al uraniului. Radonul este elementul cu numărul de ordine 86 din tabelul periodic, făcând parte din grupa a VIII-a, deci este un gaz inert, care odată format, prin dezintegrarea elementelor grele din crusta terestră, difuzează în gazele din sol sau din apă şi apoi este emanat în atmosferă. Radonul migrează spre suprafaţă prin spaţiile porilor din sol, fisuri etc.

Radonul poate pătrunde în case datorită diferenţei de presiune din clădire şi fundaţia sa din sol. Gazul migrează prin fisurile din pereţi, canale de scurgere, conducte de comunicaţii, materiale de construcţie şi apă potabilă (2).

Aportul radonului în expunerea internă şi externă a populaţiei constă în aceea, că acesta produce un şir întreg de alţi izotopi radioactivi pe de-o parte, iar pe de altă parte, fiind un gaz inert, poate ajunge în orice parte a organismului, fiind, în special, implicat în afectarea sistemului respirator (2).

Radonul este considerat o substanţă toxică din mediul ambiant şi prezintă riscuri pentru sănătate, ceea ce a condus la creşterea gradului de conştientizare a populaţiei, efectuându-se cercetări extinse, privind evaluarea concentraţiei de radon din locuinţe (3, 4). Radonul din interiorul încăperilor sporeşte riscul de dezvoltare a cancerului bronhopulmonar, poziţionându-se pe locul doi după fumatul activ, care reprezintă cel mai mare risc de apariţie a cancerului pulmonar. Mai mult de 85% din decesele cauzate de cancerul bronhopulmonar sunt printre fumători (2, 5, 6). Politica controlului tutunului este cea mai promiţătoare direcţie în realizarea obiectivelor de sănătate publică la capitolul controlului expunerii la radon (8).

Studiile epidemiologice şi ecologice, efectuate recent, demonstrează impactul radonului asupra dezvoltării cancerului bronhopulmonar. Riscul creşte în funcţie de durata expunerii şi de concentraţia radonului din interior. Expunerea totală la radon are loc în locuinţe, la şcoală, la locul de muncă, dar şi în localurile de agrement (3, 7).

Studiul prin modele statistice, aplicate celor mai recent publicate date în domeniul estimării incidenţei şi mortalităţii pentru 25 de cancere majore, efectuat în 40 de ţări ale Uniunii Europene pentru a. 2018, a demonstrat rezultate impunătoare. Astfel, au fost estimate 3,91 mln de cazuri noi de cancer (exceptând cancerul de piele non-melanom) şi 1,93 mln de decese, cauzate de cancer în Europa, cele mai uzuale fiind: cancerul de sân (523 000 de cazuri), urmat de cel colorectal (500 000), de cancerul pulmonar (470 000) şi de cancerul de prostată (450 000). Aceste patru tipuri de cancer reprezintă jumătate din rata totală a cancerului în Europa. Cele mai frecvente cauze de deces, cauzate de cancer, au fost cancerul pulmonar (388,000 de decese), colorectal (243 000), de sân (138 000) şi cancerul pancreatic (128 000). Numărul estimat de noi cazuri de maladii oncologice a constituit circa 1,6 mln la bărbaţi şi 1,4 mln la femei, cu 790 000 decese pentru bărbaţi şi 620 000 – femei (9). În vederea diminuării incidenţei maladiilor oncologice, cauzate de radon, au fost elaborate unele algoritme (modele) de informare a populaţiei despre riscul pentru sănătate, cauzat de expunerea la radon (10, 11, 12).

## MATERIAL ŞI METODE

*Scopul* studiului a constat în monitorizarea concentraţiilor de radon în aerul din diferite tipuri de locuinţe din localităţile rurale şi urbane ale principalelor Zone ale Republicii Moldova: Nord, Centru şi Sud cu evaluarea riscului expunerii populaţiei la radon. Obiectivele au constat în elaborarea ghidului de plasare a detectorilor pasivi în locuinţe, evaluarea caracteristicilor locuinţelor şi a cunoştinţelor populaţiei referitor la impactul radonului asupra sănătăţii, măsurarea concentraţiei de radon în aerul de interior din 2 500 locuinţe, prin utilizarea detectorilor pasivi; citirea rezultatelor concentraţiilor de radon în Laboratorul RADONOVA, Uppsala, Suedia; analiza statistică a rezultatelor obţinute, cartarea concentraţiilor de radon în aerul din locuinţe, pe teritoriul Republicii Moldova, cu identificarea localităţilor ţării cu risc sporit la radon. Ipoteza de cercetare: confirmarea/infirmarea existenţei pericolului pentru sănătate, în rezultatul expunerii populaţiei la radonul din aerul din locuinţe.

Ca material de studiu a servit aerul din 2500 locuinţe de diferite tipuri, plasate în zonele rurale şi urbane ale principalelor Zone ale Republicii Moldova: Nord, Centru şi Sud. Criteriul de selectare a punctelor de măsurare a concentraţiei de $^{222}$Rn – punctele de măsurare a concentraţiei de $^{222}$Rn în aerul interior au fost selectate randomizat, nemij-

locit din zonele incluse în studiu: Nord, Centru şi Sud, conform metodologiei Comisiei Europene (CE). Măsurările au fost efectuate preponderent la parter, în dormitor sau în camera pentru oaspeţi. Au fost examinate datele cu privire la vechimea locuinţelor (tip nou sau vechi), anul construcţiei, tipul materialelor de construcţie şi de finisare utilizate, adresa, prezenţa/lipsa fundamentului, conform chestionarului completat de proprietarul locuinţei. Măsurările au fost efectuate cu detectori pasivi de lungă durată RADTRAK2, perioada de expoziţie a constituit 90 de zile (fig. 1).

Figura 1. Detector RADTRAK2 de măsurare a concentraţiilor de $^{222}$Rn în locuinţe pe termen lung – de la 2 luni până la 1 an.

*Metodologia pentru realizarea hărţii de radon în locuinţele din Republica Moldova.* Pentru realizarea hărţii de radon a fost utilizat caroiajul definit în sistem Lambert – GISCO de către Centrul Comun de Cercetare (Joint Research Center (JRC), din cadrul CE. Conform caroiajului stabilit, harta Republicii Moldova este reprezentată prin 336 de celule cu laturile de 10×10 km$^2$. Pentru fiecare celulă din gridul de referinţă s-a calculat numărul de măsurări, media aritmetică, media aritmetică a valorilor logaritmate, deviaţia standard, deviaţia standard geometrică, mediana, valoarea minimă şi maximă. Gruparea rezultatelor şi redarea acestora prin anumite coduri de culoare s-a efectuat pornind de la valorile recomandate de CE. Pentru compararea şi integrarea acestor rezultate în harta europeană de radon este necesară utilizarea claselor de frecvenţă furnizate de JRC.

În vederea implementării Directivei CE Nr. 2013/59/(13) şi a estimării igienice a nivelului de expunere a populaţiei Republicii Moldova la sursele naturale de radiaţii ionizante şi a elaborării măsurilor profilactice, în perioada 2010-2015 de către specialiştii Agenţiei Naţionale pentru Sănătate Publică (ANSP) au fost efectuate circa 2982 de măsurători ale concentraţiilor de $^{222}$Rn, prin metode active, realizate astfel:

- 1779 în aerul de interior (case de locuit, grădiniţe, şcoli, Instituţii Medico Sanitare Publice (expunerea ocupaţională), blocuri locative noi date în exploatare etc.) prin metode active de măsurare a radonului;
- 891 în diverse surse de apă potabilă, inclusiv în apele din sonde şi din fântânile de mină;
- 312 la exhalarea $^{222}$Rn din sol.

*Metoda de determinare a concentraţiilor de radon în componentele mediului ambiant.* Pentru efectuarea măsurătorilor concentraţiilor de radon şi a descendenţilor săi de viaţă scurtă: $^{220}$Rn,$^{218}$Po, $^{214}$Pb, $^{214}$Bi şi $^{214}$Po în principalele componente ale mediului ambiant, cât şi în aerul din interi-orul locuinţelor a fost utilizat dispozitivul german, al companiei SARAD, Radonometru RTM 1688-2 (fig. 2).

Figura 2. Radonometru RTM 1688-2.

**REZULTATE**

În perioada de studiu cercetările au fost axate pe elaborarea metodologiei noi de investigare a radonului în interior, prin metode de măsurare de lungă durată. Metodologia în cauză a fost utilizată la măsurarea $^{222}$Rn în aerul de interior al diferitor tipuri de locuințe (n=2500), în arii rurale și urbane, ale principalelor zone ale Republicii Moldova. Detectorii RADTRACK2, oferiți de către Agenția Internațională pentru Energie Atomică (AIEA) în cadrul Proiectului de cooperare tehnică MOL9007 „Elaborarea Programului național (strategia și Planul de acțiuni) al controlului expunerii populației Republicii Moldova la radon", au fost plasate în dormitoare/camere de oaspeți pe un termen de circa 90 de zile.

În vederea realizării investigațiilor/sondajului concentrațiilor de radon în interior, prin metode de lungă durată, au fost elaborate următoarele cerințe/metodologii:

- Cerințele de plasare a detectorilor în locuință.
- Chestionarele de identificare a condițiilor/ tipului locuințelor.
- Acordul dintre investigatori ai radonului și proprietarul locuinței.
- Chestionarul de evaluare a cunoștințelor populației referitor la radon (Aprobat la Ședința Consiliului Științific al ANSP din 11.06.2019, extras din procesul verbal nr. 4).

Materialele elaborate, împreună cu detectorii, au fost repartizate medicilor șefi ai Centrelor de Sănătate Publică (CSP) regionale (n=10) în cadrul Atelierului de lucru organizat de ANSP la 04.02.2019.

În luna iunie (perioada de expunere a constituit 90 de zile) detectorii au fost colectați și expediați în laboratorul RADONOVA din Suedia pentru citirea informației (concentrația $^{222}$Rn), care a fost retrimisă în decurs de două săptămâni în Laboratorul Igiena radiațiilor și Radiobiologie, ANSP.

Rezultatele cercetărilor au demonstrat că activitatea $^{222}$Rn a variat în funcție de tipul și amplasarea locuințelor, de tipul materialelor de construcție, utilizate în construcția clădirii; tipul solului adiacent clădirii și ventilația încăperilor.

În Tabelul 1 este prezentată variabilitatea concentrațiilor de radon în aerul din locuințe pe teritoriul Republicii Moldova. Rezultatele denotă că în circa 1170 locuințe din cele investigate, adică în 49% de locuințe, concentrația radonului corespunde normelor naționale/europene.

Tabelul 1. Rezultatele măsurării concentrațiilor de radon cu detectori RADTRAK2 (măsurare pasivă, perioada de expoziție 30 zile) în 2500 locuințe în zonele rurale și urbane ale Republicii Moldova, a. 2019.

| Nr. | Concentrație radon, Bq/m$^3$ | Locuințe, număr | % |
|---|---|---|---|
| 1 | Până la 150 | 1170 | 49,00 |
| 2 | 160-290 | 615 | 25,00 |
| 3 | 300- 490 | 338 | 13,52 |
| 4 | 500-790 | 251 | 10,04 |
| 5 | 800-990 | 58 | 2,30 |
| 6 | > 1000 | 15 | 0,60 |

Totodată, în 615 locuințe (25%) concentrația radonului depășea normele naționale; în 662 locuințe (26%) concentrația radonului depășea normele europene. Astfel, în 1 277 locuințe (51%) concentrația radonului a fost mai mare ca normele naționale/europene. Rezultatele indică asupra existenței problemei radonului în locuințe pe teritoriul țării și necesitatea soluționării stringente a problemei. În legătură cu cele expuse a fost elaborat proiectul Hotărârii de Guvern „Cu privire la aprobarea Strategiei Naționale privind reducerea riscului asociat iradierii naturale, inclusiv a radonului", care a fost transmis Ministerului Sănătății, Muncii și Protecției Sociale pentru avizare. În proiectul HG este stipulată necesitatea elaborării și efectuării modificărilor la Codul construcțiilor, cu obligativitatea monitorizării radonului la darea în exploatare a clădirilor locative. Totodată, este strict necesară monitorizarea radonului în solurile pe care va fi amplasată clădirea. În țările vecine astfel de cerințe sunt deja implementate.

A prezentat interes și studierea variabilității concentrațiilor de radon în funcție de coordonatele amplasării geografice a localităților. S-a stabilit o variabilitate impunătoare pentru acest parametru, cu variații de la 150 Bq/m$^3$ în Chișinău până la 415 Bq/m$^3$ în r-nul Căușeni. Acest fapt poate fi explicat prin geologia neuniformă a teritoriului (fig. 3).

Cercetările au demonstrat variabilitatea concentrației radonului în locuințele din ariile urbane și rurale ale Republicii Moldova, în funcție de zonă. Astfel, în Zona de Sud valoarea medie a concentrației radonului a constituit 330 Bq/m$^3$, în Centru – 250 Bq/m$^3$, iar în Nord – 240 Bq/m$^3$. Rezultatele denotă că, cea mai mare concentrație a radonului a fost detectată în Sudul țării (330 Bq/m$^3$), fiind urmată de Centru (250 Bq/m$^3$) și de Zona de Nord (240 Bq/m$^3$) (fig. 4).

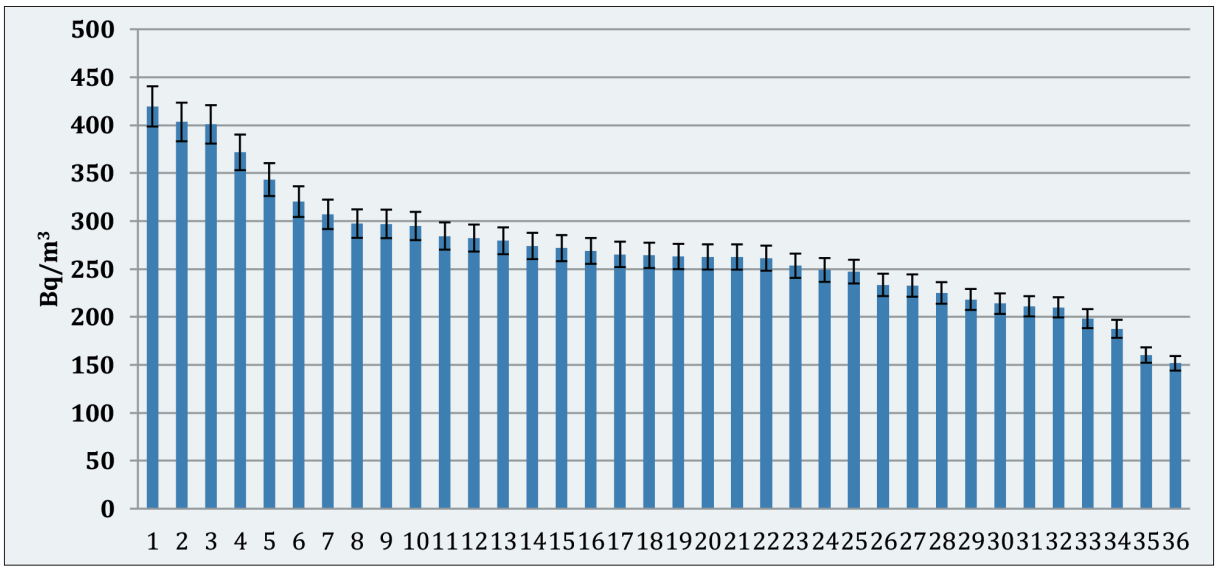


Figura 3. Variabilitatea concentrației radonului în aerul de interior al locuințelor amplasate în diferite raioane geografice ale Republicii Moldova, a. 2019.
1 – r-nul Căușeni, 2 – Comrat, 3 – Vulcănești, 4 – Ceadâr Lunga, 5 – Ștefan-Vodă, 6 – Nisporeni, 7 – Leova, 8 – Hâncești, 9 – Telenești, 10 – Cimișlia, 11 – Glodeni, 12 – Basarabeasca, 13 – Cantemir, 14 – Edineț, 15 – Bălți, 16 – Cahul, 17 – Drochia, 18 – Florești, 19 – Soroca, 20 – Călărași, 21 – Taraclia, 22 – Sângerei, 23 – Criuleni, 24 – Rezina, 25 – Strășeni, 26 – Ungheni, 27 – Ialoveni, 28 – Șoldănești, 29 – Anenii-noi, 30 – Ocnița, 31 – Dondușeni, 32 – Orhei, 33 – Briceni, 34 – Fălești, 35 – Râșcani, 36 – mun. Chișinău.

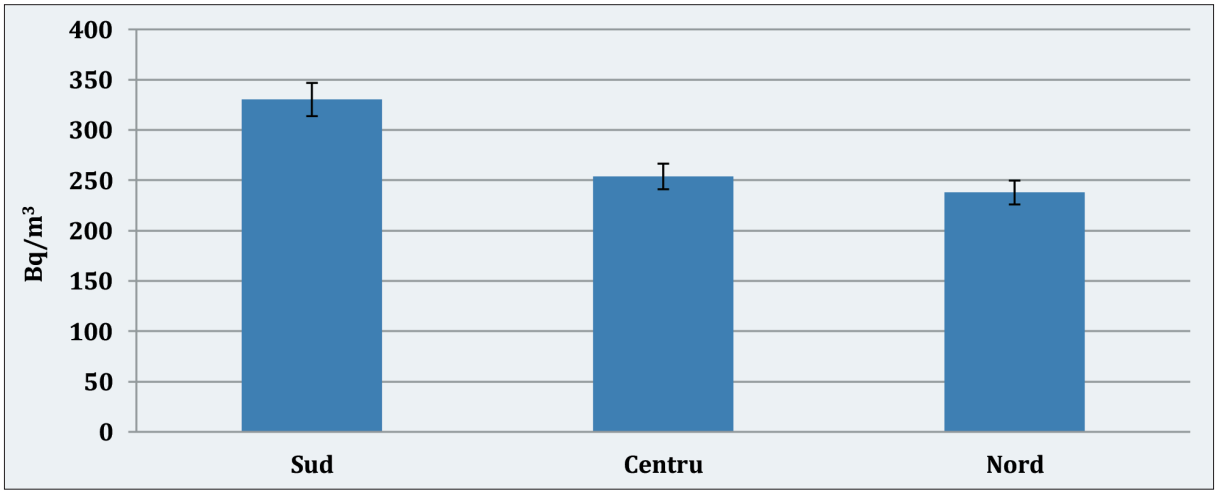


Figura 4. Variabilitatea concentrației radonului în Republica Moldova în funcție de zonă: Sud, Centru, Nord, a. 2019 (n=2500 măsurători cu detectori RADTRAK2, perioada de expoziție 90 de zile).

Concomitent s-a analizat variabilitatea concentrației radonului în aerul din locuințele, plasate pe teritoriul Republicii Moldova, în funcție de localitate: rurală sau urbană. Cercetările denotă că, valoarea medie a concentrației de radon din interior a fost mai mare în ariile rurale, constituind 260 Bq/m$^3$, în comparație cu cele urbane – 241 Bq/m$^3$. Faptul în cauză poate fi explicat prin aceea că, casele, în mediul rural, sunt amplasate direct pe sol sau au un fundament necorespunzător. Aceste condiții permit ca radonul din sol/roci să pătrundă mai ușor în încăperea de locuit (fig. 5).

În baza analizării rezultatelor măsurării concentrației de radon în aerul de interior a 2500 locuințe, prin utilizarea detectorilor pasivi RADTRAK2, s-a stabilit că media aritmetică a indicelui a constituit 254,6 Bq/m$^3$, iar media geometrică – 217,6 Bq/m$^3$. În baza rezultatelor obținute, utilizând programele Google Maps, ArcGIS și alte programe de specialitate, a fost efectuată cartarea radonului pe teritoriul țării (fig. 6). Rezultatele au fost transmise la Joint Research Comission pentru includerea acestora pe harta Europeană a radonului.

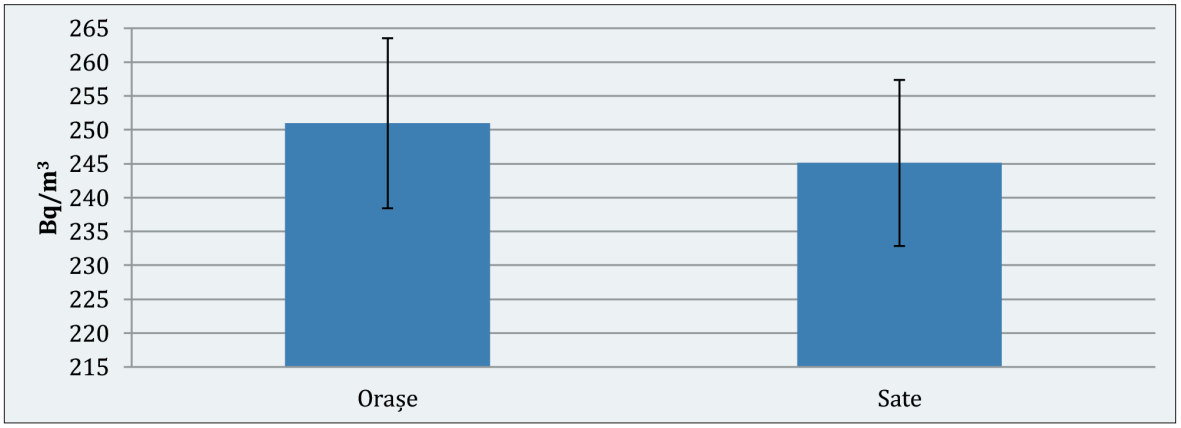


Figura 5. Concentrația radonului în aerul din locuințe, în localitățile rurale și urbane ale Republicii Moldova, a. 2019 (n=2500 măsurători cu detector RADTRAK2, perioada de expoziție 90 de zile).

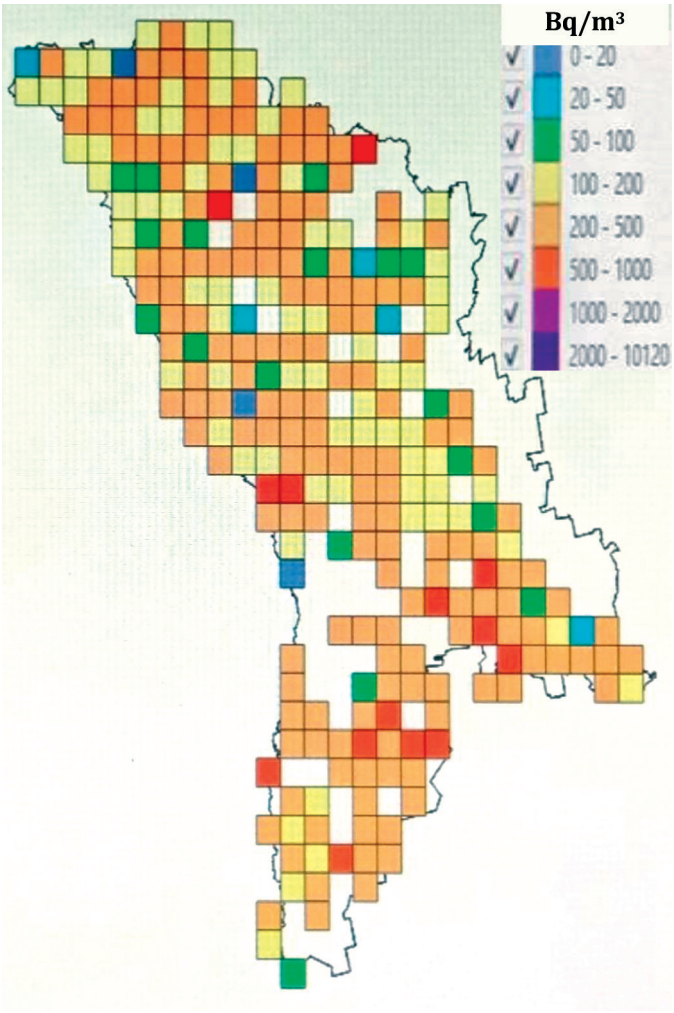


Figura 6. Cartarea radonului în aerul din diferite tipuri de locuințe, în arii rurale și urbane, în zonele de Nord, Centru și Sud a Republicii Moldova.

## DISCUȚII

În conformitate cu „Normele Fundamentale de Radioprotecție, Cerințe și Reguli Igienice" (NFRP-2000) și „Regulamentul și normele igienice privind reglementarea expunerii la radiații a populației de la sursele naturale" nivelul național de referință a $^{222}Rn$ a fost stabilit la o concentrație de 100 Bq/m$^3$ pentru clădirile noi și de 150 Bq/m$^3$ pentru clădirile existente (14).

În cazul depistării concentrațiilor sporite (peste 200 Bq/m$^3$) trebuie să fie întreprinse măsuri de radioprotecție, îndreptate spre diminuarea pătrunderii $^{222}Rn$ în aerul spațiilor locative și ameliorarea ventilării încăperilor. Dislocarea locatarilor (cu acordul acestora) și reprofilarea încăperilor și a edificiilor, poate avea loc în cazurile când este imposibilă diminuarea activității echivalente medii anuale de echilibru pe o unitate de volum a $^{222}Rn$ până la valori mai mici de 300 Bq/m$^3$ (15).

Relevant e faptul că, valorile menționate au fost stipulate ca norme naționale de referință doar teoretic, nu în baza efectuării măsurătorilor în aerul din locuințe. Recent, în perioada a. 2018-2019, în rezultatul implementării proiectului național MOL9007, finanțat de către AIEA, rezultatele fiind prezentate în lucrarea în cauză, s-a observat că în 1277 locuințe (51%) concentrația radonului depășește normele naționale/ europene. În baza acestor rezultate se propune modificarea valorilor naționale de referință – 300 Bq/m$^3$ care urmează a fi implementate în urma aprobării proiectului Hotărârii de Guvern la acest capitol.

## CONCLUZII

1. Monitoringul concentrațiilor de radon în aerul din diferite tipuri de locuințe (n=2 500), plasate în localitățile rurale și urbane, ale diferitor zone ale Republicii Moldova, prin utilizarea detectorilor *alpha* de lungă durată, de tip RADTRAK2, cu perioada de expoziție de 90 de zile, a stabilit variabilitatea indicatorului în funcție de zona geografică, condițiile abiotice, tipul casei, tipul pardoselii și a pereților.

2. Studiul a demonstrat o creștere a concentrațiilor de radon în aerul din locuințe în zona de Sud a țării, valoarea medie pe zonă constituind 330 Bq/m$^3$, fiind urmată de zona de Centru – 250 Bq/m$^3$ și Nord – 240 Bq/m$^3$.

3. Studierea variabilității concentrației radonului în aerul locuințelor, amplasate în diferite raioane geografice ale Republicii Moldova, a evidențiat valori sporite în r-nul Căușeni și diminuate în mun. Chișinău.

4. Cercetările denotă că valoarea medie a concentrației de radon din locuințe a fost mai mare în ariile rurale, constituind 260 Bq/m$^3$, în comparație cu cele urbane – 241 Bq/m$^3$.

5. Cartarea concentrațiilor de radon în aerul din locuințe pe teritoriul țării va fi utilă ministerelor și instituțiilor de resort, inclusiv specialiștilor din construcții, în vederea selectării terenurilor pentru construcția clădirilor cu risc diminuat de expunere la radon.

## CONFLICT DE INTERESE

Autorii n-au declarat conflict de interese.

## FINANȚARE

Cercetările în cauză au fost efectuate în cadrul proiectului MOL9007, susținut de Agenția Internațională pentru Energie Atomică, Viena, Austria.

## REFERINȚE

1. UNSCEAR Volume I, *Sources and effects of ionizing radiation. United Nations Scientific Committee on the Effect of Atomic Radiation*, *2008*. United Nations, New York, 2010.
2. Fran M. Exposure to radon increases your risk for lung cancer. Mass Public Health Blog. Promoting public health & wellness in Massachusetts, 2017 Available from: https://blog.mass.gov/publichealth/environmental-health/exposure-to-Radon-increases-your-risk-for-lung-cancer/ (Accessed 8th July 2019).
3. Scott BR. Residential Radon Appears to Prevent Lung Cancer. *Dose Response*. 2011; 9(4):444-464.
4. Vuchkov D, Ivanova K, Stojanovska Z, Kunovska B, Badulin V. Radon measurement in schools and kindergartens. National Center of Radiobiology and Radiation Protection. *Rom. Journ. Phys*. 2012; 58:328-335.
5. Lantz P, Mendez D, Philbert M. Radon, Smoking, and Lung Cancer: The need to refocus radon control policy. *American Journal of Public Health* 2013; 103(3):443-447.
6. Song G. et all. Indoor Radon levels in selected hot spring hotels in Guangdong, China. *Science of Total Environment*. 2005; 339(1-3):63-70.
7. WHO handbook on indoor radon: a public health

perspective/edited by Hajo Zeeb, and Ferid Shannoun. World Health Organization 2009.

8. Lantz PM, Mendez D, Philbert MA. Radon, smoking, and lung cancer: the need to refocus radon control policy. *Am J Public Health*.2013; 103(3):443-7.
9. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O, Bray F. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer*. 2018; 103:356-387.
10. Ragnar L. The communication of radon risk in Sweden: where are we and where are we going? *Journal of Risk Research*. 2019; 22(6):773-781.
11. Selim MK, James G. An Interdisciplinary Population Health Approach to the Radon Health Risk Management in Canada. *Interdisciplinary Journal of health sciences*. 2017; 3(19):1-11.
12. Biblin A. Development of the model of radiation risk-communication with the public for the arrangement of the research. Available from: https://www.researchgate.net/publication/332131134 (Accessed 10th July 2019).
13. Council Directive 2013/59/Euratom, *Official Journal of the E.U.* 2014.
14. *Normele Fundamentale de Radioprotecție, Cerințe și Reguli Igienice* (NFRP-2000) nr. 06.5.3.34 din 27.02.2001(Monitorul Oficial al Republicii Moldova, nr. 40-41, 2001).
15. RMS nr. 217: *Regulament și norme igienice privind reglementarea expunerii la radiații a populației de la sursele naturale* nr. 06-5.3.35 din 05.03.2001 (Monitorul Oficial al Republicii Moldova, nr. 92 din 03.08.2001).

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# COMPARATIVE ANALYSIS OF LONG-EARED OWL (*ASIO OTUS*) WINTER DIET FROM TWO EUROPEAN CITIES – CHISINAU (REPUBLIC OF MOLDOVA) AND BACAU (ROMANIA)

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***Key words:*** *Asio otus, urban environment, trophic spectrum, rodents, Microtus voles.*

***Introduction.*** *Many rodent species are important pests for agriculture and for urban areas. The long-eared owl is a predator that exerts constant pressure on rodent density.*
***Material and methods****. The studies were performed in winters of 2011-2013 in the cities of Chisinau and Bacau, where 599 and 82 pellets of Asio otus were collected, respectively. The prey items were identified from cranial bones extracted from pellets.*
***Results.*** *Long-eared owl's diet in both sites consists of mammals and birds, with rodents being the dominant trophic component. The Microtus species were the main prey with the abundance ≥70% in both sites. In Chisinau a high proportion of Mus species was registered in the diet. The total biomass of prey constituted 43 953 g in Chisinau and 7 038 in Bacau. The highest biomass belongs to Microtus species, with 31 710 g and 5 220 g, respectively. The trophic niche width in Chisinau constituted 0.089 and varied monthly from 0.058 to 0.28. In Bacau the WTNs was of 0.134 and varied slightly among the study months.*
***Conclusions.*** *The rodents were the main trophic source and constituted ≥96% in both sites. The prey diversity was higher in Chisinau, that was due to a larger city territory and to a higher number of wintering long-eared owls. The close values of trophic niche width in Chisinau and Bacau confirmed the high hunting specialization of the long-eared owl.*

***Cuvinte cheie:*** *Asio otus, mediu urban, spectru trofic, rozătoare, speciile genului Microtus.*

## ANALIZA COMPARATIVĂ A DIETEI DE IARNĂ A CIUFULUI DE PĂDURE (*ASIO OTUS*) ÎN DOUĂ ORAŞE EUROPENE – CHIŞINĂU (REPUBLICA MOLDOVA) ŞI BACĂU (ROMÂNIA)

***Introducere.*** *Multe specii de rozătoare sunt dăunători ai agriculturii, inclusiv şi zonele urbane. Ciuful de pădure este o specie de păsări de pradă care exercită o presiune relativ constantă asupra densităţii rozătoarelor.*
***Material şi metode.*** *Cercetările au fost efectuate în oraşele Chişinău şi Bacău, în perioada de iarnă a anilor 2011-2013, unde au fost colectate 599 şi, respectiv, 82 de ingluvii. Au fost identificate speciile pradă după oasele craniene extrase din ingluvii.*
***Rezultate.*** *Spectrul trofic al ciufului de pădure din ambele situri este format din mamifere şi păsări, rozătoarele fiind componentul trofic principal. În ambele situri speciile genului Microtus au fost prada principală cu peste 70%. Biomasa totală a prăzii a constituit 43 953 g în Chişinău, iar în Bacău – 7 038 g. Cea mai mare biomasă aparţine speciilor gen. Microtus – 31 710 g la Chişinău, 5 220 g – la Bacău. Lăţimea nişei trofice în Chişinău a fost de 0,089 şi a variat lunar de la 0,058 la 0,28. La Bacău, WTNs a constituit 0,134 şi a variat lunar în limite mici.*
***Concluzii.*** *Rozătoarele au reprezentat sursa trofică principală – peste 96% în ambele situri. În localitatea cu suprafaţa mai mare, spectrul trofic s-a dovedit a fi mai variat. Diversitatea speciilor pradă este mai mare în Chişinău şi se datorează suprafeţei mai mari a oraşului şi numărului mai mare de ciufi în colonie. Valorile apropiate ale lăţimii nişei trofice în Chişinău şi Bacău confirmă specializarea înaltă a ciufului de pădure.*

## INTRODUCTION

Many of the rodent species are important pests of agricultural crops and cereal deposits, including urban areas. The nocturnal prey birds are regulatory species that contribute to maintaining rodent density at more or less constant level. The long-eared owl (*Asio otus L.*) is a sedentary bird and one of the most widespread in Europe (1-5). During the winter period the density of species increases on the account of the migrant individuals from the northern regions and they form colonies of several tens of individuals. In most cases, the owls prefer to winter each year in the same place. The hunting sectors of the long-eared owl are open type biotopes, where they mainly hunt rodents and occasionally birds, shrews and bats. Following the digestion process, the prey birds regurgitate the indigestible remnants of eaten a-nimals (bones, hair, feathers, fur) in the form of pellets. The pellets analysis can provide important data regarding the feeding regime of the bird, the fauna of small mammals in a certain area, their density and their seasonal and annual dynamics, etc. The long-eared owl is well adapted to anthropic environment and its wintering colonies are frequently registered in urban localities (4).

Taking into consideration the huge importance of long-eared owl trophic activity in biological control of rodent pest species, especially in winter period, its diet was rather well studied in many regions of Europe (1-10). In the Republic of Moldova and Romania the diet of long-eared owl was also rather well studied in different areas of the countries (11-18). There are several studies concerning the long-eared owl's diet in urban areas (19-26).

The long-eared owl is a feeding specialist predator and not all species are equally hunted prey. The attractiveness of a prey species depends on specific qualities, the most important of which is the size (1). The long-eared owl shows strong preference for Microtus voles across Europe, but in urban areas it hunts in open type biotopes outside the city and/or use alternative prey (27). In winter, *A. otus* is capable to localize the prey under a snow cover of 40-50 cm (14). As adaptations of winter diet to urban environment can be considered the use of higher ratio of synanthropic rodent species (*Mus musculus, Rattus norvegicus*), of bird species as well as more diverse trophic spectrum (4, 24, 28-33).

The aim of the paper is to perform a comparative analysis of long-eared owl winter diet in two European cities, Chisinau and Bacau, with similar environmental – climatic conditions and rather different anthropic impact, in order to emphasize the similarities and the differences.

## MATERIAL AND METHODS

The studies were performed in winter periods of 2011-2012 and 2012-2013 in Chisinau and Bacau cities. Chisinau is a large city with the surface of 123 km$^2$, situated in the central part of the Republic of Moldova at the altitude of 82 m, coordinates 47001' N 28052' E. Bacau city is the major city of Bacau county, with the surface of 43.19 km$^2$, situated in eastern part of Romania at the altitude of 165 m, coordinates 46035' N 26055' E. The climate of both cities is humid continental with warm summers and cool, windy winters. The winter period lasts 78-80 days. The average temperature in winter is -2.3°C in Chisinau and -4 C in Bacau city, while the minimum temperature in January and February can drop below -20°C.

In Chisinau city a colony of long-eared owl (*Asio otus L.*) of 38 individuals was located in a courtyard of a school from Ciocana district with several dozens of tall coniferous and deciduous trees (*Picea abies, Populus alba, P. tremula, Salix alba*) suitable for long-eared owl individuals. In Bacau city a small colony of 7 individuals was located in the yard of the Astronomical Observatory, with several trees of *Thuja orientalis, Picea abies, Carpinus betulus*. Both locations are situated within the cities limits in heavily urbanized areas.

In Chisinau 599 pellets have been collected and in Bacau 82 pellets. Each pellet was measured, weighed and afterwards unfolded. The bone fragments were cleaned and sorted into categories. Small mammal species were determined accor-ding to cranial bones and dentition (34, 35). The sibling species *Microtus arvalis* and *M. rossiaemeridionalis*, *Mus musculus* and *M. spicilegus* that can't be differentiated morphologically were considered as genus *Microtus* and *Mus*, respe-ctively.

The ecological analysis of the prey species was performed using the indexes of abundance (A=no*100/N, where no – number of individuals of a species, N – total number of individuals); frequency (F=nop*100/N, where nop – number of pellets with certain species, N – number of pellets); total biomass of consumed prey (B=no*G,

where no – number of individuals of a species, G – mean weight of one individual). The mean weight of prey individual was calculated from our own data gathered during long term studies of small mammals and bats. The trophic niche width was estimated using the *B Levins'* index: $B=1/\Sigma p^2$, (36), in its standardized version Bs (37): *Bs*=(B-1)/(n-1), where p is the fraction of items in the diet, and n is the number of possible food categories (38). Bs ranges from 0 (100% utilization of a single food category) to 1 (equal use of all categories).

During the study none of animals was injured or sacrificed.

## RESULTS

In Chisinau the length of analyzed pellets varied from 1.16 to 6.95 cm with the average of 3.32 cm. The pellet weight varied between 1.2 and 6.07 g with the average of 2.52 g. After cleaning the bones, 1489 individuals were identified. The number of individuals per pellet varied from 1 to 6, the average constituted 2.46 individuals. In Bacau the pellet length varied between 1.2 and 5.6 cm. The minimal number of individuals/pellets was 1, the maximal number was 5 and the average was 2.56. After cleaning the bones 223 individuals were identified.

The trophic spectrum of long-eared owl in Chi-sinau consisted of mammals from 3 orders (*Soricomorpha, Rodentia, Chiroptera*) and passe-rine birds (fig. 1). In Bacau rodents and passerine birds have been identified (fig. 2). In both sites *Microtus* species dominated with 70.99% in Chi-sinau and 76.31% in Bacau. The house mouse is the second species in Chisinau pellets (10.88%), while in Bacau it constituted less than 1%. The genus *Apodemus* were represented by 4 species in Chisinau and by 3 species in Bacau. In both sites the most numerous was *A. sylvaticus* with 10.34% and 10.97%, respectively (fig. 1, fig. 2). Other *Apodemus* species constituted about 10% in Bacau pellets, while in Chisinau their ratio was less than 4%. In Chisinau the diet of long-eared owl was more diverse, probably due to much larger number of individuals that hunted in a larger variety of ecosystems. Here were identified shrews and bats in lower ratio, while the birds constituted 2.55% and in Bacau – 2.19%. Among rodents two more species have been registered – the arboreal rodent *Muscardinus avellanarius* and the synanthropic species *Rattus norvegicus* with very low ratio of 0.13%.

The diversity indexes (Shannon and Simpson) are higher in Chisinau site 0.45 and 1.89, respectively, than in Bacau 0.71 and 1.68. Although the species number is much higher in Chisinau, the difference between diversity indexes is not very high, due to more even distribution of the species in Bacau site.

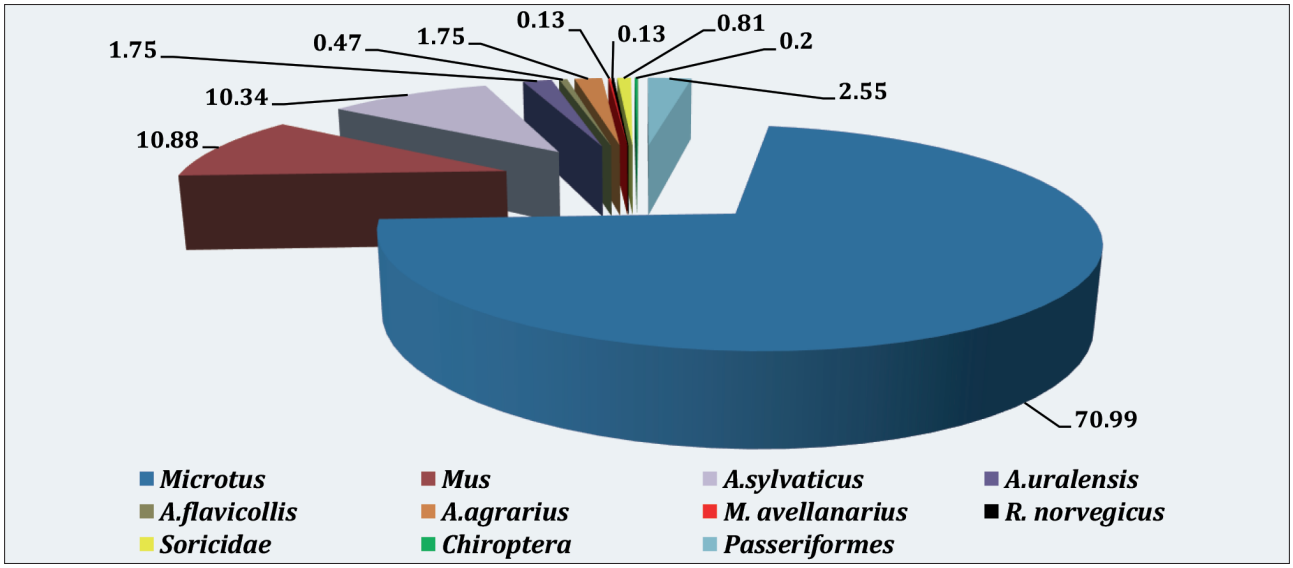


Figure 1. Trophic spectrum of long-eared owl in Chisinau city in 2011-2012.

The highest frequency in pellets from both urban areas belongs to *Microtus* species that was found in most of the pellets followed by Mus species and *A. sylvaticus* in Chisinau and by *A. sylvaticus* and *A. flavicollis* in Bacau (tab. 1). The birds, represented by Passeriformes had a frequency of 6.43% in Chisinau and 6.1% in Bacau.

The total biomass of prey items constituted 43 953 g in 6 study months in Chisinau and 7 038 in 4 months in Bacau. The highest biomass belongs to *Microtus* species, with 31 710 g in Chisinau and 5 220 in Bacau (tab. 2). In both sites a decrease of prey number and biomass was registered from November to December and from November to February in Chisinau.

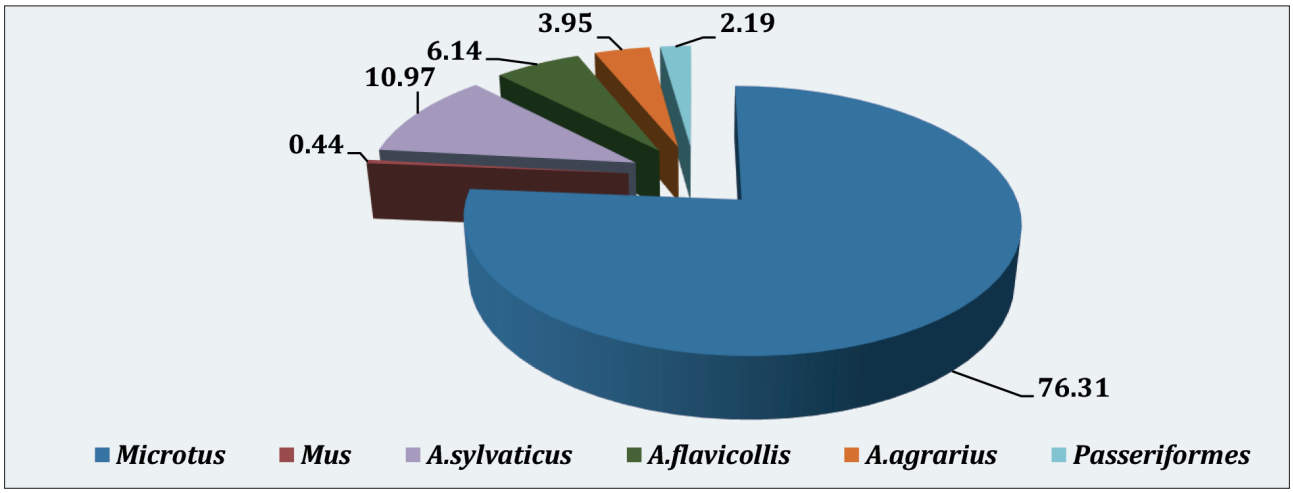


Figure 2. Trophic spectrum of long-eared owl in Bacau city in 2011-2012.

Table 1. Frequency of animal species in *Asio otus* pellets.

| Nr. | Genus/species | Chisinau | | Bacau | |
|---|---|---|---|---|---|
| | | No pellets | Frequency, % | No pellets | Frequency, % |
| 1. | ***Microtus*** | 368 | 61.44 | 62 | 75.61 |
| 2. | ***Mus*** | 136 | 22.71 | 1 | 1.22 |
| 3. | ***A. sylvaticus*** | 125 | 20.87 | 21 | 25.61 |
| 4. | ***A. uralensis*** | 25 | 4.17 | - | - |
| 5. | ***A. flavicollis*** | 6 | 1.0 | 12 | 14.63 |
| 6. | ***A. agrarius*** | 24 | 4.01 | 9 | 10.98 |
| 7. | ***M. avellanarius*** | 2 | 0.33 | - | - |
| 8. | ***R. norvegicus*** | 2 | 0.33 | - | - |
| 9. | ***Soricomorpha*** | 10 | 1.67 | - | - |
| 10. | ***Chiroptera*** | 2 | 0.33 | - | - |
| 11. | ***Passeriformes*** | 38 | 6.34 | 5 | 6.1 |

The trophic niche index in Chisinau site was 1.89 and varied monthly between 1.58 to 3.77. The standardized index was of 0.089 and varied monthly from 0.058 to 0.28 and indicate that in December 2012 the prey used belonged to many categories with more even distribution. In Bacau the trophic niche index was 1.67 with low variation degree between months. The standardized index was of 0.134 and varied slightly among the studied months (tab. 2). In Bacau WTNs index was slightly higher because only prey categories hunted there were considered.

## DISCUSSIONS

In both cities the *Microtus* voles are the most important prey item in the winter diet of Long-eared owl, as previously registered in open land ecosystems thorough Europe (1, 2, 3, 5, 7, 9, 10, 39, 40, 41, 42), as well as in urban areas (17, 21, 22, 24, 25, 29, 43, 44). *Apodemus* species constitute an important trophic source for long-eared owl in urban area. Their share can vary between 3% and 66% (17, 23, 28, 39, 40, 44) depending on location, climatic conditions, hunting sectors and prey availability. In some urban areas the *Apodemus* species even were the most abundant prey during winter period (28, 29, 43).

The difference between the ratio of *Mus* species in both cities is very high. This fact can be explained by city size and structure. In Chisinau there are many tall buildings and a massive production of waste, while in Bacau small houses are most numerous and the city is much cleaner. The higher ratio of *Mus* species (up to 10%) in winter diet of the long-eared owl was noted for large cities (9, 21, 29, 40, 45) and much lower ratio, up to 3% in small cities (17, 24, 43).

Among other rodent species in Chisinau pellets there were found *R. norvegicus* and *M. avellanarius*. The last one is arboreal species and usually hibernates during winter, therefore its share is very low in different regions of Europe, up to 1% (21, 28), but in most of studies it wasn't registered. The brown rat had a very low ratio in Chisinau pellets and wasn't registered in Bacau, although in previous studies it was found in *A. otus* diet with 0.14% (23). In many other studies in European cities the species also had a low share, up to 5% (9, 20, 21, 29, 31, 39), but in some large cities the species is one of the main preys, reaching about 20% and 60-70% biomass (28).

Table 2. Individual number and biomass of prey species in studied months.

| City | | Chisinau | | | | | | | Bacau | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Par. | XI.11 | XII.11 | I.12 | II.12 | XI.12 | XII.12 | Total | XI.11 | XII.11 | XI.12 | XII.12 | Total |
| *Microtus* | No | 346 | 242 | 175 | 174 | 67 | 53 | 1057 | 53 | 27 | 63 | 31 | 174 |
| | BM | 10 380 | 7 260 | 5 250 | 5 220 | 2 010 | 1 590 | 31 710 | 1 590 | 810 | 1 890 | 930 | 5 220 |
| *Mus* | No | 64 | 29 | 16 | 19 | 21 | 13 | 162 | - | - | 1 | - | 1 |
| | BM | 1 152 | 522 | 288 | 342 | 378 | 234 | 2916 | - | - | 18 | - | 18 |
| *A. sylvaticus* | No | 37 | 23 | 19 | 31 | 30 | 14 | 154 | 9 | 4 | 9 | 3 | 25 |
| | BM | 1 036 | 644 | 532 | 868 | 840 | 392 | 4 312 | 252 | 112 | 252 | 84 | 700 |
| *A. uralensis* | No | 5 | 7 | 4 | 7 | 3 | - | 26 | - | - | - | - | 0 |
| | BM | 115 | 161 | 92 | 161 | 69 | - | 598 | - | - | - | - | 0 |
| *A. flavicollis* | No | 2 | - | 1 | 2 | - | 2 | 7 | 5 | - | 6 | 3 | 14 |
| | BM | 70 | - | 35 | 70 | - | 70 | 245 | 175 | - | 210 | 105 | 490 |
| *A. agrarius* | No | 8 | 7 | 2 | 4 | 3 | 2 | 26 | 2 | 1 | 5 | 1 | 9 |
| | BM | 200 | 175 | 50 | 100 | 75 | 50 | 650 | 50 | 25 | 125 | 25 | 225 |
| *R. norvegicus* | No | - | - | - | - | 2 | - | 2 | - | - | - | - | 0 |
| | BM | - | - | - | - | 300 | - | 300 | - | - | - | - | 0 |
| *M. avellanarius* | No | - | 2 | - | - | - | - | 2 | - | - | - | - | 0 |
| | BM | - | 40 | - | - | - | - | 40 | - | - | - | - | 0 |
| *Soricidae* | No | - | - | 4 | 1 | 5 | 2 | 12 | - | - | - | - | 0 |
| | BM | - | - | 32 | 8 | 40 | 16 | 96 | - | - | - | - | 0 |
| *Chiroptera* | No | - | - | - | - | 3 | - | 3 | - | - | - | - | 0 |
| | BM | - | - | - | - | 36 | - | 36 | - | - | - | - | 0 |
| *Passeriformes* | No | 1 | 2 | 2 | 5 | 20 | 8 | 38 | - | 3 | - | 2 | 5 |
| | BM | 75 | 150 | 150 | 375 | 1 500 | 600 | 2 850 | - | 225 | - | 150 | 375 |
| Total ind. | No | 463 | 312 | 223 | 243 | 154 | 94 | 1489 | 69 | 35 | 84 | 40 | 228 |
| Total biomass | BM | 13 028 | 8 852 | 6 429 | 7 144 | 5 248 | 2 952 | 43 653 | 2 067 | 1 172 | 2 495 | 1 294 | 7 028 |
| WTN | | 1.71 | 1.62 | 1.58 | 1.95 | 3.77 | 2.7 | 1.89 | 1.64 | 1.61 | 1.72 | 1.63 | 1.67 |
| WTNs | | 0.071 | 0.062 | 0.058 | 0.095 | 0.28 | 0.17 | 0.089 | 0.128 | 0.122 | 0.144 | 0.126 | 0.134 |

*Note:* No – number of individuals, BM – biomass, WTN – width of trophic niche, WTNs – width of trophic niche standardized

Among other mammal groups in the diet of long-eared owl from Chisinau representatives of shrews and bats have been registered in very low percent. The shrews were represented by 4 species (*Crocidura suaveolens*, *C. leucodon*, *Sorex minutus*, *S. araneus*) and the bats – by 2 species (*Eptesicus serotinus* and *Vespertilio murinus*). The shrews are an alternative prey type for *A. otus* and are mostly hunted when the abundance of Microtus species is low. Furthermore, it was esta-blished that the share of shrews in the owl's diet depends on the abundance of Microtus species and doesn't depend on shrew abundance in certain area (46). The presence of bats in the diet of *A. otus* is usually accidental and constitutes less than 0.5%, while in the diet of other owl species (*Tyto alba*, *Bubo bubo*) Chiroptera groups can reach more than 10% (47, 48).

The passerine birds constituted about 2-3% in *A. otus* diet from both cities, as well as in other urban studies, where their share constituted 0.5-10%

(26, 29, 39, 40, 43, 49). The higher ratio of birds in some studies is conditioned by the abundant snow cover, when owls can shift their hunting areas into urban habitats, where the availability and density of bird populations, especially house sparrow, are higher (4). In the studied period in urban localities Chisinau and Bacau the snow cover did not exceed 10-20 cm and in November-December periods there was no snow cover, therefore the share of birds was rather low.

The prey diversity is higher in Chisinau site than in Bacau, which is due to larger surface of Chi-sinau city and to larger number of long-eared owl individuals. The higher diversity in larger cities was mentioned in many other studied (21, 28, 29, 40), while in smaller localities the diversity is lower (22, 26, 39).

The long-eared owl is a specialized predator and hunt individuals that weight between 15 g and 50 g, therefore, the ratio of preferred prey – *Microtus voles* remain high in spite of the availability of other prey types (1, 2). According to optimal foraging theory only the abundance of preferred prey influence upon the optimal choice of prey type, while the abundance of other prey types is not important (50). The close values of trophic niche breadth in Chisinau and Bacau prove the high hunting specialization of the long-eared owl and highlight its importance in rodent regulation in urban areas and surroundings.

## CONCLUSIONS

1. The trophic spectrum of long-eared owl in Chisinau and Bacau cities consists of mammals and birds, the rodents being the dominant trophic source (8 species in Chisinau and 5 species in Bacau with over 95%). The *Microtus* species were the main prey and constitute more than 70% in both sites.

2. The higher prey diversity in Chisinau in comparison to Bacau is due to larger surface of Chisinau city and to larger number of long-eared owl wintering individuals. In larger cities the trophic spectrum is more diverse.

3. The total biomass of prey items constituted 43 953 g in 6 study months in Chisinau and 7 038 in 4 months in Bacau. The highest biomass belongs to *Microtus* species, with 31 710 g in Chisinau and 5 220 in Bacau.

4. The trophic niche index in Chisinau site was 1.89 and varied monthly between 1.58 to 3.77. The standardized index was of 0.089 and varied monthly from 0.058 to 0.28 In Bacau the trophic niche index was 1.67 with low variation degree between months. The standardized index was of 0.134 and varied slightly among the studied months. The close values of trophic niche breadth in Chisinau and Bacau prove the high hunting specialization of the long-eared owl and highlight its importance in rodent regulation in urban areas and surroundings.

## CONFLICT OF INTERESTS

The authors do not declare any conflict of interest.

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## REFERENCES

1. Birrer S. Synthesis of 312 studies on the diet of the Long-eared Owl *Asio otus. Proc. Fourth World Owl Conf. Oct–Nov 2007, Groningen, The Netherlands. Ardea*. 2009; 97(4):615–624.
2. Goszczyński J. Connection between predatory birds and mammals and their prey. *Acta Theriol.*1977; 22:399–430.
3. Romanowski J, Żmihorski M. Effect of season, weather and habitat on diet variation of a feedingspecialist: a case study of the long-eared owl, *Asio otus* in Central Poland. *Folia Zool.* 2008; 57(4): 411–419.
4. Wijnandts H. Ecological energetics of the long-eared owl (*Asio otus*). *Ardea*. 1984; 72:1–92.
5. Korpimäki E. Diet composition, prey choice, and breeding success of Long-eared Owls: effects of multiannual fluctuations in food abundance. *Can. J. Zool*. 1992; 70:2373–2381.
6. Nilsson I. Seasonal changes in food of the long-eared owl in southern Sweden. *Ornis Scand.* 1981; 12:216–223.
7. Tome D. Diet composition of the long-eared owl in central Slovenia: seasonal variation in prey use. *J. Raptor Res*. 1994; 28:253–258.

8. Galeotti P., Canova L. Winter diet or long-eared owls (*Asio otus*) in the Po Plain (Northern Italy). *Raptor Res*. 1994; 28(4):265-268.
9. Escala C., Alonso D., Mazuelas D, Mendiburu A., Vilches A., Arizaga J. Winter diet of Long-eared Owls *Asio otus* in the Ebro valley (NE Iberia*). Revista Catalana d'Ornitologia.* 2009; 25:49-53.
10. Sergio F, Marchesi L., Pedrini P. Density, diet and productivity of Long-eared Owls Asio otus in the Italian Alps: the importance of Microtus voles: Capsule Relatively large populations, feeding predominantly upon voles, were present at higher e-levations. *Bird Study*. 2008; 55(3):321-328.
11. Schnapp B. The fauna of micromammals from Valu-lui-Traian (Dobroudja) in the years 1958-1962, according to Asio otus (L.) pellets. *Trav. Mus. Nat. His. Nat. Gr. Antipa*. 1968; 8 (2):1045-1063.
12. Аверин ЮВ., Ганя ИМ. Хищные птицы Молдавии и их роль в природе и сельском хозяйстве. Издво «Картя Молдовеняскэ», 1966, 104 стр.
13. Анисимов ЕП. Факторы, определяющие добычу ушастой совы зимой*. Вопросы экологии и практического значения птиц и млекопитающих* Молдавии. 1969; 3: с. 36-40.
14. Зубков НИ. Трофические связи сов в биоценозах Молдавии. Экология птиц и млекопита-ющих Молдавии. Кишинэу «Штиинца», 1981. с. 79-94.
15. Зубков НИ. Трофические связи и роль ушастой совы в биоценозах антропогенного ландшафта. Млекопитающие и птицы антропогенного ландшафта Молдавии и их практическое значение. Кишинэу «Штиинца», 1986. с. 41-59.
16. Petrescu A. Restes de prois de la nourriture d'*Asio otus* L. (Aves: Strigiformes) pendant l'ete dans la reserve naturelle Agigea (Roumanie*). Trav. Mus. Nat. His. Nat. Gr. Antipa*. 1997; 37:305-317.
17. Benedek A M., Sîrbu I. Dynamics of *Asio otus* L., 1758 (Aves: Strigiformes) winter-spring trophic regime in Western Plain (Romania). *Trav. Mus. Nat. His. Nat. Gr. Antipa*. 2010; 53:479 – 487.
18. Nistreanu V, Larion A, Postolachi V. Date preliminare privind dieta unor păsări răpitoare nocturne (Aves: Strigidae) în zona de nord a Republicii Moldova. *Agricultura durabilă în Republica Moldova: provocări actuale şi perspective: Culegere de articole ştiinţifice, Filiala Bălţi a Acad. de Ştiinţe a Moldovei.* Bălţi: Indigou Color, 2017. p. 356-360.
19. Barbu P, Barbu I. Colonii de ciufi (Asio otus otus L.) în câteva păduri din apropierea Bucureştiului. Necesitatea ocrotirii lor. *Ocrotirea Naturii.* 1972; 16(2):197-205.
20. Barbu P, Korodi Gal I. Despre hrana de iarnă a ciufului de pădure (Asio otus otus L.) din padurea Calcer — Cluj. *Stud. Cercet. Biol., Ser. Zool.* 1972; 24:497-504.
21. Murariu D., Andreescu I., Nesterov V. Les comprosants de la nourriture d'hiver d'*Asio otus otus* (L., 1758) du nord-est de Bucarest (Roumanie). *Trav. Mus. Nat. His. Nat. Gr. Antipa.* 1991; 31:415-420.
22. Laiu L, Murariu D. The food of the long-eared owl (*Asio otus otus* L.) (Aves: Stringiformes) in wintering conditions of the urban environment in Romania. *Trav. Mus. Nat. His. Nat. Gr. Antipa*. 1998; 40: 413-430.
23. Laiu L, Pasol P, Feneru F, Murariu D. The analysis of the winter food structure in *Asio otus otus* L. (Aves: Strigiformes) from Bacau and Iasi towns – Moldova (Romania). *Trav. Mus. Nat. His. Nat. Gr. Antipa*. 2002; 44: 423-430.
24. Banaru V, Coroiu I. Preliminary data on the micromammal fauna in the Someşul Mic basin (România) according to Asio otus otus L. pellets. *Studia Univ. "Babeş-Bolyai", Cluj-Napoca, Biol.* 1997; XLII (1-2):103-108.
25. Sike T. Hrana de iarnã a ciufului de pãdure (*Asio otus*) în Satu Mare – analize calitative si cantitative. *Studii si Comunicãri. Seria Stiintele Naturale*. 2003-2004; 4-5:222-231.
26. Nistreanu V, Larion A, Postolachi V. Small mammal diversity in steppe zone Sadaclia, Republic of Moldova. *DROBETA, Ştiinţele Naturii*. 2015; XXV:135-141.
27. Riegert J, Lövy M, Fainová D. Diet composition of Common Kestrels *Falco tinnunculus* and Long-eared Owls *Asio otus* coexisting in an urban environment. *Ornis Fenn*. 2009; 86:123-130.
28. Pirovano A, Rubolini D, Brambilla S, Ferrari N. Winter diet of urban roosting Long-eared Owls Asiootus in northern Italy: the importance of the Brown Rat Rattus norvegicus, *Bird Study,* 2000; 47:2:242-244.
29. Sharikov AV, Kholopova AV, Volkov SV, Makarova TV. The review of owls' diet in Moscow City and Moscow Region. In: Волков С.В., Шариков А.В., Морозов В.В. (ред.) Совы Северной Евразии: экология, пространственное и биотопическое распределение. М.; 2009. с. 188-203.
30. Sandor A, Kiss B. The diet of wintering Long-eared Owls (*Asio otus*) in Tulcea, Romania. *Scientific Annals of the Danube Delta Institute*. 2004; 10:49-54.
31. Sandor A, Kiss B. Birds in the diet of wintering long-eared owls (Asio otus) in the Danube Delta, Romania*. J. Raptor Res*. 2008, 42(4):292-295.
32. Kiat GY, G. Perlman, A. Balaban, Y. Leshem, I. Izhaki, M. Charter. Feeding specialization of urban Long-eared Owls *Asio otus* (Linnaeus, 1758), in Jerusalem, Israel. *Zoology in the Middle East*. 2008; 43:49-54.
33. Mori E, Bertolino S. Feeding ecology of Long-eared Owls in winter: an urban perspective. *Bird Study*. 2015; 62:2:257-261.
34. Popescu A, Murariu D. *Fauna României. Mammalia, Rodentia*. Editura Academiei Române, Vol. XVI (2), 2001, 210 pp.

35. Pucek Z. (red.) *Keys to vertebrate of Poland. Mammals.* PWN – Polish Scientific Publishers, Warszava, 1981, 370 pp.
36. Levins R. *Evolution in Changing Environments: Some Theoretical Explorations.* Princeton: Princeton University Press; 1968. 121 pp.
37. Hurlbert SH. The Measurement of Niche Overlap and Some Relatives. *Ecology*, 1978; 59(1): 67-77.
38. Krebs CJ. *Niche measures and resource preferences. Ecological Methodology*, New York: Addison-Welsey Publishers. 1999. p. 455-496.
39. Romanowski J. Trophic ecology of *Asio otus* (L.) and *Athene noctua* (Scop.) in the suburbs of Warsaw. *Pol. Ecol. Stud.* 1988; 14: 223-234.
40. Bencová V, Kašpar T, Bryja J. Seasonal and interannual changes in diet composition of the Long-eared Owl (*Asio otus*) in Southern Moravia. *Tichodroma.* 2006; 18: 65-71.
41. Drebet MV. Diet of Long-eared Owl in the Kamenetskoe Prydenstrovie, Ukraine. In: Волков С.В., Шариков А.В., Морозов В.В. (ред.) Совы Северной Евразии: экология, пространственное и биотопическое распределение. М.; 2009, с. 55-58.
42. Petrovici M, Molnar P, Sandor A. Trophic niche overlap of two sympatric owl species (*Asio otus* Linnaeus, 1758 and *Tyto alba* Scopoli, 1769) in the North-Western part of Romania. *North-Western Journal of Zoology*. 2013; 9(2): 250-256.
43. Dzemian S, Pilacinska B, Pitucha G. Winter diet composition of urban long-eared owls (*Asio otus*) in Rzeszow (SE Poland). *Biological let.*, 2012, vol. 49, isuue 2, p. 107-114.
44. Sharikov AV. Peculiarities of winter feeding in the long-eared owl (*Asio otus*) in settlements of Stavropol krai. *Zool. Zh.* 2006; 85:871-877.
45. Martelli C, Fastelli P. Svernamento e dieta del gufo commune Asio otus nella città di Grosseto. *Gli Uccelli d'Italia*. 2013; 38:85-91.
46. Korpimaki E, Norrdahl K. Avian and mammalian predators of shrews in Europe: regional diffe-rences, between year and seasonal variation and mortality due to predation. *Ann. Zool. Fenn.,* 1989; 26(4):389-400.
47. Obuch J. The representation of bats (*Chiroptera*) in the diet of owls (Strigiformes) in Slovakia. *Vespertilio*. 1998; 3:65-74.
48. Шариков АВ, Макарова ТВ. Рукокрылые в питании сов Северной Евразии. *Plecotus et al.* 2014; 17: 30-36.
49. Tulis F, Veselovský T, Birrer S. Different alternative diets within two subgroups in a winter roost of long-eared owls. *Raptor Journal.* 2019; 13. doi: 10.2478/srj20190002.
50. Pyke GH. Optimal foraging theory: a critical review. *Annu. Rev. EcoI. Syst*. 1984; 15:523-575.

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# ANTIMICROBIAL RESISTANCE AND BIOLOGICAL PROPERTIES OF *STAPHYLOCOCCUS* SPP. ISOLATED FROM PIGS

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***Key words:*** *Staphylococcus spp., plasma coagulation, biological properties, antibiotics, susceptibility, resistance.*

***Introduction.*** *Representatives of the genus Staphylococcus spp. cause a significant proportion of diseases in animals and humans. Nowadays the problem of their acquired antibiotic resistance is an urgent concern.*

***Material and methods.*** *Isolation and identification of Staphylococcus spp. carried out in accordance with DSTU EN 6888:2003 standard. The* susceptibility *of the strains to antibiotics was determined by the disk diffusion method. Interpretation of the results was carried out in accordance with the recommendations of the 8th version of EUCAST.*

***Results.*** *77 strains of Staphylococcus spp. Collected from sows at the farm No. 2, were isolated: hemolytic properties were detected in 90.6% (39 coagulases positive and 19 coagulase negative); 22 (56.4%) strains of coagulase-negative Staphylococcus spp.; 13.6% of isolates had hemolytic properties. The results of antibiograms of crops from the farm No.1: 51.8% of strains were* susceptibile *to penicillin, 47.6% – resistant; 13.62 –* susceptibile *to fluoroquinolones, 80.9% – resistant; 96.7% –* susceptibile *to chloramphenicol, no resistant strains were detected.*

***Conclusions.*** *Acquired resistance of Staphylococcus spp. to certain groups of antibiotics isolated from pigs, indicated the irrational use of antimicrobial therapy. Differences were found in the* susceptibility *of coagulase-positive and coagulase-negative Staphylococcus spp. to all groups of antibiotics.*

***Cuvinte cheie:*** *Staphylococcus spp., coagularea plasmei, proprietăți biologice, antibiotice, sensibilitate, rezistență.*

**REZISTENȚA LA ANTIMICROBIENE ȘI PROPRIETĂȚILE BIOLOGICE A *STAPHYLOCOCCUS* SPP. IZOLATE DE LA PORCINE**

***Introducere.*** *Reprezentanții genului Staphylococcus provoacă un număr semnificativ de boli la animale și la oameni. Actualmente, o problemă majoră o prezintă rezistența dobândită a acestor tulpini la antibiotice.*

***Material și metode.*** *Izolarea și identificarea Staphylococcus spp. s-a realizat în conformitate cu standardul DSTU EN 6888:2003. Sensibilitatea tulpinilor la antibiotice a fost determinată prin metoda disc-difuzimetrică, iar interpretarea rezultatelor a fost efectuată potrivit recomandărilor EUCAST, versiunea 8.*

***Rezultate.*** *De la scroafele din ferma nr. 1 au fost izolate 77 de tulpini de Staphylococcus spp: proprietăți hemolitice au fost detectate la 90,6% din probe (39 coagulazo-pozitive și 19 coagulazo-negative). De la scroafele din ferma nr.2 au fost izolate 22 (56,4%) tulpini de Staphylococcus spp. coagulozo-negative, dintre care 13,6% posedau proprietăți hemolitice. Rezultatele antibioticogramei culturilor de la ferma nr.1 ne arată că 51,8% din culturi sunt sensibile și 47,6% sunt rezistente la peniciline; 13,6% sunt sensibile și 80,9% sunt rezistente la fluorochinolone, iar 96,7% din tulpini au fost sensibile la cloramfenicol, nefiind detectate culturi rezistente.*

***Concluzii.*** *Rezistența dobândită la tulpinile de Staphylococcus spp, izolate de la porcinele din ferma nr.1, față de anumite grupuri de antibiotice, indică utilizarea irațională a terapiei antimicrobiene. Astfel, s-au constatat diferențe în sensibilitatea tulpinilor de Staphylococcus spp, coagulazo-pozitive și coagulazo-negative, la toate grupele de antibiotice.*

## INTRODUCTION

*Staphylococcus* carriers were an important source of contamination for food, raw materials, birds, pigs and pork products (1, 2). It has been repeatedly proven that pigs were sources of staphylococci, in particular methicillin-resistant *S. aureus* (MRSA) (3). Such cases were especially common in Denmark (4), Canada (4), Germany (6) and Switzerland (7). In young pigs, the disease manifests itself in the form of exudative epidermitis (EE), caused by strains of *Staphylococcus hyicus*, *Staphylococcus aureus* and *Staphylococcus chromogenes*, which produce exfoliative toxins. However, pigs in most cases were hidden carriers (7). Experimental transfer of methicillin-resistant *S. aureus* to minks during feeding of pork waste contaminated with MRSA is also known (9). In 2017, 80 methicillin-resistant staphylococcus strains were isolated in Ukraine, including 77.5% from domestic animals, 11.3% from poultry, 6.3% from cattle and 5% from pigs (10).

The purpose of the study was to investigate the biological properties and antibiotic resistance of Staphylococcus spp. isolated from pigs of two industrial pig farms located in Kyiv (No.1) and Vinnytsa regions (No.2).

## MATERIAL AND METHODS

Isolation and identification of *Staphylococcus* spp. was conducted in accordance with: DSTU EN 6888:2003 "Microbiology of food and animal feed" standard was cultivated to the Baird Parker agar (Merck) medium and were incubated at 37°C for 24-48 hours. Isolates produced on Baird Parker agar at the end of this period were defined by morphology colony, Gram stain, catalase test, and coagulation test. The hemolytic properties of staphylococci were studied on Columbia blood agar (BioMerieux). Selected colonies from Columbia blood agar introduced into Tryptone-soy broth and cultured at t 35°C for 2-4 hours. The optical density was determined using a densitometer DEN-1(Biosan) and McFarland standard (HiMedia). The susceptibility of strains to antibiotics was determined by disc-diffusion method using Müller-Hinton agar, inoculum: 0.5 according to the McFarland turbidity standard and were incubated at 35°C, for 18±2 hours. The results were assessed in accordance with the re-commendations of version 8 of the European Committee on Antimicrobial Susceptibility Testing EUCAST (11) and Guidelines "Determination of susceptibility of microorganisms to antibacterial drugs" (12).

## RESULTS

Farm No. 1 proved to be a satisfactory farm in terms of safety against contagious diseases; number of pigs was up to 2 500 heads. There were 210 pigs. Purulent diseases, abscesses and boils were not reported in piglets. Of the 77 sows sampled from the nose *Staphylococcus* spp. were isolated in 64 cases (83.1%). All strains grown on the Beard Parker agar had typical colonies of black and grey, shiny and convex with a diameter of 1 mm to 1.5 mm after incubation for 24 hours and a diameter of 1.5-2.5 mm after 48 hours of incubation. The 39 isolates (60.94%) were coa-gulated with rabbit plasma. The ability to hemo-lysis of sheep erythrocytes showed only 58 (90.6%) of strains. Of these, 39 coagulase-positive strains and 19 coagulase-negative strains did not show hemolytic properties of 6 coagulase-negative strains.

The farm No. 2 proved to be a satisfactory farm in terms of contagious diseases; number of pigs was up to 3 500 heads. Diseases caused by *Sta-phylococcus* spp. we're not registered. In 22 samples (56.4%), isolates of *Staphylococcus* spp. were isolated from 39 sows from farm No.2.

The isolates grew in the form of shiny black and grey colonies with a narrow white margin. The isolates did not coagulate plasma. The 3 strains (13.6%) from the isolated strains had hemolytic properties.

*Staphylococcus* had a natural susceptibility to penicillins, but subsequently acquired resistance to them (13). In the study, were used: Natural Penicillin, Benzylpenicillin; Semisynthetic Oxacillin, Gentamicin, Tobramycin from the aminoglycosides group, and Erythromycin from the macrolids group (13). The susceptibility to natural Tetracycline and Semisynthetic Doxycycline; Lincomycin and Clindamycin from the group of lincosamides was investigated. Susceptibility for norfloxacin was recommended for screening susceptibility for all fluoroquinolones (11). There were studied Norfloxacin, Ciprofloxacin, Ofloxacin, Pefloxacin, Lomefloxacin, Levofloxacin, Sparfloxacin and Gatifloxacin (12, 13). Susceptibility to Chloramphenicol, and Rifampicin was investigated (11).

51.87% of the selected strains obtained at the farm No.1 were susceptible to Penicillin antibiotics,

and 47.59% were resistant. 78.48% of bacterial strains showed susceptibility to aminoglycosides, and 21.52% of the strains were resistant. 87.1% of strains showed susceptibility to macrolides. 70.45% of the isolates were susceptible to tetracyclines. 70% of isolates showed susceptibility, and 30% were resistant to lincosamides. 13.62% were susceptible, and 80.93% – resistant to fluoroquinolones. 96.72% of revealed strains were susceptible to chloramphenicol. 73.53% of the studied isolates were susceptible to rifampicin.

10 strains from 22 pigs, on farm No. 2, were isolated and an antibiogram was determined (tab. 1). It was established that 10 isolated strains were resistant to oxacillin and clindamycin. 3 strains were susceptible, and 7 – resistant to benzylpenicillin and norfloxacin. 1 culture was susceptible, and 9 – resistant to tetracycline and erythromycin. 4 strains were susceptible, and 6 – resistant to chloramphenicol.

Table 1. Ranges of growth inhibition diameters of the studied cultures isolated from pigs on farm No. 2, mm. Min-Max.

| Name of antibiotic | Eucast interpretation (version 8.0) and Guidelines "Determination of susceptibility of microorganisms to antibacterial drugs" | Ranges of diameters of cultures growth inhibition mm. Min-Max | S strains (n) | R strains (n) |
|---|---|---|---|---|
| **Benzylpenicillin** | 26>s; 26<r | 0-29 | 3 | 7 |
| **Oxacillin** | 18≥s; 17<r | 0-16 | 0 | 10 |
| **Erythromycin** | 21>s; 18<r | 16-25 | 1 | 9 |
| **Clindamycin** | 22>s; 19<r | 0-17 | 0 | 10 |
| **Norfloxacin** | 24>s; 24<r | 0-28 | 3 | 7 |
| **Tetracycline** | 22>s; 19<r | 17-24 | 1 | 9 |
| **Chloramphenicol** | 18>s; 18<r | 15-22 | 4 | 6 |

*Note:* "0" – continuous growth, "15-22" – the minimum and maximum value of growth inhibition of the test culture

Number of coagulase-positive and coagulase-negative strains isolated from the farm No. 1 was showed in Table 2.

There was 100% resistance to benzylpenicillin among 18 coagulase-positive strains of *Staphylococcus* spp. from isolated strains. Oxacillin: intermediate resistanse (12) – 3% of isolated strains, resistant – 6%, susceptibile – 91% of strains. All resistant strains. Doxycycline: 28.5% – intermediate resistanse; 28.5% – resistant and 43% susceptible strains.

There were 7.8% of the strains from which 92% were resistant to lincomycin. Clindamycin: 42% – resistant, 58% of susceptible isolates. 100% of the coagulase-positive *Staphylococcus* spp. were resistant to Norfloxacin and Ciprofloxacinum. 4.7% and 95.3% of resistant strains were susceptible to Ofloxacin. Pefloxacin: 10% – resistant, 60% – intermediate resistanse and 30% susceptible isolates. Lomefloxacin was found to be resistant in 87% of strains, intermediate in 6.5% and susceptible in 6.5% of strains. 6.7% of the isolated strains were susceptible to Levofloxacin and the remaining 93.3% were resistant There were 97% cultures resistants and 3% isolates which showed susceptibility to Sparfloxacin. Gatifloxacin: intermediate resistanse strains – 13% and 87% – resistant strains. The 84.6% demonstrated resistance to rifampicin 7.7% and intermediate resistanse 7.7% of strains.

In the group of coagulase-negative *Staphylococcus* spp. isolated from pigs in farm No. 1, there were 25% resistant strains and 75% strains showed susceptibility to Benzylpenicillin. All 100% strains were susceptible to Oxacillin. Ampicillin: 13% of strains were susceptible, 87% of strains – resistant. Gentamicin: 46% of strains were susceptible and 54% – resistant. There were 92% susceptible and 8% resistant strains to Tobramycin. Erythromycin: 6.9% of strains were resistant and 6.9%-intermediate resistanse, the rest all isolated strains (86.2%) showed susceptibility.

Tetracycline: 81.5% of strains were intermediate resistanse, 11% – resistant, and 7.5% strains were susceptible. Doxycycline: 4% of strains were intermediate resistanse, 4% – resistant, and 92% susceptible isolates. Lincomycin: 84% of the strains were susceptible and 16% were resistant.

3% intermediate resistanse strains to Clindamycin, the remaining 97% – susceptible. Norfloxacin: susceptible – 3% and intermediate resistanse – 3% and 94% – resistant strains. 80% of strains were 20% susceptible to Ciprofloxacin. Ofloxacin: susceptible – 25%, resistant – 75% of isolates. Pefloxacin: resistant – 50%, intermediate resistanse – 23%, susceptible – 27% of strains.

Lomefloxacin: susceptible – 16%, intermediate resistanse – 8%, and resistant – 76% isolates. 8% of the strains were susceptible to Levofloxacin and 92% were resistant. 64.5% of the strains showed susceptibility to Sparfloxacin and 35.5% was resistant. There were 23% intermediate resistanse and 8% resistant and 69% of strains susceptible to Gatifloxacin. Chloramphenicol: intermediate resistanse – 7% of strains from 93% of strains. Rifampicin: 66.5% were susceptible, 24% were intermediate resistanse, and 9.5% of isolates were resistanse.

Table 2. Number of studied cultures of *Staphylococcus* spp. isolated in the farm No.1.

| Name of antibiotic | The number of cultures of *Staphylococcus* spp. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Coagulase-positive | | | | Coagulase-negative | | | |
| | S | I | R | Total | S | I | R | Total |
| **Penicillins** | | | | | | | | |
| **Benzylpenicillin** | 0 | 0 | 18 | 18 | 7 | 0 | 21 | 28 |
| **Oxacillin** | 30 | 1 | 2 | 33 | 31 | 0 | 0 | 31 |
| **Ampicillin** | 0 | 0 | 14 | 14 | 3 | 0 | 20 | 23 |
| **Aminoglycosides** | | | | | | | | |
| **Gentamicin (I)** | 13 | 0 | 1 | 14 | 12 | 0 | 14 | 26 |
| **Tobromycin (II)** | 14 | 0 | 0 | 14 | 23 | 0 | 2 | 25 |
| **Macrolides** | | | | | | | | |
| **Erythromycin** | 29 | 2 | 2 | 33 | 25 | 2 | 2 | 29 |
| **Tetracyclines** | | | | | | | | |
| **Tetracycline** | 10 | 0 | 11 | 21 | 22 | 3 | 2 | 27 |
| **Doxycycline** | 6 | 4 | 4 | 14 | 24 | 1 | 1 | 26 |
| **Linkosamides** | | | | | | | | |
| **Lincomycin** | 1 | 0 | 12 | 13 | 21 | 0 | 4 | 25 |
| **Clindamycin** | 18 | 0 | 13 | 31 | 30 | 1 | 0 | 31 |
| **Fluoroquinolones** | | | | | | | | |
| **Norfloxacin (II)** | 0 | 0 | 27 | 27 | 1 | 1 | 28 | 30 |
| **Ciprofloxaine (II)** | 0 | 0 | 15 | 15 | 5 | 0 | 20 | 25 |
| **Ofloxacin (II)** | 1 | 0 | 20 | 21 | 7 | 0 | 21 | 28 |
| **Pefloxacin (II)** | 3 | 6 | 1 | 10 | 7 | 6 | 13 | 26 |
| **Lomefloxacin (II)** | 1 | 1 | 13 | 15 | 4 | 2 | 19 | 25 |
| **Levofloxacin (III)** | 1 | 0 | 14 | 15 | 2 | 0 | 23 | 25 |
| **Sparfloxacin (III)** | 1 | 0 | 32 | 33 | 11 | 0 | 20 | 31 |
| **Gatifloxacin (IV)** | 0 | 2 | 13 | 15 | 6 | 2 | 18 | 26 |
| **Others** | | | | | | | | |
| **Chloramphenicol** | 32 | 0 | 0 | 32 | 27 | 2 | 0 | 29 |
| **Rifampicin** | 11 | 1 | 1 | 13 | 14 | 5 | 2 | 21 |

## DISCUSSIONS

The obtained results indicate a significant colonization of coagulase-positive and coagulase-negative staphylococci in the nasal passages of sows. A significant percentage of isolated crops possesses "pathogenicity factors". Plasma coagulation and hemolysis were particularly important. The high circulation of staphylococci in the herd leads to an increase of antibiotic resistance. Natural penicillin is known to have little effect on staphylococci, while synthetic and semisynthetic penicillin antibiotics of other groups inhibit their growth.

The results showed that in the farm No. 1 polyresistant strain of coagulase-positive and coagulase-negative staphylococci was present. A major

component of beta-lactam resistance is the so-called *mecA* gene, which encodes the formation of modified penicillin-binding protein and thus interferes with the incorporation of beta-lactam into the cell wall. When the cell is methicillin-resistant *Staphylococcus aureus* in contact with β-lactam antibiotics, the additional β-lactam-resistant penicillin binding protein (PBP2a) takes on the biosynthetic functions of normal PBPs.

*Staphylococcus aureus* resistance to methicillin (Oxacillin) may be due to the production of additional PBP-2a, which is encoded by the chromosomal *mecA* gene, inactivation through hyper production of β-lactamases and modification of normal PBPs. The presence two resistant strains of coagulase-positive staphylococci and one intermediate resistanse in pigs may indicate the possible presence of the *mecA* gene. Also, the resistance of the isolated strains to Ampicillin indicates the synthesis of staphylococcal penicillinase. The higher resistance to Tetracycline was shown by coagulase-positive staphylococci, which was confirmed by the resistance of these strains to Doxycycline. Also, indicative is the resistance of some coagulase-positive staphylococci to both Lycomycin and Clindamycin, which is not observed among coagulase-negative strains. Practically all staphylococci have been shown to be resistant to Fluoroquinolones of different generations, including Gatifloxacin. Two intermediate resistanse strains of coagulase-negative staphylococci were detected for Chloramphenicol while all coagulase-positive strains were susceptible.

As a result, the circulation of polyresistant coagulase-positive staphylococcus strains proved to be resistant to almost all antibiotic groups, especially to Fluoroquinolones – 4.6% susceptible, 6.0% – intermediate resistanse, and 89.4% – resistant. Among coagulase-negative staphylococci, 20% were susceptible, 5.0% were intermediate resistanse, and 75% were resistant to Fluoroquinolines. Coagulase-negative strains from farm No. 2 also had poly resistant properties in tested antibiotics of all groups.

## CONCLUSIONS

1. High percentage of staphylococcus circulation in pigs of experimental farms was detected.

2. The selected strains possessed “pathogenicity factors” by hemolysis and plasma coagulation.

3. Some staphylococci showed resistance to three or more antibiotics at the same time.

4. All these factors testify the misuse of antibiotics and the rapid manifestation of resistance to their individual representatives.

5. To confirm the emergence of the mechanism of resistance, it is necessary to carry out molecular genetic studies of isolated strains.

6. It is necessary to change fundamentally the pattern of antibiotic use in pigs in order to prevent staphylococcus resistance and their subsequent transfer to humans.

## CONFLICT OF INTERESTS

All authors declare no competing interests.

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## REFERENCES

1. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, Cartwright RA, et al. Recent human – to – poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. *Proc Natl Acad Sci USA*. 2009; 106(46):19545–19550.
2. Kadariya J, Smith T.C, Thapaliya D. *Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. *Biomed Res Int*. 2014; 2014:827965.
3. Armand-Lefevre L, Ruimy R, Andre Mont A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg. infect dis*. 2005; 11(5):711-714.
4. Lewis H, Molbak K, Reese C, et al. Pigs as source of methicillin-resistant *Staphylococcus aureus* cc398 infections in humans, Denmark. *Emerg infect dis*. 2008; 14(9):1383-1389.
5. Khanna T, Friendship R, Dewey C, et al. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary microbiology*. 2008; 128(3-4):298-303.

6. Cuny C, Nathaus R, Layer F, et al. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without Exposure to Pigs. *Plos one*. 2009; 4(8):e6800.
7. Oppliger A, Moreillon P, Charrière N, et al. Antimicrobial Resistance of *Staphylococcus aureus* Strains Acquired by Pig Farmers from Pigs. *Appl Environ Microbiol*. 2012; 78(22):8010-8014.
8. Duijkeren E, Jansen MD, Flemming CS, et al. Methicillin-Resistant *Staphylococcus aureus* in Pigs with Exudative Epidermitis. *Emerg Infect Dis*. 2007; 13(9):1408-1410.
9. Fertner M, Pedersen K, Chriél M. Experimental exposure of farmed mink (Neovisonvison) to livestock-associated methicillin-resistant *Staphylococcus aureus* contaminated feed. *Veterinary Microbiology*. 2019; 231:45-47.
10. Kozytska T, Garkavenko T. *Circulation of Methicillin-Resistant Staphylococcus (MRS) in Livestock and Domestic Animals*. Proceedings of the BTRP Ukraine Regional One Health Research Symposium. 2019, Kyiv.
11. Eucast. The european committee on antimicrobial susceptibility testing (2018). Available from: http://www.eucast.org/ [Accessed 10th September 2019].
12. Ministry of health of Ukraine. Determination of susceptibility of microorganisms to antibacterial drugs (2009). Available from: https://zakon.rada.gov.ua/rada/show/v0167282-07 [Accessed 10th September 2019].
13. Chambers H, Deleo F. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat rev microbiol*. 2009; 7(9):629-641.
14. Nicola F, McDougal L, Biddle J, et al. Characterization of Erythromycin-Resistant Isolates of *Staphylococcus aureus* Recovered in the United States from 1958 through. *Antimicrob Agents Chemother.* 1998; 42(11):3024-3027.

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# DIALDEHIDA MALONICĂ – UN POTENȚIAL MARCHER AL TOXICITĂȚII NANOPARTICULELOR ÎN MEDIUL ACVATIC

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***Key words:*** *nanoparticles, toxicity tests, malondialdehyde, Porphyridium cruentum.*

**MALONDIALDEHYDE – A POTENTIAL MARKER OF NANOPARTICLE TOXICITY IN AN AQUATIC ENVIRONMENT**

***Introduction.*** *As a result of increased production and spread in the environment, nanoparticles can pose a significant risk to public health. To date, the toxicity data of nanoparticles collected, using traditional models and methods, are contradictory and inconsistent. Highlighting the significant methods and markers of nanoparticle toxicity is a current research direction.*

***Material and methods.*** *The strain of red microalgae Porphyridium cruentum CNM-AR-01, known as a lipid manufacturer, was used as object of study. The toxic effect of CdSe (3-7 nm), ZnSe (40 nm) and ZnS (30-35 nm) nanoparticles was tested. The amount of malondialdehyde was determined based on thiobarbituric acid reactive substances.*

***Results.*** *A close correlation between the amount of biomass and malondialdehyde in the cells of red microalgae Porphyridium cruentum has been established for nanoparticle concentration ranges which have a toxic effect on Porphyridium cruentum.*

***Conclusions.*** *Malondialdehyde can be considered as a marker of nanoparticle toxicity.*

***Cuvinte cheie:*** *nanoparticule, teste de toxicitate, dialdehidă malonică, Porphyridium cruentum.*

***Introducere.*** *Nanoparticulele pot prezenta un real pericol pentru sănătatea publică, ca urmare a creșterii producției și a răspândirii lor în mediu. Până în prezent, datele de toxicitate a nanoparticulelor, colectate cu ajutorul modelelor și al metodelor tradiționale, sunt contradictorii și inconsistente. Relevarea metodelor și a marcherilor semnificativi ai toxicității nanoparticulelor constituie o direcție de actualitate în domeniul cercetărilor.*

***Material și metode.*** *Ca obiect de studiu a servit tulpina microalgei roșii Porphyridium cruentum CNM-AR-01, cunoscută în calitate de producător de lipide. A fost testat efectul toxic al nanoparticulelor de CdSe (3-7 nm), ZnSe (40 nm) și ZnS (30-35 nm). Cantitatea de dialdehidă malonică a fost determinată în baza substanțelor reactive ale acidului tiobarbituric.*

***Rezultate.*** *A fost stabilită relația strânsă între cantitatea de biomasă și cantitatea de dialdehidă malonică în celulele microalgei roșii Porphyridium cruentum pentru acele domenii de concentrații de nanoparticule, care au efect toxic asupra obiectului studiat.*

***Concluzii.*** *Dialdehida malonică poate fi considerată marcher al toxicității nanoparticulelor.*

## INTRODUCERE

Ultimele două decenii se remarcă printr-o dezvoltare vertiginoasă a nanotehnologiei – domeniu de cercetare eminamente inedit, atât din punctul de vedere al cunoașterii fundamentale, cât și din cel al aplicărilor practice, având înregistrate la activ succese spectaculoase. De exemplu, s-au obținut unele nanometriale cu proprietăți deosebite: nanotuburi de carbon, fulerene, puncte cuantice, dendrimere, nanoparticule ale diferiților oxizi metalici, nanoparticule ceramice, nanoparticule polimerice etc. (1). Pe lângă domeniile tradiționale de utilizare, cum ar fi: electronica, optoelectronica, nanotehnologia, nanoprodusele s-au afirmat în medicina practică și în produsele farmaceutice, contribuind semnificativ la ameliorarea sănătății oamenilor, dar și la înțelegerea proceselor biologice în celulele sănătoase și în cele afectate. Dezvoltarea nanotehnologiei a influențat inclusiv asupra producerii de medicamente principial noi (mai eficiente și cu efecte secundare minime) și asupra elaborării de noi metode de diagnostic precoce și de tratament al maladiilor grave, cum ar fi: cancerul, diabetul zaharat, maladiile neurodegenerative etc. (2-4).

Creșterea producției de nanoparticule și răspândirea lor în mediu pot prezenta un risc semnificativ pentru sănătatea publică. În urma activității fiziologice înalte și a diversității căilor de pătrundere în organism (prin inhalare, administrare orală, transdermic, prin injectare), efectele nanoparticulelor se pot amplifica enorm. Afară de eficiență terapeutică, nanoparticulele pot prezenta un nivel sporit de toxicitate pentru sistemele biologice. În scopul anticipării și al contracarării efectelor negative ale nanoparticulelor pentru om, este necesar un studiu prealabil, care ar demonstra efectele impuse de nanomaterialele străine în organism (5).

Studiile de acest tip cel mai des sunt efectuate pe culturi celulare și pe obiecte-model, printre care microorganismele se regăsesc destul de rar. Investigațiile ce țin de domeniul interacțiunii nanomaterialelor cu microorganismele au în vizor studiul formării sistemelor hibride, în scopul obținerii vectorilor pentru preparatele medicamentoase și a dispozitivelor microelectronice, precum și pentru efectuarea imobilizării dirijate a nanomaterialelor pe celulele microbiene (6, 7, 8). În majoritatea lucrărilor de acest fel, celulele vii nu sunt apreciate ca sisteme vii funcționale, motiv pentru care va apărea, în mod evident, problema influenței nanoparticulelor asupra activității sistemelor vitale ale celulei, care pot reacționa diferit la prezența xenoparticulelor.

Sistemele hibride nanomateriale – microorganisme oferă posibilitatea de a efectua un studiu perfect al toxicității nanoparticulelor asupra organismului, dar și al posibilelor efecte benefice ale lor. Microorganismele acvatice, printre care cianobacteriile și microalgele, se prezintă ca obiecte de studiu foarte comode și reprezentative, oferind facilități enorme în modelarea diferitelor efecte și în stabilirea mecanismelor de acțiune a diferiților compuși asupra proceselor vitale în celulele vii.

Afară de obiectele de studiu, o importanță majoră pentru testele de toxicitate o au și metodele aplicate. Metodele de testare a efectelor toxice ale nanoparticulelor asupra diferitelor tipuri de celule sunt foarte diverse – de la determinarea lactat dehidrogenazei (LDH), care semnalizează apoptoza, până la detectarea stresului oxidativ. Ele furnizează informații valoroase pentru identificarea biomarcherilor, care relevă daunele induse de nanoparticule (9).

În studiile de toxicitate a nanomaterialelor sunt foarte răspândite testele bazate pe aplicarea tetrazoliumului (testul MTT cu utilizarea 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromidului, testul MTS cu utilizarea 3-(4,5-dimetiltiazol-2-il)-5-(3-carboximetoxifenil)-2-(4-sulfofenil)-2H-tetrazoliumului și testul WST cu utilizarea uneia dintre sărurile solubile ale tetrazoliumului, de exemplu a 2-(2-metoxi-4-nitrofenil)-3-(4-nitrofenil)-5-(2,4-disulfofenil)-2H-tetrazoliumului), precum și testele care evidențiază răspunsul inflamator celular, indus de nanoparticule (cuantificarea biomarcherilor inflamatori, cum ar fi IL-8, IL-6 și factorul de necroză tumorală). Diferite tipuri de culturi de celule, inclusiv linii de celule canceroase, au fost folosite ca modele de toxicitate in vitro.

Până în prezent, datele de toxicitate a nanoparticulelor, colectate cu ajutorul modelelor și al metodelor menționate mai sus, sunt contradictorii și inconsistente. Prin urmare, pe baza modelelor experimentale disponibile, poate fi dificil de a trage concluzii despre potențialul toxic al nanoparticulelor (10).

Stresul oxidativ, indus de anumite substanțe, este considerat drept un indicator sigur al toxicității acestora. Pe lângă modificarea activității enzime-

lor antioxidante, stresul oxidativ este caracterizat şi prin degradarea macromoleculelor. Pentru multe nanoparticule a fost determinată creşterea dependentă de doză a gradului de deteriorare a AND-ului, a peroxidării lipidelor şi a carbonilării proteinelor (5). Lipidele, ca element component esenţial al membranelor biologice, care asigură nu numai funcţionalitatea, dar şi integritatea acestora, sunt grav afectate de factorii nocivi. Dialdehida malonică, care se formează în calitate de produs final al peroxidării lipidice, este unul dintre marcherii moleculari universali ai stării de stres.

Generalizând cele expuse mai sus, relevăm scopul acestui studiu – evidenţierea posibilităţii de aplicare a testului dialdehidei malonice în calitate de marcher al toxicităţii nanoparticulelor pentru organismele acvatice.

## MATERIAL ŞI METHODE

În cercetare au fost incluse nanoparticule de CdSe, ZnSe şi ZnS, care au fost sintetizate la Institutul de Inginerie Electronică şi Nanotehnologii *D. Ghiţu*. Naoparticulele luminiscente CdSe, cu dimensiunea de 3-7 nm, au fost obţinute prin metoda coloidală. Nanoparticulele de ZnSe, cu dimensiunea de 40 nm şi nanoparticulele de ZnS, cu dimensiunea de 30-35 nm, au fost obţinute prin sinteza hidrotermală. Nanoparticulele au fost adăugate la mediul de cultivare sub formă de soluţie hidrică din prima zi. Domeniul de concentraţii a constituit: pentru nanoparticulele CdSe – de la 0,01 la 12,0 mg/l; pentru nanoparticulele de ZnSe – de la 0,01 la 0,6 mg/l şi pentru nanoparticulele de ZnS – de la 0,01 la 8,0 mg/l.

În calitate de obiect-model a fost utilizată tulpina microalgei roşii Porphyridium cruentum CNM-AR-01, cunoscută în calitate de producător de lipide şi, în special, de acid eicosapentaenoic (11). Microalga a fost cultivată pe mediul nutritiv mineral cu următoarea componentă: în g/l – NaCl-7,0; KCl-7,5; $MgSO_4 \cdot 7H_2O$-1,8; $Ca(NO_3)_2 \cdot 4H_2O$-0,15; KBr-0,05; KI-0,05; $K_2HPO_4$-0,2 şi 1,0 ml/l soluţie de microelemente, ce conţine în mg/l: $FeCl_3 \cdot 6H_2O$-2,7; $NaVO_3$-0,05; $ZnSO_4 \cdot 5H_2O$-0,02; $CuSO_4 \cdot 5H_2O$-0,05; $MnSO_4 \cdot 5H_2O$-0,3; $H_3BO_3$-0,6; $MoO_3$-0,02, în baloane Erlenmeyer, cu volumul de lucru de 100 ml, la temperatura de 28°C, cu iluminare constantă cu fluxul de fotoni de 40,5 $\mu M/m^2s$ şi agitare periodică. Durata cultivării a fost de 14 zile. La sfârşitul ciclului de cultivare, biomasa se separă de lichidul cultural prin centrifugare. Conţinutul de biomasă algală a fost determinat spectrofotometric conform curbei de calibrare, care reflectă dependenţa absorbanţei la 465 nm de cantitatea de biomasă.

Conţinutul produselor degradării oxidative a lipidelor a fost stabilit în baza substanţelor reactive ale acidului tiobarbituric – testul dialdehidei malonice (MDA – malondialdehyde assay) (12). Concentraţia dialdehidei malonice a fost calculată cu utilizarea coeficientului extincţiei molare a complexului dialdehidei malonice sau în % inhibiţie faţă de proba martorului pozitiv.

## REZULTATE

Unul dintre indicatorii esenţiali ai adaptabilităţii microalgei la componenta mediului de cultivare este productivitatea, prin urmare monitorizarea acestui parametru poate servi ca factor de bază în procesul de stabilire a influenţei diferiţilor xenobiotici, inclusiv a nanoparticulelor asupra organismului. Pentru toate tipurile de nanoparticule studiate, a fost determinat conţinutul de biomasă, obţinută la finele ciclului de cultivare a microalgei.

Concentraţia particulelor CdSe a fost calculată în mg/l mediul de cultivare. Au fost efectuate 3 serii de experienţe, în diferite intervale de concentraţii (de la 0,01 la 0,1 mg/l; de la 0,1 la 1,0 mg/l şi de la 1 la 12 mg/l). În figurile 1 şi 2, rezultatele conţinutului de biomasă sunt prezentate în %, prin comparare cu probele control.

Concentraţiile de la 0,01 până la 0,1 mg/l au produs abateri minime de la valorile martorului, iar în cazul concentraţiei de 0,6 mg/l a fost observată o creştere statistic semnificativă ($p<0,01$) cu 20% comparativ cu martorul (fig. 1).

Concentraţia de 0,09 mg/l CdSe a avut efect de reducere a productivităţii (cu până la 16%, $p<0,05$) comparativ cu martorul.

O reacţie similară a culturii de *Porphyridium cruentum*, la introducerea CdSe, a fost constatată şi în variantele experimentale cu aplicarea concentraţiilor de la 0,1 până la 1,0 mg/l CdSe. Conţinutul de biomasă a crescut cu 18-19% la concentraţia CdSe de 0,6 şi 0,7 mg/l. Concentraţiile mai mici, precum şi cele mai mari, s-au manifestat ca inerte, productivitatea menţinându-se la nivelul probelor martor.

Pentru experienţa cu utilizarea concentraţiilor mari de particule, rezultatele obţinute (fig. 2) au indicat un spor al productivităţii cu 33,7-47,5% ($p<0,001$), în cazul concentraţiilor de 4,0-6,0 mg/l CdSe şi cu 18% ($p<0,01$) pentru concentraţia de

8,0 mg/l. Creşterea în continuare a concentraţiei CdSe în mediul de cultivare a redus drastic productivitatea: cu 37%, în cazul concentraţiei de 10 mg/l şi cu 77,3 %, în cazul concentraţiei particulelor de 12 mg/l.

Concentraţiile mai înalte de CdSe în mediul de cultivare induc moartea celulelor în primele 5 zile (faza exponenţială de creştere), ceea ce indică asupra implicării toxicităţii nanoparticulelor la acest nivel de concentraţie.

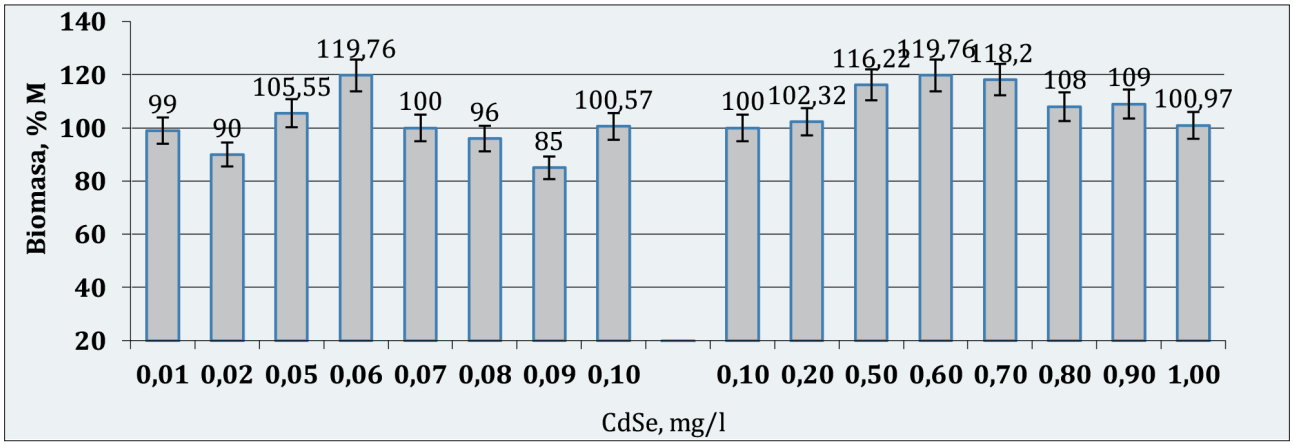


Figura 1. Biomasa *Porphyridium cruentum*, % faţă de proba martor (M) în prezenţa nanoparticulelor CdSe (0,01-0,1 mg/l; 0,1-1,0 mg/l).

Un alt compus studiat au fost particulele de ZnSe. În cadrul experienţelor preliminare, a fost stabilit că depăşirea concentraţiei de 0,8 mg/l provoacă moartea celulelor în primele 5 zile de cultivare, cu agregarea şi sedimentarea lor. Rezultatele experienţelor cu aplicarea concentraţiilor de la 0,01 mg/l la 0,6 mg/l ZnSe sunt prezentate în Figura 3.

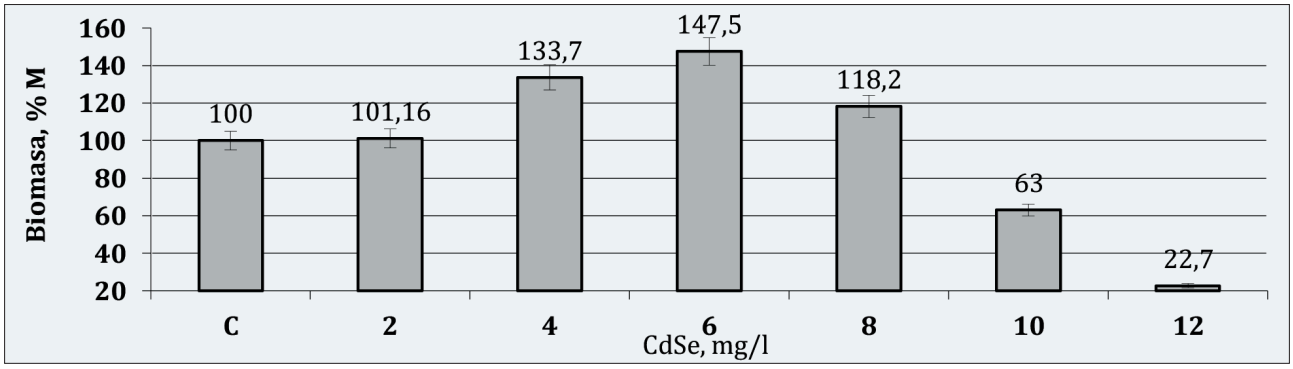


Figura 2. Biomasa *Porphyridium cruentum*, % faţă de proba martor (M) în prezenţa nanoparticulelor CdSe (2,0 – 12,0 mg/l).

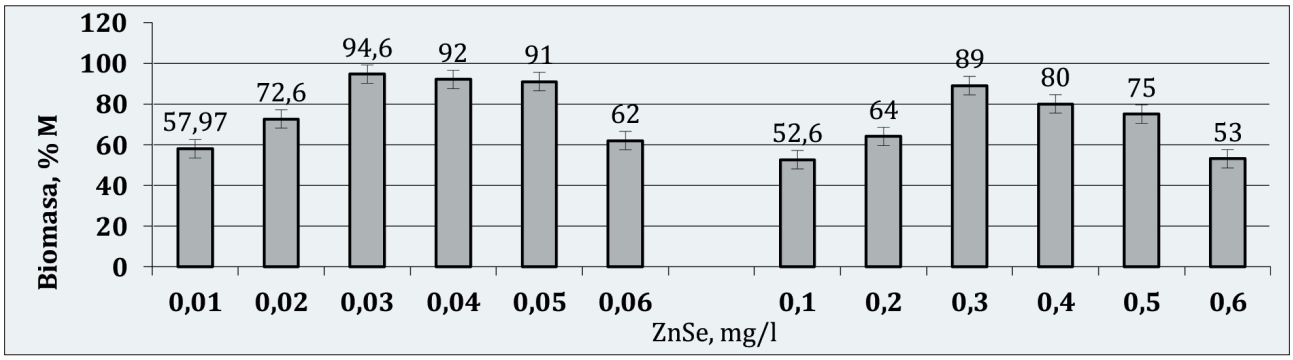


Figura 3. Biomasa *Porphyridium cruentum*, % faţă de proba martor (M) în prezenţa nanoparticulelor ZnSe (0,01-0,06 mg/l; 0,1 – 0,6 mg/l).

Rezultatele indică o reducere a productivităţii cu 42-28%, în comparaţie cu proba martor, în cazul concentraţiilor de 0,01-0,02 mg/l ZnSe şi cu 47-36%, în cazul concentraţiilor de 0,1-0,2 mg/l ZnSe. Pentru concentraţiile de 0,03-0,05mg/l ZnSe productivitatea este la nivelul probelor martor (90-95%), urmată de o scădere cu 38% în varianta aplicării concentraţiei de 0,06 mg/l ZnSe.

În seria experimentală cu utilizarea concentraţiilor de 0,1-0,6 mg/l ZnSe, cea mai mare productivitate a fost determinată în cazul concentraţiei de 0,03 mg/l ZnSe, care este de 94,6% faţă de proba martor.

Experienţele cu adăugarea nanoparticulelor de ZnS la mediul de cultivare a microalgei *Porphyridum cruentum* au pornit de la determinarea concentraţiilor maximale toxice pentru microalge. A fost stabilit că ZnS reduce productivitatea, indiferent de concentraţia utilizată, 8,0 mg/l nanoparticule generează o scădere a productivităţii cu 46% faţă de probele martor (fig. 4).

Concentraţiile mai mici au manifestat, de asemenea, un grad pronunţat de toxicitate, excepţie prezentând concentraţiile de 0,03 şi 0,2 mg/l, unde productivitatea a fost la nivelul probelor control.

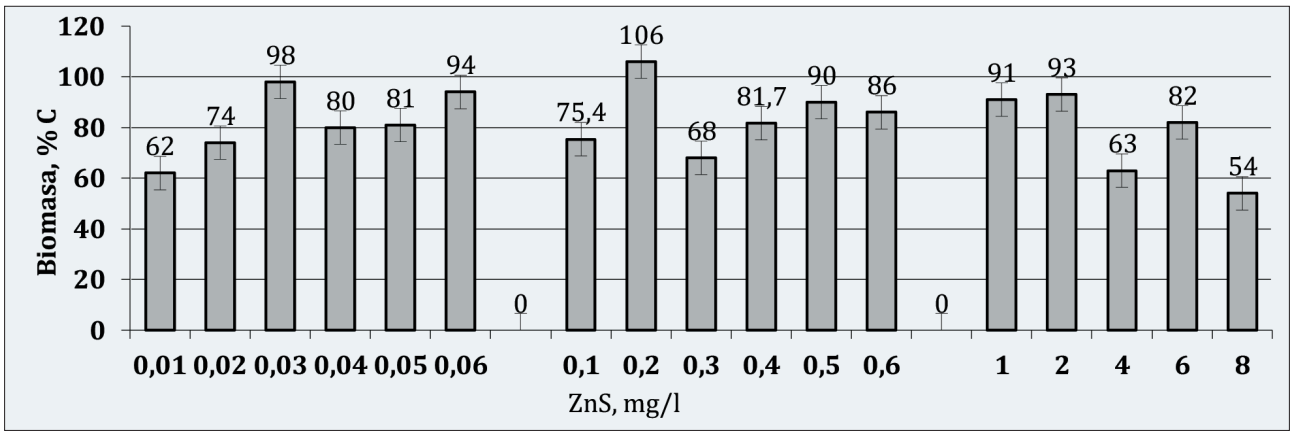


Figura 4. Biomasa *Porphyridium cruentum*, % faţă de proba martor (M) în prezenţa nanoparticulelor ZnS (0,01-0,06 mg/l; 0,1 – 0,6 mg/L; 1,0-8,0 mg/l).

Un efect de reducere a nivelului de acumulare a biomasei s-a manifestat în probele cu concentraţia nanoparticulelor de 0,01 (cu 35%), 0,02 (cu 25%), 0,1 (cu 25%) şi 0,3 (cu 32%).

Unul dintre cele mai semnificative teste ale evoluţiei stresului oxidativ în celulă este testul nespecific de determinare a produselor peroxidării lipidelor, testul dialdehidei malonice (MDA). Testul de acumulare a radicalilor acizilor graşi indică influenţa negativă a condiţiilor de cultivare asupra celulelor vii (fig. 5).

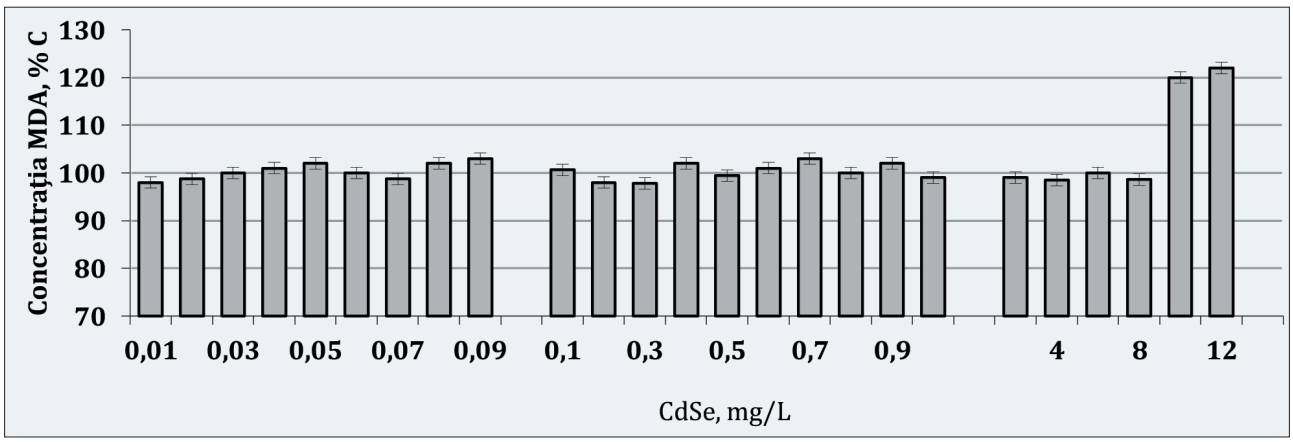


Figura 5. Dialdehida malonică, % C în biomasa de *Porphyridium cruentum* la cultivare în prezenţa nanoparticulelor CdSe.

Rezultatele obţinute demonstrează că pentru majoritatea concentraţiilor de CdSe aplicate nu are loc afectarea structurilor membranare ale porfiridiumului. Prin urmare nu se produce deteriorarea statutului oxido-reducător, cu formarea radicalilor lipidelor structurale. Concentraţiile toxice ale CdSe de 10-12 mg/l au indus oxidarea lipidelor membranare. Astfel, mecanismul toxicităţii nanoparticulelor de CdSe se manifestă prin implicarea lor în deteriorarea structurilor membranare. Radicalii formaţi participă activ în procesul biosintetic al algelor, urmat de micşorarea productivităţii.

Determinarea produselor de oxidare a lipidelor prin testul acumulării dialdehidei malonice a scos în evidenţă impactul toxic al nanoparticulelor ZnSe asupra celulelor prin inducerea proceselor de oxidare a lipidelor şi acumularea radicalilor acili. Rezultatele obţinute (fig. 6) indică o acumulare a dialdehidei malonice în celulele de porfiridium. Valorile testului MDA relevă o creştere cu 20-30%, comparativ cu proba de control.

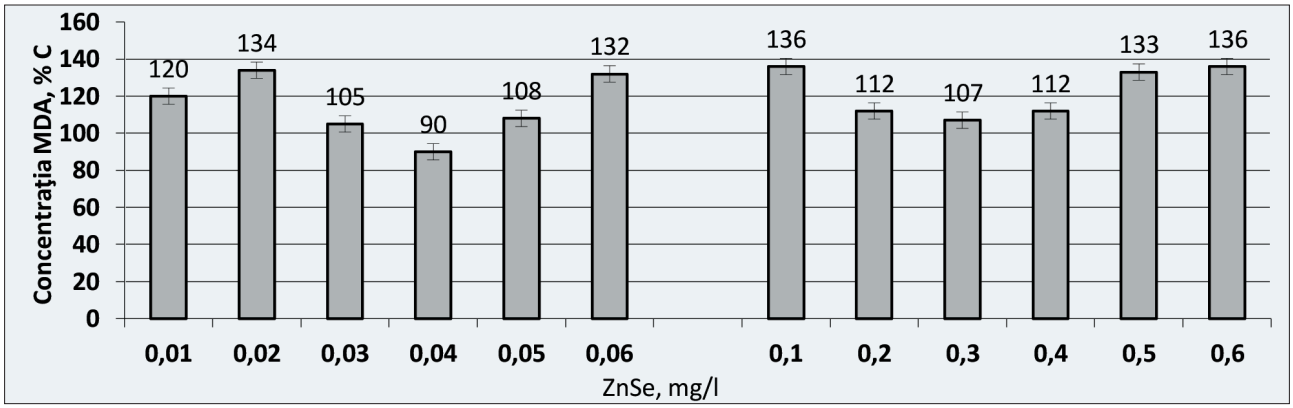


Figura 6. Dialdehida malonică, % C în biomasa de *Porphyridium cruentum* la cultivare în prezenţa ZnSeNP.

Prin urmare, toxicitatea nanoparticulelor ZnSe se manifestă prin implicarea lor în oxidarea lipidelor structurale ale membranelor celulare, ceea ce duce la modificarea permeabilităţii membranelor şi la afectarea proceselor metabolice celulare.

A fost constatată, de asemenea, o majorare a conţinutului dialdehidei malonice, produse în rezultatul aplicării concentraţiilor de 0,01-0,02 mg/l ZnS; 0,1 şi 0,3 mg/l ZnS şi 4-8 mg/l ZnS (fig. 7).

Valori mai mici ale testului MDA, comparativ cu proba de control, nu au fost determinate.

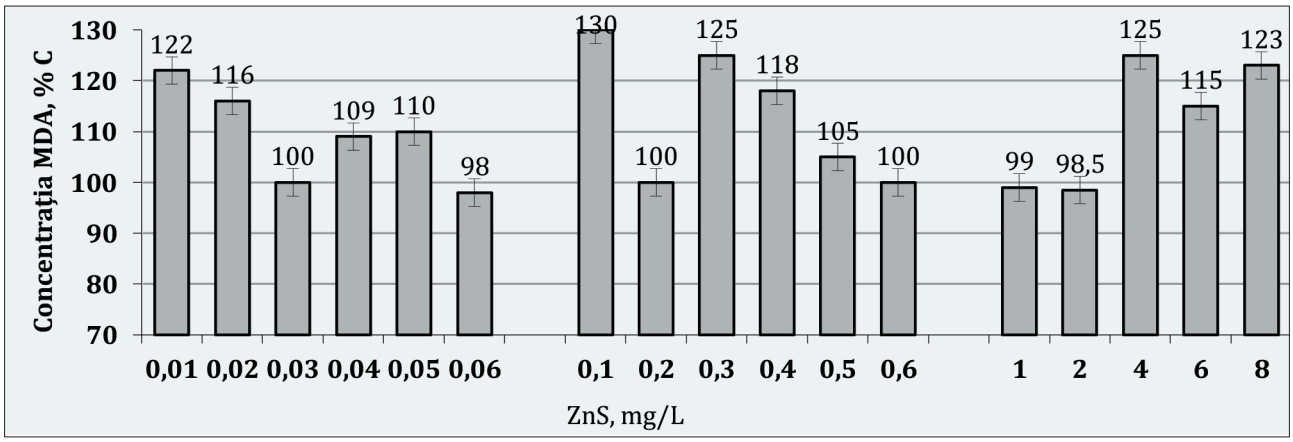


Figura 7. Dialdehida malonică, % C în biomasa de *Porphyridium cruentum* la cultivare în prezenţa ZnSNP.

## DISCUŢII

Analizând influenţa pe care o are concentraţia nanoparticulelor de CdSe asupra productivităţii culturii de *Porphyridium cruentum,* putem afirma că dependenţa dată, în limitele studiate, poartă un caracter de undă, cu efecte de stimulare a producerii de biomasă la unele concentraţii, urmat de

scăderi şi de creşteri ulterioare, fenomen atestat destul de frecvent în lumea vie.

Productivitatea microalgei obţinute, prin aplicarea nanoparticulelor de ZnS, este redusă, valorile testului MDA sunt crescute, prin urmare mecanismul acţiunii toxice este rezultatul implicării lor în activitatea biosintetică.

În scopul evaluării impactului nanoparticulelor asupra culturii de porfiridium, a fost calculat coeficientul de corelare între productivitate si cantitatea dialdehidei malonice.

Rezultatele prezentate în Figura 8 demonstrează că, în cazul manifestării reacţiilor toxice de către *Porphyridium cruentum,* se înregistrează o corelare inversă puternică dintre conţinutul de biomasă si valorile dialdehidei malonice, produsă în rezultatul peroxidării lipidelor.

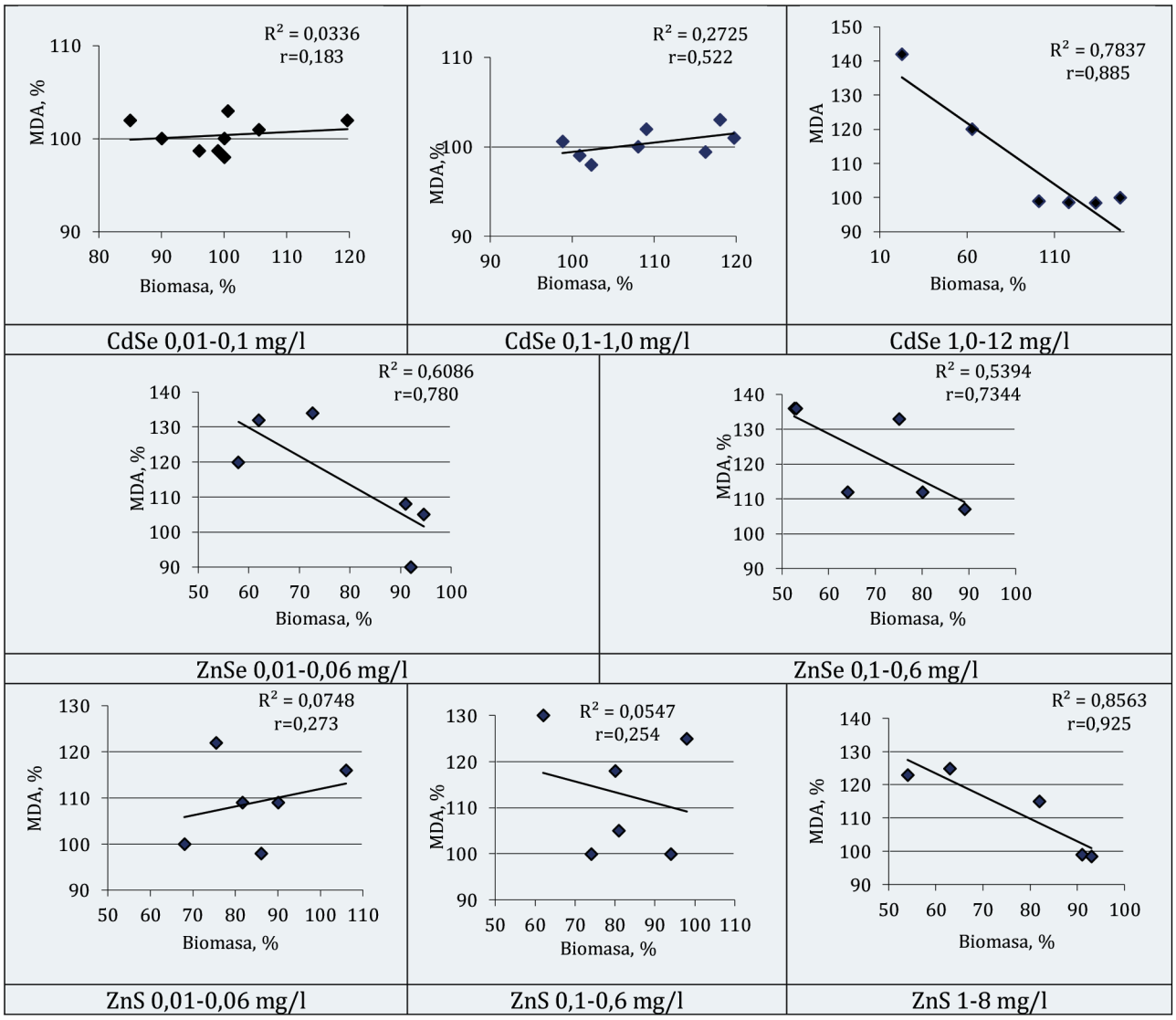


Figura 8. Corelarea între productivitatea culturii de porfiridium şi procesul de peroxidare a lipidelor în biomasă la acţiunea nanoparticulelor CdSe, ZnSe şi ZnS.

Au fost stabilite manifestări toxice pentru concentraţiile 1,0-12 mg/l CdSeNP, cu un coeficient de corelare r=0,885. Dependenţa corelaţională este una inversă, pentru care reducerea conţinutului de biomasă este asociată cu valori crescute ale dialdehidei malonice, determinate pentru concentraţia nanoparticulelor de peste 8,0 mg/l. Un raport corelaţional mare a fost stabilit şi pentru varianta experimentală, având concentraţiile CdSeNP între 0,1 şi 1,0 mg/l. În cazul dat se observă o corelare directă, unde conţinutului sporit de biomasă algală îi corespund valori joase ale testului MDA. Valorile moderat majorate ale dialdehidei malonice pot fi rezultatul unei activităţi biosintetice intensive, cu formare de specii reactive ale oxigenului, nefiind vorba despre manifestarea to-

xicității nanoparticulelor în concentrațiile determinate.

Coeficientul Pearson ridicat, cu valori de peste 0,7, a fost determinat în cazul aplicării nanoparticulelor ZnSe. Raportul corelațional este puternic și invers, reducerea conținutului de biomasă fiind asociată cu valori crescute ale dialdehidei malonice. Putem confirma existența efectului toxic al nanoparticulelor ZnSe, în limita concentrațiilor aplicate asupra microalgei.

Manifestări toxice au fost determinate pentru concentrațiile 1,0-8,0 mg/l ZnS NP, având coeficientul de corelare r=0,925 foarte puternic. Dependența corelațională este una inversă, pentru care biomasa redusă este asociată cu valori ridicate ale dialdehidei malonice. Coeficientul de corelare mic r=0,273 a fost stabilit pentru concentrațiile 0,01-0,06 mg/l și r=0,254, pentru concentrațiile de 0,1-0,6 mg/l a nanoparticulelor de ZnS. În cazul concentrațiilor 0,1-0,6 mg/l ZnS se stabilește corelarea inversă, astfel că putem releva manifestarea efetului toxic al nanoparticuleor în limita concentrațiilor date, ceea ce nu a fost stabilit pentru seria experimentală având concentrațiile de 0,01-0,06 mg/l ZnS NP.

## CONCLUZII

1. Dialdehida malonică poate fi considerată drept marcher al toxicității nanoparticulelor. Metoda nespecifică de determinare a procesului de peroxidare a lipidelor structurale permite a stabili efectul toxic al nanoparticulelor studiate, în cazul existenței unei corelări dintre cantitatea de dialdehidă malonică și biomasa acumulată.

2. Toxicitatea tipurilor de particule menționate descrește în șirul ZnSe >ZnS>CdSe.

3. Nivelul înalt de corelare între cantitatea de biomasă și produsele degradării oxidative a lipidelor evidențiază mecanismul acestei influențe, care constă în degradarea membranelor biologice, în modificarea permeabilității și în dereglarea proceselor vitale.

## REFERINȚE

1. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem. 2019; 12(7):908-931.
2. Wang EC, Wang AZ. Nanoparticles and their applications in cell and molecular biology. *Integr Biol (Camb).* 2014*; 6*(1):9–26.
3. Blasiak B, van Veggel FCJM, Tomanek B. Applications of Nanoparticles for MRI Cancer Diagnosis and Therapy. *J Nanomater* 2013, Article ID 148578, doi: org/10.1155/2013/148578.
4. Prasad M, Lambe UP, Brar B, Shah IJM, Ranjan K, Prasad G. Nanotherapeutics: An insight into healthcare and multi-dimensional applications in medical sector of the modern world. *Biomed Pharmacother*. 2018; 97:1521-1537.
5. Dubey A, Goswami M, Yadav K, Chaudhary D. Oxidative Stress and Nano-Toxicity Induced by TiO2 and ZnO on WAG Cell Line. In Vitro Toxicity Evaluation of Metallic Nanoparticles. *PLoS ONE.* 2015; 10(5). doi.org/10.1371/journal.pone.-0127493.
6. Ma J, Wong H, Kong LB., Peng KW. Biomimetic processing of Nanocrystallite bioactive apatite coating on titanium. *Nanotechnology.* 2003*;* 14:619-623.
7. De la Isla A, Brostow W, Bujard B, Estevez M, Rodriguez JR, Vargas S, et al. Nanohybrid scratch resistant coating for teeth and bone viscoelasticity manifested in tribology. *Mat Resr Innovat.* 2003*;* 7:110-114.
8. Parak WJ, Boudreau R, Gros ML, Gerion D, Zanchet D, Micheel CM, et al. Cell motility and metastatic potential studies based on quantum dot imaging of phagokinetic tracks. *Adv Mater.* 2002*;* 14:882-885.
9. Savage DT, Hilt JZ, Dziubla TD. In vitro methods for assessing nanoparticle toxicity. *Methods in Molecular Biology*. 2019; 1894:1–29.
10. Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of nanoparticles and an overview of current experimental models. *Iran Biomed J*. 2016; 20(1):1-11.
11. Rebolloso MM, Fuentes GG, Acién Fernández JA, Sánchez Pérez JL, Guil G. Biomass nutrient profiles of the microalga Porphyridium cruentum. *Food Chemistry*, 2000**;** 70(3): 345-353.
12. Hodges DM, DeLong JM, Forney F, Prange RK. Improvind the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*. 1999; 207:604-611.

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ARTICOLE DE SINTEZĂ – ОБЗОРНЫЕ СТАТЬИ – SYNTHESIS ARTICLE – ARTICLES DE SYNTHÈSE



# MEDICAL AND SOCIAL ASPECTS OF FLOODS AND THEIR MEDICAL RISK MANAGEMENT

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***Key words:*** *flood, calamities, victims, medical management.*

***Introduction.*** *Currently, there is a steadily increasing trend in losses due to flooding. Nevertheless, global warming, followed by an inevitable overuse of river valleys might further contribute to an increase in the frequency and destructive power of floods. It is quite important to trace the cause and effect chain of the economic, social and ecological impacts during the flood hazard.*
***Material and methods.*** *A bibliographic research was carried out by using historical and descriptive methods based on the keywords, underlining the medical, social and economic significance of the present issue.*
***Results.*** *There are 57 natural lakes and about 3 400 artificial water reservoirs found on the territory of the Republic of Moldova, including 90 amounting to over 1 million $m^3$ per each. The accumulation basins help in preventing and combating overflows and flooding during spring and summer periods. Most of the dams do not comply with the technical norms, as they do not have channels of respite and drainage, thus, a dam failure on top dike might generate the collapse of the others at the lower watercourse, resulting in disastrous consequences. Therefore, individual health protection measures and appropriate population behavior are vitally important in catastrophic floods.*
***Conclusions.*** *Over the last decades, the floods occurring in the Republic of Moldova have conditioned the need to increase interventions for prevention and reduction of both economic and human losses both at national and international levels.*

***Cuvinte cheie:*** *inundații, calamități, victime, management medical.*

**ASPECTE MEDICO-SOCIALE ALE INUNDAȚIILOR, MANAGEMENTUL MEDICAL ŞI AL RISCURILOR**
***Introducere.*** *În prezent, există o tendință de creștere a prejudiciilor provocate de inundații. În același timp încălzirea globală a climei și creșterea inevitabilă a va-lorificării văilor râurilor va contribui pe viitor la creșterea frecvenței și puterii distructive a inundațiilor. În timpul inundațiilor, este importantă constituirea lanțului cauză-efect a consecințelor economice, sociale și ecologice.*
***Material și metode.*** *Au fost studiate surse bibliografice care redau informații ample cu referire la inundațiile ce au avut loc în Republica Moldova și în regiunile din vecinătate.*
***Rezultate.*** *Pe teritoriul Republicii Moldova sunt amplasate 57 de lacuri naturale și circa 3400 de rezervoare artificiale de apă, inclusiv 90 cu un volum de peste un milion de $m^3$ fiecare. Lacurile de acumulare servesc pentru prevenirea și combaterea revărsărilor și inundațiilor în timpul viiturilor de primăvară și vară. Barajele multora din ele sunt construite fără respectarea normelor tehnice, nu au canale de degrevare și scurgere, de aceea ruperea unuia, în partea de sus, generează ruperea celorlalte din cursul inferior, urmările fiind catastrofale. O importanță deosebită în inundațiile catastrofale au măsurile de protecție medicală individuală și comportamentul corect al populației.*
***Concluzii.*** *Inundațiile din ultimele decenii în Republica Moldova au condiționat necesitatea intensificării activităților de prevenire și minimizare a consecințelor materiale și umane, atât la nivel național cât și internațional.*

## INTRODUCTION

Humanity faced flooding since ancient times, a fact confirmed by the biblical Great Flood. According to Robert Ballard, a marine explorer, there is an evidence of a catastrophic flood that happened 7500 years ago in the Black Sea at about 170 meters deep that is possible effects of "Noah's Flood".

Floods are both natural disasters and technological and social phenomena (1), considered the most widespread hazard on earth. According to the Centre for Research on the Epidemiology of Disasters of the Catholic University of Louvain (Belgium), they make up 37% of the total number of natural hazards resulting in severe outcomes and extremely severe human losses with considerable economic damages, followed by negative economic, social and ecological impacts (2, 3, 4).

The study analysis of the floods throughout the last century confirms a steadily increasing tendency of damages caused by floods worldwide, one of the main causes being the irrational use of the river valleys and the increase of the economic activity in risk-prone areas (5). Nevertheless, global warming and the inevitable overuse of river valleys might further contribute to an increase in the frequency and destructive power of floods. Floods can be caused by natural phenomena such as: water flows (flash floods, clogging of the running water streams and sloping into the riverbed, crowding of glaciers or other floating objects in narrow curves or sections, the increase of the sea or ocean level in the area of the watercourse overflow due to high tides or strong winds); rain or stagnant melting snow, dripping from the slopes; an increase in groundwater level due to high infiltration rates; hurricanes or typhoons; and submarine volcanic eruptions. Floods triggered by accidental scenarios are due to a dam failure and collapse or other hydrotechnical constructions, as well as due to inappropriate and non-compliant actions related to the hydrological situation at the water drainage systems and sudden sliding slopes into the accumulation basins.

The human-induced floods occur due to the overfilling of the artificial basins, the deliberate cutting of the dam defense structures, inadequate irrigation system performance, characterized by great water loss with no proper drainage measures, and earthquake-triggered floods due to accumulations (6).

## MATERIAL AND METHODS

The purpose of the bibliographic study was to underline both the medical and social aspects and flood-health risk management. The studied bibliographic sources provide ample data on floods occurring both in the Republic of Moldova and in the neighboring regions. A bibliographic study was carried out by using the historical and descriptive methods based on the keywords, emphasizing the medical, social and economic impacts of the present problem.

## RESULTS

Catastrophic floods make up about 33-35% of total floods. They can result from heavy rainfalls; overflow of accumulation basins of hydroelectric stations and lakes; sudden melting of snow and glaciers; and coastal water waves and tsunami. Dam and levee failure might cause landslides and earthquakes, etc. The size of the flood outbreak depends on the amount of water, the affected area and the rainfall duration and intensity.

There are several types of floods depending on their triggering factors and the flood-affected areas. The most commonly occurring floods are as following (7,8):

- fluvial flooding is the overflowing of water body, generated by a high flow over the boundaries of the minor riverbed to the major one due to several causes, such as: heavy rainfall, an increased water level, resulting from the degradation of the riverbed by alluvium, ice blockages, dam and levee collapse etc.;
- coastal flooding might affect the coastal areas, caused by strong storms, which trigger large wind waves that might sometimes lead to a dam failure and storm surges, magnified by rising sea levels. Depending on the generating factors, coastal areas might be flooded with salty or brackish water;
- urban flooding occurs due to improper sizing or poor maintenance of urban drainage systems, which cannot provide a proper rainwater runoff during heavy rainfall episodes.

Other types of floods occur due to accumulation of the running water in the low-lying areas at the base of the slope and because of groundwater excess (in areas where groundwater is close to the topographic surface).

The most severe floods that have caused major human losses were as following:

China (1931) – 3,700,000-4,000,000 victims,
China (1887) – 900,000-2,000,000 victims,
China (1938) – 500,000-700,000 victims,
China (1975) – 231,000 victims,
Indonesia (2004) – 230,000 victims,
China (1935) – 145,000 victims,
Vietnam (1971) – 100,000 victims,
China (1911) – 100,000 victims.

The Republic of Moldova has 57 natural lakes and about 3400 artificial water reservoirs, including 90 with over one million $m^3$ per each (3, 10). Accumulation basins might prevent and combat overflows and floods during spring and summer seasons. The largest storage tanks are found in Costinesti Stînca (735 mln $m^3$), situated on the Prut river and in Dubăsari (277.4 mln $m^3$), on the Dniester river. Most dams do not comply with normal technical standards and do not have respite and drainage channels, thus, a dam failure on top dike might generate the collapse of the others at the lower watercourse, resulting in disastrous consequences. In 1991, the heavy torrential rains led to catastrophic floods in Şoldanesti, Orhei district. As a result, 21 people died, 8 thousand houses were damaged, of which 516 were completely destroyed, and 400 thousand ha of agricultural land was flooded. In 1994, the Republic of Moldova experienced one of the most unfavorable episodes throughout the last century (11).

On August 26-27, 1994, the downpour intensity was over 40 mm/hour, accompanied by strong winds and hail, which caused enormous damage ($ 100 million) and human comorbidities (29 people).

Over the last years, the major floods occurring on the territory of the Republic of Moldova were on June 16-17, 2003 and on August 7, 18-19, 2005, being caused by heavy rainfalls. These led to stream flows, as well as the formation of intensive leakage from the slopes, causing huge financial losses within different national economic sectors. The total land area of the Republic of Moldova, which are periodically flooded makes up about 20% of the entire country's surface or more than 600 thousand ha (3, 11).

At the end of May and the beginning of June 2016, following the heavy lasting downpours across Central Europe, the floods affected dozens of locations in Austria, Belgium, France, Germany, the Republic of Moldova and Romania (1). On June 1, torrential rains caused massive flooding in the Republic of Moldova. A great number of streets and basements of flat blocks were flooded in Chisinau, including Albisoara Street, where the water was over one and a half meter high due to the sewer system failure. 200 houses were flooded in Iargara village, Leova district. The water flooded into the courtyards and borderlines of dozens of houses in Logăneşti, Hînceşti district (6).

In June-August 2008, the Ukraine, Romania and the Republic of Moldova were hit by one of the worst floods over the last two centuries. In July 22-28, 2008, in Western Ukraine, where the Dniester and Prut upper courses are located, the amount of precipitation was 63-260 mm, which is 1-3-month norm fell. In the third decade of July 2008, precipitations fell everywhere on the territory of the Republic of Moldova. The largest amount of precipitation during the decade, was 225 mm in Ocniţa, exceeding 10 times the de-cade normal amount, which was first reported during the entire period of instrumental mea-surements. In the northern and in some of the central and southern regions of the republic the amount of precipitation per decade made up 85-185 mm or 440-800% of the decade norm. The remainder of territory amounted precipitation of 15-70 mm or 100-420% of the decade norm. As a result, the floods occurring during the period from July to August 2008 on the Dniester and Prut rivers were historical flash floods (6, 11, 12, 13).

It is crucial to establish the cause and effect chain during flooding while considering the following aftermath (13, 14, 15, 16):

- economic destruction or damage of: industrial buildings, roads and railways, locations, oil, water or gas system supply, electrical and telecommunications lines, bridges and culverts, and the zootechnical sector;
- negative social impacts: human comorbidities, people evacuation, risks of epidemics, educational process disruption, damage to cultural assets, panic disorder, reducing the disaster rate of flood-affected areas and income of the population;
- negative ecological consequences: environmental degradation, pollution of the surface or groundwater, soil pollution, excess humidity, slope degradation, destruction of fauna and flora.

Additionally, to the direct effects, there is a number of indirect effects that might interrupt the manufacturing processes, delay goods delivery, trigger expenditures for defense works and for normalization of life after floods, as well as reduce exports.

Most floods might lead to considerable financial and human losses, being termed as catastrophic flood events, faced by a community or region. For a better objective determination of financial and human losses, the territory is divided into four flood zones with the following determinants: the height of the water level, the flood onset, the water drainage velocity and the flooded surface area (17).

The catastrophic floods occurring on large areas might influence the strategies of the health and civil protection bodies and the healthcare services of the Ministries and Departments of defense appropriations for liquidation of disaster consequences, as well as on the organization and use of the trained forces and means of medical assistance. In these cases, great attention is paid to the flooded land surface, since the population might be deprived of sheltering, food and water supply, as well as of medical or social assistance that should be commonly provided during the first flood hours (hours, days). The population in this area might be subjected to the action of low-temperature water, winds, air humidity and other meteorological factors (18).

A series of factors might influence the volume and structure of health losses in catastrophic flood outbreak, such as: timely notification of the population in the area at risk of flooding; the degree of preparedness for people evacuation; the population density in the risk-prone areas; the building patterns; the day time (night, day); season; the distance between different locations (village, city) and the dams; the flood wave height and duration, water temperature; exacerbation of area – based socio-economic status (19).

The efficiency of medical assistance and the use of the trained forces and means of emergency healthcare services will increase considerably, if the flood outbreak is divided conventionally into four zones (9). The volume and structure of the health losses (tab. 1) vary depending on the speed of the water, the height of the water wave, the distance from localities to the hydrological object with flood phenomenon (typhoon, tsunami, disturbances of the sea, the ocean, etc.) The amount and structure of the health losses (tab. 1) vary according to water velocity, water wave height, the distance between the locations and the flood-related hydrological objects. (typhoon, tsunami, sea and ocean disturbances, etc.)

The volume and structure of the health damages (tab. 2) will differ in different floodplains depending on the day and night time.

Table 1. Characteristics of flood pains.

| Flood plains | Distance between the dam and water wave | Water wave height | Flow velocity | Wave duration |
|---|---|---|---|---|
| **I. Catastrophic flood** | 6-12 km | > 3 m | > 30 km /h | 30 min |
| **II. Fast flow** | 15-20 km | 1.5-2 m | 15-20 km/h | 50-60 min |
| **III. Medium flow** | 30-50 km | <1 m | 10-15 km / h | 2-3 hours |
| **IV. Slow flow (overflow)** | 36-70 km | - | 6-10 km / h | 5-6 hours |

In order to eliminate the catastrophic effects of floods, the local healthcare services (civil protection, the Ministry of Health, Labor and Social Protection) develop early health-action planning for medical assistance of the population and the affected – area victims.

The presidents of the commissions for exceptional affairs will administer the Special Forces and the emergency medical assistance services that help in evacuation and rescue of the population, and who otherwise administer the local institutes, district and republican medical esta-blishments.

It is highly important to be aware of the sanitary and epidemiological conditions of the risk-prone areas for the liquidation of the aftermath calamities, it might worsen due to the destruction of the water supply and sewerage systems, the pol-lution of the wells etc.

Wastes and garbage that spread to other neighboring places might pose a threat on spreading infectious morbidity within the affected floodplain.

Overcrowding of people on a small area, as well as exacerbation of the social and economic status can also cause an outbreak and spread of communicable infections and zoonotic diseases (decay of animal bodies and rodents).

Additionally, to civil protection and Ministry of Health, Labor and Social Protection means, the liquidation of aftermath catastrophic floods will require medical services of the Armed Forces, according to the relevant directive documents, available in the National Army and documents, regulating medical assistance under exceptional circumstances. Specially trained teams will provide premedical aid (paramedics) and first medical aid (doctors) during the outbreak.

Table 2. The characteristics of the losses within the flood plains (in %, according to the number of population in the flood-affected areas).

| Flood plains | Overall losses | | Out of the total loss amount | | | |
|---|---|---|---|---|---|---|
| | day | night | Irretrievable losses | | Health losses | |
| | | | day | night | day | night |
| I | 60.0 | 90.0 | – | – | – | – |
| II | 13.0 | 25.0 | 10.0 | 20.0 | 90.0 | 80.0 |
| III | 5.0 | 15.0 | 7.0 | 15.0 | 93.0 | 85.0 |
| IV | 2.0 | 10.0 | 5.0 | 10.0 | 95.0 | 90.0 |
| Mean loss value | 20.0 | 35.0 | 15.0 | 30.0 | 85.0 | 70.0 |

Persons joining the immediate rescue operations of the victims must be well trained and provided with various salvation (belts, circles, lifejacket) and floating means (boats). Emergency medical assistance brigades (paramedics and doctors), mobile detachments of the Ministry of Health, Labor and Social Protection, as well as Ministries of Armed Forces and Civil Protection will also be activated during the outbreak.

Specially trained teams and health-epidemic assistance brigades from various preventive medicine centers (district, city, republican) will be provided for flood- affected areas.

In many cases, flooding can be a very serious testing of the community's responsiveness. The ability and capacity to effectively cope with a flood has become increasingly relevant nowadays due to a higher-risk flood tendency, especially in recent years.

The major objective of risk identification refers to flood risk management (20). It determines the application of policies, procedures and even practices that would tend towards this objective. The flood risk management is aimed at analysis and assessment, treatment, monitoring and re-assessment of risks in order to reduce them, so that human communities and all citizens would survive, work and meet their needs and aspirations within a sustainable physical and social environment.

The experience of Moldova on the reduction of negative flood aftermaths in small river basins, indicates that the optimal economical effect can be achieved when applying both the passive methods (digging, forest improvement works, etc.) and the active methods of protection by regulating the water flowing into a system of maximum water storage capacity tanks, followed by a subsequent water evacuation amounted at a 1-3% insurance volume (19).

The planning schemes for flood mitigation works in the river basins consist of correlative and cumulative effects of the following works: embankments, works for the regularization of the riverbeds, non-permanent accumulations and polders, permanent accumulation basins provided with volume-control storage to mitigate floods.

The first stage on reducing floods by embankments was actually a mistake because it eliminates the natural effect of mitigation of meadows and leads to the increase of maximum overflows. As the lower sectors of the large watercourses are embanked, new embankment works should be avoided without being compensated in the accumulation basins or into polders.

Although most of the extreme phenomena cannot be completely overcome yet, a prior knowledge on the area patterns and the possible intervals between the disaster episodes would significantly reduce their destructive impact (21). Farmers should be aware and familiar with the achievements of modern science, as well as timely access

of qualitative forecasting services, which would ensure a considerable increase in the productivity of agricultural crops, by significantly reducing the losses and negative consequences of various natural hazards.

Currently, the perfection of the long-term weather forecasting methods on negative phenomena occurrence has become vitally important for the Republic of Moldova since both the amplitude and the incidence rate of the natural disasters have obviously increased over the last years. This step can be defined and highlighted as regional trends of climate change and variability that refers to long-term climate predictions, being categorized as more qualitative, rather than quantitative ones (including the daily weather forecasts) (22, 23).

In order to reduce the risk of flooding, the following measures are recommended:

- Mapping of the land surfaces and river course that are mostly exposed to flooding, as well as the quasi-horizontal surfaces of the plains, where the water can stagnate during periods of excess humidity.
- Overall embankment works of the major riverbeds and meadows, as well as polder formation should be avoided in order to provide "breathing" spaces during flood periods.
- Anti-erosion works in the accumulation basins should be carried out, as well as the unclogging of minor riverbeds.
- Any kind of construction near the minor ri-verbeds, which are at risk of flooding and are marked red, should be prohibited. Strong involvement of local and national authorities regarding the environmental protection is required, in order to apply flood prevention measures to protect the population and environment.
- Information system efficiency regarding the warnings for hydrometeorological hazards.
- Education of the population on the protection of the riverbeds from solid wastes pollution.

The Republic of Moldova has developed the "Regulation on Flood risk Management" that provides (24):

- Normative framework for flood risk management.
- Preliminary Flood Risk Assessment.
- Flood hazard and flood risk mapping.
- Flood Risk Management Planning.
- Public consultations and plan approval and revision.
- Reports on implementation action plans.

The measures on population individual health protection and appropriate behavior is of particular importance in catastrophic floods. Prior to a flood episode, the population should be trained and urged to follow and carry out certain recommendations:

- To show interest in the possible local floodplains, found at the town halls.
- To avoid building houses nearby the risk-prone flood areas.
- To use waterproof walls for the basements in order to prevent water flow.
- To build barriers to prevent water from flooding into the houses.
- To clean the ditches and storm sewers, to prevent clogging of the rainwater.
- To safely dispose the household waste and vegetable debris not to contaminate the running streams.
- To prepare the emergency backpack in advance.
- To participate to simulated emergency exercises organized by emergency professional services and local authorities.
- To learn about the warning alarm signals and the evacuation procedures.
- In case of imminent flood risk, to turn off the heating, gas and electricity devices.
- To place the toxic substances in a safe place so as to avoid pollution.

In case of flooding:

- To stay calm.
- To warn the neighbors and support the disabled people, children and the elderly.
- To be aware about the danger and its evolution.
- To use the phone only in emergencies as not to overload the network.
- To follow the radio or television broadcast recommendations given by the authorities.
- In case of evacuation, to move towards an immediate or nearby high level area, specified by the authorities; in case of flash floods, people should move up towards the upper parts of the house or on the roof, until the rescue teams arrive.
- To move the essential objects to the upper floors of the house, if there is enough time.
- To disconnect the general power switch. To disconnect all the electrical devices. Not to touch the electrical devices if they are dirty or in water.

- To switch off water and gas supply systems.
- To evacuate animals and valuable assets into specific prior- established refuge places.
- To avoid moving through the water flows, as people might lose the balance in about 15 cm high waters.
- To avoid traveling by car across the flooded area: in case of about 20 cm of floodwaters, water may enter the vehicle, resulting in loss of control. At about 40 cm of floodwaters, the vehicle starts floating. In 60 cm of water, most vehicles are carried away by floodwater.
- To be prepared of quick evacuation at short notice of those in charge of rescue operations.
- To use the itinerary indicated by those in charge.
- To avoid shortcut pathways as not to get into a dangerous place.
- To take only the strictly necessary belongings (identity documents, medicines).

After the flooding:

- To help the injured.
- To listen to local authorities regarding the information on the local drinking water supply.
- To avoid areas with still water: the water may be contaminated with gasoline, diesel or sewage debris; it can also be electrically charged with high-voltage power lines or grounded electrical cables.
- To avoid water currents.
- To learn about the water withdrawal areas: roads or bridges may exhibit low resistance and collapse due to vehicle overloading.
- To be careful when entering the flooded buildings since their resistance might be affected, particularly the foundations.
- To avoid using the electricity, water, gas supply equipment unless specialized services approve.
- To clean and disinfect all objects that have been in contact with floodwater: there is a life-threatening situation of disease contamination due to the lack of hygiene and the affected sewers.

If any suspicions arise regarding the contamination of the drinking water, bottled or boiled water should be used.

Prevention and reduction of the negative effects, as well as disaster preparedness, including flooding are crucially important issues and policies promoted by the World Health Organization (25, 26) – a specialized agency within the United Nations that is responsible for coordinating global health problems. Another key aspect of the "One health" approach concerns the need to include the health institutions, all the available private sectors, the military medical services, etc., within the planning process. Risk reduction and emergency preparedness are the responsibility of all national actors. At the national level, the Ministry of Health, Labor and Social Protection is the leading agency responsible for the healthcare sector, which apart from the medical services of the Armed Forces, includes the Red Cross society, the non-governmental healthcare organizations, the private health facilities and professional associations. Emergency preparedness requires a multi-sectoral approach. This approach might be applied to all types of emergencies and crises including those of health origin such as major epidemics, food poisoning, waterborne diseases or toxic chemical leaks and spills.

## CONCLUSIONS

1. Floods remain some of the most frequent and powerful natural phenomena that have aggravated the situation of many countries, including the Republic of Moldova, especially in the last decades and have conditioned the need to increase interventions for prevention and reduction of both economic and human losses both at national and international levels.

2. While assessing the tremendous increase in direct flood damages, the expenses involved in their liquidation and rehabilitation of the affected population, as well as the experience of the developed countries worldwide, we can conclude that the problem of the flood risk management requires enormous financial costs that show an unprecedented growth rate along with the increased level of security.

3. The major purpose of the health care system, while liquidating the medical flood consequences, is to provide primary emergency medical care to the injured, according to the medical indications and to evacuate people safely to health-care institutions away from the flood outbreak.

4. Since floods, particularly the catastrophic ones, lead to a sudden exacerbation of the sanitary-hygienic and anti-epidemic status within the flooded areas, medical assistance to the injured, along with sani-

tary-hygienic and anti-epidemic measures should be necessarily carried out by both the regional and national health care systems, for the purpose of maintaining the health status of the affected population as well as to prevent the occurrence and spread of infectious diseases.

## CONFLICT OF INTERESTS

All authors declare no competing interests.

## REFERENCES

1. Boian I. Ploile torenţiale abundente – fenomen de risc pentru Republica Moldova. *Materialele Conferinţei Internaţionale Diminuarea impactului hazardelor naturale şi tehnogene asupra mediului şi societăţii. Ministerul ecologiei şi resurselor naturale.* Chişinău: Academia de Ştiinţe a Moldovei; 2005.
2. Cazac V, Boian I, Prepeliţă A. Principalele tipuri de hazarduri naturale şi impactul lor asupra mediului şi societăţii. *Mediul Ambiant*. 2005; 5:18-25.
3. Bogdan O, Marinică I. *Hazarde meteo-climatice din zona temperată. Factori genetici şi vulnerabilitate cu aplicaţii la România*. Sibiu: Ed. Lucian Blaga, 2007.
4. Ciulache S. *Dezastrele naturale în contextul dezvoltării globale. Riscuri şi catastrofe*. Cluj-Napoca: Ed. Casa Cărţii de Ştiinţă, 2005.
5. Bogdan O. Caracteristici ale hazardurilor/riscurilor climatice de pe teritoriul Romaniei. *Mediul Ambiant.* 2005; 5(23):26-36.
6. Domenco R. Riscuri pluviale din sezonul cald pe teritoriul Republicii Moldova. *Materialele Conferinţei Ştiinţifice cu participare Internaţională, Biodiversitatea în contextul schimbărilor climatice*. Chişinău. 2016; 126-129.
7. Cazac V, Boian I. Riscul inundaţiilor în Republica Moldova. *Revistă ştiinţifică, de informaţie şi cultură ecologi*că. 2008; 4(40):43-48.
8. Stănescu V, Drobot R. *Măsuri nestructurate de gestiune a inundaţiilor*. Bucureşti; 2002.
9. Şteiner N, Mănăstireanu D. *Managementul medical al dezastrelor*. Bucureşti; 2003.
10. Cazac V, Boian I, Volontir N. *Hazardurile naturale. Colecţia Mediul geografic al Republicii Moldova*. Chişinău; 2008.
11. Constantinov T, Nedealcov M. et al. *Calamităţile naturale. Starea Mediului în Republica Moldova în anul 2005 (Raport Naţional).* Chişinău, 2006.
12. Boian I. Hazarde meteo-climatice din zona temperată. Geneză şi vulnerabilitate cu aplicaţii la România. *Revistă ştiinţifică, de informaţie şi cultură ecologică*. 2008; 5(41):47-48.
13. Domenco R. *Dinamica precipitaţiilor excedentare pe teritoriul Republicii Moldova în anii 1960-2015*. tz de doct. Chişinău. 2017.
14. Mihailescu C. *Clima şi hazardurile Moldovei – evoluţia, starea, predicţia*. Chişinău; 2004.
15. Mihailescu C, Boian I, Galiţchi I. Hazardurile climatice. *Revistă ştiinţifică, de informaţie şi cultură ecologi*că. 2007; 5(35):39-43.
16. Nedealcov M. *Resurse agroclimatice în contextul schimbărilor de climă*. Chişinău; 2012.
17. Gavrilescu M. *Estimarea şi managementul riscului*. Iaşi: Ed. ECOZONE, 2008.
18. Melniciuc O, Lalîkin N, Bejenaru G. *Probleme de studiu a inundaţiilor în Republica Moldova*. Chişinău: CIAPI-Moldova; 2002.
19. Dumitraş V, Dediu I, Cîrstea N, Grigorean D, Cebotar D. *Managementul medical al dezastrelor*. Chişinău; 2010.
20. Boian I. Riscul ploilor torenţiale abundente în Republica Moldova. *Revistă ştiinţifică, de informaţie şi cultură ecologică.* 2009; 3(45):43-45.
21. Goţiu D, Surdeanu V. *Notiuni fundamentale în studiul hazardelor naturale*. ClujNapoca: Presa Universitară Clujeană, 2007.
22. Boian I, Sandu M. Inundaţiile pe teritoriul Republicii Moldova şi măsurile de reducere a lor. *Mediul ambiant*. 2006; 2(26):47-48.
23. Boian I, Serenco L, Bejenaru Gh, Moldovanu N. Evaluarea inundaţiilor catastrofale din vara anului 2010 pe teritoriul Republicii Moldova. *Serviciul Hidrometeorologic de Stat*. Available from: http://old.meteo.md/mold/inundatii.htm [Accessed 21 November 2019].
24. Capcelea A. Managementul riscurilor hazardelor: abordarea integrativa a Bancii Mondiale. *Mediul Ambiant.* 2005; 5(23):42-49.
25. https://www.who.int/water_sanitation_health/publications/9789241598422_cdrom/en/ [Accessed 10th January 2020].
26. https://www.who.int/news-room/fact-sheets/detail/climate-change-and-health [Accessed 10th January 2020].

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OPINII ALE EXPERȚILOR – МНЕНИЯ ЭКСПЕРТОВ – EXPERTS› OPINIONS – AVIS DES EXPERTS



# STRENGTHENING GLOBAL BIOSAFETY & BIOSECURITY

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## INTRODUCTION

The International Federation of Biosafety Associations (IFBA) is a global community of 46 biosafety associations, 774 certified biosafety professionals, and other partners with the mission of "*safe, secure and responsible work with biological materials*". The IFBA works globally to enhance multi-sectoral collaboration and foster partnerships between its members, government ministries, and other stakeholders in the deve-lopment and implementation of comprehensive national biosafety/biosecurity strategies. This includes the implementation of the Global Health Security Agenda's Action Package 3, International Health Regulations, UN Security Council Resolution 1540, Biological Weapons Convention, and other health security initiatives.

The effectiveness of public health functions including surveillance, diagnosis and research are influenced by reliable laboratory services, of which biosafety and biosecurity are central elements. Yet, many laboratories still lack sufficient biosafety and biosecurity practices, equipment and infrastructure to conduct their work in a safe and secure manner. Safe disposal of potentially contaminated laboratory waste also remains a challenge. Attention must be drawn to the serious dangers that can arise from the fai-lure to implement effective biosafety and biosecurity and, importantly, must highlight the signi-ficant benefits offered by the implementation of sound biosafety and biosecurity practices including:

- minimising the socio-economic impact of human and animal disease outbreaks and better protection of laboratory staff, the wider community and the environment; and,
- contributing to better biosecurity through control of access to dangerous pathogens, tracking their use, and improving reporting of incidents.

The IFBA and its members are assisting national authorities in integrating biosafety into policies and programs, to improve sustainable laboratory infrastructure and equipment, and to increase biosafety skills and competencies among those working with infectious diseases. Our objective is to build and operate safe, yet, cost-effective laboratories and prevent laboratory exposures to infectious diseases. IFBA's members understand local challenges in operating safe laboratories and are facilitating simple, yet, effective approaches that can be cost-effectively sustained over the long term. Rather than simply taking a high technology approach of focusing on engineering and equipment, approaches to laboratory containment facilities must balance engineering controls with operational, scientific and management controls. There is no "one size fits all" approach to BSL-2 and BSL-3 facilities. Every biocontainment laboratory should be based primarily on a solid risk assessment and the specific laboratory program requirements.

### Bridging the Policy Implementation Gap

While some progress is being made in the deve-lopment of national biorisk management and biosecurity strategies, there is an increasing awareness on whether these policies do not succeed or fail on their own merits; rather their progress is dependent upon the process of implementation (i.e. turning policy into practice). The space between government's motivation behind the passage of new legislation and how that intent is translated into reality in many countries becomes an implementation gap when that policy remains on paper only or is implemented poorly. More needs to be done to try to ensure intentions for the safe and secure handling of biological materials are turned into results – in short, that policy failure is avoided.

The IFBA has been advancing ways in which the implementation phase can be strengthened and supported. We recognize that in addition to government accountability and funding investments, implementation is highly dependent on local context and that high-level decision makers cannot succeed without having some understanding of what happens on, or close to, the front line. In many cases, biosafety professionals and those who work directly with biological materials in laboratories on the front line of global health security know a great deal about the challenges and solutions of implementing national biosecurity strategies. A key success factor for bridging the policy implementation gap, therefore, is to tap into the experiences and competencies of these individuals who can positively shape the implementation process.

Moving beyond the dichotomy, between the top-down and bottom-up approaches, the IFBA's approach to implementing biosecurity policy is a synthesis of both perspectives. We encourage a strong collaboration between decision makers and front line biosafety professionals to develop strategies and innovations that will be successful and sustainable over the long term. Additionally, participation in local, national, and international networks allow for a multilateral type of exchange and collaboration between individuals and a vehicle for innovation in best practices. A range of virtual exchange and on-line collaboration formats allows for an information flow across institutions, nations and regions.

Using the internet and social media, the IFBA is connecting individuals that might not otherwise be able to share best practices and sustainable solutions. These settings also give individuals opportunities to acquire new skills that may not be offered by traditional capacity building approaches. What's more, participation in these initiatives fuels their intention to stay in the bio-security and biorisk management field.

### Risk-based Sustainable Laboratories

Laboratories are an integral component of global health security and play a major role in the safe and secure handling of biological materials. Building laboratory infrastructure that is highly dependent on engineering controls and technology presents a challenge in many countries where construction and maintenance costs are prohibitive. Rather than taking a high technology approach, the IFBA adopts a risk-based approach to designing "built-to-purpose" laboratory equipment and infrastructure that is:

- relevant to local circumstances;
- tailored to the actual risks of an individual laboratory;
- economically feasible and cost-effective to maintain.

In 2010, the IFBA's Biocontainment Engineering Working Group (BEWG) was created to serve as a "think-tank" to identify practical and sustainable solutions for biocontainment laboratories around the world. This network of biocontainment engineers and private industry partners are working together on sustainable laboratory design approaches to reduce initial capital and on-going operational costs. Laboratories in lower resource countries often struggle to implement containment solutions, which have been designed for use in other parts of the world where different working conditions prevail. Compounding the problem is a lack of well-trained biocontainment engineers that can adequately maintain and operate laboratories and critical containment equipment (e.g. biological safety cabinets) over the longer term. Effective supplier networks, maintenance provision and other basic measures are often unavailable to those most in need.

To meet these challenges, the IFBA promotes risk-based approaches to laboratory and equipment design that are cost-effective, locally driven, and can be practically implemented over the long term. The vision for risk-based approaches is not to lay out the requirements for a BSL2 or BSL3 laboratory, but rather to describe "how" these facilities should be planned and designed, based on a local biocontainment risk assessment. The resulting facilities would be built-to-purpose, utilizing a more nuanced set of requirements, and would allow for investment in infrastructure, equipment and precautions suited to the type of procedures performed. It is important to note that building sustainable laboratories also requires a strong focus on procedural and human factors, including trained and competent engineering and maintenance staff.

### Professional Competency

Ensuring that individuals, who handle biological materials, demonstrate competencies on the safe and secure handling of biological materials is an

essential component of the overall effort of reducing biosafety and biosecurity risks. The IFBA's certification program is the only internationally recognized program to certify the competency of individuals in biorisk management and a variety of related technical disciplines. The program is structured in compliance with the policies and procedures of ISO/IEC 17024: 2012 *Conformity assessment – General Requirements for Bodies Operating Certification of Persons.* Examinations are delivered to candidates worldwide in the following disciplines:

- Biorisk Management *(Pre-requisite certification for all others);*
- Biological Waste Management*;*
- Biocontainment Facility Design, Operations & Maintenance;
- Biosafety Cabinet Selection, Installation and Safe Use*;*
- Biosecurity.

To date, the IFBA has issued 959 Professional Certifications to individuals in 65 countries worldwide, a milestone for our growing program. The organization recently collected feedback from their network of certified professionals in Southeast Asia to assess the program's impact in the region. Many respondents said the IFBA's certification program is recognized internationally as a high standard of competency in managing biological risks and was a pathway to enhanced responsibilities at their workplace. The survey also showed a number of ways in which certification has a positive impact on enhancing biosafety and biosecurity practices in the region. The IFBA credential sets biosafety and biosecurity competency standards and recognizes professionals who have demonstrated the knowledge and skills to safely and securely handling of biological materials. With the continued dedication and commitment from IFBA's global community of biosafety associations, and other key partners, the momentum is expected to grow through 2020.

**Global Mentorship Program**

The newly launched IFBA Global Mentorship Program recruits biosafety and biosecurity champions across all regions of the world to provide regionally relevant peer mentorship to developing professionals in their geographic region. Mentors and their mentees discuss foundations of biosafety and biosecurity as it pertains to global and regional standards of practice, as well as emerging trends and threats in health security across diverse professional disciplines in the human, animal and security sectors. Working collaboratively with governments, strong partnerships are being forged between decision makers and frontline workers in turning policy into practice. Feedback from mentorship teams is collected as an informal horizon scan of current norms in biosafety and biosecurity practices across regions.

The pool of mentor/mentee pairs, from an array of professional disciplines and sectors, have been sharing knowledge, skills and experiences towards translating policy objectives into action on the frontlines. Mentorship pairs use One Health approach to harmonize health security approaches across the human and animal health sectors. Mentees are learning how to best translate principles in global health security into strategies and innovations that will be successful and sustainable on the front lines over the long term. In addition to meaningful mentee-mentor collaboration, pairs are participating in regional and international networks to gain additional skills and knowledge for strengthening health security implementation at the local level. The IFBA's south-to-south mentoring program has demonstrated its success as a vehicle for forming crucial links between frontline biosafety professionals, laboratory workers and government decision makers. By supporting regionally relevant peer mentorship programs, the gap between health security policy development and implementation can be narrowed.

## CONCLUSIONS

Over the past years, the IFBA's network of biosafety associations, certified professionals and mentees/mentors have exercised considerable initiative, ingenuity, and drive to implement and sustain biosafety, biosecurity and biorisk management programs and activities in their respective countries. Our activities have formed crucial links between front line biosafety professionals and governments and are participating in the policy-making process and providing input to their governments about biosecurity best practices. They also serve to monitor government actions, helping to hold officials accountable and keep them responsive to actual needs. In this way, biosafety associations can assist government to ensure that practical and locally relevant solutions are reflected in biosecurity laws and their implementation.

Finally, we must remember that the most important aspects of biosafety and biosecurity are the practices and procedures used by trained laboratory staff. The World Health Organization's Laboratory Biosafety Manual states "no biosafety cabinet or other facility or procedure alone guarantees safety unless the users operate safe techniques based on informed understanding." It is the responsibility of everyone, including managers and laboratory workers, to ensure their work is performed in a safe manner. Whether you are new to the field or an experienced biosafety professional, a policy maker or a bench scientist, we need to work together to increase biosafety awareness, leadership, and support for the implementation of national biosafety strategies and laboratory capacity building.

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## EVENIMENTE/ANIVERSĂRI – СОБЫТИЯ/ЮБИЛЕИ – EVENTS/ ANNIVERSARIES– ÉVÉNEMENTS/ANNIVERSAIRES

# CONSTANTIN SPÎNU – PILONUL CERCETĂRII, PERSONALITATE NOTORIE DEVOTATĂ ȘTIINȚEI MEDICALE

*Sănătatea este un cuvânt mare. Ea cuprinde nu numai corpul, mintea și spiritul, ci și perspectiva unui om.*

*James H. West*

Medicina este nu numai o știință, ci este, de asemenea, o artă. Ea nu consistă doar din prescrierea medicamentelor, ea se ocupă cu adevărat de procesele vieții, care trebuie bine cunoscute înainte ca acestea să fie bine călăuzite. Prin urmare, cei care își dedică întreaga carieră pentru a aduce un progres la menținerea sănătății oamenilor, trebuie cunoscuți și apreciați cu cele mai lăudabile cuvinte. Profesorul Constantin SPÎNU este unul dintre cei care s-a dedicat pe parcursul mai multor decenii acestei misiuni importante.

Domnul Constantin SPÎNU – academician AȘM (2018), profesor universitar (1996), doctor habilitat în științe medicale (1991) și actualmente șef Direcție cercetare și inovare, din cadrul Agenției Naționale pentru Sănătate Publică, s-a născut la 19 martie 1950 în comuna Nicoreni, raionul Râșcani, Republica Moldova într-o familie de intelectuali.

În anul 1967 absolvește cu medalie de aur școala medie din satul natal, iar în 1973 – Facultatea de sanitarie a Institutului de Stat de Medicină din Chișinău.

După absolvirea cu mențiune a facultății, lucrează în sfera de supraveghere sanitaro-epidemiologică, activând succesiv în funcțiile de cercetător științific stagiar la Institutul de Cercetări în Igienă și Epidemiologie (Chișinău, 1973-1974), doctorand la Institutul de Virusologie „D.I. Ivanovski" (Moscova, 1974-1977), cercetător științific stagiar, superior, șef de laborator, șef de sector la Institutul de Cercetări în Igienă și Epidemiologie (Chișinău, 1977-1988). Din anul 1988 deține funcția de șef de laborator la Institutul de Cercetări Științifice în Medicina Preventivă și Curativă, iar începând cu 1995 până în 2018 – activează în calitate de prim-vicedirector, vicedirector în probleme științifice și de inovare la Centrul Național de Sănătate Publică.

În anul 1977 Constantin Spînu susține cu succes teza de doctor în medicină la specialitatea „Epidemiologie", iar în anul 1991 – teza de doctor habilitat la specialitatea „Virusologie". În anul 1996 i se conferă titlul de profesor universitar.

Munca asiduă s-a soldat cu rezultate remarcabile ale activității sale: sute de publicații științifice, multiple brevete de invenții, manuale, monografii, un preparat medicamentos „Pacovirina" folosit în medicina autohtonă și o mulțime de discipoli în știință. Realizările profesorului Constantin SPÎNU au fost pro-

movate şi apreciate de foruri şi instituţii ştiinţifice naţionale şi internaţionale. Datorită performanţelor obţinute în domeniul de cercetare, i-au fost acordate cele mai înalte distincţii şi titluri onorifice din ţară şi de peste hotare.

Profesorul universitar Constantin SPÎNU se bucură de popularitate şi în străinătate: este Coordonator Naţional în problemele de poliomielită (Organizaţia Mondială a Sănătăţii), Coordonator Naţional în activitatea de inventică şi transfer tehnologic pentru saloanele internaţionale (România – Cluj-Napoca, Bucureşti, Iaşi) şi membru activ al Academiei de Ştiinţe din New-York, SUA.

Pentru merite deosebite în dezvoltarea medicinii şi rezultate remarcabile în activitatea profesională şi de inovare a fost menţionat cu următoarele distincţii de stat: Diplome de Onoare ale Parlamentului şi Guvernului Republicii Moldova, titlul „Om Emerit" (1998), Medalia „Meritul Civic" (2005), laureat al Concursului Naţional de Susţinere a Ştiinţei şi Inovării „Savantul Anului în domeniul ştiinţelor reale" (2008), laureat al Premiului Organizaţiei Mondiale pentru Proprietatea Intelectuală, Medalia „60 ani ai Academiei de Ştiinţe a Moldovei", Crucea Regatului Belgia (Bruxelles) în grad de Cavaler (2007), Ofiţer (2008) şi Comandor (2009), Diplomă de Onoare a Guvernului Republicii Moldova (2020).

Cunoştinţele profunde, abilităţile manageriale, fructificate cu rezultate remarcabile în domeniul cercetărilor ştiinţifice şi controlul infecţiilor virale au servit drept temei pentru a fi promovat în calitate de coordonator naţional al OMS pentru aspecte legate de combaterea poliomielitei, gripei şi hepatitelor virale.

Profesorul Constantin SPÎNU a inspirat şi încurajat colegii săi cu idei, care fiind aplicate, au adus plus valoare.

Este onorabil pentru noi, consiliului de redacţie, să colaborăm împreună cu academicianul, profesorul universitar, doctorul habilitat, Constantin SPÎNU. Îl apreciem, deoarece a devenit unul din cei mai valoroşi şi notorii specialişti în virusologia medicală, este fondatorul şcolii de virusologie medicală, recunoscută la nivel mondial.

Cu ocazia jubileului de 70 ani, din partea consiliului de redacţie, îi dorim multă sănătate, noi succese şi realizări frumoase în domeniul cercetării şi inovării, realizarea a cât mai multe proiecte de cercetare valoroase pentru asigurarea bunăstării sănătăţii populaţiei şi a sănătăţii publice.

**Mulţi ani prosperi, Domnule Academician!**

Cu profund şi deosebit respect, consiliului de redacţie al Revistei *One Health & Risk Management*

# PROFESORUL RODICA STURZA: MUNCA ŞI MODESTIA SUNT CREZUL VIEŢII SALE

*A fi om înseamnă în chip neîndoielnic a fi responsabil. Înseamnă a simţi, punând şi tu piatră la temelie, că astfel contribui la clădirea lumii.*

*Antoine de Saint-Exupery*

Se spune că timpul trece – mai corect, noi trecem prin timp – şi doar Omul poate să contribuie la atingerea scopului său în viaţă prin insistenţă, efort şi muncă asiduă, prin omenie şi dăruire faţă de tot ce face şi gândeşte, şi încă prin multe altele din care să-şi realizeze Eul în viaţă. Toate acestea dau sens şi bogăţie împlinirilor.

Doamna Rodica STURZA s-a născut în comuna Caracuşenii Vechi, raionul Briceni, Republica Moldova, la 6 ianuarie 1960 şi a urmat studiile gimnaziale şi liceale la şcoala din localitate. Anii de şcoală i-au marcat profund destinul: descendentă dintr-o familie de profesori, Rodica STURZA a dat dovadă de perseverenţă şi capacităţi remarcabile, a fost membru al lotului olimpic la fizică şi chimie, fiind laureat al olimpiadelor internaţionale la aceste discipline. Ulterior, studiilor medii, a devenit studentă la facultatea de chimie a Universităţii de Stat din Chişinău. În anii de studenţie, Rodica STURZA a participat activ la activităţile ştiinţifice studenţeşti şi proiecte de cercetare, fiind menţionată cu burse de merit republicane.

Cariera doamnei Rodica STURZA debutează în acelaşi an cu absolvirea cursurilor universitare (1981), în cadrul catedrei absolvite – Chimie fizică, în calitate de colaborator ştiinţific inferior, unde a activat până în anul 1983, când s-a înscris la doctorat în laboratorul de electroflotaţie al Institutului de Fizică Aplicată al Academiei de Ştiinţe a Moldovei. În anul 1989 a obţinut titlul de doctor în chimie susţinând teza: „Proprietăţi electrochimice ale anozilor titan-dioximanganici obţinuţi prin metoda cu scântei electrice" la Institutul de Chimie şi Tehnologie Chimică din Vilnius, Lituania, iar doi ani mai târziu, în anul 1991, a îmbrăţişat cariera universitară, devenind conferenţiar la catedra de Chimie din cadrul Facultăţii de Tehnologie şi Management în Industria Alimentară, Universitatea Tehnică a Moldovei. Începând din această perioadă, cariera ştiinţifică şi universitară a doamnei Rodica STURZA sunt nedespărţite, cercetările sale ştiinţifice fiind axate pe chimia şi ingineria alimentelor.

Prodecan al Facultăţii de Tehnologie şi Management în Industria Alimentară (1993-2005), şef catedră Chimie (2005-2007), doamna Rodica STURZA participă la realizarea a numeroase proiecte de cercetare naţionale şi internaţionale, implicând în aceste activităţi studenţii, doctoranzii şi colaboratorii tineri din cadrul facultăţii. Activitatea sa este încununată de succes, fiind menţionată cu titlul de „Cel mai bun profesor al anului 2005" de către Senatul UTM, iar doctoranzii sub conducerea doamnei Rodica Sturza fiind menţionaţi cu burse de merit republicane.

Începând cu anul 1997, doamna Rodica STURZA devine profesor al filierei francofone „Technologies Alimentaires", unde realizează numeroase proiecte internaţionale de cercetare şi formare.

În anul 2007 doamna Rodica STURZA este aleasă prin concurs în postul de director al Centrului Naţional de verificare a calităţii produselor vinicole, înfiinţat prin Hotărâre de Guvern în toamna anului 2006. Pe

parcursul a doi ani a creat un laborator de excelenţă, care a permis organizarea controlului calităţii la un nivel recunoscut prin acreditare europeană drept laborator de referinţă.

În anul 2009 doamna Rodica STURZA a susţinut teza de doctor habilitat în tehnică „Principii teoretice şi practice de fortificare a alimentelor cu micronutrimente: iod, fier, calciu", fiind notificată cu menţiunea „Cea mai bună lucrare de doctor habilitat a anului" de către Consiliul Naţional pentru Acreditare şi Atestare a cadrelor ştiinţifice (CNAA), iar în anul 2011 obţine titlul de profesor universitar.

În calitate de profesor invitat prezintă cursuri speciale la Universitatea de Tehnologie Chimică şi Metalurgie din Sofia, Bulgaria; Universitatea de Tehnologii Alimentare (UTA) din Plovdiv, Bulgaria; Universitatea Paul Sabatier, Toulouse, Franţa. Recunoaşterea internaţională a meritelor ştiinţifice ale doamnei Rodica STURZA rezultă din participarea sa în calitate de membru al colegiului de redacţie a unor reviste ştiinţifice internaţionale şi naţionale cu circulaţie internaţională.

Savant cu pregătire universală, autor a peste 300 de lucrări ştiinţifice, inclusiv peste 150 de lucrări publicate în reviste internaţionale recenzate, preşedinte al Seminarului ştiinţific de profil al UTM la specialităţile ştiinţifice: Tehnologia produselor alimentare, Procese şi aparate în industria alimentară, Control şi certificare a produselor alimentare, doamna profesor Rodica STURZA a contribuit la promovarea imaginii Republicii Moldova pe plan internaţional şi la integrarea ei în spaţiul european.

Pentru merite deosebite în dezvoltarea ingineriei şi controlului calităţii alimentelor, în anul 2012 doamnei profesor universitar doctor habilitat Rodica STURZA i s-a acordat titlul de *Doctor Honoris Causa* de către Universitatea Ştefan cel Mare din Suceava.

Din anul 2019 şi până în prezent doamna Rodica STURZA deţine funcţia de şef Departamentul Oenologie şi Chimie din cadrul Universitpţii Tehnice din Moldova.

Cu ocazia împlinirii frumoasei vârste, Vă urăm să atingeţi treptele cele mai înalte ale împlinirilor şi realizărilor. Vă dorim multă sănătate, bucurii în familie, activitate prodigioasă şi noi realizări în activitatea pedagogică şi cercetarea ştiinţifică.

**Mulţi ani prosperi, Doamnă Profesor!**

Cu profund şi deosebit respect, consiliului de redacţie al Revistei *One Health & Risk Management*

# CERINȚE PENTRU AUTORI

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Pregătirea manuscrisului (elaborat în limbile română, engleză, franceză și rusă) va fi în conformitate cu instrucțiunile publicate în: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Manuscrisele trebuie să fie cu font Cambria, dimensiune 11 puncte, spațiat la interval 1,5, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colțul de jos, în partea dreaptă) și să includă numerotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariție în text și nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depășească (fără a număra titlul, afilierea, rezumatul și referințele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3 000 de cuvinte; pentru opinii ale experților – 2 500 de cuvinte; prezentare de caz și imagini din practica clinică/laborator – 1 700 de cuvinte; note experimentale și clinice – 1 300 de cuvinte; recenzii și prezentări de carte – 2 000 de cuvinte; articole didactice – 4 000 de cuvinte. Volumul tabelelor și figurilor nu trebuie să depășească ⅓ din volumul manuscrisului. Revista își rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

**Toate manuscrisele transmise spre publicare trebuie să fie însoțite de două rezumate: în limba de origine al articolului și în limba engleză.**

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Titlul ar trebui să fie cât mai scurt posibil (maximum – 120 de semne cu spații), elocvent pentru conținutul manuscrisului. Numele autorilor vor fi scrise deplin: prenume, nume de familie (*ex:* Ion RUSU). Afilierea va include: Secția/Departamentul/Catedra, Universitatea/Spitalul, Orașul, Țara pentru fiecare autor. Se vor menționa obligatoriu, mai jos, datele autorului corespondent și informațiile de contact – adresa de e-mail (*ex:* autor corespondent: Ion Rusu, e-mail: ion.rusu@gmail.com).

## Structura manuscrisului

Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

- **REZUMAT** (vezi cerințele mai jos)
- **INTRODUCERE**
- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE**
- **REFERINȚE**

**Rezumatul** va conține până la 1 600 de semne cu spații și va cuprinde:

- **Introducere**
- **Material și metode**
- **Rezultate**
- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

În rezumat nu vor fi incluse tabele, grafice și note bibliografice; informații care nu sunt prezentate în studiu.

**Figuri.** Textul inclus în figuri trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare figură trebuie să fie însoțită de titlu și legendă. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) și legenda figurii trebuie să fie scrisă centrat, sub figură.

**Tabele**. Textul inclus în tabele trebuie să fie scris cu font Cambria, dimensiune 10 puncte. Fiecare tabel trebuie să fie însoțită de titlu. Tabelele vor fi inserate în text, fără a depăși lățimea unei pagini. Ele vor fi numerotate cu cifre arabe și vor fi menționate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziționat deasupra tabelului centrat (*ex:* Tabelul 1).

**Referințele** trebuie să fie numerotate în ordinea apariției în text. Citarea sursei de referință va fi conform stilului *Vancouver*, plasată la sfârșitul articolului și va include doar referințele citate în text (menționând numărul de referință în paranteză rotundă). Dacă aceeași referință este citată de mai multe ori, ea va fi trecută în text cu același număr ca la prima citare. Numărul total de referințe nu va depăși 50 de surse. Acuratețea datelor ține de responsabilitatea autorului.

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# ТРЕБОВАНИЯ ДЛЯ АВТОРОВ

**Правила составления**

Подготовка рукописи (разработанной на румынском, английском, французском и русском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,5, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронумерованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названии, принадлежности, резюме и литературы): для обзорных статей/рефератов – 4 500 слов; для научных статей – 3 000 слов; для экспертных заключений – 2 500 слов; для презентации случаев из клинической/лабораторной практики – 1 700 слов; для экспериментальных и клинических заметок – 1 300 слов; для рецензий и презентаций книг – 2 000 слов; для учебных статей – 4 000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

**Все рукописи, представленные для публикации, должны сопровождаться двумя резюме: на языке оригинала статьи и на английском языке.**

**Название и авторы**

Название должно быть как можно короче (максимум – 120 знаков с пробелами), но достаточно информативным для содержания рукописи. Фамилии авторов будут написаны полностью: имя, фамилия (например: Иван ИВАНОВ). Принадлежность будет включать: Отделение/Департамент/Кафедра, Университет/Больница, Город, Страна для каждого автора. Данные соответствующего автора и контактная информация – адрес электронной почты (например: контактная информация: Иван Иванов. e-mail: ivan.ivanov@gmail.com) будут обязательно ниже.

**Структура Рукописи**

Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ**
- **МАТЕРИАЛЫ И МЕТОДЫ**
- **РЕЗУЛЬТАТЫ**
- **ДИСКУССИИ**
- **ВЫВОДЫ**
- **КОНФЛИКТ ИНТЕРЕСОВ**
- **БЛАГОДАРНОСТИ И ИСТОЧНИКИ ФИНАНСИРОВАНИЯ**
- **ЛИТЕРАТУРА**

Резюме должно содержать 1 600 знаков с пробелами и будет включать в себя следующие подзаголовки:

- **Введение**
- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова: 3-5 слов**

Резюме не должно включать таблицы, диаграммы и библиографические заметки, а также информацию, не представленную в исследовании.

**Рисунки** (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (например: Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (например: рис. 1).

**Таблицы.** Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (например: таб. 1). Название таблицы должно располагаться над таблицей в центре (например: Таблица 1).

**Литература** должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *Vancouver*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точность данных лежит на авторе. Будут цитироваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

Для получения дополнительной информации см.: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

# REQUIREMENTS FOR AUTHORS

## Rules of drafting

The manuscript (written in Romanian, English, French and Russian) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.5, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

**All manuscripts submitted for publication should be accompanied by two abstracts: in the language of origin of the article and English.**

## Title and authors

The title should be as short as possible (maximum – 120 signs with spaces), relevant for the manuscript content. The names of the authors should be written in full: name, surname (*e.g.:* Jon JONES). Affiliation should include: Department/ Unit/Chair, University/Hospital, City, Country of each author. Beneath the affiliation, the author`s details and contact information – e-mail address (*e.g.:* corresponding author: Jon Jones, e-mail: jon.jones@gmail.com).

## The structure of the manuscript

The manuscript should comprise the following subheadings (capitalized):

- **SUMMARY**
- **INTRODUCTION**
- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT**
- **REFERENCES**

The **summary** should contain 1 600 signs with spaces:

- **Introduction**
- **Material and methods**
- **Results**
- **Conclusions**
- **Key words:** 3-5 words

The summary should not include tables, charts, and bibliographic notes; information not included in the article.

**Figures.** The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

**Tables.** The text included in tables should be written in font Cambria, 10 point. Each table should be accompanied by a heading. Tables should be inserted into the text and adjusted to the width of the page. The tables are numbered in Arabic numerals and mentioned in body text in parentheses (*e.g.* tab. 1). The title of the table is centred on the top of the table (*e.g.* Table 1).

**References** are numbered in the order they appear in the paper. The reference sources are cited at the end of the article by using *Vancouver* style and will include only the references cited within the text (the reference is numbered within round parentheses). The in-text citations that appear more than once are numbered similarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

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# EXIGENCES POUR LES AUTEURS

## Normes de rédaction

La préparation des manuscrits (rédigés en roumain, anglais, français et russe) sera conforme aux instructions publiées dans *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org).

Les manuscrits doivent être en police Cambria, taille 11 points, espacés à l'intervalle 1,5, alignement justifié, champs 2 cm de tous les côtés. Toutes les pages doivent être numérotées consécutivement (dans le coin inférieur droit) et inclure une numérotation continue des pages. Les abréviations doivent être expliquées lors de la première apparition dans le texte et ne doivent pas être utilisées de manière excessive.

Les manuscrits ne doivent pas dépasser (sans mentionner le titre, l'affiliation, le résumé et la bibliographie) le volume suivant: pour articles de synthèse/ rapports – 4 500 mots; pour les articles de recherche – 3 000 mots; pour les opinions d'experts – 2 500 mots; présentation de cas et photos de la pratique clinique/de laboratoire – 1 700 mots; notes expérimentales et cliniques – 1 300 mots; commentaires et présentations de livres – 2 000 mots; articles pédagogiques – 4 000 mots. Le volume des tableaux et des figures ne doit pas dépasser ⅓ du volume du manuscrit. La revue se réserve le droit d'apporter toute autre modification de formatage. Les manuscrits rejetés ne sont pas retournés.

**Tous les manuscrits à publier doivent être accompagnés par deux résumés : dans la langue originale et en anglais.**

## Titre et auteurs

Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets : prénom, nom (*ex :* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.le-brun@gmail.com*)* seront obligatoires ci-dessous.

## Structure du manuscrit

Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

- **RÉSUMÉ** (voir les exigences ci-dessous)
- **INTRODUCTION**
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **REFERENCES**

Le **résumé** contiendra 1 600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

**Figures.** Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

**Tableaux.** Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (ex : tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *Vancouver*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

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