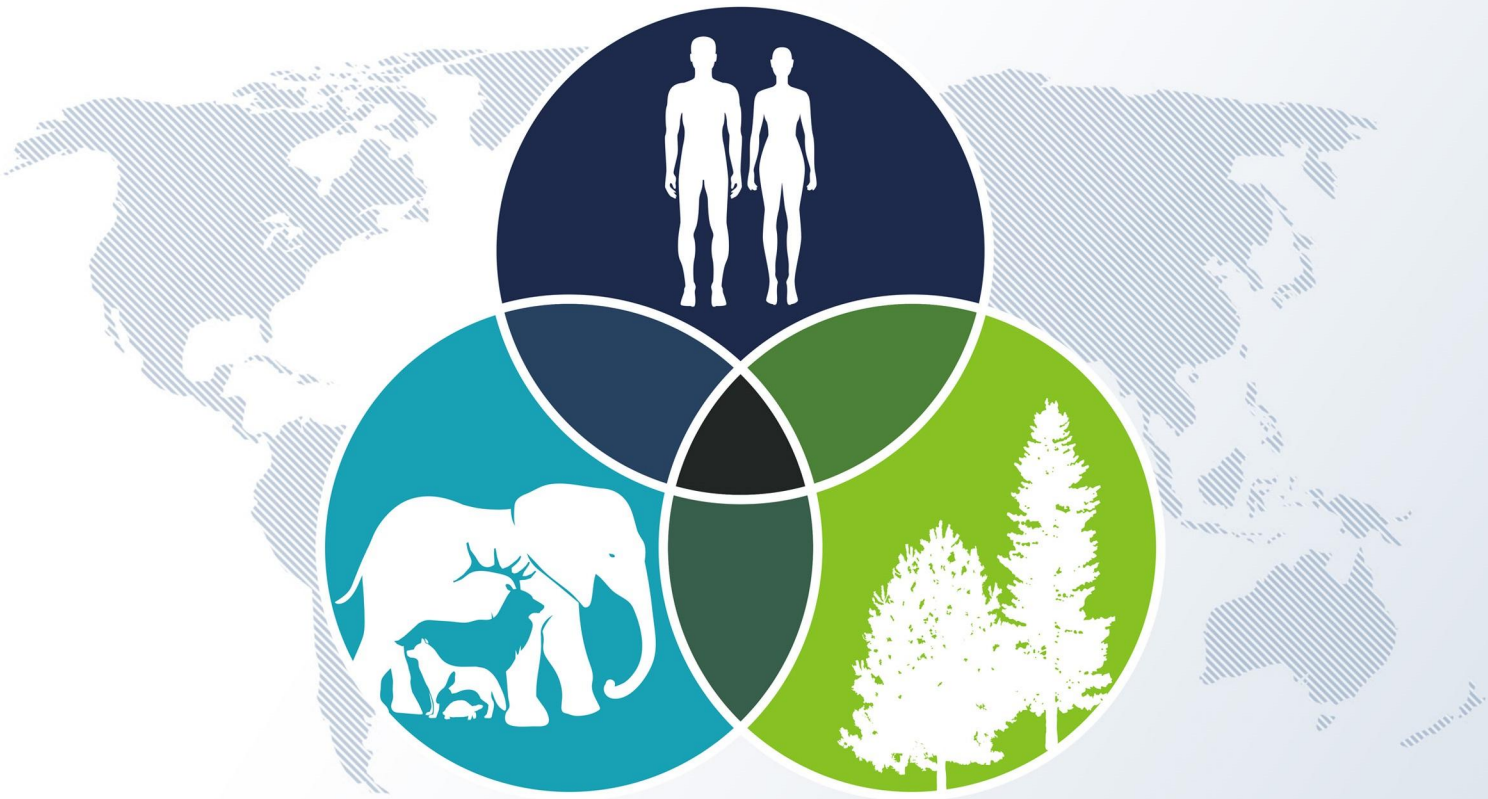
OPEN ACCESS

ISSN 2587-3458
e-ISSN 2587-3466

OH&RM ONE HEALTH & RISK MANAGEMENT

THE SCIENTIFIC JOURNAL OF THE
MOLDAVIAN BIOSAFETY AND BIOSECURITY ASSOCIATION



VOLUME 1, ISSUE 2/OCTOMBER 2020

Asociația de Biosiguranță și Biosecuritate din Republica Moldova (ABBRM) este o organizație profesională cu caracter științifico-practic și instructiv-educativ, neguvernamentală, apolitică și nonprofit, creată în 2017.

Obiectivul principal al asociației este dezvoltarea bunelor practici și culturii în domeniul biosiguranței și biosecurității și promovarea cunoștințelor în cadrul grupurilor profesionale și de cercetare-inovare.

Biosiguranța – include principii de securizare, tehnologii şi reguli ce trebuie urmate pentru a preveni expunerea neintenţionată la agenţi patogeni şi toxine sau eliberarea/scurgerea lor accidentală.

„*Prote*jarea personalului, populației de expunerea neintenţionată la patogeni/material cu biohazard".

Biosecuritatea – include un spectru larg de măsuri (politici de biosecuritate, regim de reglementări, măsuri ştiinţifice şi tehnice) aplicate într-un cadru organizat, necesar minimalizării riscurilor (prevenirea acţiunilor, atentatelor teroriste de eliberarea intenţionată de patogeni sau toxine precum şi a pierderii, furtului sau folosirii greşite a acestora).

„Protejarea și prevenirea furtului, abuzului intenționat a patogenilor/materialului cu biohazard".

Managementul riscului – este un proces de luare a deciziilor în urma căruia rezultatele din evaluarea riscului (procesul de estimare a pericolelor la locul de muncă) sunt integrate cu principii economice, tehnice, sociale şi politice pentru generarea unor strategii de reducere a riscului.

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Biannual edition
Languages of publication: English, Romanian, French, Russian
Founder: Moldavian Biosafety and Biosecurity Association

ISSN 2587-3458 (Print)
e-ISSN 2587-3466 (Online)
Edited by: Typography "Print-Caro", **Edition:** 300 ex.
Registered at the Ministry of Justice with no. 476676, 5th of July, 2017

Lansarea revistei „One Health & Risk Management” și provocările mileniului

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O nouă apariție în spațiul excelenței

Actualmente pe mapamond în parcursul de promovare a rolului cercetării și inovării pentru societate s-a realizat importanța incontestabilă a platformelor dedicate utilizării rezultatelor științifice. Schimbul de informații între cercetători impulsionează creșterea performanțelor instituțiilor academice, permanent în competiție întru atingerea obiectivelor, ce oferă soluții la provocările stringente ale timpului. Totodată, aceste facilități de mediatizare și diseminare a produsului inovațional au calea lor de evoluție. Relevant este și numărul în ascendență a publicațiilor cu participarea autorilor din diferite colțuri ale lumii, realitate ce denotă conștientizarea efectului benefic al concepției de internaționalizare a științei. În regimul unui asemenea progres, în avalanșă, a volumului de date, este neevitată implementarea unor principii orientate de a oferi suport societății.

Civilizația umană întotdeauna e preocupată de asigurarea condițiilor pentru existența sa. Utilizarea reușită a resurselor naturale pentru a-și asigura hrană, apă și energie a dictat evoluția științifică și tehnologică.

Dar cercetările fundamentale și aplicative în cadrul disciplinelor științelor vieții și inginerești au scos la iveală efecte duale asupra sănătății: pozitive și negative. Specificul evoluției științelor moderne introduce abordări stricte pentru managementul sănătății, riscului răspândirii patogenilor și a materialului cu biohazard, reieșind din mediul unic al lumii organice, unde nu rămâne izolată nicio specie sau grup. În laboratoare se produc organisme cu mutații genetice și aceasta necesită un control riguros. Biosiguranța și Biosecuritatea sunt arii de maximă importanță în cadrul concepției *One Health*, promovată de instituțiile din sfera de sănătate în cadrul OMS,

acoperind un vast set de acțiuni la nivel de politici, legi și norme, tehnologii și aparate, reguli, măsuri științifice și tehnice, instrumente de finanțare, toate elaborate în scopul prevenirii căilor de expunere a sănătății umane și a mediului înconjurător la patogeni și toxine, iar neproliferarea armei biologice, contracararea bioterorismului și biocrimelor reprezintă subiecte de cea mai înaltă îngrijorare pentru întreaga omenire.

Agenda Comisiei Europene pentru Excelență în Cercetare stabilește priorități vis-a-vis de fortificarea competitivității economice, cu rezultate scalabile de progres, ce asigură creșterea calității vieții cetățenilor, inclusiv prin securitate alimentară, sănătate publică și ocrotire a mediului ambiant cu suport financiar decent a cercetărilor în disciplinele biologice. În Programele Europene (PC7, PC8) este de mare rezonanță internaționalizarea abordării sănătății. De asemenea, a fost conștientizat pericolul global ce se manifesta prin atacuri biologice cu o serie de agenți patogeni, afectând culturile, consecințe fiind îmbolnăvirea și decesele oamenilor și a animalelor ce consumă aceste produse de origine vegetală, iar prejudiciul economic și social este colosal.

În această lume modernă, ne împărțim orele unei zile în foarte multe direcții, încât uităm de sănătatea noastră. A citi revista „One Health & Risk Management" este o plăcere intelectuală, o destindere cu entuziasm a cercetării, o metodă de informare despre un viitor sănătos.

Avem încrederea, că Asociația de Biosiguranță și Biosecuritate din Republica Moldova (ABBRM) prin lansarea revistei științifice „One Health & Risk Management" va deschide noi perspective în sensul aderării reprezentanților domeniilor sănătate, biologie și protecția mediului din Republica Moldova la spațiul de cercetare paneuropean și internațional, profitând de valoarea adăugată pe tărâm științific întru beneficiul comun.

„Entuziasmul nestăvilit, susținut de bun simț și perseverență,
este calitatea care aduce, cel mai adesea, succesul"

Dale Carnegie

Succes în continuare !

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

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

MEDICAL SCIENCES

EPIDEMIOLOGY OF THE INITIAL PERIOD OF NOVEL CORONAVIRUS (COVID-19) PANDEMIC IN THE REPUBLIC OF MOLDOVA

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DOI: 10.38045/ohrm.2020.1.11 UDC: 616.98:578.834.1(478)

Keywords: *COVID-19, infection, Republic of Moldova, prevention, epidemiological study.*

Introduction. *In the Republic of Moldova, the first case of COVID-19 was confirmed on March 7, followed by a significantly increasing incidence across the country. It is important to describe the clinical and epidemiological aspects that were adjusted to the national context in order to develop and implement optimal public health care measures.*

Material and methods*. A cross-sectional descriptive study was conducted from March 7 to April 6, 2020, using the surveillance system data of the Republic of Moldova on COVID-19 case incidence. All cases of COVID-19 were confirmed by Real-Time PCR.*

Results. *During the reference period, 965 cases of COVID-19 were registered, whereas the urban incidence rate was 1.4 times higher than the rural one. The mean age of infected population was 45.2 years, whereas the most affected age group was 50-59 years, found in 232 cases. Healthcare workers made up 26.6±1.4% out of 965 diseased patients. They included nurses – 34.2±3.0%, auxiliary medical staff – 29.6±2.8%, doctors – 27.2±2.8%, first-aid assistants – 7.4±1.6%, pharmacists – 1.2±0.7%, paramedics – 0.4±0.4%. The health status of people diagnosed with COVID-19 was severe in 8.0±0.9% of cases; moderate severity – in 34.7±1.5% and satisfactorily severe – in 57.3±1.6% of cases.*

Conclusions. *The identified clinical and epidemiological aspects allowed readjusting the public health policies in order to prevent the spread of COVID-19 infection among the elderly and medical workers.*

Cuvinte cheie: *COVID-19, infecție, Republica Moldova, prevenire, studiu epidemiologic.*

EPIDEMIOLOGIA INFECȚIEI CU NOUL TIP DE CORONAVIRUS (COVID-19) ÎN PERIOADA INIȚIALĂ A PANDEMIEI ÎN REPUBLICA MOLDOVA

Introducere. *Primul caz de infectare cu noul tip de coronavirus COVID-19 în Republica Moldova a fost confirmat la data de 7 martie 2020, ulterior a fost înregistrată o creștere semnificativă a îmbolnăvirilor. Aspectele clinico-epidemiologice ajustate contextului național sunt necesare a fi descrise pentru elaborarea și implementarea acțiunilor optime de sănătate publică.*

Material și metode. *Am realizat un studiu descriptiv transversal în perioada 7.03.2020 – 6.04.2020, folosind datele din sistemul de supraveghere a Republicii Moldova cu privire la cazurile de infecție COVID-19, care au fost confirmate prin tehnici de biologie moleculară (Real-Time PCR).*

Rezultate. *În perioada de referință au fost înregistrate 965 cazuri de COVID-19, mediul urban fiind afectat de 1,4 ori mai mult decât cel rural. Vârsta medie a celor infectați a fost de 45,2 ani, iar cel mai afectat grup de vârstă a fost cel de 50-59 ani – 232 cazuri. Dintre cele 965 persoane infectate, lucrătorii medicali reprezintă 26,6±1,4%: asistenți medicali – 34,2±3,0%; personal medical auxiliar – 29,6±2,8%; medici – 27,2±2,8%; felceri – 7,4±1,6%; farmaciști – 1,2±0,7%; paramedici – 0,4±0,4%. Starea de sănătate a persoanelor diagnosticate cu COVID-19 a fost gravă în 8,0±0,9%, de gravitate medie în 34,7±1,5% și satisfăcătoare în 57,3±1,6%.*

Concluzii. *Aspectele clinico-epidemiologice identificate permit reajustarea politicelor de sănătate publică referitor la prevenirea răspândirii infecției COVID-19 printre persoanele în vârstă și lucrătorii medicali.*

INTRODUCTION

Coronavirus belongs to a large family of viruses that can cause various symptoms such as pneumonia, fever, shortness of breath and lung infection (1). These viruses are common in animals around the world but are known to affect humans in some cases. Although coronaviruses are a large family of viruses, it is considered that only six (229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV) could infect humans. Thus, 2019-CoV became the seventh (2, 3).

The World Health Organization (WHO) used the term new coronavirus 2019 to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China, on December 29, 2019 (4, 5, 6). The WHO has announced that the official name of the new type of coronavirus in 2019 is COVID-19 – coronavirus disease (6).

And the current reference name for the virus is Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This is a single-stranded RNA coronavirus with positive polarity. The virus underwent a genomic sequence, following the nucleic acid testing on specimens from a patient with pneumonia during the 2019-2020 Wuhan coronavirus epidemic. Wuhan beta-coronavirus sequences have similarities to the beta-coronaviruses identified in bats. However, the virus is genetically distinct from other coronaviruses, such as SARS-CoV and MERS-CoV (7).

At the end of December 2019, a group of patients was hospitalized with an initial diagnosis of pneumonia with an unknown etiology. These patients have been epidemiologically related to a seafood and wet animals wholesale market in Wuhan, Hubei Province, China (8). Early reports predicted the emergence of a potential Coronavirus outbreak, by estimating the reproduction number of the novel Coronavirus (COVID-19, named by the WHO on 11 February 2020) as being significantly higher than 1 (intervals from 2.24 to 3.58) (9).

By April 6, 2020, more than 134,901 people were infected worldwide, by affecting almost every country across the globe. Moreover, at the beinning of April, the mortality rate was calculated in the following countries like Germany, this index being of 1.2%, compared to Italy – 11.9%, Spain – 8.6%, the Netherlands – 8%, the United Kingdom – 7.1% and France – 9.0% (10, 11). However, the overall mortality rate accounted for 2.2-3.4% (12).

Globally, about 3.4% of reported COVID-19 cases have died. By comparison, seasonal flu generally kills much less than 1% of those infected (13, 14). Virus susceptibility appears to be associated with age, gender and other health conditions (15). COVID-19 has now been declared by the WHO as public health emergency of international concern (16).

Due to the rapid spread of the new coronavirus and its effects on human health, the scientific community responded quickly to the new virus and many early research studies have already been published on this epidemic (9-14). This article is aimed to provide information and evidence on the evolution of the COVID-19 epidemic in the Republic of Moldova at its initial stage. This article can provide meaningful information for future research on this topic and justify the governmental decision-making on management strategies regarding this public health emergency at both community and national level. The combination of epidemiological data can provide an early understanding of the pandemic situation, thus promoting a balanced and well-targeted action from public health perspective.

MATERIAL AND METHODS

The present cross-sectional descriptive study was conducted from March 7 – April 6, 2020. The national surveillance system data of the Republic of Moldova have been reported cases based on standard case definition, namely, COVID-19 suspected, possible or confirmed cases during the initial stage of the epidemic. All cases of COVID-19 were confirmed via molecular biology (Real-Time PCR) techniques. The confirmed case definition was used according to the national legislation in force, namely "A person with laboratory confirmation of COVID-19 infection, regardless of clinical signs and symptoms." The statistical data have been processed via MicrosoftExcel and EpiInfo 7.2.

RESULTS

The first suspected COVID-19 case in the Republic of Moldova was reported on March 7, fol lowed by laboratory confirmation on 08 March in a person who entered the country. In the following

week, there was a steady increasing daily tendency, with unique cases being registered. A constant increase in the number of cases were reported over the next 2 weeks, viz. tens of newly-infected people per day, so that over the last week there was a sudden increase in the number of cases, being estimated to around 100 cases per day. On April 6.965 cases were registered, whereas 101 cases had been reported a day before (fig. 1).

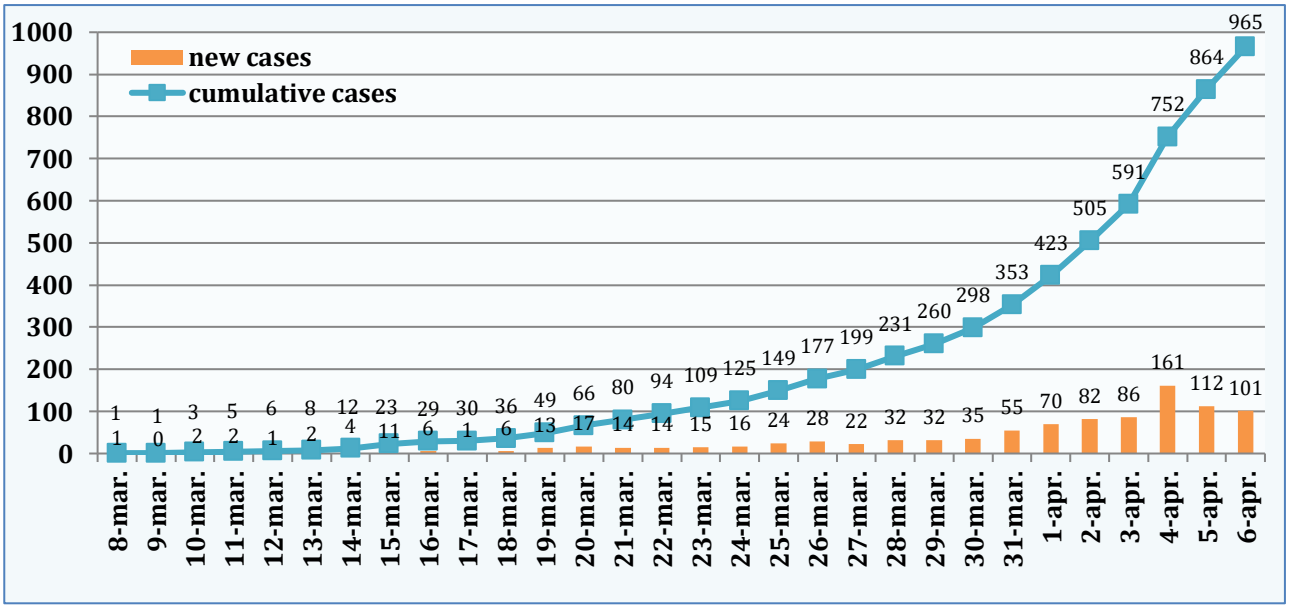

Figure 1. Number of new and cumulative cases of COVID-19 reported in the Republic of Moldova during the reference period (abs.).

However, along with the increasing number COVID-19 cases, various laboratory diagnostic activities were conducted in this regard (fig. 2).

The present data confirms that according to the case definition for COVID-19 infection, a signifycant number of people underwent testing. The number of investigations was continuously increasing until April 4, 2020, when it was at highest, when 681 investigated samples were performed. Afterwards on April 5 and 6, the number of tests decreased, more likely because these were the weekend days. It should be mentioned that an overall number of 4,598 tests have been performed so far, within the Virology laboratory of NAPH.

The geographical distribution of COVID-19 cases was also assessed, in order to identify the virus spreading intensity. Therefore, on April 6, 2020, almost all the administrative territories of the country were affected, except for Soldanesti and Rezina districts.

The highest levels of COVID-19 incidence were recorded in the following regions: Ștefan Vodă – 213.27‰ (148 cases), Soroca – 125.73‰ (125 cases), and Glodeni – 61.34‰ (36 cases). A high incidence rate was found in Chisinau – 37.94‰ (316 cases). As regarding the geographical distribution, the most affected were the Southern areas – 41.64‰ (219 cases), followed by the Northern areas – 37.94‰ (222 cases), and the Central areas – 14.51‰ (152 cases). The incidence rate in the administrative territories with special status, namely, ATU Gagauzia was 1.24‰ (2 cases), and Transnistria – 11.53‰ (54 cases) (tab. 1).
Therefore, the major outbreaks registered at this stage of the epidemic, also called as community transmission, occurred mainly in Chisinau, and Soroca and Stefan-Voda districts. The massive spread of disease was reported in Glodeni district.

During the initial period of the epidemic, the total number of people infected with the novel coronavirus accounted for 385 patients from rural areas and 539 patients from urban areas. Therefore, the incidence rate made up 41.7±1.6% cases – rural areas and 58.3±1.6% – urban areas, respectively. This phenomenon is probably due to the massive internal and exter

nal migration of people from urban areas, as well as due to a higher contact level among people in places with high population density and urban activities associated with people behavior and mass-gathering (fig. 3).

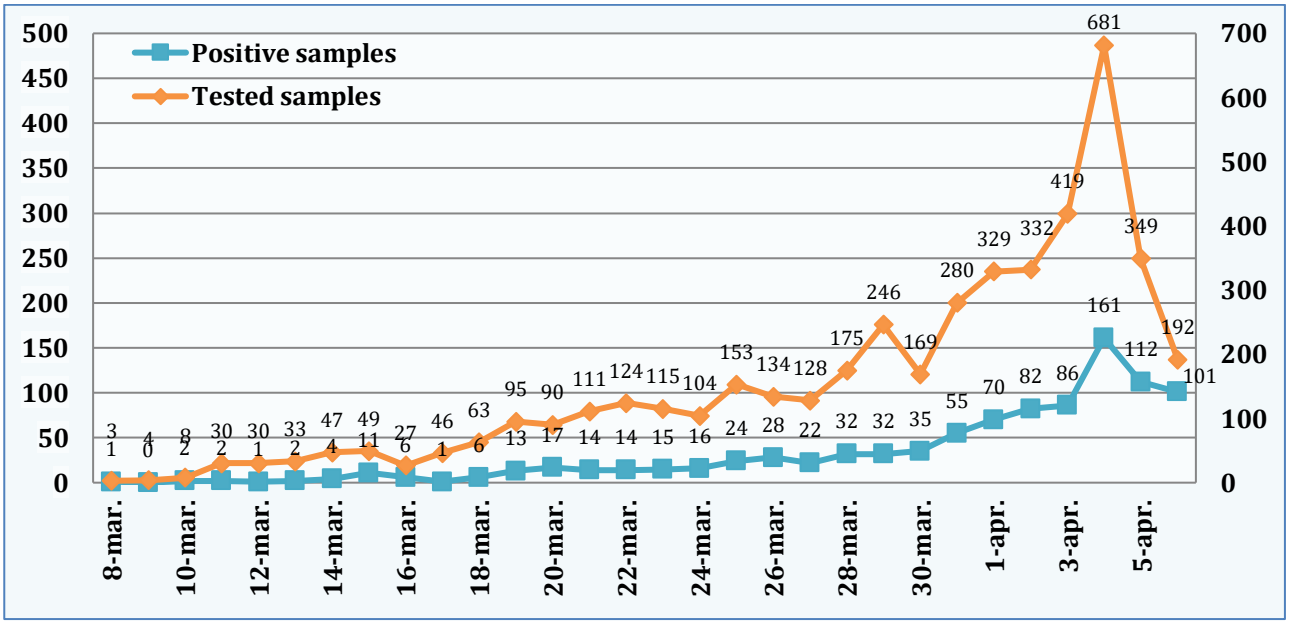

Figure. 2. Number of persons tested and found positive to COVID-19 in the Republic of Moldova, during the reference period (abs.).

Table 1. Geographical distribution of COVID-19 cases in the early stages of the epidemic in the Republic of Moldova (abs. and ‰).

Administrative territory	Number of population	Number of cases	Incidence
Chisinau	**832,865**	**316**	**37.94**
Northern areas	**974,558**	**222**	**22.78**
Balti	151,791	10	6.59
Briceni	71,447	4	5.60
Donduseni	41,719	1	2.40
Drochia	85,558	2	2.34
Edinet	79,160	5	6.32
Falesti	90,275	20	22.15
Floresti	85,643	2	2.34
Glodeni	58,691	36	61.34
Ocnita	52,948	1	1.89
Riscani	66,498	5	7.52
Singerei	91,412	11	12.03
Soroca	99,416	125	125.73
Central areas	**1,047,681**	**152**	**14.51**
Anenii Noi	82,998	12	14.46
Calarasi	76,551	4	5.23
Criuleni	73,371	6	8.18
Dubasari	34,969	1	2.86
Hincesti	118,619	32	26.98
Ialoveni	101,797	36	35.36
Nisporeni	64,797	10	15.43
Orhei	124,007	30	24.19
Rezina	49,891	0	0.00
Straseni	92,052	5	5.43

Soldanesti	40,942	0	0.00
Telenesti	70,982	10	14.09
Ungheni	116,705	6	5.14
Southern areas	**525,928**	**219**	**41.64**
Basarabeasca	27,997	1	3.57
Cahul	124,091	32	25.79
Cantemir	61,317	8	13.05
Causeni	89,356	13	14.55
Cimislia	58,604	3	5.12
Leova	51,990	2	3.85
Stefan-Voda	69,394	148	213.27
Taraclia	43,179	12	27.79
ATU Gagauzia	**161,676**	**2**	**1.24**
Transnistria	**468,414**	**54**	**11.53**
Total cases in cities	**984,656**	**326**	**33.11**
Total cases in districts	**2,558,052**	**585**	**22.87**
Total cases per Republic	**3,542,708**	**965**	**27.24**

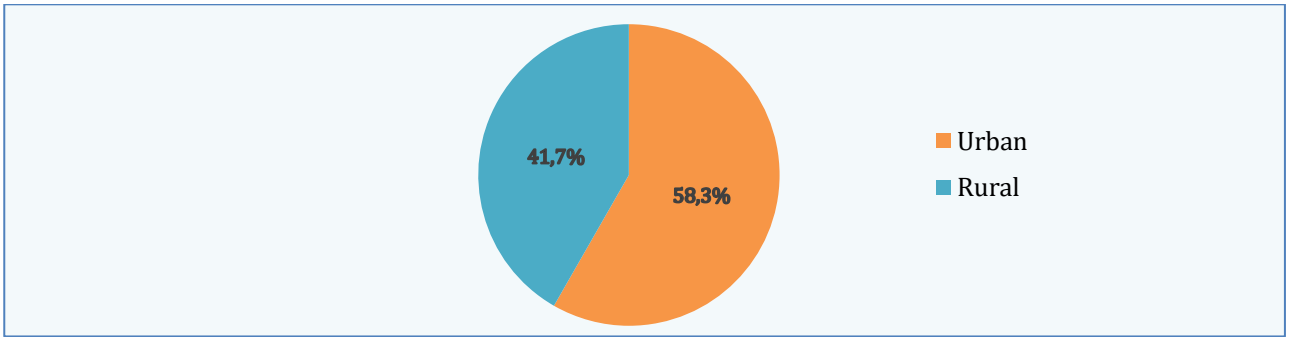

Figure 3. Distribution of COVID-19 cases depending on the living areas of the infected persons during the reference period in the Republic of Moldova (in %).

The average age of those infected was 45.2 years; the mean age for COVID-19 infected people from urban areas was 44.6 years, while those from rural areas – 46.1 years. The mean age of COVID-19 infected men was 42.9 (386 people) and infected women – 46.7 years (575 people).

According to gender distribution, 244 infected women and 141 infected men were from rural areas, which made up 63.4±2.5% vs. 36.6±2.5%, respectively. 316 COVID-19 infected women and 223 men were from urban areas, accounting for 58.6±2.1% vs. 41.4±2.1%, respectively. Thus, these data confirmed that gender distribution depending on the living environment presents a statistically significant difference ($p \leq 0.05$) (fig. 4).

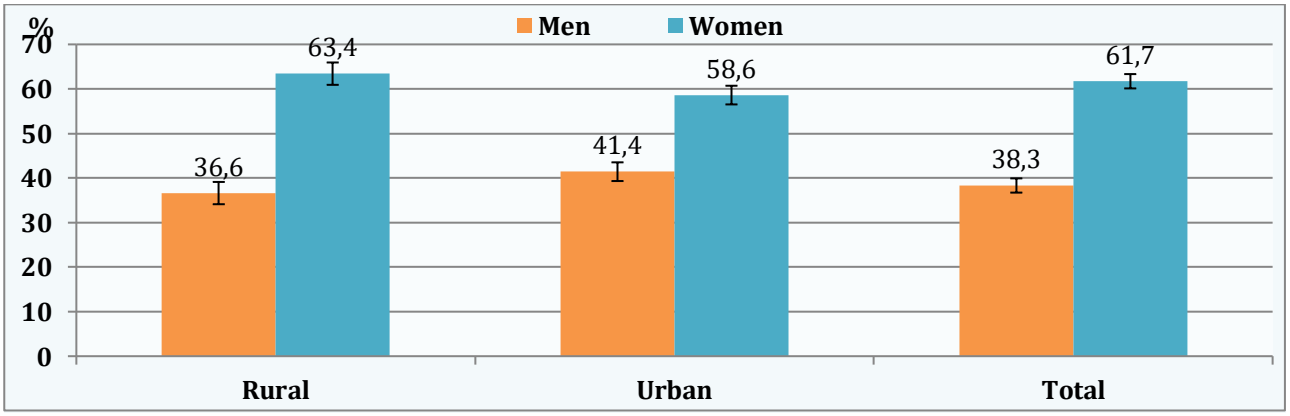

Figure 4. Gender distribution of infected persons depending on their living areas in the initial pandemic stages in the Republic of Moldova (in %).

Out the total number of women, 11 women were found pregnant, which made up 1.9% of pregnancy cases. Furthermore, 2 people pregnant were reported as being in the first trimester (25%), 5 pregnant women were in the second trimester of pregnancy (62.5%), and only one woman was in the third trimester of gestation (12.5%).

The study of age-related COVID-19 distribution showed that the most affected were people aged 50-59 years, which included 232 cases, followed by people aged between 40-49 years found in 170 COVID-19 cases. People aged 30-39 years were ranked third among total number, found in 152 confirmed cases, followed by a small difference in-group of people aged 60-69 years, accounting for 147 of infected cases. The least number of infected cases were reported in children (0-9 years) with a total of 37 cases and in those older than 80 years, where only 6 cases were recorded (fig. 5).

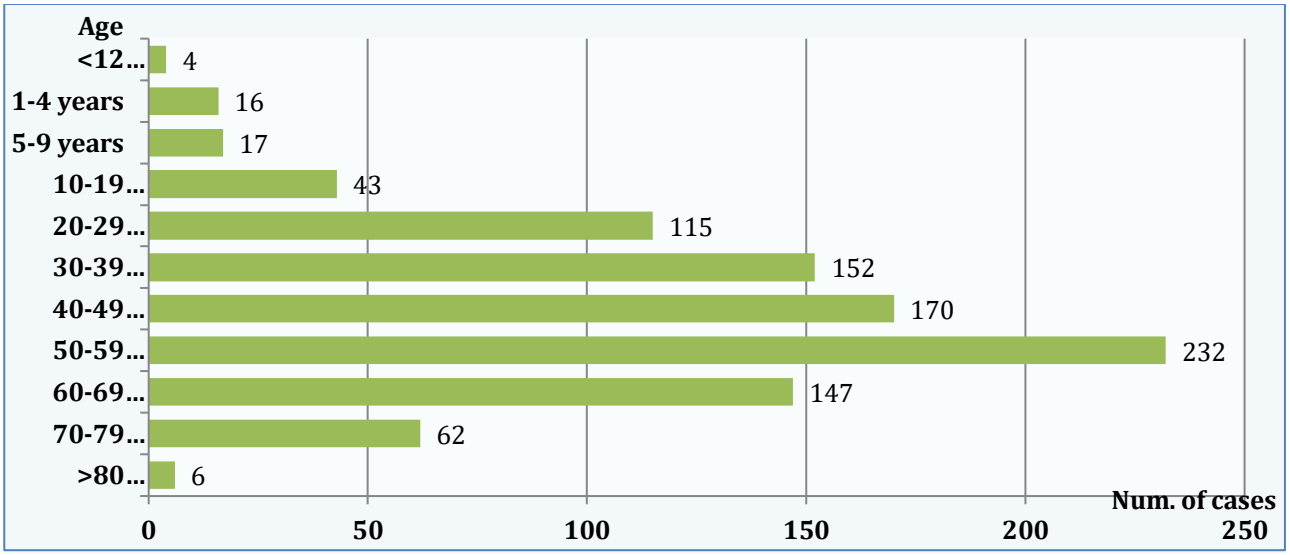

Figure 5. Age distribution of COVID-19 cases recorded during the reference period in the Republic of Moldova (abs.).

Another important epidemiological aspect included within the study refers to people traveling abroad in the last 14 days before the onset of disease. Therefore, the distribution of people who traveled abroad within 14 days before the onset of the disease was 20.2±1.6% (129 cases); those who did not travel abroad 79.8±1.6% (510 cases); those who traveled or admitted to have been in contact with a confirmed case of COVID-19 abroad – 22.7±4.0% (25 cases); 43.7±4.7% (48 cases) could not confirm or deny being in contact with infected people; 33.6±4.5% (37 cases) stated that they were not in contact with a confirmed case of COVID-19 abroad (fig. 6).

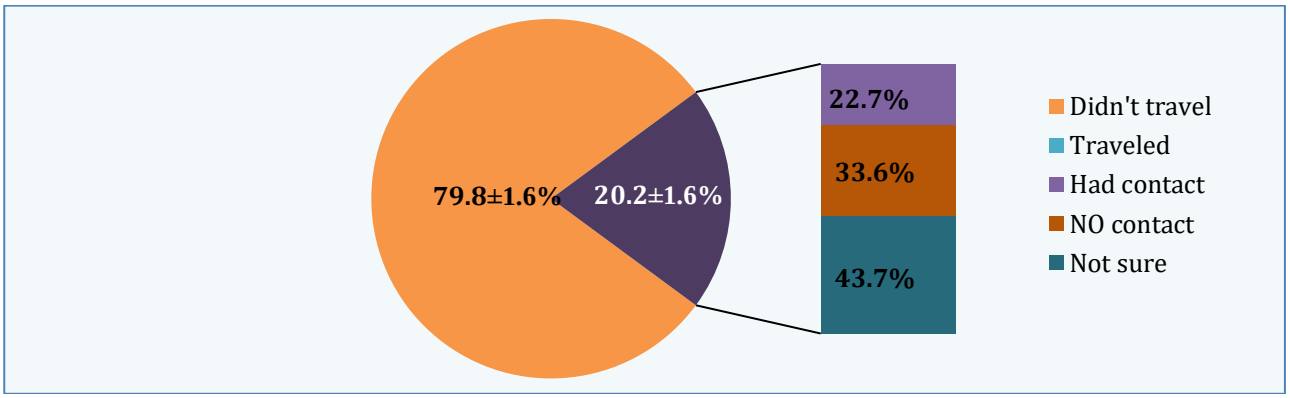

Figure 6. Distribution of people traveling abroad and being in contact with confirmed COVID-19 cases (in %).

Of those who traveled abroad during the last 14 days, most people were found to return from the UK (33 people), followed by those who returned from Italy (21 cases), and from Ukraine (14 cases), which are the most visited countries by Moldovans for business or travelling purposes. Other countries included France – 13 cases, Russia – 10, Romania 7, Dubai – 4, Czech Republic and Germany – per 2 cases each, Austria, Belarus, Bulgaria, Switzerland, Turkey – 1 case per each country. 18 epidemiological investigations did not reveal data upon the country visited by those who claimed to have traveled abroad.

All people diagnosed with COVID-19 and who had not traveled abroad during the last 14 days (being considered as local transmission of infection) were asked about their contacts with already confirmed COVID-19 cases. Therefore, based on epidemiological surveys, it was found that 430 people were in contact with a confirmed case (54.3±2.0%), 164 people stated that they were not in contact with COVID-19 positive people (20.7±1.5%) and 198 people did not know for sure (25.0±1.5%). Epidemiological data did not reveal any relevant information in 173 people (fig. 7).

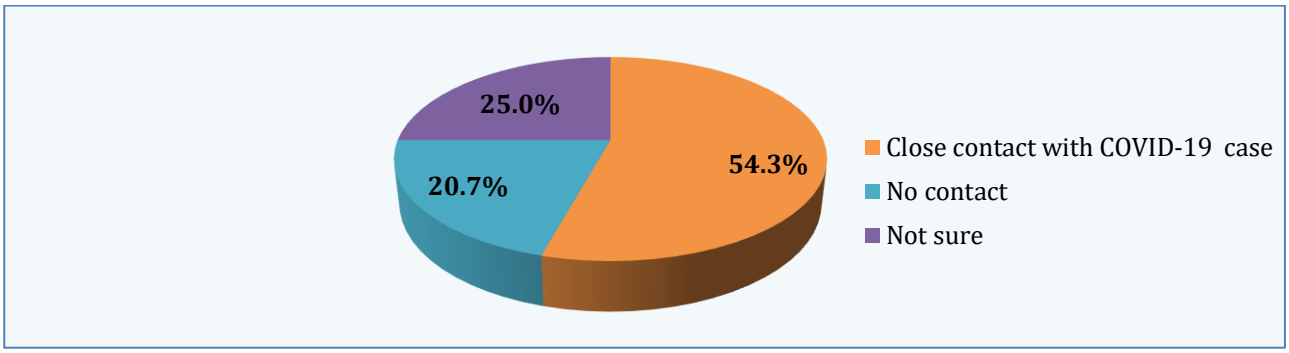

Figure 7. Distribution of cases that were in/no contact with people diagnosed with COVID-19 (in%).

The Republic of Moldova, as well as other countries registered a number of SARS-CoV-2 – infected health care workers. The HCWs were a subject of specific testing strategy, therefore some selection bias could influence the figures regarding the HCWs infection prevalence. The HCWs as general population have been tested in case of COVID-19 suspecting, when they were exposed to the risk in the health care facilities also when they were identified as being in contact with a confirmed case. The local initial epidemic stage revealed that out of the total of 965 infected people, 257 were healthcare workers, which made up 26.6±1.4% of cases. Out of these cases, 88 (34.2±3.0%) were nurses, 76 (9.6± 2.8%) – auxiliary medical staff, 70 (27.2±2.8%) – doctors, 19 (7.4±1.6%) – first-aid men, 3 (1.2± 0.7%) – pharmacists and a paramedic (0.4± 0.4%)(fig. 8).

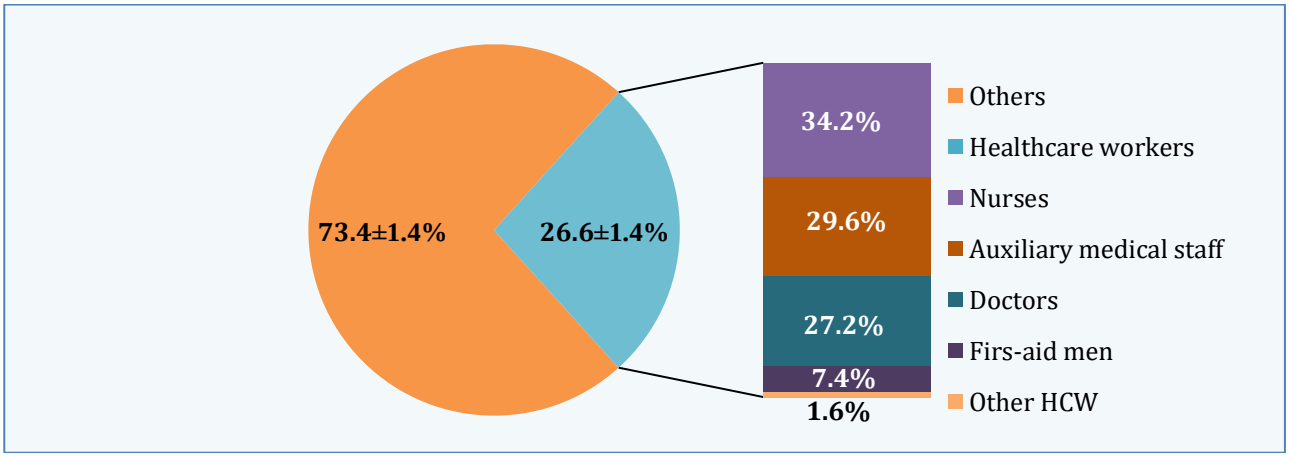

Figure 8. Distribution of infected healthcare workers out of the total number of cases and distribution depending on their professional category (in%).

Most cases of infections among healthcare workers were registered in Soroca – 86, Stefan Voda – 46, Glodeni – 15, Republican Clinical Hospital (Chisinau) – 14, Emergency Hospital Chisinau – 13, Clinical Psychiatric Hospital – 8, Orhei District Hospital – 7, National Center for Pre-Hospital Emergency Medical Assistance – 6. Of those 37 (14.4±2.2%) healthcare workers had contracted the infection within medical institutions, whereas the remaining 220 (85.6±2.2%) cases could not determine the path of transmission (fig. 9).

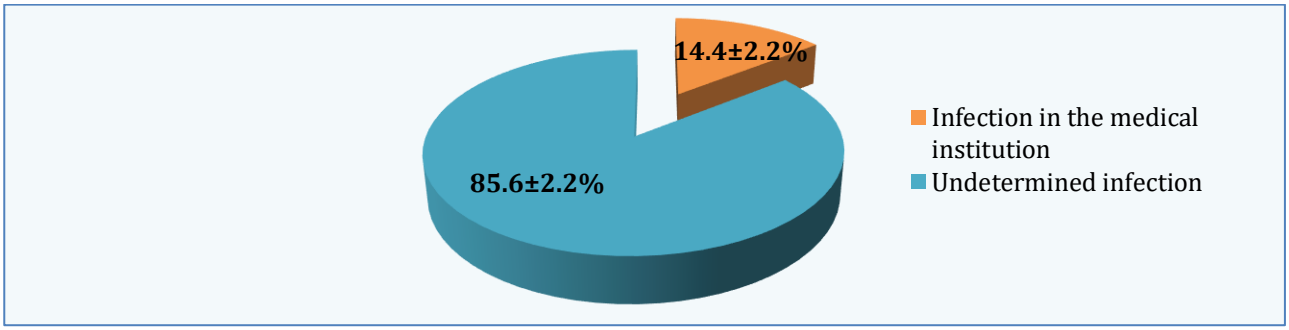

Figure 9. Distribution of COVID-19 cases within medical institutions during the reference period (in %).

Clinical manifestation of the diseases and health status of people diagnosed with COVID-19 have been assessed. Thus, it was established that on April 6, 2020 there were 77 (8.0±0.9%) people hospitalized with severe disease, 335 (34.7±1.5%) people had a medium severe disease, and 553 (57.3±1.6%) cases showed mild symptoms (fig. 10).

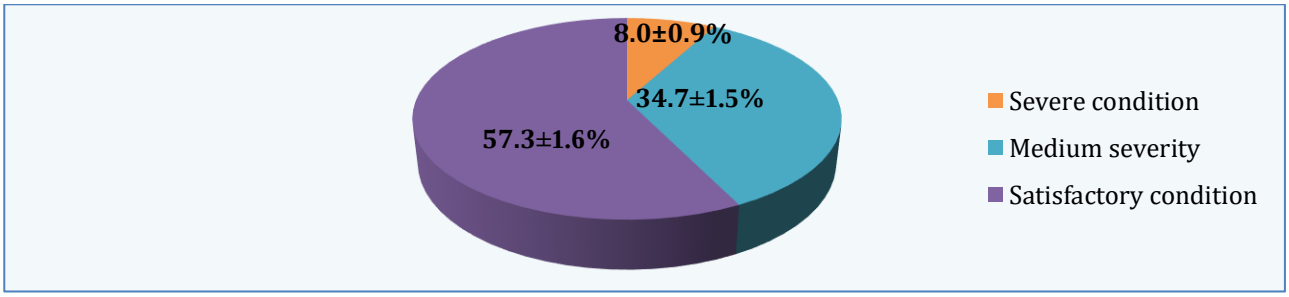

Figure 10. Distribution of patient health status with COVID-19 in the initial epidemic period in the Republic of Moldova (in %).

To date, the total number of people treated for COVID-19 is 40 (65.6±6.1%) and 21 (34.4±6.1%) people died. 904 (93.7±0.8%) cases are active and 6.3±0.8% are closed cases (deceased, discharged) (fig. 11).

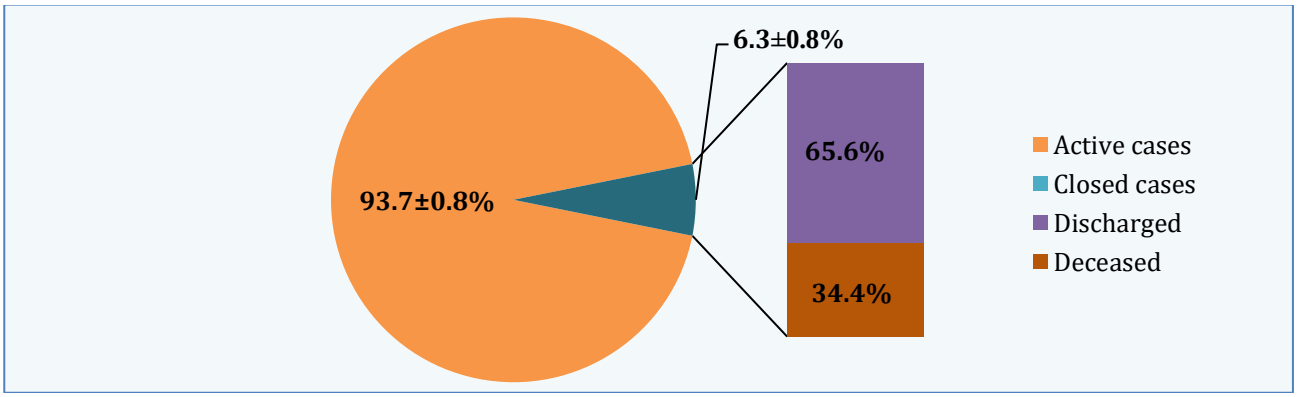

Figure 11. Distribution of closed cases and their classification in the initial COVID-19 epidemic period in the Republic of Moldova (in %).

DISCUSSIONS

The epidemiological situation in the Republic of Moldova has evolved following WHO well-known scenarios: ”zero”, imported cases, clusters and community transmission. The first COVID-19 case, which was confirmed on 8 March 2020, did not surprisingly occur. Furthermore, the infection transmitted among people through close contact, including family members. Clusters and later community transmission were registered.

The present study findings are similar to other studies that found a higher percentage of infected people in urban living environment. Thus, Fan J. et al. (17) found that in China's Gansu Province, the share of urban COVID-19 infected people was 58.3%. The same findings were reported by another study conducted in China in Tianjin Province by Cao C et al. (18) that determined COVID-19 infection in 52.4% of cases from urban areas and in suburban, whereas 47.6% cases were from rural areas. These findings as well as those of the present study can be explained by the fact that SARS-CoV-2 is more easily spread in urban areas due to higher population density, closer contact between subjects and less social distancing compared to rural living environment.

Regarding the age of patients with COVID-19 in the initial period, studies in outbreaks in China found that in Gansu Province, the patients identified in the early period were younger than those identified in the late period, but in general 54 of patients were younger (mean age 38 years) than patients identified in the early stage of the outbreak in Wuhan (mean age 59 years) (4). In this context, the mean age of 45.2 years was recorded in the early period of the COVID-19 epidemic in the Republic of Moldova, which tends to resemble the indices recorded in other (secondary) outbreaks, other than the initial one in the Chinese city of Wuhan. By assumption, COVID-19 have been registered among middle age group, because cases at the beginning of pandemic have been mainly imported, and represent middle-age people who are working abroad with following transmission of the virus among their family members, In the study of Fan J. (17) the distribution of the disease by sex did not differ significantly between the early and late periods, but number of female patients slightly predominated. In contrast, Chen and colleagues reported more men than female patients in the Wuhan outbreak (19). Cao C. and co-workers also reported higher prevalence of infection in men in their Tianjin study, accounting for 51.64% and women in 48.06% (18). In the context of these confusing data, it is still premature to interpret the obtained study data where the COVID-19 incidence in women is obviously higher than in men.

As regarding the most affected age group, almost all the studies showed that middle-aged people were the most affected, especially those aged 40-49 years, followed by people aged 50-59 (17, 18, 19). It could be assumed that this is associated with people migration particularly in Italy and Great Britain, middle-aged females being the most affected ones. The study conducted during the reference period revealed a tendency of increasing COVID-19-associated morbidity in people aged 50-59 years. However, further additional information is required to trace out final conclusions.

As regarding the population travelling, different studies divide information into the early epidemic stages of travelling to China and the later stages of the COVID-19 pandemic. Studies have drawn particular attention to the citizens who returned from the so-called "red zones" or countries with a large number of confirmed COVID-19 cases. Thus, according to Hien Lau et al. (20), 51.89% of those interviewed traveled to China in the initial stage and 28.26% travelled to other countries in the second stage. These data correspond to our study findings, where 20.2% of people reported travelling abroad in the last 14 days. The significant difference between the number of people who traveled to China in the initial pandemic stage and those who traveled to other countries during the second stage can be explained by an increase in number of cases of local (community) SARS-CoV-2 transmission.

An important feature of COVID-19 pandemic is the infection spread among healthcare workers, particularly of first-line medical assistance. A series of studies have been referred to this phenomenon, showing different incidence among infected healthcare workers. Thus, in the USA, the state of Ohio reported at least 16% of cases involving healthcare workers, while in Minnesota, this index was 28% (21) In the province of Brescia, the central outbreak in Italy, 10-15% of doctors and nurses were infected during the initial period of the pandemic (22). Subsequently, it appears that the share of infected health-

care workers in Italy has increased to 20% (23). Spain reported the latest figures for infected healthcare workers on March 30. At that time, around 12 thousand of healthcare workers were estimated to be infected, out of over 85 thousand cases, i.e. the incidence rate being of 14% (24).

In Romania, the last official and valid report was made on April 3. By that time, 474 doctors and other medical staff were infected in the neighboring country, out of a total 3,183 cases, the incidence rate being of 14.8%. It should be noted that 318 cases were reported within a hospital in Suceava. On March 30, the incidence rate was 14.5% (25). Compared to these data, our study revealed that the percentage of COVID-19 – confirmed cases among healthcare workers accounted for 26.6±1.4%.

Therefore, there is a great difference in the incidence of infected healthcare workers across the countries worldwide due to economic and socio-cultural differences, access to protective equipment, medical staff training and compliance to preventive and control measures etc.

As regarding the clinical aspects of COVID-19 infection worldwide, particularly of patient's condition, 4% of cases were reported as severe, followed by milder or moderate severity level (26). If compared to this index from the Republic of Moldova, our study revealed 8% of patients with severe clinical evolution; therefore, all suspected cases were admitted to the hospital. However, due to the differences among counties regarding hospitals admission criteria as well as assigning of the severity of cases it is difficult to affirm that there were any specific issues regarding the higher diseases' severity. Another indicator evaluated at this stage was the share of closed cases. The global indicator is 32.03% compared to 6.3% of closed cases from the Republic of Moldova. This gap is probably due to the late onset of the pandemic in the Republic of Moldova, considering that the recovery period of patients with COVID-19 lasts up to a month, however, it might vary, depending on the clinical form and manifestations. There are also great concerns regarding the high rate of lethal cases among patients in the Republic of Moldova – 34.4% compared to 21.0% worldwide, thus implicitly displaying a lower rate of discharged patients from Moldova – 65.6% compared by 79.0% globally (26).

CONCLUSIONS

1. The first month of COVID-19 epidemic exhibited a dynamic evolution, showing an increasing tendency to affect all the administrative territories of the Republic of Moldova.
2. The most COVID-19 affected age groups in the Republic of Moldova correspond to those from the other countries worldwide, thus, the prevailing female infected cases made up 63.4±2.5%, then people living in urban areas – 58.3±1.6%, the mean age of people was 45.2 years, with a tendency to increase among people aged up to 70 years.
3. The high rate of infected health care workers accounted for 26.6±1.4%, which was relatively higher, compared to other states, where this indicator was as following: Spain – 14.0%, Romania – 14.8%, Italy 20.0%.
4. The incidence of severe clinical forms, which required assisted ventilation, was higher in the Republic of Moldova compared to this index worldwide, thus being of 8% compared to 4% in other countries. Additional clinical resource management and practices are needed.

CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

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Date of receipt of the manuscript: 24/04/2020
Date of acceptance for publication: 05/08/2020

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OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

MEDICAL SCIENCES

OPEN ACCESS

CHARACTERISTICS OF MENINGOCOCCAL INFECTION MORBIDITY IN THE REPUBLIC OF MOLDOVA OVER THE PERIOD 2000-2019

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DOI: 10.38045/ohrm.2020.1.12 UDC: 616.98:579.845(478)

Keywords: *meningococcal infection, epidemiology, morbidity.*

Introduction*. Meningococcal infection (MI) and N. meningitidis carriage are widespread. The global incidence represents a total of 500,000-1,200,000 cases per year, of which 50,000-135,000 are fatal. In European countries the incidence is 0.6-2.0 per 100,000. Infants and young people are more likely to get affected due to different genotypes of meningococci. Thus, MI surveillance is required.*

Material and methods. *The research purpose was to perform an epidemiological analysis of MI in the Republic of Moldova between 2000 and 2019. In the descriptive retrospective epidemiological study were analysed the total MI incidence of a population of 100,000 people, the number of cases in urban and rural areas, the morbidity rate among 1000 children aged between 0 and 17 years, and patients' age structure.*

Results. *A decreased incidence, without cyclicity, was registered over the past 5 years from 2.57 to 0.55-1.01 per 100,000. The number of carriers accounted for 0.48-0.26 per 100,000 between 2000 and 2005, and zero during 2016-2019. MI vaccination is not carried out. The MI incidence in urban and rural areas was the same. In children aged between 0 and 17 years, during 2000 and 2005, MI incidence was 0.05–0.07, compared to 2016–2019 when it constituted 0.02–0.04 per 1000. In children aged between 0 and 2 years these indices were 0.08 per 1000 in 2004, and 0.44 per 1000 in 2018. Thus, the indices were lower among children aged between 3 and 6 years.*

Conclusions. *Incomplete detection of MI has been noted. Children aged between 0 and 2 years are prone to develop MI, the most vulnerable ones being children aged up to one year. The age structure of patients depends on the intensity of the epidemic process. Hence, it is necessary to improve MI surveillance with genotyping of circulating strains.*

Cuvinte cheie: *infecție meningococică, epidemiologie, morbiditate.*

PREZENTARE GENERALĂ A ASPECTELOR CARACTERISTICE ALE MORBIDITĂȚII PRIN INFECȚIA MENINGOCOCICĂ ÎN REPUBLICA MOLDOVA ÎN PERIOADA ANILOR 2000-2019

Introducere. *Infecția meningococică (IM) și portaj de N. meningitidis sunt larg răspândite, incidența globală fiind de 500.000-1.200.000 cazuri anual; 50.000-135.000 dintre ele sunt fatale; în țările europene incidența este de 0,6-2,0 la 100.000. Cei mai afectați sunt sugarii, adolescenții și adulții tineri. Din cauza variațiilor genetice ale meningococilor, este necesară o supraveghere permanentă a IM.*

Material și metode. *Scopul lucrării - analiza epidemiologică a IM în Republica Moldova în aa. 2000-2019. În cadrul studiului descriptiv-retrospectiv epidemiologic au fost analizate morbiditatea generală prin IM la 100 mii populație, numărul de cazuri atestate în zonele urbane și în cele rurale, rata morbidității, înregistrate pe un eșantion de 1000 de copii, având vârsta de 0-17 ani și structura de vârstă a bolnavilor.*

Rezultate. *Este evidentă diminuarea aciclică a morbidității, de la 2,57 la 100 mii populație, până la 0,55-1,01 în ultimii 5 ani. Numărul purtătorilor în 2000-2005 era 0,48-0,26 la 100 mii, și zero în 2016-2019. Vaccinarea împotriva IM nu se realizează. Intensitatea IM în zonele urbane și în cele rurale este la același nivel. La copiii de 0-17 ani, IM era de 0,05-0,07 în 2000-2005 și de 0,02-0,04 la 1000 copii în 2016-2019; la cei de 0-2 ani era de la 0,44 în 2004 și de la 0,08 la 1000 în 2018. La copiii de 3-6 ani este o incidență mai joasă.*

Concluzii. *Se constată evidențierea incompletă a IM. Cei mai afectați sunt copiii de 0-2 ani, în special cei de până la un an. Structura de vârstă a bolnavilor depinde de intensitatea proceslui epidemic. Se impune perfecționarea sistemului de supraveghere a IM cu genotiparea tulpinilor circulante.*

INTRODUCTION

Meningococcal infection (MI) caused by *Neisseria meningitidis* bacterium, at least 13 genotypes, as well as the asymptomatic carriage of the causative agent, are widespread throughout the world. The distribution of meningococcal serogroups varies according to world regions (1-4). The overall incidence of invasive meningococcal disease varies between 500,000 and 1,200,000 cases each year; of which, from 50,000 to 135,000 cases are fatal (5). In the European countries, the overall MI notification rate is from 0.6 to 2.0 per 100,000 people and varies in different regions. For example, in 2016, 3280 confirmed cases of invasive meningococcal disease, including 304 deaths, were reported in 30 EU Member States. In some countries the epidemiological situation of MI is quite dramatic, such as the tenfold increase in MenW type IM in the United Kingdom, recorded between 2009-2010 – 2016-2017 (6). Serogroups B and C are the most common causes of MI in Europe, but an increase in serogroup W has been observed in recent years. Generally, serogroup B causes the highest burden in Europe, followed by serogroups C, W and Y (1, 7).

Nasopharyngeal colonization with *Neisseria meningitidis* ranges from 5-10% to 25% in certain populations (the highest is found in adolescents and closed groups). Invasive meningococcal disease is a major cause of meningitis and septicemia. It often has a rapid evolution, with a ratio of 8-15% case-fatality. Some age groups are disproportionately affected by MI, with major incidence peaks occurring in infants, adolescents, and young adults (1, 2, 3, 8).

In recent years within the Global Meningococcal Initiative (GMI), the global importance of meningococcal disease, the ever-changing epidemiology, and the importance of proper surveillance of this evidence-based disease have been emphasized. Due to large geographical variations of circulating meningococcal serogroups, each country should be further monitored in order to change major disease-causing serogroups, as well as to spread information on vaccination and control policies. Likewise, the laboratory capacity must be properly adapted to better understand local epidemiology and disease burden, as well as its impact (9-12).

Inactivated meningococcal vaccines are authorized. There are conjugate polysaccharide and polysaccharide vaccines containing capsules of serotypes A, C, Y and W. Antibiotics are used to eliminate infection transmission and to treat the disease (1, 2, 3).

It can be concluded that analysis of epidemiological data on MI in different regions is necessary and relevant for a multilateral assessment. Such analyses have not been performed, which makes prevention difficult. The ongoing surveillance of MI is important for the development of future vaccination and surveillance policies.

MATERIAL AND METHODS

The purpose of the research is to analyze the epidemiological data collected in the Republic of Moldova over a period of about 20 years (2000-2019) in order to estimate the incidence and characteristics of meningococcal infection. In order to reveal the problems related to meningococcal infection worldwide, 212 bibliographic sources were analyzed as well as the WHO, CDC, ECDC materials published in the last five years.

The analysis of meningococcal infection morbidity in the Republic of Moldova over the period 2000-2019 was performed in the descriptive retrospective epidemiological study based on official statistical data (F.2) with the presentation of intensive and extensive indices, such as general morbidity per 100 thousand people (population), number of cases in urban and rural areas, morbidity of children aged 0-17 years by age groups per 1000 children, age structure of patients (%), including adults, children up to one year, 0-2; 3-6; 7-17 years. There were analyzed MI cases of 71 patients hospitalized over the period 2010-2019 in the Municipal Clinical Children's Hospital of Infectious Diseases.

RESULTS

The statistical data on the spread of meningococcal infection in the Republic of Moldova before 2000, revealed that between 1945 and1969 the morbidity on average was recorded at 0.98 cases per 100,000 people, between 1970 and 1989 it considerably increased (220-230 times compared to 1963). Over the period 1990-1999, 2-4 cases per 100,000 people were registered (13, 14). Vaccination against meningococcal infection is neither included in the National Immunization Program, nor carried out.

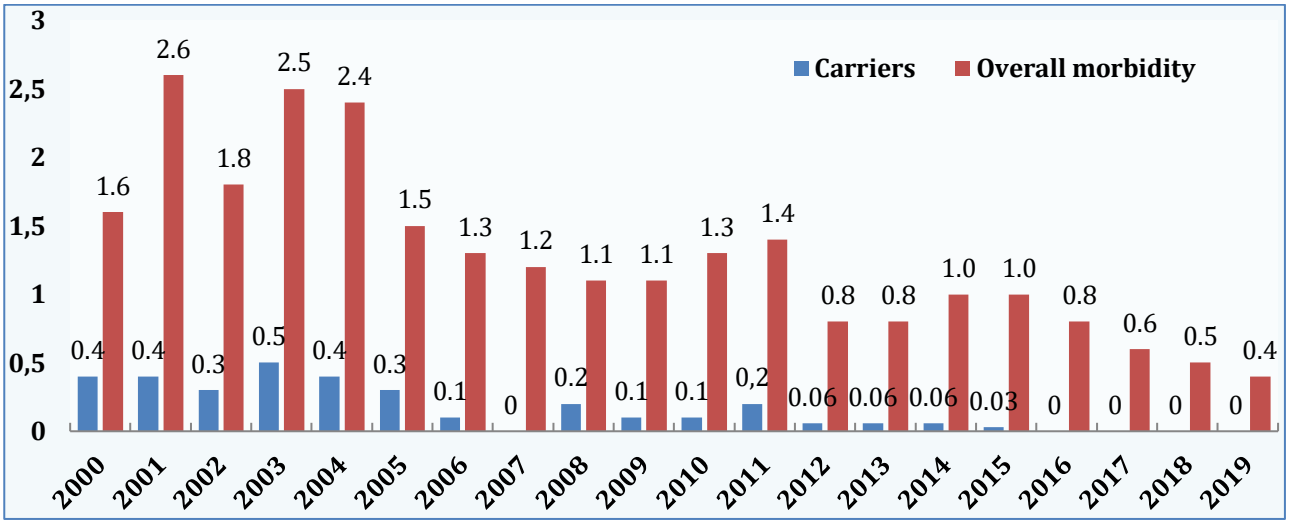

Figure 1. Data on the evolution of meningococcal infection morbidity and the level of *N. meningitidis* carriage, Republic of Moldova, 2000-2019.

Analysis of morbidity indices of meningococcal infection during the reference period found (fig.1) a gradual decrease from 2.57 to 100 thousand people (111 cases) at the beginning of the period, up to 0.55-1.01 over the last 5 years (on average 23.6 cases per year). Analysis of multi-annual morbidity of meningococcal infection did not highlight any cyclicity. The number of *N. meningitidis* carriers over the period 2000-2005 accounted for 0.48-0.26 per 100 thousand people (14.5 cases on average annually), then suddenly decreased to 0 (zero) during 2016-2019.

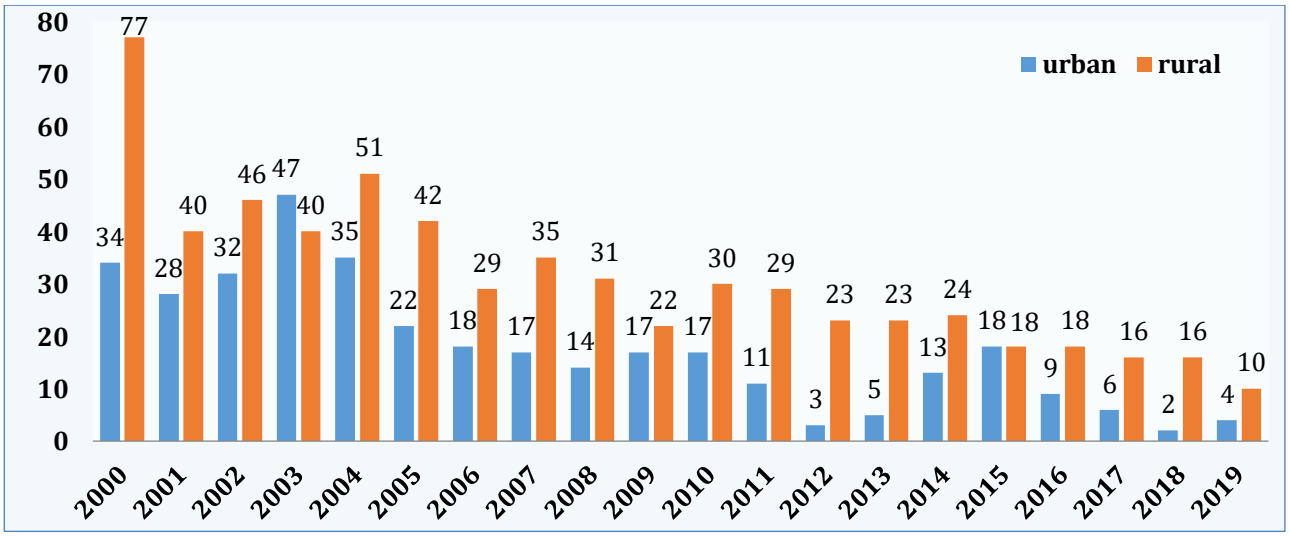

Figure 2. Data on the number of cases of meningococcal infection in urban and rural areas, Republic of Moldova, 2000-2019.

The data presented in Figure 2 show that the ratio of the number of cases of meningococcal infection in rural and urban areas ranges between 0 and 8 (2015 and 2018). The average annual ratio of cases of meningococcal infection in rural and urban areas is equal to 2.54, which broadly reflects the ratio of the population in these two regions.

In order to take adequate surveillance and control measures of meningococcal infection, it is important to analyze how frequently and intensively different age groups of the population are affected, considering the fact that according to multiple scientific publications a higher incidence is observed in infants, adolescents, and young adults (1, 2, 3, 8, 13). The data recorded in

the Republic of Moldova during the reference period are presented in Figure 3.

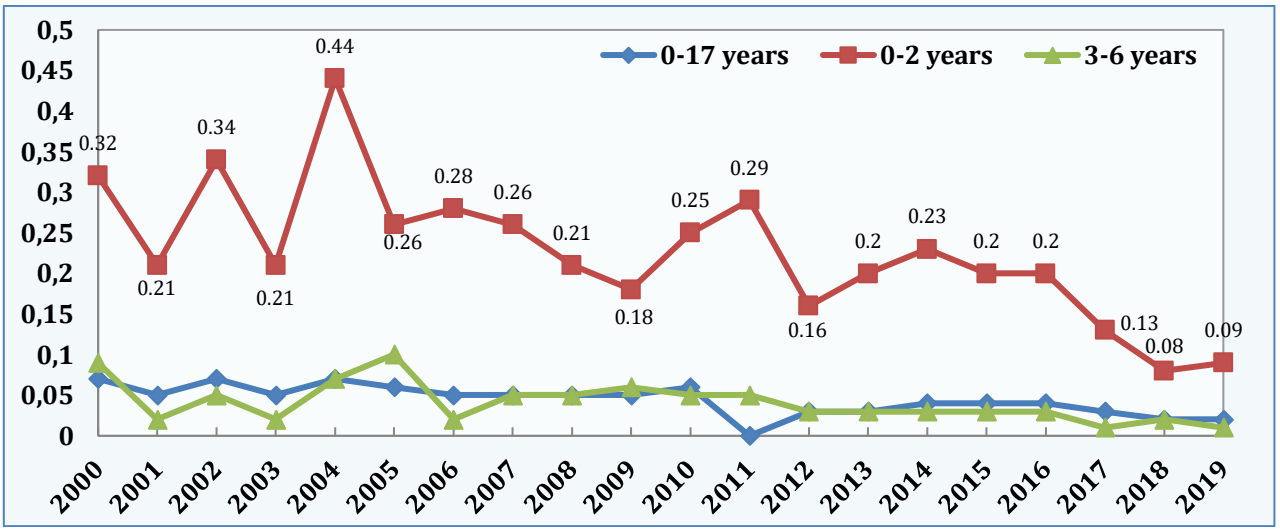

Figure 3. Data on meningococcal infection morbidity in children of different ages, Republic of Moldova, 2000-2019.

Data presented in Fig. 3 show that the morbidity of meningococcal infection in children aged between 0 and 17 years was 0.05-0.07 per 1000 children over the period 2000-2005 and 0.02-0.04 per 1000 children over the period 2016-2019. The morbidity level in children aged between 0 and 2 years ranged from 0.44 to 1000 children in 2004 and 0.08 to 1000 children in 2018. In children aged 3-6 years there was a lower incidence, 0.01 per 1000 children over the period 2017-2019 and 0.1 per 1000 children in 2005.

Table 1. Age distribution of MI patients in the Republic of Moldova, 2000-2019.

Period	Total number of cases	Average number of cases per year	Average age rate of MI patients (%) per year				
			adults	children's age, years			
				0-17	0-2 (including < 1 year)	3-6	7-17
2000-2004	430	86	27.4	72.6	43.3 (17.9)	12.1	17.3
2005-2009	247	49.4	9.7	90.3	55.5 (20.2)	17.8	17.0
2010-2014	177	35.4	5.6	94.4	71.7 (20.1)	14.2	8.5
2015-2019	118	23.6	8.5	91.5	64.4 (32.2)	12.6	14.5

Age structure analysis of patients with meningococcal infection (tab. 1) shows that among the total number of patients the disease predominated in children 72.6-94.4%, in different periods. The rate of children aged between 0 and 2 years was the highest compared to other age groups, being within 43.3% in the first period and 94.4% during 2010-2014. Particular attention was drawn to children up to one year that constituted a rather large number among patients with meningococcal infection; the rate ranged from 17.9% in the first period and 32.2% in the last one. The epidemic incidence, with expressed intensity, was higher among adult patients, 27.4% during 2000-2004, compared to 5.6%-9.7% in other researched periods.

During 2010-2019, in the Municipal Clinical Children's Hospital of Infectious Diseases in Chisinau, 71 patients with meningococcal infection were admitted (23.3% of the total number of patients – 305 people). Annually, the number of children hospitalized with MI fluctuated, 10-16 patients per year (44.8-71.7% of the total number of patients) during 2010-2015 and 2-4 per year (7.4-14.8% of the total number of patients) during 2016-2019. Respectively, MI share was 0.02-0.2% per hospitalized patients. All patients developed meningococcemia, a severe form of MI; about 75% had purulent meningitis, of which over 2/3 of cases had toxic shock syndrome and/

or cerebral edema. Four children died (5.6%), all of them being up to 1 year old. The etiological diagnosis in most cases was assessed according to clinico-epidemiological pattern. As a result, the bacteriological examination (CSF, blood and nasopharynx exudate) was negative. In 2019, in two cases, of the total number of 3 children with MI, Neisseria meningitidis DNA in CSF was detected using PCR.

DISCUSSIONS

During the researched period, 2000-2019, in the Republic of Moldova due to the absence of MI vaccines there was a gradual decrease in morbidity of this infection (on average 23.6 cases per year) without obvious cyclicity. The number of *N. meningitidis* carriers dropped to 0 (zero) in the last three years. It is known that on average 1% to 10% of the population are asymptomatic carriers of meningococci, but in epidemic situations this rate ranges between 10% and 25% (1, 14). This accounts for the incomplete recording of Neisseria meningitides cases and its carriers. In addition, it is known that different age groups are disproportionately affected by MI, with major incidence peaks occurring in infants, adolescents and young adults (1, 2, 3, 8). In the Republic of Moldova, according to the analysed data, there is only one, so-called epidemic peak, in children aged between 0 and 2 years, except for adolescents and young adults.

It should be noted that IM surveillance is necessary to reduce the impact of meningococcal disease, monitor morbidity incidence and prevent epidemics. This practice should include detection, application of evidence-based measures, and etiological investigation of each suspected case of meningococcal disease. In addition, modern surveillance includes the identification of circulating serogroups of *N. meningitidis*, this being the first step in implementing strategies to prevent MI by vaccination (1-3). These principles formulated by WHO and Global Meningococcal Initiative specialists should be laid on the organization of meningococcal infection surveillance in the Republic of Moldova.

CONCLUSIONS

1. Based on the data analysis related to morbidity of meningococcal infection and the number of *N. meningitidis* carriers, an incomplete highlighting of the affected persons was found.
2. Children aged between 0 and 2 years, especially infants up to one year, are most affected by meningococcal infection.
3. The age structure of patients with meningococcal infection depends on the epidemic process intensity. Thus, due to progressive involvement, there might occur a higher morbidity rate among adolescents and adults.
4. In the Republic of Moldova, it is necessary to improve the surveillance and control of meningococcal infection as well as to carry out the permanent examination of circulating *N. meningitides* strains, including genotyping.

CONFLICT OF INTERESTS

No conflicts of interest were reported.

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Date of receipt of the manuscript: 28/03/2020
Date of acceptance for publication: 05/05/2020

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

BIOLOGICAL SCIENCES

OPEN ACCESS

RECENT EVOLUTIONS OF NATURAL FOCI OF LEPTOSPIROSIS AND SMALL MAMMAL COMMUNITIES (RODENTIA, INSECTIVORA) IN THE REPUBLIC OF MOLDOVA

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DOI: 10.38045/ohrm.2020.1.13 UDC: 616.98:579.834.115:599.32/.38(478)

Keywords: *small mammals, Leptospira spp., leptospirosis, ecosystem, biotope.*

Introduction. *Leptospirosis is a zoonosis caused by microorganisms of the genus Leptospira with a global spread. The main reservoir of leptospires are the small mammals, which survive after infection, spreading the causative agent in the environment with urine.*

Material and methods. *The studied were conducted in 2017-2019 in different ecosystems, where 1617 small mammals were collected, investigated for the presence of specific antibodies to Leptospira spp. Ecological analysis of small mammal communities was performed and leptospire-carrier species were identified.*

Results. *17 species of the order Rodentia and Soricomorpha were identified. The most widespread are A. sylvaticus, A. flavicollis and A. agrarius with a frequency of 100%, abundant and dominant in most of the studied ecosystems. Antibodies specific to Leptospira spp. were detected in 9 species, the highest share of the species with leptospire belongs to A. agrarius (53.85%) and C. glareolus (11.54%). The serogroups Leptospira grippotyphosa, L. icterohaemorrhagiae and L. pomona were highlighted.*

Conclusions. *The epizootiological process in leptosrosis is mentained active in the nature by 9 species of small mammals, including one of the euritope species A. agrarius, the leptospire rate was determined at 53.85%. The intense circulation of leptospires in the small mammal population determines the need to monitor the multiannual dynamics of their in order to establish changes in natural foci of leptospirosis, forecast the epizootological situation and the risk of spreading of disease among the human population.*

Cuvinte cheie: *mamifere mici, Leptospira spp., leptospiroza, ecosistem, biotop.*

EVOLUTIILE RECENTE A FOCARELOR NATURALE DE LEPTOSPIROZĂ ŞI COMUNITĂŢILE MAMIFERELOR MICI (RODENTIA, INSECTIVORA) ÎN REPUBLICA MOLDOVA

Introducere. *Leptospiroza este o zoonoză cauzată de microorganismele din genul Leptospira cu o răspândire globală. Rezervorul de bază al leptospirelor sunt mamiferele mici care după infectare supravieţuiesc, diseminând agentul cauzal în mediul înconjurător prin urină.*

Material şi metode. *Cercetările au fost efectuate în perioada 2017-2019 în diverse ecosisteme, de unde au fost colectate 1617 mamifere mici, care au fost investigate la prezenţa anticorpilor specifici Leptospira spp. S-a efectuat analiza ecologică a comunităţilor de mamifere mici şi identificate speciile purtătoare de leptospire.*

Rezultate. *Au fost identificate 17 specii din ordinul Rodentia şi Soricomorpha. Cele mai răspândite sunt A. sylvaticus, A. flavicollis şi A. agrarius cu o frecvenţă de 100%, fiind abundente şi dominante în majoritatea ecosistemelor cercetate. Anticorpi specifici la Leptospira spp. s-au evidenţiat la 9 specii, dintre care cea mai înaltă pondere îi revine speciei A. agrarius (53,85%) şi C. glareolus (11,54%). S-au evidenţiat serogrupele Leptospira grippotyphosa, L. icterohaemorrhagiae şi L. pomona.*

Concluzii. *Procesul epizootologic la leptosroză este menţinut activ în natură de 9 specii de mamifere mici, inclusiv la una din speciile euritope A. agrarius s-a determinat portajul de leptospire în cote de 53,85%. Circulaţia intensa a leptospirelor în populaţia mamiferelor mici determină necesitatea monitorizării în dinamică multianuală a acestora pentru stabilirea modificărilor in focarele naturale de leptospiroză, prognozarea situaţiei epizootologice şi riscului de răspândire a bolii în populaţia umană.*

INTRODUCTION

Leptospirosis is a zoonosis caused by representatives of the genus *Leptospira* with a global spread, which affects the human population on continents with tropical and temperate climates. It occurs in urban areas of industrialized and developing countries, as well as in rural areas around the world. This disease is recognized as an emerging global public health problem, due to the increased incidence in many economically advanced and developing countries. For the first time, living leptospires were observed under dark-field microscopy, during the examination of a water sample from a pool, an observation communicated in 1914 by Wolbach and Binger. After the discovery of leptospires, the Japanese researchers (1) described the role of rats as carriers of the causative agent of leptospirosis, which led to the establishment of the mechanism and ways of transmission of the causative agent from animals to humans. The representatives of the genus Leptospira persist in the kidneys and genital tract of carrier animals and are excreted in the urine. Human infection occurs accidentally, through direct contact with the infected animal, or indirectly through contaminated environment, such as soil or water (1-8).

The leptospire reservoir includes a large number of species of wild and domestic animals (cattle and pigs) that survive infection with the causative agent of leptospirosis, spreading the causative agent in the environment for a long time with urine. All terrestrial mammals can be indicated as a source of leptospira, but the most important species are those from the orders Rodentia and Insectivora, which can be true living environments particularly favorable for hosting, multiplying and eliminating leptospires in the environment. A potential risk of human infection is maintained in the territories where leptospire-carrying status have been recorded in wild animals, and in domestic animals – diseases or asymptomatic state. These territories are considered as foci of leptospirosis and, depending on the reservoir, can be classified into natural and anthropogenic (1-6, 9, 10, 11).

According to literature data, as a reservoir and source of leptospira infection the orders Marsupialia, Insectivora, Chiroptera, Primates, Edentata, Lagomorpha, Rodentia, Carnivora and Artiodactyla are of major importance, which are taxonomically included in the class of mammals, but the main carriers are the representatives of 4 orders: Marsupialia, Insectivora, Rodentia and Carnivora. At the same time not all the species of an order are carriers of leptospires. As a reservoir of leptospires a significant role was assigned to mammals of the order Rodentia – 20%, Marsupialia – 39% and Chiroptera – 35% (12). The role of the species *Mus musculus* and *Rattus norvegicus* as a reservoir and source of leptospires is well known in Portugal, Azores (13). It is known that small mammals are indispensable elements of terrestrial ecosystems and are important links in the food chains of the living world. Most species of small mammals, especially rodents, are very prolific, reach high densities in various types of ecosystems and have wide limits of ecological valence. All these peculiarities favor the wide spread of this group of mammals and their rapid adaptation to anthropogenic and environmental changes (14-17). In France, the beaver (*Myocastor coypus*) is involved in the spread of leptospirosis, a species that inhabits clean stagnant waters and is common in the last decade. The seroprevalence of leptospires in the population of *Myocastor coypus* varied from 16.5% to 66.0%, being detected the serogroups: *L. icterohaemorrhagiae* and *L. sejroe* (18). In Germany, the wild boar (*Sus scrofa*) has been identified as the possible source of leptospirosis infection, a fact confirmed by a number of European researchers who have highlighted the serogroup *L. pomona*, frequently isolated from domestic pigs and wild boars (19).

In the Republic of Moldova, the study of leptospirosis began in the 1950s, where leptospirosis was first established in cattle in Tiraspol city, and then the disease was recorded in pigs, horses and cattle in all districts of the republic. Later, during 1969-1971, natural outbreaks of leptospirosis were registered in the northern area, with the involvement in the epizootic process of *Microtus* species and the etiological agent *Leptospira grippotyphosa*. During 1987-1988 when 2 cases of leptospirosis were registered in the workers of a meat processing plant, as a source of leptospirosis infection the brown rat was established, in which specific antibodies to the serogroup *L. icterohaemorrhagiae* were highlighted (20). Another outbreak of leptospirosis was recorded in 1996 in 7 fishermen from the local fish farm of Costesti village, Ialoveni district and it was shown that the source of infection were the small mammals, in which the serogroup

L. icterohaemorrhagiae was reported (21). Studies conducted during 2006-2008 demonstrated that in the leptospirosis foci the infection reservoir are the species of the order Rodentia, where the highest share of positive results in leptospirosis was found in *Rattus norvegicus* (37.7%), *Apodemus sylvaticus* (21.8%) and *A. agrarius* (20.9%) (9). During this period, positive serological results were established in 10 species of the order Rodentia (*Muscardinus avellanarius*, *Clethrionomys glareolus*, *A. terrestris*, *Microtus sp.*, *A. uralensis*, *A. sylvaticus*, *A. flavicollis*, *A. agrarius*, *Mus musculus*, *R. norvegicus*) and 2 species of the order Insectivora (*Sorex minutus*, *S. araneus*). Studies conducted in our country showed that the main source of infection is rodents, and human infection occurs both during bathing, using water from unauthorized sources in the field and after using food contaminated by rodents (9, 10, 20, 21-25).

The study of the ecological peculiarities of small mammals in leptospirosis outbreaks aims to elucidate the species involved in the epizootic process, followed by determining the etiological structure of circulating leptospirosis in the ecosystems of the Republic of Moldova. The data obtained are used to develop epizootic prognoses, public health risk assessment and argumentation of disease prevention and control measures.

MATERIAL AND METHODS

The studies were conducted during 2017-2019 in the North, Center and South of the Republic of Moldova, districts Glodeni, Edinet, Ocnita, Orhei, Ialoveni, Chisinau, Cahul and Stefan Voda, in 147 natural and anthropogenic biotopes that were grouped in 6 types: forest, paludous, agrocenoses, forest belt, forest edge and rural-paludous ecotone. The data are presented for winter - spring (December, February-May) and summer-autumn (June - November) periods. Snap traps were used to collect small mammals baited with pieces of bread soaked in sunflower oil. The traps were installed either in line or randomly, with the distance of 7-10 m between the traps. About 40-50 traps were used per biotope and only the functional ones were taken into account (26). A total of 8593 night-traps were processed, 1617 small mammals were captured and 1572 individuals were investigated for the presence of specific antibodies to *Leptospira* spp. in the microbiological laboratory within the National Agency for Public Health. Each individual was identified, except for sibling species of the genus *Microtus* (*M. arvalis* and *M. rossiaemerdionalis*), which were considered as *Microtus sp*.

The ecological analysis of small mammal communities was performed using the indexes of capture (Ic), abundance (A), frequency (F), diversity (Shannon, H'; Simpson) and dominance (Naughton-Wolf, I_d). The ecological significance (Wa) of the species in studied ecosistems was assessed. The statistical analysis and graphic presentation of the results were performed via the Microsoft Excel and BioDiversity Pro Programs. The data for the Statistical Report regarding the morbidity caused by leptospirosis in the Republic of Moldova for 2017-2019 were processed.

RESULTS

A total of 1617 small mammals were collected, 13 species of the order Rodentia: *M. glis, A.sylvaticus, A. flavicollis, A. uralensis, A. agrarius, Mus spicilegus, M. musculus, R. norvegicus, M. minutus, Microtus* sp., *M. subterranus, C. glareolus, A. terrestris* and 4 species of the order Soricomorpha: *S. minutus, S. araneus, C.suaveolens* and *N. anomalus.*

The capture index of small mammals varied depending on the year, season and geographical area. In the study period 2017-2019, the average multiannual capture index was 18.81%. In 2017 this index was the highest (23.37%), compared to 2018 (17.38%) and 2019 (12.77%).

In the northern area 808 small mammals were collected from 10 rodents species: *A. sylvaticus, A. flavicollis, A. uralensis, A. agrarius, M. spicilegus, M. musculus, R. norvegicus, M. minutus, Microtus* sp., *M. subterranus* şi *C. glareolus* and 4 shrew species: *S. minutus, S. araneus, C. suaveolens* and *N. anomalus* (tab.1). For the period of 2017-2019, the multiannual average capture index was 24.92%. In 2017 this index was 30.61%, in 2018 - 18.62% and in 2019 – 11.90%. The highest multiannual average capture index was recorded in the rural-poaludosu ecotone (62.4%) and very close values were recorded in the paludous biotope and forest belt (30.52%-30.83%), while the lowest index was in forests and at forest edge (17.42%-17.83%) (tab.1).

The total diversity of small mammal species according to the Shannon index was 0.643 and the Simpson index – 4.225. The greatest diversity was registered in the forest belt (0.793; 4.182), agrocenosis (0.782; 3.783) and paludous (0.714; 4.007) biotopes with the dominant species *A. flavicollis* (I_d - 86.40%) and *A. agrarius* (I_d – 73.94%-90.85%), which constituted more than half of the small mammal community. The lowest values of diversity were recorded in the forest biotopes (0.569; 1,801), the dominant being *A. flvicollis* (80%) (tab.1).

Throughout the study period, the dominant species of *A. sylvaticus*, *A. flavicollis* and *A. agrarius* were the most widespread and present in all the biotopes with a frequency of 100%. *A. sylvaticus* was abundant in forest belt and rural-paludous ecotone with eudominant ecological significance, and low values of abundance in forest with a dominant significance. *A. flavicollis* was abundant in forest, forest edge and forest belt with eudominant ecological significance, and low values of abundance in agrocenoses and rural-paludous ecotone (about 6%) with dominant significance (tab.1).

Table 1. Abundance (A), ecological significance (Wa) and diversity of small mammal species in studied ecosystems from the northern area of the Republic of Moldova.

No.	Species	Forest		Agrocenosis		Paludous		Forest edge		Shelter belt		Rural-paludous		Total
		A	Wa	A	Wa	A	Wa	A	Wa	A	Wa	A	Wa	A
1	*S. araneus*	2.36	1.57	2.42	1.61	2.45	1.64	-	-	-	-	4.76	3.17	1.61
2	*S. minutus*	-	-	-	-	1.84	0.61	-	-	-	-	1.59	0.53	0.50
3	*C. suaveolens*	-	-	-	-	-	-	0.54	0.09	-	-	-	-	0.12
4	*N. anomalus*	-	-	-	-	0.61	0.10	-	-	-	-	-	-	0.12
5	*A. sylvaticus*	5.51	5.51	16.94	16.94	9.20	9.20	25.41	25.4	8.22	8.22	20.63	20.63	14.23
6	*A. flavicollis*	73.2	73.23	6.45	6.45	20.86	20.86	32.97	32.97	49.32	49.32	6.35	6.35	33.66
7	*A. uralensis*	-	-	11.29	3.76	2.45	0.82	-	-	-	-	-	-	2.23
8	*A. agrarius*	11.8	11.81	45.97	45.97	42.94	42.94	23.24	23.2	24.66	24.66	46.03	46.03	30.94
9	*M. spicilegus*	-	-	9.68	3.23	-	-	11.89	3.96	-	-	-	-	4.21
10	*M. musculus*	-	-	1.61	0.81	-	-	-	-	0.68	0.34	1.59	0.79	0.50
11	*R. norvegicus*	-	-	-	-	1.23	0.41	-	-	-	-	1.59	0.53	0.37
12	*M. minutus*	-	-	-	-	0.61	0.20	-	-	-	-	1.59	0.53	0.25
13	*Microtus sp.*	-	-	5.65	4.70	9.82	8.18	3.243	2.7	2.05	1.71	1.59	1.32	4.08
14	*M.subterraneus*	-	-	-	-	-	-	-	-	4.11	1.37	1.59	0.53	0.87
15	*C. glareolus*	7.09	5.91	-	-	7.98	6.65	2.70	2.25	10.96	9.13	12.70	10.58	6.31
Capture index, %		17.42		26.96		30.52		30.83		17.83		62.38		24.92
Shannon index		0.569		0.782		0.714		0.793		0.713		0.692		0.643
Simpson index		1.801		3.783		4.007		4.182		3.122		3.749		4.225

A. agrarius had the highest abunance in agrocenosis, paludous and rural-paludous biotopes with eudominant ecological significance. *A. uralensis* with a frequency of 33.33% was registered in agrocenosis with dominant ecological significance and in paludous biotope with recedent significance (tab.1).

The forest vole species *C. glareolus* was registered in all type of biotopes except the agrocenosis with a frequrency of 83.33% and dominant ecological significance, while *M. subteraneus* was recorded at forest edge and in rural-paludous ecotone with 33.33% frequency and recedent or subrecedent signify cance. The field voles *Microtus* sp. were mostly spread in all types of biotopes, except the forest ones, showing a dominant ecological significance in agrocenosisi and paludous biotopes, and with subdominant significance in other habitats (tab.1; fig.1).

M. musculus is a typical synanthropic species and was registered in anthropyzed biotopes with 50% frequency, low abundance and subrecedent significance. *M. spicilegus* (F-33.33%) was recorded in agrocenosis and at forest edge near agrocenoses with recedent ecological significance. The species *R. norvegicus* and *M. minutus* had the same biotopic distribution and subrecedent ecological significance (tab.1; fig,1).

Among shrews, the most spread was *S. araneus* (F-66.67%) with recedent ecological significance. Other three species were observed only in paludous biotopes (*S. minutus*, *N. anomalus*) or at forst edge (*C. suaveolens*) with subrecedent ecological significance (tab.1; fig.1).

In the central area, 479 small mammals were collected from 13 species: *M. glis*, *A. sylvaticus, A. flavicollis, A. uralensis, A. agrarius, M. spicilegus, M. musculus, R. norvegicus, M. minutus, Microtus* sp.*, M. subterraneus*, *C. glareolus* and *S. araneus* (tab.2). The average multiannual capture index for the study period was 14.83%. In 2017 the capture index was 13.70%, in 2018 – 18.11% and in 2019 the lowest index – 12.98%. The highest multiannual average catch index was recorded in the agrocenosis (21.55%) and at forest edge (16.26%), and the lowest index was reported in the paludous biotope (10.25%) (tab.2).

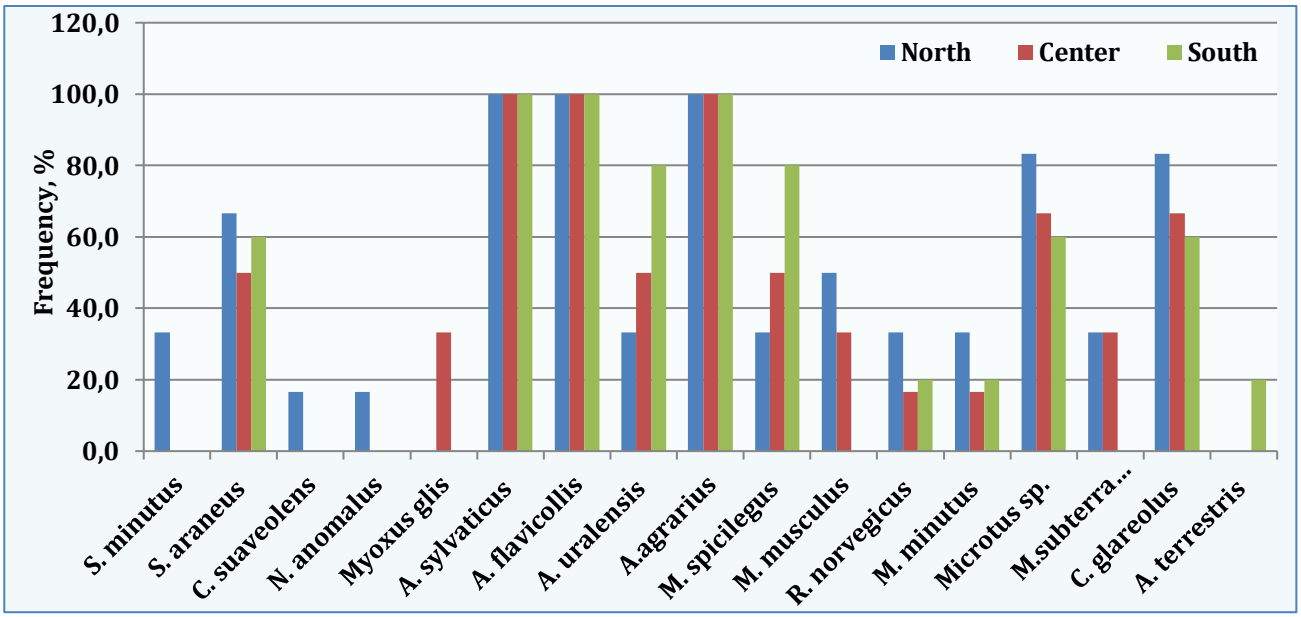

Figure 1. Frequency (%) of small mammal species in studied ecosystems from the northern, central and southern areas of the Republic of Moldova.

The total diversity of small mammal species according to Shannon index was 0.744 and the Simpson index – 5.064. The greatest diversity of species was recorded in the agrocenosis (0.854; 5.317) and forest belt (0.816; 4.29), and the lowest diversity – in the forest (0.543; 2.102), similar with the results obtained in the northern area (tab.2).

The dominant species of *A. sylvaticus*, *A. flavicollis* and *A. agrarius,* found in the northern area were present in all studied biotopes with a frequency of 100%. *A. sylvaticus* was more abundant and do-minant in agrocenosis (I_d-62.94%) and in forest belt (I_d-56.14%) with eudominant ecological significance. *A. flavicollis* was more abundant and dominant in forest biotopes (I_d-84.35%) and at forest edge (I_d-57.67%) with eudominant ecological significance, while in the paludous biotope, it had a low abundance and a recedent significance. *A. agrarius* was most abundant and dominant in the paludous biotope (I_d-68%) and in the rural-paludous ecotone (I_d-40.91%) with eudominat significance. The species *A. uralensis* with a frequency of 50% was reported in agrocenosis and forest belt with dominant ecological significance (tab.2; fig.1).

The voles *Microtus* sp. and *C. glareolus* had a frequency of 66.67% and *M. subterraneus* had a 33.33% frequency, recorded only in forest biotopes with recedent ecological significance. *M. spicilegus* showed a dominant ecological significance in agrocenosis, whereas the *M. musculus* had a dominant ecological significance in rural-paludous ecotone. The species of *M. minutus* and *R. norvegicus* were recorded only in rural-paludous ecotone (F-16.67%) with recedent ecological significnance (tab. 2; fig. 1).

The shrew of *S. araneus* had a 50% frequency, showing a dominant ecologcal significance in paludous biotopes and a recedent significance at forest edge (tab.2; fig.1).

In the southern area**,** 329 small mammals from 11 species were collected: *A. sylvaticus, A. flavicollis, A. uralensis, A. agrarius, M. spicilegus, M. musculus, R. norvegicus, M. minutus, Microtus* sp.*, C. glareolus* and *S. araneus* (tab.3).

Table 2. Abundance (%), ecological significance (Wa) and diversity of small mammal species in studied ecosystems from the central area of the Republic of Moldova.

No.	Species	Forest		Agrocenosis		Paludous		Forest edge		Shelter belt		Rural-paludous		Total
		A	Wa	A	Wa	A	Wa	A	Wa	A	Wa	A	Wa	A
1	*S. araneus*	-	-	2.11	1.06	12.00	6.00	-	-	1.52	0.76	-	-	1.46
2	*M. glis*	0.87	0.29	-	-	-	-	16.09	5.36	-	-	-	-	3.13
3	*A. sylvaticus*	18.26	18.26	27.46	27.46	8.00	8.00	36.78	36.78	16.67	16.67	11.36	11.36	22.96
4	*A. flavicollis*	66.09	66.09	23.94	23.94	4.00	4.00	4.60	4.60	62.12	57.67	-	-	32.57
5	*A. uralensis*	-	-	17.61	8.80	-	-	13.79	6.90	-	-	4.55	2.27	8.14
6	*A. agrarius*	0.87	0.87	9.86	9.86	56.00	56.00	24.14	24.14	6.06	6.06	56.82	56.82	16.49
7	*M. spicilegus*	-	-	13.38	6.69	-	-	-	-	1.52	0.76	-	-	4.80
8	*M. musculus*	-	-	1.41	0.47	-	-	-	-	-	-	15.91	5.30	1.88
9	*M. minutus*	-	-	-	-	-	-	-	-	-	-	4.55	0.76	0.42
10	*R. norvegicus*	-	-	-	-	-	-	-	-	-	-	2.27	0.38	0.21
11	*Microtus sp.*	-	-	4.23	2.82	12.00	8.00	-	-	1.52	1.01	4.55	3.03	2.51
12	*M.subterraneus*	1.74	0.58	-	-	-	-	3.45	1.15	-	-	-	-	1.04
13	*C. glareolus*	9.57	6.38	-	-	8.00	5.33	1.15	0.77	10.61	7.07	-	-	4.38
Capture index, %		12.53		21.55		10.25		13.41		16.26		12.39		14.83
Shannon index		0.543		0.854		0.763		0.816		0.613		0.703		0.744
Simpson index		2.102		5.317		3.03		4.29		2.378		2.832		5.064

The diversity of small mammals according to the Shannon index was 0.786 and the Simpson index – 5.575. The highest diversity was reported at forest edge and agrocenosis, whereas the lowest – in the forest (tab. 3).

The average multiannual capture index was 15.53%. In 2017 the index was 17.81% and practically similar values were regisered in 2018 and 2019 making up 12.81% and 12.91% respectively. The highest multiannual average catch index was recorded at the forest edge (43.88%), and the lowest index was reported in the paludous biotope – 11.22% (tab.3).

The species *A. sylvaticus, A. flavicollis* and *A. agrarius* are the most spread and dominant in various types of biotopes: *A. sylvaticus* was dominant in paludous biotope (I_d-58.88%), *A. flavicollis* was dominant in forest (I_d-56.64%), at forest edge (I_d-36,93%) and forest belt (I_d-39.78%). *A. agrarius* had dominant ecological significance in forest and paludous biotopes. *A. uralensis* and *M. spicilegus* were largely distributed with a frequency of 80%. *A. uralensis* was most abun- dant and dominant in agrocenosis (I_d-44.08%) *M. spicilegus* – in agrocenosis and forest belt with eudominant ecological significance. The vole *C. glareolus* had a frequency of 60% and showed a dominant and eudominant significance in forest biotopes.

The field voles had recedent significance in agrocenosis, paludous and shelter belts. The hygrophilous species *M. minutus*, *R. norvegicus* and *A. terrestris* were registered only in paludous biotopes with recedent significance (tab. 3; fig.1).

In the northern area, in 5 species of small mammals of 15 registered specific antibodies to *Leptospire* spp. were detected. The highest share belonged to the species *A. agrarius*, which constituted 57.14%, followed by *C. glareolus* – 21.43%, while *A. sylvaticus*, *A. flavicollis* and *Microtus* sp. had equal share – 7.14% (fig. 2). Two serological groups were highlighted: *L. icterohaemorrhagiae* and *L. pomona* in equal shares of 50%. The serogroup *L. grippotyphosa* was hosted by the species *A. agrarius*, *Microtus sp*. and *C. glareolus*, and *L. pomona* – by *A. sylvaticus*, *A. flavicollis* and *A. agrarius*. Only in *A. agrarius* were detected both serological groups. The presence of the natural outbreak of leptospirosis in the forest ecosystem located near Hijdieni village of Glodeni district has been established with the involvement of *A. sylvaticus* and *A. agrarius* species. Another outbreak of leptospirosis was reported in paludous ecosystems, agrocenoses and rural-paludous ecotone near Hadarauti, Cores-tauti and Valcinet localities of Ocnita district with the involvement of the species *A. agrarius*, *C. glareolus* and *Microtus* sp.

In the central area among identified 13 species in the epizootic process were involved *A. agrarius*, *M. spicilegus*, *R. norvegicus* and *S. araneus*. The highest carrier rate among Leptospira-positive animals belongs to *A. agrarius* (60.0%), *M. spici-*

legus – 20.0%, *R. norvegicus* and *S. araneus* – 10.0% per each (fig.2). 2 serological groups were found: *L. icterohaemorrhagiae* – 90% and *L. grippotyphosa* – 10%. The serogroup *L. icterohaemorrhagiae* was hosted by the species *A. agrarius*, *M. spicilegus*, *R. norvegicus* and *S. araneus*, and *L. grippotyphosa* by one species – *A. agrarius*. Both serological groups were detected in *A. agrarius*.

Table 3. Abundance (%), ecological significance (Wa) and diversity of small mammal species in studied ecosystems from the southern area of the Republic of Moldova.

No.	Species	Forest		Agrocenosis		Paludous		Forest edge		Shelter belt		Total
		A	Wa	A	Wa	A	Wa	A	Wa	A	Wa	A
1	*S. araneus*	1.89	1.13	9.23	5.54	7.69	4.62	-	-	-	-	4.26
2	*A. sylvaticus*	5.66	5.66	23.08	23.08	41.76	41.76	20.78	20.78	20.93	20.93	24.62
3	*A. flavicollis*	64.15	64.15	16.92	16.92	4.40	4.40	24.68	24.68	37.21	37.21	25.53
4	*A. uralensis*	-	-	32.31	25.85	20.88	16.70	20.78	16.62	9.30	7.44	18.24
5	*A. agrarius*	22.64	22.64	1.54	1.54	13.19	13.19	5.19	5.19	9.30	9.30	10.03
6	*M. spicilegus*	-	-	15.38	12.31	4.40	3.52	14.29	11.43	2.33	1.86	7.90
7	*R. norvegicus*	-	-	-	-	1.10	0.22	-	-	-	-	0.30
8	*M. minutus*	-	-	-	-	2.20	0.44	-	-	-	-	0.61
9	*Microtus sp.*	-	-	1.54	0.92	2.20	1.32	1.30	0.78	-	-	1.22
10	*A. terrestris*	-	-	-	-	2.20	0.44	-	-	-	-	0.61
11	*C. glareolus*	5.66	3.40	-	-	-	-	12.99	7.79	20.93	12.56	6.69
Capture index, %		19.06		16.21		11.22		14.50		43.88		15.53
Shannon index		0.63		0.843		0.752		0.9		0.866		0.786
Simpson index		2.18		4.837		4.196		5.66		4.426		5.575

The activation of the natural outbreak of leptospirosis has been reported in forests, paludous ecosystems and in agrocenoses located near Danceni vill., Ialoveni ds., being a possible infectious area for 2 leptospirosis patients in autumn 2018. Compared to the northern and southern areas, the leptospirosis outbreak was active only in the central area for two consecutive years 2018-2019.

In the southern area among 11 species in *R. norvegicus* and *A. terrestris* specific antibodies to *L. icterohaemorrhagiae* (100%) were determined in equal shares – 50% (fig.2). The outbreak of leptospirosis was reported in paludous ecosystems of Prut meadow, near Cahul town in 2018. In September 2008 (Cahul, Prut meadow), the species *A. agrarius* and *A. uralensis* were detected with the serotypes *L. grippotyphosa* and *L. pomona*. Over a decade of monitoring, there is an active leptospirosis outbreak, being substituted by leptospira serotype.

Thus, in 9 species of small mammals from 17 collected specific antibodies to *Leptospira* spp. were found. The presence of leptospires was determined in 4.10% of total investigated small mammals. In the northern area, positive results were registered in 3.39%, in the central area – 5.88% and in the southern area – 4.0% (fig. 2). The highest rate belonged to the species *A. agrarius* (53.85%) and *C. glareolus* (11.54%), followed by *M. spicilegus* and *R. norvegicus* (7.69% each) and equal weight – *A. sylvaticus*, *A. flavicollis*, *Microtus* sp., *A. terrestris* and *S. araneus* (3.85% each). The presence of 3 serogroups were determined in small leptospira-infected mammals, viz. *L. icterohaemorrhagiae*, *L. grippotyphosa* and *L. pomona*. The results obtained indicate a great diversity of species involved in the epizootic process of leptospirosis in the country's ecosystems.

DISCUSSIONS

Leptospirosis is one of the most common zooanthroponoses in the Republic of Moldova, being a nosology, first diagnosed in humans and reported in 1963 (9, 24, 25). The primary detailed study (1950-1960) of the peculiarities of leptospirosis was preceded by determining the cases of disease in cattle registered in Tiraspol (1950). Subsequently, in 1970-1990, this disease was registered in pigs, cattle and horses from all districts (9, 24, 25).

In the Republic of Moldova, there are 2 types of leptospirosis foci: natural and anthropogenic (9, 23, 27, 28, 29). Currently, the obtained study results confirm the existence of natural and anthropogenic foci of leptospirosis. However, a larger part of the natural ecosystems of the coun-

try underwent major anthropic changes, this excludes the possibility of strict delimitation of natural foci of leptospirosis from anthropogenic ones. Therefore, in the leptospirosis foci in Moldova the reservoir of infection is the species of the orders Rodentia and Insectivora (9, 10, 20-23, 27-29). The present study obtained similar data. Thus, during the study period, 13 rodent and 4 insectivore species were determined on the territory of the country, and 9 of them showed positive results to *Leptospira* spp. Most of the listed species are widespread in the studied ecosystems (9, 10, 27-29). The results obtained on the diversity of small mammal populations, including species involved in the epizootic process, showed a decreasing tendency, which correspond to other published study results (9, 10, 20-23, 27, 28, 30, 31, 32).

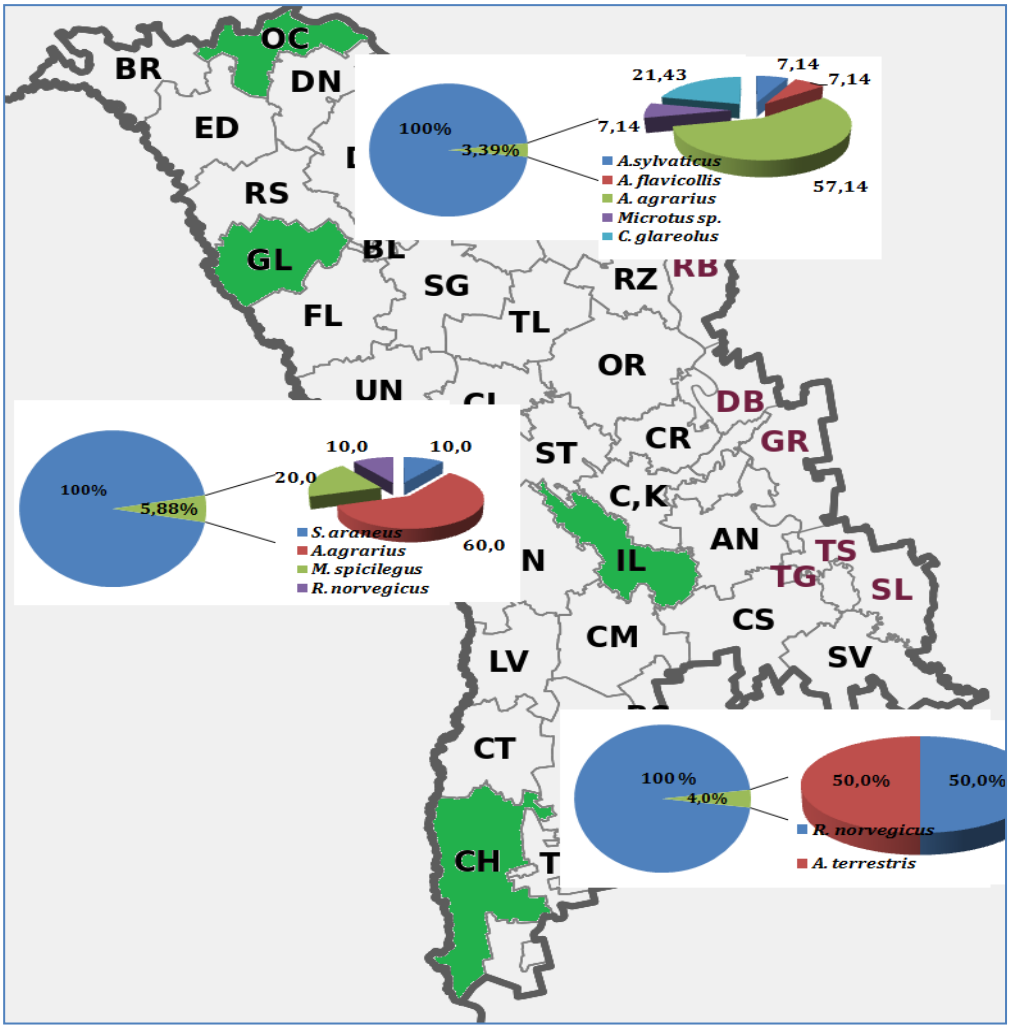

Figure 2. Share (%) and diversity of small mammal species found with leptospires in northern, central and southern areas of the Republic of Moldova.

An important role in the formation of leptospirosis foci belongs to the species reported as leptospirosis carriers, such as *A. sylvaticus*, *A. flavicollis* and *A. agrarius*, which are widely distributed, abundant and dominant in most of the ecosystems of the country. The obtained results confirm the importance of these species as reservoirs that maintain the etiological agent in the environment (9, 10, 27-29); however not all the aforementioned species are leptospira carriers (12). The analysis of the etiological structures of leptospires during the period 2017-2019 showed a lower diversity of serogroups (*L. grippotyphosa*, *L. icterohaemorrhagiae*, *L. pomona*) compared to 11-13 serogroups detected in previous studies (9, 10, 20, 21).

The obtained data confirm the relatively intense circulation of the causative agent of leptospirosis in small mammal populations. Human infection occurs accidentally during direct contact with

objects from the environment, contaminated with the urine of leptospira-carrier small mammals. Some authors claim that the level of morbidity from leptospirosis is influenced by flood-related outbreaks, recreational activities such as bathing in unauthorized water basins, boating or other water sports, as well as the tendency of disease urbanisation (3, 13, 33-36). In many countries, leptospirosis is considered an occupational disease previously encountered in farmers and agricultural workers (33, 37). Leptospirosis is recognized as a potential hazard in water sports or other recreational activities related to the exposure of the population to potentially contaminated water sources (3). In the Republic of Moldova, during 2017-2019, a total of 15 cases of leptospirosis disease were reported, registered in 8 administrative territories. A higher number of cases of disease were reported mainly in the northern districts: Briceni – 5 cases, Drochia – 2, Soroca, Edinet and Floresti – 1 case each; followed by the central area: Chisinau city – 3, Ialoveni – 1 case and the southern area: Tiraspol city – 1 case (38). In addition to some of the mentioned factors, leptospirosis morbidity in Moldova is determined by non-compliance with hygiene rules for people with high risk of contracting the disease, by consuming products kept in poor conditions, as well as through rodent and other animal urine – contaminated water etc.

Based on the aforementioned, we can conclude that in the current conditions of the Republic of Moldova all key elements persist for the formation and activation of natural foci of leptospirosis such as biotic, abiotic environmental factors, ecological characteristics of the reservoir, the causative agent of leptospirosis and as a result the risk of spreading this disease in the human population.

CONCLUSIONS

1. 1617 small mammals were collected from the ecosystems of the Republic of Moldova that belong to 13 species of the order Rodentia and 4 of the order Soricomorpha. The greatest diversity of species was registered in the northern area: 11 rodents, 4 insectivores; center – 11 and 2 respectively; south – 9 and 2 respectively. The most widespread and euritope species are *A. sylvaticus*, *A. flavicollis* and *A. agrarius*, found in all areas of the country.
2. Of the 17 small mammal species 9, showed positive serological results for *Leptospira* spp. (*A. agrarius*, *C. glareolus*, *M. spicilegus*, *R. norvegicus*, *A. sylvaticus*, *A. flavicollis*, *Microtus* sp., *A. terrestris*, *S. araneus*), which proves a great diversity of species involved in the epizootic process in leptospirosis. The highest rate belongs to the species *A. agrarius* (53.85%) and *C. glareolus*. The presence of leptospires was determine in 4.10% of small mammals studied. The highest rate was determined in the central area (5.88%), the southern area (4.0%) and the northern area (3.39%). Among the leptospirosis-carrier species, *A. sylvaticus*, *A. flavicollis*, *A. agrarius* have a wide distribution (F-100%) and high values of the dominance and abundance index in all the studied ecosystems.
3. In the northern area, the presence of natural leptospirosis foci has been established in forests, paludous, agrocenoses and the rural-marsh ecotone with the involvement of *A. flavicollis*, *A. sylvaticus*, *A. agrarius* species, *C. glareolus* and *Microtus* sp. The presence of 2 serogroups *L. grippotyphosa* and *L. pomona* was determined; both serogroups being detected in *A. agrarius*.
4. In the central area, the presence of natural leptospirosis foci was established in forests, paludous biotopes and agrocenoses. The main species involved were *A. agrarius*, *M. spicilegus*, *R. norvegicus* and *S. araneus*. The presence of 2 serogroups was determined: *L. icterohaemorrhagiae* and *L. grippotyphos;* both serogroups were detected in *A. agrarius*.
5. In the southern area the presence of the natural outbreak of leptospirosis in thepaludous ecosystems with the involvement of *R. norvegicus* and *A. terrestris* species has been established. Antibodies specific to the serotype *L. icterohaemorrhagiae* were determined in both species.
6. The study of the leptospira circulation in the environment and in the population of small mammals allows the prediction of the epizootological situation and the risk of spreading this disease in the human population in the Republic of Moldova.

CONFLICT OF INTERESTS

The author does not declare any conflict of interest.

ACKNOWLEDGEMENTS

The author is grateful to dr. Victoria Nistreanu and dr. Natalia Caterinciuc for valuable comments and suggestions, and for improving the English text of the paper. The study was performed within the doctoral project, State Program project 20.80009.7007.02 and within collaboration contract between the Institute of Zoology and the National Agency for Public Health.

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Date of receipt of the manuscript: 22/07/2020
Date of acceptance for publication: 12/08/2020

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OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

BIOLOGICAL SCIENCES

OPEN ACCESS

THE INFLUENCE OF CULTIVATION TEMPERATURE ON SOME PHENOTYPIC TRAITS OF *YERSINIA PSEUDOTUBERCULOSIS*

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DOI: 10.38045/ohrm.2020.1.14 UDC: 616.98:578.834.1(478)

Keywords: *Yersinia pseudotuberculosis, cultivation temperature, phenotypic traits, antibiotics, resistance, sensitivity, biofilms.*

Introduction*. Some causative agents of sapro-zoonotic infections can multiply in the external environment (water reservoirs, soils, plants), as well as in animals, including microorganisms of the genus Yersinia.*

Material and methods. *Isolation and identification of Y. pseudotuberculosis was carried out in accordance with the instructions on "Epidemiology, laboratory diagnosis of yersiniosis, organization and conduct of preventive and anti-epidemiological measures". Antibiotic sensitivity was performed via the disc diffusion method in accordance with EUCAST and national guidelines. Biofilm formation was tested using the spectrophotometric assay.*

Results. *It was established that the studied cultures showed a decrease in the level of saccharolytic activity during cultivation at a temperature of +37°C in comparison with the results obtained at 25°C, changes in sensitivity to antibiotics depending on the temperature of cultivation were revealed. It was established that Y. pseudotuberculosis cultures were able to form denser (λ 570) biofilms when cultured at 25°C, in comparison with biofilms formed at 37°C.*

Conclusions. *Biological characteristics of the studied Y. pseudotuberculosis isolates (changes in the saccharolytic activity, the level of sensitivity to antibiotics and the formation of biofilms were revealed) depends on the cultivation conditions.*

Cuvinte cheie: *Yersinia pseudotuberculosis, temperatura de cultivare, trăsături fenotipice, antibiotice, rezistență, sensibilitate, biofilme.*

INFLUENȚA TEMPERATURII DE CULTIVARE ASUPRA UNOR PROPRIETĂȚI FENOTIPICE ALE *YERSINIA PSEUDOTUBERCULOSIS*

Introducere. *Unii agenți cauzali ai sapronozelor se pot multiplica atât în mediul extern (rezervoare de apă, sol, plante), cât și în organismul animalelor, la astfel de agenți patogeni atribuindu-se și bacteriile din genul Yesinia.*

Material și metode. *Izolarea și identificarea Y. pseudotuberculosis a fost efectuată în conformitate cu instrucțiunea „Epidemiologia, diagnosticul de laborator al yersiniozei, organizarea și desfășurarea măsurilor preventive și antiepidemice". Sensibilitatea la antibiotice a fost efectuată prin metoda difuzimetrică, în conformitate cu EUCAST și gidul național. Capacitatea de a forma biofilme, cât și densitatea lor, au fost determinate prin metoda spectrofotometrică după densitatea optică.*

Rezultate. *Culturile studiate au demonstrat o scădere a nivelului activității zaharolitice, în timpul cultivării la temperatura de +37°C, în comparație cu rezultatele obținute la 25°C, totodată înregistrându-se și diferențe ale sensibilității la antibiotice în funcție de temperatura de cultivare. S-a mai stabilit că culturile de Y. pseudotuberculosis au fost capabile să formeze biofilme mai dense (λ 570) la temperatura de 25°C, decât cele formate la 37°C.*

Concluzii. *S-a stabilit că, manifestarea proprietăților biologice ale izolatelor studiate de Y. pseudotuberculosis (modificări ale activității zaharolitice, nivelul de sensibilitate la antibiotice și formarea de biofilme) depinde de condițiile de cultivare.*

INTRODUCTION

Some causative agents of sapro-zoonotic infections are capable of reproduction in the external environment (water reservoirs, soils, plants), as well as in the body of animals, including microorganisms of the genus *Yersinia*. The disease-causing pathogens occurring in humans and animals *Yersinia pestis*, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica* are quite common. Yersiniosis is a foodborne gastrointestinal tract zoonotic disease that can be transmitted via contaminated food or water. In Ukraine, there are active natural reservoirs of *Y. pseudotuberculosis*. Bacteriocarrier is recorded among synanthropic and agricultural animals, which determines the source of the pathogen and the factors of its transmission. Contamination of livestock products and/or food causes human diseases. There are evidences, suggesting that *Y. pseudotuberculosis* serotype O1b has been isolated due to intragenomic rearrangements and deletions of *Y. pestis*. The pathogenicity of *Y. pseudotuberculosis* is determined by genes that dictate invasive and cytotoxic properties of the pathogen (1). Therefore, the isolation of pure culture, as well as the identification of *Y. pseudotuberculosis* for a particular serovar, creates the basis for diagnosing pseudotuberculosis.

Pseudotuberculosis is an infectious disease of various animal species, accompanied by intoxication, the formation of caseous nodules and granularnecrotic lesions like tuberculosis in various organs. The disease is characterized by fever, intoxication, damage to the small intestine and liver, scarlet fever-like rash.

The main route of infection is the alimentary one.

Yersinia spp. are bipolar-staining, gram-negative facultative anaerobic bacteria. The optimum cultivation temperature is 28-29°C, and can vary from 2 to 40°C. They retain mobility at 22-28°C (except *Y. pestis*) and become motionless at 37°C. It should also be noted that *Y. enterocolitica* has a higher motility compared to other *Yersinias*. At low temperature conditions (4-8°C), bacteria form smooth (S) colonies with slow accumulation.

At temperatures above 28°C, *Y. Pseudotuberculosis* dissociates into a rough (R) form. When isolated from the body of the patient or from animals, *Y. pseudotuberculosis* and *Y. enterocolitica* exhibit an S-form. However, sometimes, the cultures of *Y. pseudotuberculosis* are isolated in R, S-R-, R-S-forms (1, 2).

Demidova GV, Zyuzina VP, et al. (2009) examined the pathogenic strains of *Y. pseudotuberculosis* and found that, due to their sensitivity to polymyxin B, the studied strains, belonging to different serotypes can be divided into groups, particularly in cultures of serotypes I and IV, which were sensitive to polymyxin B at 28°C and resistant to 37°C (3).

Titareva GM, Fursova NK, Balakhonov SV. (2003) examined strains of *Y. pestis* and reported that the strain resistance, cultured at 25°C is higher than that of strains cultured at 37°C. Therefore, the study of the biological properties of cultures under incubation temperature conditions of 23-28°C and 37°C is an important feature of the differential diagnosis of *Yersinia spp*. (4). The ability to form biofilms is considered as a factor providing selective advantages of microorganisms in biological niches (according to CDC data, nearly 80% of human bacterial infections are caused by polymicrobial biofilms) (5, 6). The aim of the study was to study the biological properties of *Y. pseudotuberculosis* strains.

MATERIAL AND METHODS

The study carried out the *Y. pseudotuberculosis* isolation and identification in accordance with “Epidemiology, laboratory diagnosis of yersiniosis, the organization and conduct of preventive and anti-epidemiological measures" instructions (7). Five cultures of *Y. pseudotuberculosis* were investigated, which have been observed in synanthropic rodents.

The morphological features and mobility of Y*ersinia* cultures were determined according to the instructions (7). The morphological features were determined via microscope of Gram-stained smears. To determine the motility of bacteria, preparations such as “crushed drop” were prepared from daily agar cultures grown at 23°C and 37°C.

The enzymatic properties such as the ability of microorganisms to form acetyl methyl carbinol (acetoin) during glucose fermentation in the Foges-Proskauer reaction were determined; phenylalanine deaminase, the capacity for esculin hydrolysis, indole/urea, urease and ornithine de carboxylase were detected; the saccha-

rolytic properties on the Hiss medium with sugars were also revealed.

Antimicrobial susceptibility testing was performed using the EUCAST disk diffusion method. (version 10) and MU on "Determination of microorganisms susceptibility to antibacterial drugs" (MOH, 2007) (8, 9).

The ability of isolates to form biofilm (by method C. C. Heilmann E, 1996) and the biofilm optical density indexes (by spectrophotometrically) in three cultures of *Y. pseudotuberculosis* were evaluated, as well as in *Pasteurella multocida*, *Salmonella* Typhimurium, and *Salmonella* Enteritidis, *E. coli*, *S. aureus* (10, 11) under different cultivation regimes (25°C and 37°C).

The nutrient media, commercial tests, and discs with antimicrobial drugs manufactured by HiMedia were used within the study.

RESULTS

The morphological features of the obtained cultures, as well as the motility in a "crushed drop" type drug have been determined via the microscope of the Gram-stained smears. To determine the bacterial motility, the one-day agar cultures have been prepared under two cultivation regimes (23°C and 37°C).

Over 24 hours, reddish transparent convex (sometimes greyish-yellow oily colonies with 0.5–1mm diameter), and granular or tubular colonies of S-, O- and R-forms are formed on meat-and-peptone agar, Endo agar, blood agar. The S-forms were represented by smooth, convex colonies. The R-forms had granular colonies with darkened center and thin lace-type periphery; this pattern was also found in the pathogen of zoonotic plague (camel plague).

The gram-negative organisms of ovoid (coccoid-like) form (0.8–5.0μm length, 0.4–0.8μm width), as well as the organisms with rounded tails (1.5–6 μm length, 0.4–0.8μm width) were observed in the smears. Some bacterial cultures exhibited bipolarity properties. The study of motility in cultures showed that they were stationary at 37°C and mobile at 23°C.

Studies of bacterial enzyme properties showed that the studied cultures did not exhibit the ability to form acetyl methyl carbinol in the Foges-Proskauerre action at +23°C and +37°C; they hydrolyzed the esculin and did not form phenylpyruvic acid (determination of phenylalanine deaminase) and ornithine decarboxylase (tab. 1). A decrease in saccharolytic activity of the cultures was recorded when cultivated at +37°C (doubtful reaction).

Table 1. Enzymatic properties of *Y. pseudotuberculosis.*

Isolates	Enzymatic properties																			
	Mobility		Hydrolysisofesculin	Phenylalanine	Foges-Proskauer23°C	Foges-Proskauer37°C	Ornithine decarboxylase	Maltose		α-Arabinose		Glucose		Ramnos		Mannitol		Mannose	
	23°C	37°C						23°C	37°C	23°C	37°C	23°C	37°C	23°C	37°C	23°C	37°C	23°C	37°C
Y. p 1	+	–	+	–	–	–	–	+	±	+	±	+	±	+	±	+	±	+	±
Y. p 2	+	–	+	–	–	–	–	+	±	+	±	+	±	+	±	+	±	+	–
Y. p 3	+	–	+	–	–	–	–	+	±	+	±	+	±	+	±	+	±	+	±
Y. p 4	+	–	+	–	–	–	–	+	±	+	±	+	±	+	±	+	±	+	–
Y. p 5	+	–	+	–	–	–	–	+	±	+	±	+	±	+	±	+	±	+	–

Antibiotic resistance was determined in 5 obtained isolates via the disc diffusion method (tab. 2), and the results were interpreted according to EUCAST (version 10) and MU on "Determination of microorganism susceptibility to antibacterial drugs" (MOH, 2007).

Table 2. Susceptibility to antibacterial drugs *Y. pseudotuberculosis*.

The name of the drug	*Y. pseudotuberculosis*, diameter of crop growth inhibition, mm, (n = 5)									
	Y. p 1		Y. p 2		Y. p 3		Y. p 4		Y. p 5	
	23°C	37°C	23°C	37°C	23°C	37°C	23°C	37°C	23°C	37°C
Piperacillin <17; 20≤;	35	26	34	25	34	28	28	20	37	28
Ampicillin <14; 14≤;	31	17	30	16	20	11	13	6	20	11
Cefalexin <14; 14≤;	31	23	30	22	36	24	28	20	28	20
Cefuroxime <19; 19≤;	31	24	30	23	28	21	30	22	30	23
Cefotaxime <17; 20≤;	37	23	36	22	32	23	28	20	36	22
Ceftriaxone <22; 25≤;	40	30	40	30	34	25	34	25	34	25
Cefepime <24; 27≤;	22	22	21	21	34	34	26	26	28	28
Imipenem <17; 22≤;	24	24	24	24	36	36	30	30	32	32
Meropenem <16; 22≤;	22	36	23	37	24	36	28	40	24	37
Gentamicin <17; 17≤;	22	22	23	23	24	24	26	26	28	28
Nalidixic acid	30	30	31	31	36	36	42	42	30	30
Ciprofloxacin <22; 25≤;	30	30	31	31	26	40	34	34	34	34
Norfloxacin <22; 22≤;	26	34	27	35	32	40	20	28	30	38
Ofloloxacin <22; 24≤;	34	43	36	38	26	43	30	38	26	36
Levofloxacin <19; 23≤;	30	26	32	36	22	26	30	40	40	35
Chloramphenicol <17; 17≤;	32	23	31	20	23	20	30	21	30	20
Polymyxin B	6	16	6	18	6	16	6	12	6	6

The examined cultures were predominantly sensitive to penicillins, cephalosporins, carbapenems, quinolones, gentamicin, and chloramphenicol, both at +23°C and at +37°C. Individual cultures exhibited resistance (Y. p 4 to Ampicillin; Y. p 1 and Y. p 2 to Cefepime) and moderate resistance (Y. p 4 to Cefepime) under given conditions.

The cultures of Y. p 3 and Y. p 5 exhibited sensitivity to Ampicillin at +23°C and resistance at +37°C. At +23°C, a culture Y. p 4 showed resistance to Norfloxacin. The Y. p 3 culture showed moderate level of resistance to Levofloxacin at +23 °C but remains uniformly susceptible to it at +37°C.

The culture sensitivity to Cefepime, Imipenem, Gentamicin, Nalidixic acid was the same under the given temperature conditions. In general, most bacterial cultures demonstrated changes in drugs susceptibility depending on temperature conditions. Thus, the lower level of bacterial susceptibility was recorded at +37°C – to Piperacillin, Ampicillin, Cefalexin, Cefuroxime, Cefotaxime, Ceftriaxone, Chloramphenicol and at 23°C – to Meropenem, Norfloxacin, Ofloxacin, Levofloxacin, Polymyxin B.

The ability to form biofilms and their density (tab. 3) were determined in *Y. pseudotuberculosis* cultures (Y. p 1, Y. p 2, Y. p 3) at 25°C (S-form) and 37°C (R-form); the obtained results were compared with similar indicators of *Pasteurella*

multocida culture (cultivation on heart-brain broth (HBB)), as well as *Salmonella* Typhimurium and *Salmonella* Enteritidis (cultivation on meat-peptone broth (MPB), *E. coli (STX2)* and associated *E. coli* culture *(STX2)* + *Proteus mirabilis* (cultivation on MPB), *S. aureus* (cultivation on tryptone-soy broth (TSB)).

Table 3. Indicators of biofilm optical density produced by cultures under different cultivation conditions.

Culture	The environmental conditions	T, °C cultivation	λ 570	λ 570, control	actual value (λ 570 – λ 570 control)
Y. p 1	MPB	25°C	2.8781	0.1423	2.7358
		37°C	1.6416		1.4993
Y. p2		25°C	2.9901		2.8478
		37°C	1.6734		1.5311
Y. p 3		25°C	3.6737		3.5314
		37°C	1.4557		1.3134
P. multocida	SMB	25°C	1.4218	0.2244	1.1974
		37°C	2.5968		2.3734
***S.* Typhimurium**		37°C	0.9048		0.6804
***S.* Enteritidis**		37°C	1.1209		0.8965
E. coli	MPB	37°C	0.3798	0.1423	0.2375
E. coli+P. mirabilis		37°C	1.3265	0.1423	1.1842
S. aureus	TSB	37°C	4.6778	0.3719	4.3049

Y. pseudotuberculosis were reported to form biofilms. All studied bacterial cultures (S colonies) formed high density bacterial associations at 25°C (Y. p1 - λ 2.8781, Y. p2 - λ 2.9901, Y. p3 - λ 3.6737). When cultured at 37°C (in R-form), the biofilm optical density was significantly lower (Y. p1 - λ 1.6416, Y. p2 - λ 1.6734, Y. p3 - λ 1.4557), which is respectively 45.2%, 46.2%, 62.8% of the optical density of the cultures at 25°C.

The evaluation of the optical density in other cultures (S colonies) showed significantly lower optical densities of biofilms compared to cultures of *Y. pseudotuberculosis*, namely in *S.* Typhimurium – λ 0,6804, *S.* Enteritidis – λ 0.8965, *E. coli* λ – 0.2375, and in the associated culture of *E. coli* O723 (STX2) + *Proteus mirabilis* λ – 1.1842, which was 23.89%, 31.48%, 8.34%, and 41.58% respectively, from the optical density index of the S-shaped culture (at 25°C) *Y. pseudotuberculosis* 2 (λ 2.8478).

The coagulase-positive culture of *S. aureus* formed a much higher density biofilm than *Y. pseudotuberculosis* cultures. The optical density of *S. aureus* was λ 4.3049, which made up 151.17% of optical density (at 25°C) *Y. pseudotuberculosis* 2 (λ 2,8478).

The ability to form biofilms in *P. multocida* cultures was slightly lower than in *Y. pseudotuberculosis* cultures. At 25°C and 37°C, the optical density of *P. multocida* cultures were λ 1.1974 and λ 2.3734, respectively, that is, at 25°C the *P. multocida* optical density was 50.5% of that of *P. multocida* at 37°C.

DISCUSSIONS

The obtained results proved that the studied cultures of *Y. pseudotuberculosis* didn't show the ability to form acetyl methyl carbinol at +23°C and +37°C; they hydrolyzed the esculin and did not form phenyl pyruvic acid and ornithine decarboxylase; a constant decrease in the level of saccharolytic activity of cultures was observed during cultivation at +37°C.

An analysis of the results showed that, *Y. pseudotuberculosis* antibiotic susceptibility changes to

most drugs depending on the cultivation temperature, decrease of sensitivity level to Piperacillin, Ampicillin, Cefalexin, Cefuroxime, Cefotaxime, Ceftriaxone, Chloramphenicolwasrecorded, and at 23°C – to Meropenem, Norfloxacin, Ofloloxacin, Levofloxacin, Polymyxin B at +37°C.

Biofilm formation is regarded as a pathogenicity factor. This study investigated the ability to form biofilmsin *Y. pseudotuberculosis* cultures compared with *Pasteurella multocida*, *S.* Typhimurium*, S.* Enteritidis*, E. coli, S. aureus.*

The obtained isolates of *Y. Pseudotuberculosis* were capable to form denser bacterial biofilms at cultivation temperature of 25°C. At 25°C and 37°C, the *Pasteurella multocida* culture (S colonies), whereas at 37°C, it formed a denser biofilm. The biofilm-forming capacity of *Y. Pseudotuberculosis* cultures *(*S and R colonies*)* to form biofilms is higher than S-form in other studied cultures of enterobacteria (*S.* Typhimurium*, S.* Enteritidis, *E. Coli*) and *Pasteurella multocida,* compared to the culture of coagulase-positive *S. aureus.*

CONCLUSIONS

1. It was established that the manifestation of the biological properties of the studied *Y. pseudotuberculosis* isolates (in particular, changes in the saccharolytic activity, antibiotic susceptibility and biofilm formation capacity were revealed) depends on the cultivation conditions.
2. The obtained isolates of *Y. pseudotuberculosis* were able to form denser bacterial biofilms at a culture temperature of 25°C, compared with the formation of biofilms at cultivation temperature +37°C.
3. The biofilm-forming capacity *Y. pseudotuberculosis* cultures (S and R colonies) is higher than S-form in other studied cultures of enterobacteria (*S.* Typhimurium*, S.* Enteritidis, *E. Coli*) and *Pasteurella multocida,* compared to the culture of coagulase-positive *S. aureus.*

CONFLICT OF INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENT

Research was carried out with the financial support of the Ministry of Education and Science of Ukraine.

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Date of receipt of the manuscript: 03/04/2020
Date of acceptance for publication: 30/05/2020

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OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

MEDICAL SCIENCES

OPEN ACCESS

LEFT VENTRICULAR REMODELING PATTERNS IN CHILDREN WITH METABOLIC SYNDROME

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DOI: 10.38045/ohrm.2020.1.15 UDC: 616.124.2:616-056.52-053.2

Keywords: *metabolic syndrome, children, left ventricular remodeling patterns.*

Introduction. *Pathological left ventricular (LV) remodeling in children with metabolic syndrome (MS) is associated with a significant increase in cardiometabolic risk. However, data regarding the prevalence of LV remodeling patterns in children with MS are limited.*
Material and methods. *An observational analytical cohort study was conducted on 145 children. The diagnosis of MS was established according to the International Diabetes Federation (IDF) criteria. We analyzed the echocardiography, as well as clinical and paraclinical data. Participants were distributed, depending on LV mass index and relative wall thickness into four LV geometric patterns as recommended by American and European Society of Echocardiography: normal geometry, concentric left ventricular remodeling (cLVR), concentric left ventricular hypertrophy (cLVH), and eccentric left ventricular hypertrophy (eLVH).*
Results. *The pathological remodeling patterns were distributed as follows: 62.1% (n=90) participants showed a normal LV geometry pattern, 27.6% (n=40) – cLVH, 5.5% (n=8) – cLVR and 4.8% (n=7) – eLVH. In terms of presence/absence of MS, 54.7% (n=29) participants from the research group showed a normal LV geometry pattern, 32.1% (n=17) – cLVH, 5.7% (n=3) – cLVR and 7.5% (n=4) – eLVH, whereas 66.3% (n=61) participants from the control group presented normal LV geometric appearance, 25% (n=23) – cLVH, 5.4% (n=5) – cLVR and 3.3% (n=3) – eLVR ($\chi 2=0.52$; $p>0.05$).*
Conclusions. *Concentric left ventricular hypertrophy was the commonest LV geometric pattern among the subjects with metabolic syndrome. Concentric left ventricular remodeling and eccentric left ventricular hypertrophy were rare among the study population.*

Cuvinte cheie: *sindrom metabolic, copii, modele de remodelare a ventriculului stâng.*

MODELE DE REMODELARE VENTRICULARĂ STÂNGĂ LA COPIII CU SINDROM METABOLIC

Introducere. *Remodelarea patologică a ventriculului stâng (VS) la copiii cu sindrom metabolic (SM) este asociată cu o creștere semnificativă a riscului cardiometabolic. Cu toate acestea, date privind prevalența paternelor de remodelare ale VS la copiii cu SM sunt limitate.*
Material și metode. *Studiu analitic, observațional, de cohortă. Au fost incluși 145 de copii. Diagnosticul de SM a fost stabilit conform criteriilor Federației Internaționale de Diabet (FID). Am analizat datele clinice, paraclinice și ecocardiografice. Participanții au fost stratificați în patru tipare geometrice, folosind indicele de masă a VS și grosimea relativă a PPVS, așa cum recomandă Societatea Americană și Europeană de Ecocardiografie: geometrie normală VS, remodelare concentrică VS (RC VS), hipertrofie concentrică VS (HC VS) și hipertrofie excentrică VS (HE VS).*
Rezultate. *Tipurile de remodelare patologică s-au repartizat în felul următor: 62.1% (n=90) participanți au prezentat aspect geometric normal al VS, la 27,6% (n=40) dintre ei s-a înregistrat HC VS, la 5,5% (n=8) participanți s-a atestat RC VS, iar 4,8% (n=7) din acest lot au prezentat HE VS. În funcție de prezența/absența SM, în lotul de bază, 54,7% (n=29) participanți au prezentat aspect geometric normal, la 32,1% (n=17) a fost înregistrată HC VS, 5.7% subiecți (n=3) au manifestat RC VS, iar 7,5% (n=4) au prezentat HE VS, iar în lotul de control – la 66,3% (n=61) participanți s-a atestat aspect geometric normal al VS, 25% (n=23) au prezentat HC VS, în 5,4% (n=5) cazuri s-a determinat RC VS, iar 3,3% (n=3) participanți au prezentat HE VS ($\chi 2=0,52$; $p>0,05$).*
Concluzii. *Hipertrofia concentrică VS a fost cel mai frecvent model geometric al VS în rândul subiecților cu sindrom metabolic, iar remodelarea concentrică VS și hipertrofia excentrică VS au fost rar depistate în rândul populației din studiu.*

INTRODUCTION

Metabolic syndrome (MS) is considered a recent major health problem, although scientific advances and therapeutic strategies have provided lots of opportunities for its management. It gets even more important if it occurs in children since the therapeutic possibilities in pediatric patients are quite limited due to the considerable side effects and/or no sufficient studies so far.

Metabolic syndrome is a pediatric pathology, leading to several early disorders, including cardiovascular disease (CVD)(1), showing an increased risk due to its early onset and duration. The prevalence rate varies between 10% and 84%, depending on the geographical region, environment, individual demographic characteristics (gender, age, race, and ethnic origin), as well as other criteria used for its defining (2). In terms of gender, most studies show a higher frequency in males (3, 4), whereas the differences also vary depending on the age gaps, being higher in males than adolescent females (10.9% vs 6.29%), subsequently being reversed in adulthood (18% vs 20% at the age of 20-39 years and 42% vs 51% at the age ≤ 60 years) (5).

Pediatric MS correlates with cardiac structural and geometrical changes, leading to a cardiac pathological remodeling, which is considered a substrate for developing heart failure, being also a strong predictor of arrhythmia, characterized by an impaired heart function (systolic and diastolic) and an early risk of sudden death (6).

While considering the aforementioned arguments and the impact of childhood health on further adult health, we considered to choose the following research on left ventricular remodeling patterns in children with MS, that will contribute to the opening of new perspectives for identifying a single and effective approach, as well as for preventing cardiovascular complications of this syndrome, based on IDF criteria adapted for children, to reduce the morbidity and mortality rates at a young age.

MATERIAL AND METHODS

The purpose of the research: to study the left ventricular remodeling patterns in children with metabolic syndrome.

General design and study population. The study project was carried out within the IMPH IMC, at the Department of Pediatrics of the Pediatric Cardiology Clinic, to which 161 children were admitted, aged from 10 to 17 years 11 months and 29 days, from both urban and rural areas, the patients being selected electively during the 2016 – 2019 period. An observational analytical cohort study was planned to achieve the research purpose.

The research comprised several stages. The 1st stage included 145 children (out of 161 participants, 16 ineligible), who were selected based on the inclusion/exclusion criteria, and made up the research group according to *the following criteria*: the age of 10 – 17 years 11 months and 29 days (inclusive); with abdominal obesity (waist circumference (WC) ≥ 90th percentile (7)); the child's parent or guardian consent, as well as children's assent (age ≥ 14 years) on research participation; being a citizen of the Republic of Moldova; ability to effectively communicate with the researcher; ability to understand and follow the study requirements; sufficient understanding in signing the informed agreement and written assent.

The *study exclusion criteria* for the patients were the following: secondary obesity: endocrine, genetic and neurological type, having a suggestive clinical examination, confirmed by specialized examinations; secondary high blood pressure: renal, endocrine, neurogenic, drug - induced, etc., patients having a suggestive clinical examination, confirmed by specialized examinations; acute conditions, whether or not accompanied by fever, whether or not undergoing treatment; chronic respiratory, cardiovascular, gastrointestinal, renal, neurological, endocrine, etc., disorders, whether or not undergoing treatment; the child's parents or legal representative disagreement, child's refusal to participate in the research, with a difficult ultrasound window, low compliance, patient's refusal to be included in the study.

The selected participants underwent a complex examination, which included: filling in a specific questionnaire (food and physical activity survey), the clinical examination on systems, laboratory testing for lipid status (total cholesterol (TCh), triglycerides (TG), high-density cholesterol (HDLc)), the glucose (Glu) spectrum (basal glucose, oral glucose tolerance test (OGTT) – selectively) and uric acid.

Following the clinical and paraclinical findings,

the 2nd stage included the respondent's self-division into 2 groups, by using the criteria of MS according to the Consensus of the IDF adapted for children [1], namely: the research group *(L_1)* – 53 de children with MS (including 3 – 5 criteria) and control group *(L_0)* – 92 children without MS (including 1 – 2 criteria). The ratio of the study groups was 1:2. Subsequently, subjects from both groups were investigated by transthoracic echocardiography.

The 3rd stage included a comparative study of the two groups, in terms of lifestyle, symptoms, demographic, anamnestic and biochemical profile, cardiac function and morphology, etc., as well as a statistical analysis of the obtained results. Practical conclusions and recommendations, based on the obtained results, were traced out at the 4th stage of the study.

All the participants were selected and informed about the research stages, being enrolled only by personal informed consent, following a detailed explanation of the requirements and procedures of necessary investigations by discussing with each subject individually. All the procedures were performed, based on children's parent and legal representative consent, as well as on written assent of children ≥ 14 years old. They were not paid and have not suffered any financial costs for participation.

Ethical considerations. The study complied with the international standards of medical ethics, developed by the Declaration of Helsinki, regarding confidentiality and personal data protection of the participants. The research was approved by the Research Ethics Committee of State University of Medicine and Pharmacy *Nicolae Testemitanu* (report no. 59 of 03.06.2016). The resulting data were revealed only to the concerned participant, the personal data of each subject were not used and will not be used for any other purpose. The study applied the following research methods: historical, comparative, biostatistics ones, and others.

Metabolic syndrome. MS was defined according to the IDF consensus definition of metabolic syndrome in children (8): WC≥90th percentile or adult cut-off if lower, plus any two of the following four factors: TG≥1.7 mmol/L, HDLc<1.03 mmol/L, BP systolic ≥130 and diastolic ≥85 mmHg (> 95th percentile for age, height, and sex), Glu≥5.6 mmol/L (If≥5.6 mmol/L (or known T2DM) recommend an OGTT) for age group 10 – <16 years, and use existing IDF criteria for adults: central obesity (defined as WC≥94 cm for Europid men and ≥ 80cm for Europid women, with ethnicity specific values for other groups) plus any two of the following four factors: TG≥1.7mmol/L or specific treatment for high TG, reduced HDLc: <1.03mmol/L in males and <1.29 mmol/L in females, or specific treatment for low HDLc, systolic BP≥130 mmHg or diastolic BP≥85 mmHg or treatment of previously diagnosed Arterial Hypertension, and Glu≥5.6 mmol/L or known T2DM for age group >16 years.

Left ventricular geometry pattern. To define the LV geometry pattern, we measured the myocardial mass index by M-mode echocardiography using the Devereux formula and normalized to height ^{2,7}, as well as the relative wall thickness (RWT) defined as LV wall thickness + septal thickness relative to the internal dimensions of the LV. The LVH was diagnosed by cutoff value utilized in adults (51 g/m) corresponding to the 97.5th percentile in children (9). The LV geometric patterns (normal, eccentric or concentric LV hypertrophy, and concentric LV remodeling) were calculated according to the *left ventricular mass index* (*LVMI*) and RWT. All echocardiographic measurements were conducted using the Toshiba Aplio 300, MODEL TUS-A300 Cardiac Ultrasound Machine, by a specialized sonographer, who was unaware of the patients' diagnosis.

Covariates. Apart from standard biochemical parameters to confirm the diagnosis of MS, uric acid level, and LDLc (TCh - HDLc - TG/5), were determined in every patient. All blood specimens were taken after an overnight fasting. Additional clinical evaluation included medical history, lifestyle questionnaire, anthropometric measurements, physical examination, etc.

Statistical analysis. The data collected from the primary material were introduced into the electronic database, whereas the statistical processing was performed using the Statistical Package for the Social Sciences (SPSS) version 20.

RESULTS

According to the inclusion and exclusion criteria, there was formed a general group, including 145 participants, of which 36.6% (53 pts) children with MS (research group) and 63.4 % (92 pts) children with non-MS (control group). Gender groups were divided into 55.9% boys (81 pts) and 44.1% girls (64 pts) of the total number of enrolled children. MS was present in 39.5% (32 pts) boys vs 32.8% (21 pts) girls, and non-MS in 60.5% (49 pts) boys vs 67.2% (43 pts) girls ($\chi 2=0.69$; $p>0.05$).

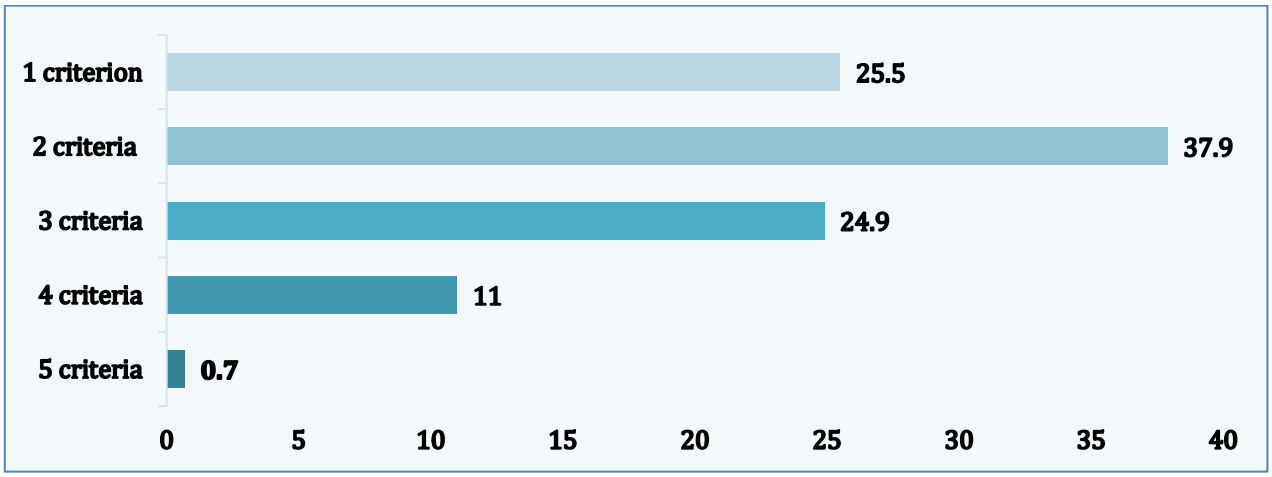

Figure 1. The participant distribution based on the number of criteria and IDF consensus for children (%), n=145.

According to *MS criteria* and based on the IDF agreement, 24.9% (35 pts) subjects presented 3 criteria, 11% (17 pts) – 4 criteria and 0.7% (1 pts) – 5 criteria, whereas 25.5% (37 pts) of the participants presented 1 criterion, and 37.9% (55 pts) – 2 of the mentioned criteria (fig. 1). Gender distribution revealed that 24.7% (20 pts) boys and 25% (16 pts) girls had 3 positive criteria, and 14.8% (12 pts) boys and 7.8% (5 pts) girls – 4-5 criteria for metabolic syndrome ($\chi 2=1.77$; $p>0.05$).

Also, there were selected *5 factors of MS* (according to the IDF consensus, adapted for children). The first factor (F1) - obesity, was recorded in 100% (145 pts) of cases in order of prevalence (it might be because children included in the study exhibited WC ≥ 90th percentile), the second factor (F2) found in 47.6% (69 pts) of cases, showed low values of HDLc and the third factor (F3), found in 25.5% (37 pts) of cases, which were defined by high TG was named the lipidic factor. The fourth factor (F4), recorded in 42.8% (62 pts) of cases with high BP values (BP systolic and/or BP diastolic), was called the blood pressure factor, and the fifth factor (F5), reported in 7.6% (11 pts) of cases with higher than normal blood Glu levels, was related to carbohydrate metabolism, is less prevalent.

The assessment of pediatric MS was also performed by detecting the components and their association. The prevalence of cases with defined MS was assessed using *clustering patterns,* which was estimated according to the number of criteria (tab.1).

Cluster WC+HDLc+HBP was found in 10.3% of cases (15 pts), WC+TG+HDLc was registered in 8.3% (12 pts), WC+TG+HDLc+HBP – 7.6% (11 pts), WC+TG+HBP – 5.5% (8 pts) of cases. The clusters WC+HDLc+Glu+HBP and WC+TG+ HDLc+ Glu were found to exhibit the same frequency of 1.4% (2 pts), whereas WC+Glu+HBP, WC+TG+ Glu+HBP and CA+TG+HDL+Glu+HBP in 0.7% (1 pts) of cases. Gender-based clustering showed higher rate of WC+HDL+HBP in females vs male 15.6% (10 pts) vs 6.2% (5 pts) ($p<0.01$); higher WC+TG+HDL cluster rate in males vs females 9.9% (8 pts) vs 6.3% (4 pts) ($p<0.01$); high rate of WC+TG+HDLc+HBP cluster in males vs females 9.9% (8) vs 4.7% (3 pts) ($p<0.01$); WC+TG+HBP cluster prevailing in males vs females 8.6% (7 pts) vs 1.6% (1 pt) ($p<0.01$). Obesity cases were found to be associated with dyslipidemia, high BP and glycoregulation disorders (similar data was found in specialized literature among adult population, whereas no data were recorded for pediatric population).

Table 1. The study of metabolic syndrome components, their association, and clustering.

MS, components	Total (no. %)	Gender (no. %) ($\chi 2 = 27.35$; $p<0.01$)	
		Males	**Females**
WC	37 (25.5%)	20 (24.7%)	17 (26.6%)
WC+HBP	23 (15.9%)	19 (23.5%)	4 (6.3%)
WC+Glu	4 (2.8%)	2 (2.5%)	2 (3.1%)
WC+TG	2 (1.4%)	1 (1.2%)	1 (1.6%)
WC+HDLc	26 (17.9%)	7 (8.6%)	19 (29.7%)
WC+Glu+HBP	1 (0.7%)	-	1 (1.6%)
WC+TG+HDLc	12 (8.3%)	8 (9.9%)	4 (6.3%)
WC+HDLc+HBP	15 (10.3%)	5 (6.2%)	10 (15.6%)
WC+TG+HBP	8 (5.5%)	7 (8.6%)	1 (1.6%)
WC+HDLc+Glu+HBP	2 (1.4%)	1 (1.2%)	1 (1.6%)
WC+TG+HBP+Glu	1 (0.7%)	1 (1.2%)	-
WC+TG+HDLc+HBP	11 (7.6%)	8 (9.9%)	3 (4.7%)
WC+TG+HDLc+Glu	2 (1.4%)	2 (2.5%)	-
WC+TG+HDLc+Glu+HBP	1 (0.7%)	-	1 (1.6%)

Note: *The values are presented as absolute values (and percentage) for statistically significant categorical data p<0.01; WC* – waist circumference*; TG – triglyceride; HBP – high blood pressure values; Glu – glucose; HDLc – high-density cholesterol.*

The results of the selective analysis of some anthropometric parameters. The studied groups were characterized by the following values (tab.2): 80.5±2.05 kg in the study group vs 73.4±2.3 kg in the control group, with the statistical difference ($p<0.01$); the height, values were 168.4±1.7 cm in MS group, and 161.4±1.5 cm in non-MS group, with a true statistical difference; the mean BMI index showed absolute values in the study group – 28.2±0.4 kg/m^2, and in the control group 27.1±0.5 kg/m^2 ($p>0.05$), according to the percentiles – 94.7±0.6 vs 93.9±0.4 ($p>0.05$), and according to the Z score both groups had the same mean value of 1.7±0.05 ($p>0.05$); the mean values of WC were 94.5±1.2 cm in the MS group and 90.5±1.07 cm in the control group (according to the percentiles, in 100% of WC cases ≥90th percentile), with a statistically significant difference ($p<0.05$); hip circumference was 103.5±1.3 cm in children with MS and 98.7±1.3 cm in those with non-MS, with a statistically significant difference ($p<0.05$); abdominal index – 0.9±0.01 and the waist-to-hip-ratio was higher in the study vs control group 1.88±0.03 m^2 vs 1,76±0.03 m^2, but showing no significant statistical difference ($p>0.05$).

The results of the evaluation of some biochemical parameters. A comparative study between the biochemical indices in children with MS vs non-MS revealed the following mean values (tab.2): TG – 1.97±0.2 mmol/l, compared to 1.16±0.03 mmol/L ($p<0.001$); TCh – 4.09±0.14 mmol/L, compared to 4.06±0.09 mmol/L ($p>0.05$); HDLc – 1.03±0.03 mmol/L vs 1.33±0.03 mmol/L ($p<0.001$); LDLc – 2.11±0.12 mmol/L vs 2.05±0.17 mmol/L ($p<0.001$); LDLc/HDLc – 2.04±0.04 mmol/L vs 1.54±0.05 mmol/L ($p<0.001$); TCh/HDLc – 3.97±0.01 mmol/L vs 3.05±0.01 mmol/L ($p>0.05$); ß-lipoproteins 45.15±1.78 mmol/L, compared to 46.74±1.21 mmol/L ($p>0.05$) and uric acid – 315.41±10.65 mmol/L, compared with 292.88±8.56 mmol/L ($p>0.05$).

The results of the evaluation of the types of remodeling of the left ventricular myocardium (fig.2). The distribution of LV remodeling types was carried out as recommended by the American and European Society of Echocardiography, based on the mea-surements of LVMI and RWT. The types of pathological remodeling were distributed as follows: 62.1% (n=90) participants showed a normal geometry pattern of LV, 27.6% (n=40) – concentric LV hypertrophy, 5.5% (n=8) – concentric LV remodeling, and 4.8% (n=7) – eccentric LV hypertrophy.

In terms of presence/absence of MS, 54.7% (n=29) participants from the research group showed a normal LV geometry pattern, 32.1% (n=17) – concentric LV hypertrophy, 5.7% (n=3) – concentric LV remodeling and 7.5% (n=4) – eccentric LV hypertrophy, whereas 66.3% (n=

61) participants from the control group presented normal LV geometric appearance, 25% (n=23) – concentric LV hypertrophy, 5.4% (n=5) – concentric LV remodeling and 3.3% (n=3) – eccentric LV hypertrophy ($\chi 2=0.52$; $p>0.05$) (fig. 3).

Table 2. The values of some anthropometric and biochemical parameters in children included within the research.

Variables (M±m)	MS	non-MS	p-value
Weight, M±m, (kg)	80.5±2.05	73.4±2.3	<0.01
Height, M±m, (cm)	168.4±1.7	161.4±1.5	<0.01
BMI, M±m, (kg/m²)	28.2±0.4	27.1±0.5	SI
BMI, M±m, (percentiles)	94.7±0.6	93.9±0.4	SI
BMI, M±m, (Z score)	1.7±0.05	1.7±0.05	SI
WC, M±m,(cm)	94.5±1.2	90.5±1.07	<0.05
HC, M±m,(cm)	103.5±1.3	98.7±1.3	<0.05
WHR, M±m,	0.9±0.01	0.9±0.01	SI
AI, M±m,	0.6±0.01	0.6±0.01	SI
BSA (m²) M±m,	1.88±0.03	1.76±0.03	SI
TG (mmol/l)	1.16±0.03	1.97±0.2	<0.001
TCh (mmol/l)	4.06±0.09	4.09±0.14	SI
HDLc (mmol/l)	1.33±0.03	1.03±0.03	<0.001
LDLc (mmol/l)	2.05±0.17	2.11±0.12	<0.001
TCh/HDLc	3.05±0.01	3.97±0.01	SI
LDLc/HDLc	1.54±0.05	2.04±0.04	<0.001
ß-lipoproteins (mmol/l)	46.74±1.21	45.15±1.78	SI
Uric acid (mmol/l)	292.88±8.56	315.41±10.65	SI

***Note**: Values are presented as mean ± standard deviation for a number of values; SI – statistically insignificant (p>0.05); the value of p<0.05 = considered significant; the value of p<0.001 = considered significant; BSA – body surface area; AI – abdominal index; WHR –waist-to-hip-ratio; HC – hip circumference; WC* – waist circumference*; BMI – body mass index; TG – triglycerides, TCh – total cholesterol, HDLc – high density cholesterol, LDLc – low density cholesterol*

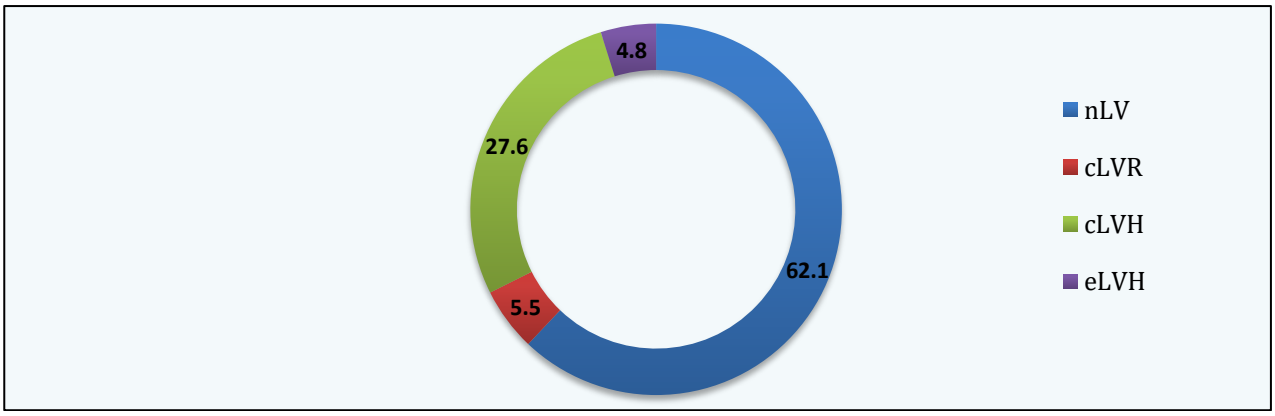

Figure 2. Left ventricular remodeling patterns (%).

***Note**: nLV – normal LV appearance, cLVR – concentric LV remodeling; cLVH – concentric LV hypertrophy; eLVH – eccentric LV hypertrophy.*

The gender-related distribution revealed the following results in boys: 63.4% (n=52) – normal LV geometric appearance, 24.4% (n=20) – concentric LV hypertrophy, 4.9% (n=4) – concentric LV remodeling and 7.3% (n=6) – eccentric LV hypertrophy and in girls: 60.3% (n=38) – normal LV geometric appearance, 31.7% (n=20) – concentric LV hypertrophy, 6,3% (n=4) – concentric LV remodeling and 1.6% (n=1) showed eccentric LV hypertrophy ($\chi 2=0.34$; $p>0.05$).

Depending on the number of MS criteria, a normal LV geometry appearance was found in

25.8% (n=24) of MS children with 1 positive criterion, 38.7% (n=37) – 2 criteria, 24.2% (n=21) – 3 criteria and in 11.3% (n=8) cases with 4-5 criteria. Concentric LV remodeling was recorded in 10% (n=1) subjects with 4-5 MS criteria, and in 30.0% (n=2) of cases with 1, 2 and 3 criteria. Concentric LV hypertrophy was recorded in 15.2% (n=8) of MS children with 1 positive criterion, 27.3% (n=15) – 2 criteria, 32.6% (n=11) – 3 criteria and 24.9% (n=6) – 4-5 criteria. Eccentric LV hypertrophy was found in 11.7% (n=1) of MS children with 1 positive criterion, 19.1% (n=2) – 2 criteria, 27.9% (n=1) – 3 criteria and 41.3% (n=3) – 4-5 criteria ($\chi 2=3.58$; $p>0.05$) (fig. 4)

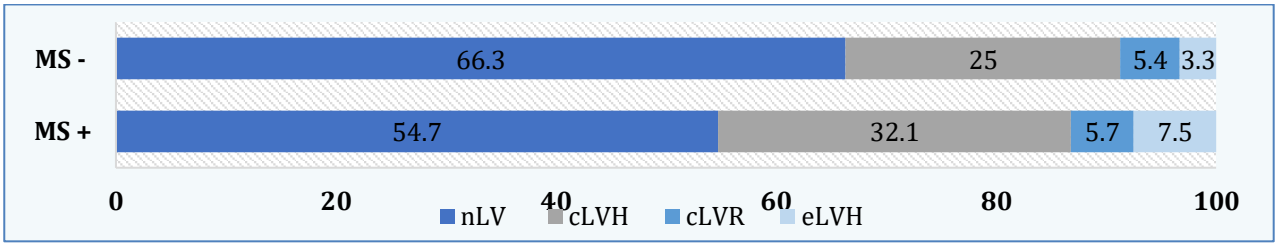

Figure 3. Left ventricular remodeling patterns depending on the presence/absence of metabolic syndrome.

Note: *nLV – normal LV appearance, cLVR – concentric LV remodeling; cLVH – concentric LV hypertrophy;eLVH – eccentric LV hypertrophy.*

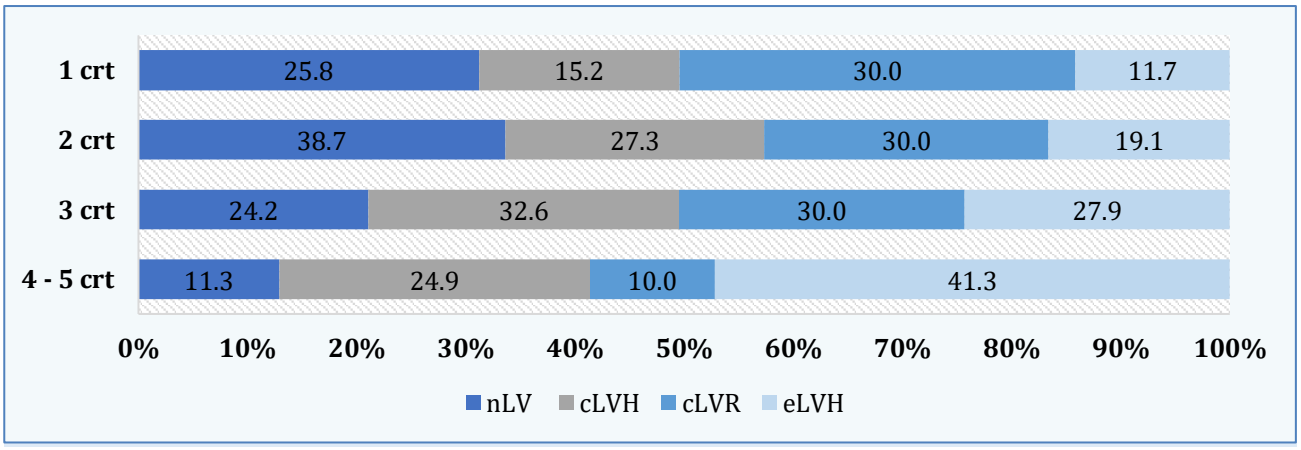

Figure 4. Left ventricular remodeling patterns depending on the number of MS criteria and according to IFD Consensus for children.

Note*: nLV – normal LV appearance. cLVR – concentric LV remodeling; cLVH – concentric LV hypertrophy; eLVH – eccentric LV hypertrophy.*

According to MS clustering**,** concentric LV remodeling was recorded in 15% (n=1) of subjects with WC+TG+HBP, WC+HDLc+HBP and WC+HDLc+TG+HBP clustering patterns. Eccentric LV hypertrophy was recorded in 14.7% (n=1) of participants with WC+HDLc+HBP, WC+HDLc+TG+HBP+Glu and 27.9% (n=2) of subjects with WC+HDLc+TG+HBP cluster patterns. Concentric LV hypertrophy was found in 18.7% (n=6) of participants with WC+HDLc+HBP clusters, 6.1% (n=4) of cases with WC+TG+HBP and WC+TG+HDLc+HBP and in 1.6% (n=1) – WC+TG+HDLc, WC+TG+Glu+HBP, WC+TG+HDLc +Glu. The LV with normal geometric appearance had a different overall distribution, being registered in all clinical patterns ($\chi 2=11.96$; $p>0.05$).

The risk for installing of left ventricular myocardial remodeling. During the research, the risk of installing the LV myocardium remodeling patterns in children with MS has also been determined, initially being formed as "table 2x2", afterwards the necessary indicators were calculated and the obtained results were interpreted. Respectively, RR (relative risk) = 1.7 was obtained, ranging between 1.7 – 2.5, which was considered a moderate risk. Thus, MS is a risk factor for the development of LV myocardium remodeling, whereas taking into account that

confidence interval (CI) does not include value 1 (CI is between 1.3 and 4.2) it can be concluded that there is a positive association between these two.

DISCUSSIONS

MS is a clinical condition, which is associated with an increased risk of cardiovascular diseases (10). The present study investigated a well-defined general population, aged between 10–18 years, the prevalence of the MS and that of its contributors, as well as the relationship with non-invasively measured markers of cardiac involvement.

The syndrome was found to have a considerable prevalence in the pediatric population, averaging 36.6% of all subjects aged between 10-18 years, showing greater prevalence among boys (39.5% vs 32.8%), which was similar to previous studies (10).
Regarding the MS-related factors, apart from visceral adiposity present by definition in 100% of cases, high TG (25.5%), high BP (42.8%), and low HDLc (47.6%) values were the most frequently detected.

The assessment of pediatric MS was also performed by detecting the components and their association. The prevalence of cases with defined MS was assessed by using the clustering patterns, being estimated according to a number of criteria. The clusters WC+HDLc+HBP, WC+TG+HDLc, WC+TG+HDLc+HBP, WC+TG+HBP were the most frequently registered. Obesity cases were found to be associated with dyslipidemia, high BP, and glucose regulation disorders (similar data were found in specialized literature among the adult population, whereas no data were recorded for the pediatric population).

In our study, MS subjects-compared to non-MS had significantly higher values of weight, waist circumference, hip circumference, triglycerides, as well as lower levels of HDL-cholesterol. The anthropometric and biochemical data altogether are the most important features of metabolic syndrome in this present research.

The analysis of cardiac remodeling types showed a higher incidence in pediatric subjects with normal LV geometric appearance – 62.1%. The three pathological LV remodeling subtypes (eccentric hypertrophy, concentric hypertrophy, and concentric remodeling) showed a higher rate for concentric LV hypertrophy – 27.6%, followed by concentric LV remodeling type – 5.5% and eccentric LV hypertrophy – 4.8%, which was predominantly found in males, for three positive criteria, and within the following clusters WC+HDLc+HBP, WC+TG+HDLc, WC+TG+HDLc+Glu, WC+TG+ HDLc+HBP, showing no statistically significant difference ($p>0.05$; similar data were found in specialized literature among the adult population, whereas no data were recorded for pediatric population).

Finally, during the research, the potential risk of installing remodeling patterns of LV myocardium in children with MS was also estimated, after calculating the necessary indicators. The syndrome was found to be a risk factor, as well as an association between MS and remodeling of the LV myocardium, which has been identified.

Some limitations of this study must be taken into account. The current study included a relatively small number of patients, particularly of subjects aged between 16-18 years, although metabolic syndrome is considered to have a high incidence rate. The main study limitation regarding the patients'enrollment was the fact that we aimed at identifying pediatric subjects with MS from within the Cardiology Clinic. Another reason for a relatively small number of participants was the careful selection of patients, as to obtain an optimal ultrasound window, for an accurate analysis of the echocardiographic data. The short-term MS installation is another study limitation.

CONCLUSIONS

1. According to the International Diabetes Federation definition, 24.9% of the subjects presented 3 criteria, 11% – 4 criteria and 0.7% – 5 positive criteria (25.5% – 1 criterion, and 37.9% – 2 criteria) for metabolic syndrome. Waist circumference ≥ 90th percentile was recorded in 100% of cases, high densi ty cholesterol value <1.03 mmol/L/1.29 mmol/L – in 47.6%, triglyceride value ≥1.7 mmol/L – in 25.5%, systolic blood pressure value ≥130 mmHg/diastolic blood pressure ≥85 mmol/L – in 42.8%, and glucose values ≥5.6 mmol/L – in 7.6%.
2. The analysis of cardiac remodeling types showed a higher incidence in pediatric subjects wi th nor

mal left ventricular geometric appearance – 62.1%. The three pathological remodeling subtypes (eccentric hypertrophy, concentric hypertrophy, and concentric remodeling) showed a higher rate for concentric left ventricular hypertrophy – 27.6%, followed by concentric left ventricular remodeling type – 5.5% and eccentric left ventricular hypertrophy – 4.8%.

3. In children aged 10-18 years, metabolic syndrome is a risk factor and has a positive association with the development of left ventricular myocardial remodeling (relative risk=1.7, confidence interval=1.3-4.2).

PRACTICAL RECOMMENDATIONS

1. Screening of the metabolic syndrome is recommended in children with abdominal obesity, aged 10 – 18 years in order to detect those cases, who are at risk of developing complications.
2. Echocardiography is recommended to assess the presence of structural remodeling patterns of left ventricular myocardium, which will allow detecting children with metabolic syndrome and those who are at higher risk for developing cardiovascular complications.

CONFLICT OF INTERESTS

The authors do not declare any conflict of interest.

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Date of receipt of the manuscript: 24/04/2020
Date of acceptance for publication: 08/08/2020

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OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

BIOLOGICAL SCIENCES

OPEN ACCESS

ALTERATIONS OF TOTAL ANTIOXIDANT CAPACITY OF PATHOGEN CULTURES UNDER THE INFLUENCE OF NOVEL CHEMICAL COMPOUNDS

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DOI: 10.38045/ohrm.2020.1.16 UDC: 579.61:546-3

Keywords: *antimicrobial activity, antioxidant activity, chemical compounds, copper, reference strains.*

Introduction. *The major and most common mechanisms for almost all antimicrobial substances is the induction of oxidative stress responses within the pathogen cells by accumulation of free radicals, which mediate a whole range of classical antimicrobial mechanisms of action observed by researchers. Thus, this present study was conducted to reveal some biochemical changes regarding the antioxidant status of pathogenic microorganisms under the influence of novel chemical compounds selected.*

Material and methods. *The copper (II) coordination compounds were included as in vitro study material, namely, Co (II), Zn (II) and aromatic propenones, synthesized at the Department of Inorganic Chemistry, the State University of Moldova. The antimicrobial properties were tested on five reference strains. The ABTS test was applied to determine the antioxidant activity, thus determining the total antioxidant capacity using the ABTS˙⁺ cation radical.*

Results. *The most vulnerable to high-intensity oxidative stress are the reference strains of Staphylococcus aureus ATCC 2592 and Bacillus cereus ГИСК 8035, the most resistant being the reference culture Salmonella enterica (S. Abony ГИСК 03/03y).*

Conclusions. *The study results showed that the selected compounds might substantially reduce the total antioxidant capacity in the studied reference cultures.*

Cuvinte cheie: *activitate antimicrobiană, activitate antioxidantă, compuși chimici, cupru, tulpini de referință.*

MODIFICAREA CAPACITĂȚII ANTIOXIDANTE TOTALE A CULTURILOR DE MICROORGANISME PATOGENE SUB INFLUENȚA COMPUȘILOR CHIMICI NOI

Introducere. *Unul dintre mecanismele, generale și comune, pentru majoritatea preparatelor antimicrobiene, este inducerea stresului oxidativ în celulele patogene, exprimat prin acumularea în exces a radicalilor liberi, care mediază întreg spectrul clasic de mecanisme de acțiune antimicrobiană, observate de cercetători. Astfel, a devenit oportună realizarea unui studiu, care ar elucida unele modificări biochimice, ce reflectă statutul antioxidant al microorganismelor patogene sub influența compușilor chimici noi selectați.*

Material și metode. *În calitate de obiecte de studiu in vitro au servit compușii coordinativi ai Cu (II); Co (II), Zn (II) și propenonele aromatice, sintetizate la Catedra de chimie anorganică de la Universitatea de Stat din Moldova. Efectele antimicrobiene au fost testate pe 5 tulpini de referință. Pentru determinarea activității antioxidante a fost aplicat testul ABTS, care stabilește capacitatea antioxidantă totală, cu utilizarea radicalului cation ABTS˙ +.*

Rezultate. *Cele mai vulnerabile, din punctul de vedere al instalării stresului oxidativ de înaltă intensitate, sunt culturile de referință Staphylococcus aureus ATCC 2592 și Bacillus cereus ГИСК 8035, cea mai rezistentă fiind cultura de referință Salmonella enterica (S. Abony ГИСК 03/03y).*

Concluzii. *Analizând rezultatele prezentate mai sus, putem afirma că, sub acțiunea compușilor selectați, se produce o reducere substanțială a capacității antioxidante totale în culturile de referință studiate.*

INTRODUCTION

Currently, there is an increasing interest regarding the synthetic chemical compounds, including several perspective categories. Recent studies have reported that different metals might cause multiple disorders of microbial cells. The oxidative stress, protein dysfunction and cell membrane damage are the most common causes of that, being generated by the action of metal ions (1).

Copper is a transition metal with pronounced biological effects. Its biological activity has been confirmed by its copper ion-binding ability to combine with biomolecules, particularly to proteins and nucleic acids to form various complexes (2, 3, 4).

Current interest in Cu complexes has derived from their potential use as antimicrobial, antiviral, anti-inflammatory, antitumor, inhibitory agents of different enzymes (5). It is well-known that Cu (II) complexes of non-steroidal anti-inflammatory drugs show both a high anti-inflammatory activity and a low toxicity degree towards healthy tissues. Although the anti-inflammatory effect of Cu compounds are quite promising, the latest researches have been focused on the potential chemotherapeutic properties of copper-based compounds. Theoretical studies related to the development, synthesis and testing of the copper complexes are considered promising in obtaining active antiviral and antimicrobial drugs, including for HIV, H1N1 treatment, as well as for multidrug-resistant bacteria (6, 7, 8).

Copper complexes exhibit diverse in vitro biological activities, ranging from antibacterial and anti-inflammatory to cytostatic and some enzyme inhibitory properties. Copper compounds (due to copper ion) interact directly with proteins and DNA at the molecular level, which leads to their structural distortion. The indirect mode of action of copper compounds shows the same effects on biopolymers, as in direct coupling, however generated by reactive oxygen species, formed in presence of Cu (II) ions (5).

The aforementioned arguments were the selection criteria for the present study, namely the coordination copper (II) compounds with different ligands and aromatic propenones, showing potential antimicrobial effects on reference strains and clinically isolated strains of pathogenic microorganisms. It is highly important to highlight the characteristics and mode of action of new antimicrobials both in assessing the therapeutic effects and in promoting a new pharmaceutical product by its implementing into therapy practice (5, 6, 7, 8).

MATERIAL AND METHODS

The copper (II) coordination compounds were included as *in vitro* study material, namely, Co (II), Zn (II) and aromatic propenones, synthesized at the Department of Inorganic Chemistry, at State University of Moldova. The antimicrobial properties were tested on five reference strains viz. *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ГИСК 8035, *Escherichia coli* ATCC 25922, *Shigella sonnei* ATCC 25931, and *Salmonella enterica (S.* Abony ГИСК 03/03), which come from two culture collections, recognized as quality biological material used for research studies: American Type Culture Collection (ATCC, USA) and the State Collection of Pathogenic Microorganisms of L. A. Tarasevici State Institute of Scientific Research on Standardization and Control of Biological medical preparations ГИСК, Russian Federation.

The ABTS assay (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) to determine the antioxidant activity (9) was carried out. The ABTS˙+ cation radical testing was used to identify the total antioxidant capacity, as it allows assessing the multicomponent extracts, as in the studied cell lysate.

Oxidized ABTS (ABTS˙+) (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was generated by the radical ABTS. The formed radical was reduced by the mechanism of electron addition. Trolox, a water-soluble compound with antiradical activity similar to tocopherol was used as an equivalent for quantitative assessment. The test findings were expressed in terms of percentage inhibition (in order to compare the test results) and TEAC (trolox equivalent antioxidant activity) to compare with other antioxidants.

An ABTS˙+ cation radical was generated by oxidation of ABTS with potassium persulfate. Therefore, 7 mM ABTS stock solution in deionized water was prepared with 2.45 mM potassium persulphate in a ratio of 1:1. The ABTS radical formation occurred for 16 h at room temperature in darkness. The working solution was

prepared from ABTS stock solution dissolved in ethanol or distilled water until the absorbance value ranged between at 0.700±0.020 units to the wavelength of 734 nm.

0.3 ml of bacterial extract and 2.7 ml of ABTS solution were assessed. The reduction reaction occurred for 6 min at room temperature, whereas the inhibition percentage was calculated according to the following equation: % inhibition = $(Abs_{t=0} - Abs_{t=6\ min.})/Abs_{t=0} * 100$, Whereas, $Abs_{t=0\ min.}$ is the absorbance value ranging between 0.700±0.020 at 734 nm ABTS˙+ solution, and $Abs_{t=6\ min}$ is the absorbance value after incubition. The TEAC index was expressed as µM Trolox/mg biomass and calculated using Trolox standard calibration curve.

To avoid separation, drying and weighing of pathogenic cultures, the pathogenic dry weight was determined indirectly by calculating the average cell volume and applying the density value ρ = 1. The average cell size, volume and weight used within the study are presented in Table 1. The following table also includes the cell mass found in McFarland standard in ml for each culture.

Table 1. Estimated parameters of microbial biomass.

Culture	Cell parameters				Cell mass, mg	Culture mass (mg) per 0.5McF ml standard
	r, µm	h, µm	V, µm³	V, mm³		
Staphylococcus aureus ATCC 25923	0.64±0.038	0	0.6200	$6.2*10^{-10}$	$6.2*10^{-10}$	0.093
Bacillus cereus ГИСК 8035	0.54±0.061	3.5±0.2	2.7475	$2.7*10^{-9}$	$2.7*10^{-9}$	0.405
Escherichia coli ATCC 25922	0.55±0.036	2.7±0.3	2.1195	$2.12*10^{-9}$	$2.12*10^{-9}$	0.318
Shigella sonnei ATCC 25931	0.37±0.020	2.6±0.1	0.73476	$7.3*10^{-10}$	$7.3*10^{-10}$	0.1095
Salmonella enterica (S. Abony ГИСК 03/03y)	0.54±0,010	3.9±0.1	3.0301	$3.03*10^{-9}$	$3.03*10^{-9}$	0.4545

The necessary biomass amount was determined according to the method described in Table 1 in order to obtain the cell extracts. Cell extracts were obtained from biomass after being washed with phosphate buffered solution (pH 7.0). Afterwards, the biomass (100 mg) was resuspended in phosphate buffer to destroy the cell wall and cytoplasmic membrane. Therefore, 0.1 g of glass beads, sized 150-212 µm (Sigma) were applied. The beads were mixed with cell suspension in a vortex shaker for one minute, at 5-minute interval with ice cooling. The lysate was centrifuged for 10 min at 8000 rpm at 4°C, whereas the supernatant was refriger ated at 4°C until being used (10).

Bacterial cultures corresponding to 0.5 McFarland turbidity standard were subjected to new chemical compounds activity, being selected in a concentration equal to the MIC for each compound and reference culture. After a 2-hour contact time of the culture with the compounds, the concentration of the cells was adjusted according to the turbidity standard. Biomass was collected and processed according to the methods described above. New antibacterial chemical compounds were assessed. ABTS assay values were expressed as percent inhibition of ABTS˙+ cation radical.

RESULTS

The study results obtained in assessing the new antimicrobial chemical compounds against the *Staphylococcus aureus* ATCC 25923-reference strain are shown in Figure 1.

There is a significant decrease in the antioxidant capacity of *Staphylococcus aureus* strains ATCC 25923, used in the treatment of cells with both Furacillin and selected new chemical compounds. The percentage inhibition of ABTS cation radical, when treated with Furacillin, accounted for 59.1% of the cell extract capacity of staphylococcus, untreated with antibacterial substances. The total antioxidant activity of the

cell extracts obtained from the staphylococcus biomass treated with the 12 newly-selected chemical compounds ranged from 6.1 to 30.5% of the antioxidant capacity of the control strains.

13 new chemical compounds with high antibac terial effect were chosen for *Bacillus cereus* ГИСК 8035 reference culture. The study results obtained for the total antioxidant capacity of cell lysates are presented in Figure 2.

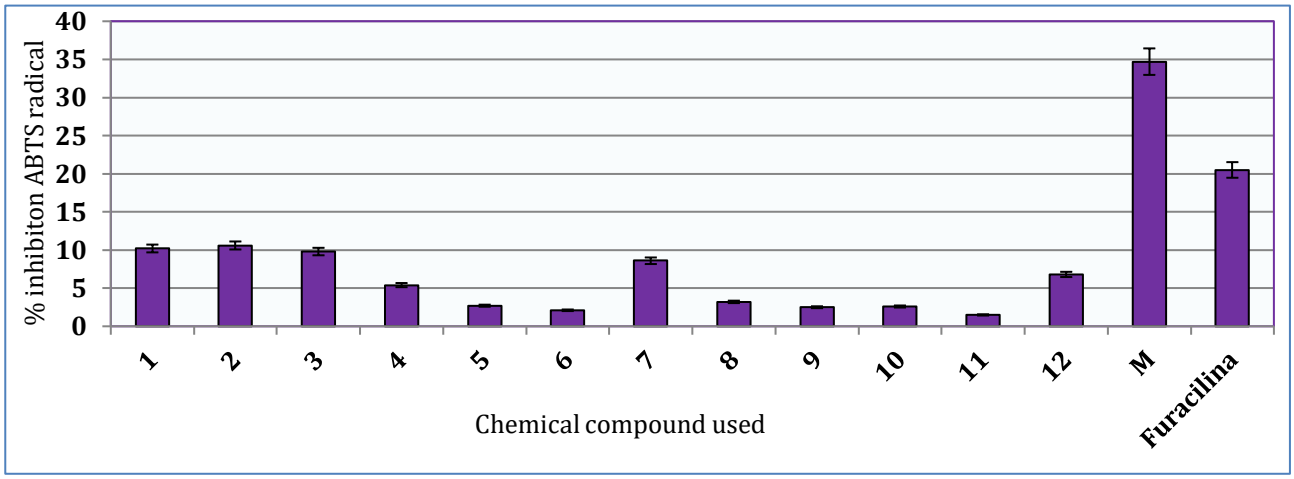

Figure 1. Alterations of total antioxidant capacity in *Staphylococcus aureus* strain ATCC 25923 under the influence of new chemical compounds: 1 – $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 – $C_{42}H_{42}Cu_2N_{14}O_{12}S_4$; 3 – $C_{46}H_{46}Cu_2N_{18}O_{10}S_6$; 4 – $C_{46}H_{42}Cu_2N_{18}O_{10}S_4$; 5 – $C_{15}H_{19}ClCuN_4O_2S$; 6 – $C_{15}H_{19}CuN_5O_5S$; 7 – $C_{15}H_{17}ClCuN_4OS$; 8 – $C_{15}H_{19}CuN_5O_5S$ (2,5); 9$C_{15}H_{19}CuN_5O_5S$ (3,4); 10 – $C_{15}H_{19}CuN_5O_5S$ (2,4); 11 – $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 12 – $C_9H_{11}ClCuN_4S$; M – untreated culture.

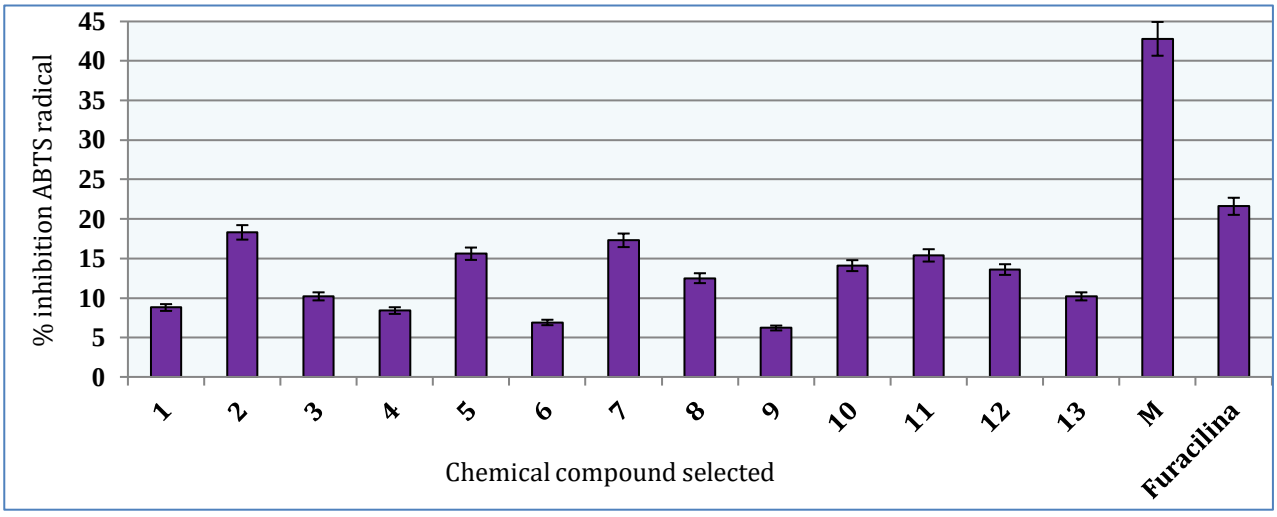

Figure 2. Alterations of total antioxidant capacity in *Bacillus cereus* ГИСК 8035S strain under the influence of new chemical compounds: 1 – $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 – $C_{42}H_{42}Cu_2N_{14}O_{12}S_4$; 3 – $C_{46}H_{46}Cu_2N_{18}O_{10}S_6$; 4 – $C_{46}H_{42}Cu_2N_{18}O_{10}S_4$; 5 – $C_{15}H_{19}ClCuN_4O_2S$; 6 – $C_{15}H_{19}CuN_5O_5S$; 7 – $C_{15}H_{17}ClCuN_4OS$; 8 – $C_{15}H_{19}CuN_5O_5S$ (2,5); 9 – $C_{15}H_{19}CuN_5O_5S$ (3,4); 10 – $C_{15}H_{19}CuN_5O_5S$ (2,4); 11 – $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 12 – $C_9H_{11}ClCuN_4S$; 13 – $C_{10}H_{13}CuN_5O_4S_2$; M – control strains.

The total antioxidant capacity of cell lysate from untreated *Bacilus cereus* culture made up 43.8% of inhibited ABTS cation radical. This index is quite high even for plant extracts, which are considered the most active one. Treatment of *Bacillus cereus* culture with Furacillin caused a two-fold decrease in the total antioxidant capacity of the cell extract. The antioxidant capacity shows a higher decrease under the influence of new chemical compounds. Thus, the antioxidant capacity of the lysates made up 14.5-40.4% of the total capacity of the control sample, depending

on the compound used.

Bacillus cereus ГИСК 8035 reference strain showed a significant decrease in the ability to remove radicals produced in the biological system, which suggested the inability of the culture to protect itself effectively under oxidative stress caused by the impaired activity of new chemicals.

The study results of the total antioxidant capacity assay of the lysates in *Shigella sonnei* ATCC 25931 that underwent treatment with equal MIC value dosage of new chemical compounds are presented in Figure 3.

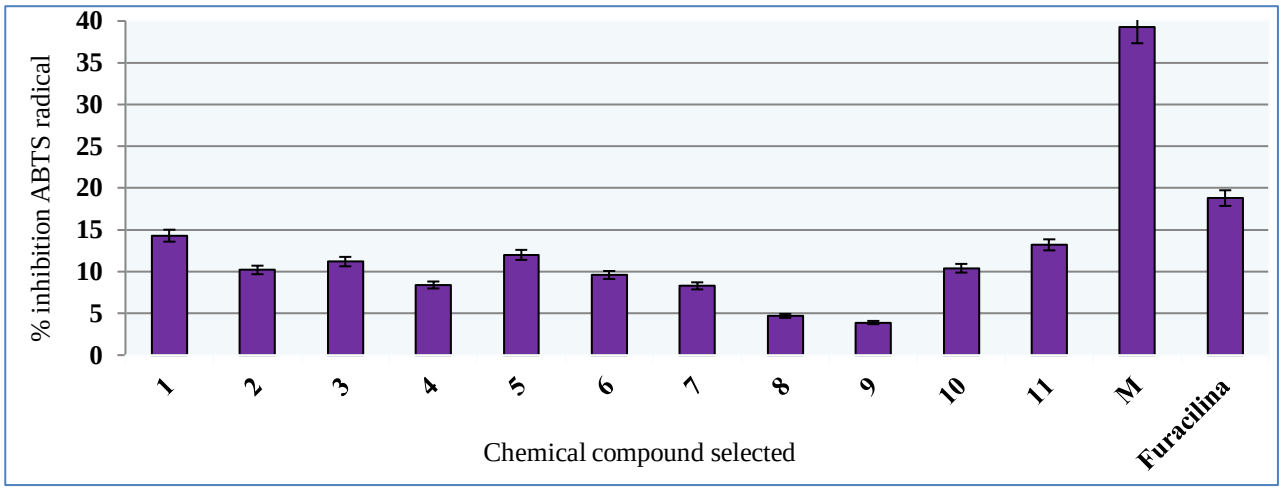

Figure 3. Total antioxidant capacity in standard *Shigella sonnei* ATCC 25931 culture under the influence of the following new chemical compounds: 1 – $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 – $C_{42}H_{42}Cu_2N_{14}O_{12}S_4$; 3 – $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; 4 – $C_{15}H_{19}ClCuN_4O_2S$; 5 – $C_{15}H_{19}CuN_5O_5S$; 6 – $C_{15}H_{17}ClCuN_4OS$; 7 – $C_{15}H_{19}CuN_5O_5S(2,5)$; 8 – $C_{15}H_{19}CuN_5O_5S(3,4)$; 9 – $C_{15}H_{19}CuN_5O_5S(2,4)$; 10 – $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 11 – $C_9H_{11}ClCuN_4S$; M – untreated culture.

The total antioxidant capacity assay in Gram-negative bacterial cultures showed the same decreasing effects in the tested compounds. Thus, *Shigella sonnei* ATCC 25931 reference strain under normal conditions exhibited a reduced ABTS cation radical capacity of about 40%, however, being a very good indicator. The level of antioxidant activity in Furacillin-treated biomass was twice lower compared to the control sample activity, accounting for 47.8%. The biomass treated with MIC doses of new chemical compounds, the values varied between 3.9 and 14.3% inhibition that was 9.9-36.4% of the values characteristic to the control sample.

The results of ABTS assay for di (μ-S) - bis {nitrate-[2-picoliden-4-(3,4-dimethylphenyl) thiosemicarbazide-(1-)] copper} tetrahydrate and di (μ-S) - bis {nitrate-[2-picoliden-4-(2,4-dimethylphenyl) thiosemicarbazide- (1-)] copper} tetrahydrate, were 4.7 and 3.9% inhibition of ABTS radical cation, respectively. In these cases, the total antioxidant capacity of the culture was reduced to 88-90%, thus leading to blockage of almost all cellular protection responses and structural damage of micromolecular components with antioxidant properties. Therefore, it is obviously clear that under such conditions, the oxidative stress had a highest value in the tested culture, which is crucial for vital processes of the pathogen cells.

The test results regarding the action of the selected compounds on the antioxidant activity of *Escherichia coli* ATCC 25922-reference culture are presented in Figure 4.

The *Escherichia coli* ability to reduce the ABTS cation radical under normal conditions made up 36% inhibition. About a twofold decrease of the total antioxidant capacity was reported in the culture treated with Furacillin, the inhibition percentage being of 18.9 in absolute values and 52.5%, compared to the control sample activity. A significant decrease in antioxidant activity was found in treatment of *Escherichia coli* ATCC 25922 – reference strain with selected chemical compounds, however, not as significant as in *Shigella sonnei* strain.

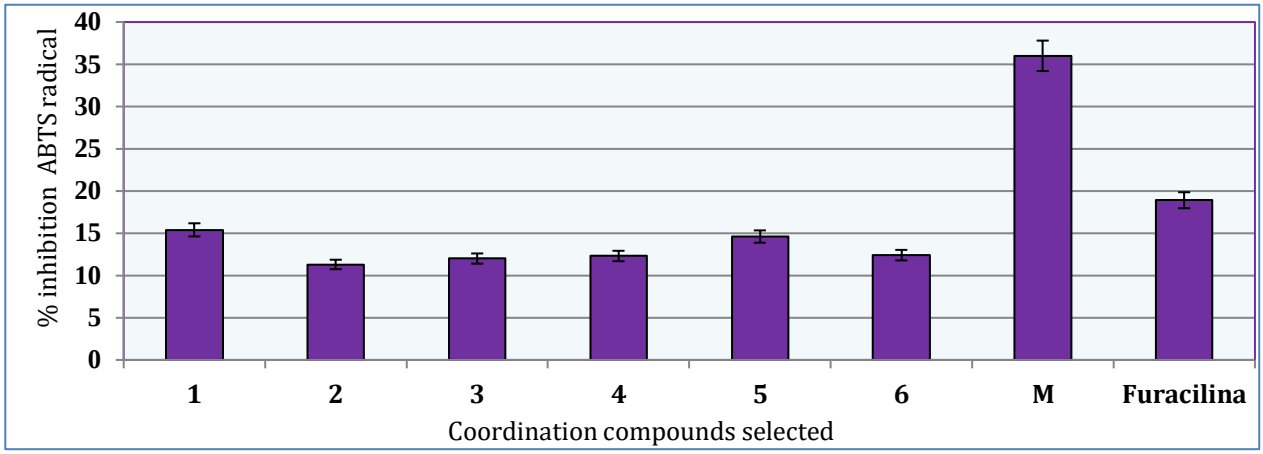

Figure 4. Total antioxidant capacity in standard culture of *Escherichia coli* ATCC 25922 under the influence of the following new chemical compounds: 1 – $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 – $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; 3 – $C_{46}H_{46}Cu_2N_{18}O_{10}S_6$; 4 – $C_{46}H_{42}Cu_2N_{18}O_{10}S_4$; 5 – $C_{18}H_{22}Cl_2Cu_2N_8S_2$; 6 – $C_9H_{11}ClCuN_4S$; M – untreated culture.

Therefore, the reduction capacity of the ABTS cation radical in the bacterial lysate obtained from cultures treated with new chemical compounds decreased by 57.6-68.6% compared to the lysate capacity obtained from the control biomass samples. This reduction is characteristic for active compounds used against *Escherichia coli.*

The *Escherichia coli* reference culture partially retains the antioxidant protection capacity, which has been confirmed by previous studies where the MIC and CBI values of the active compounds compared against this culture were higher compared to other studied strains. However, a reduced total antioxidant capacity of the bacterial lysate by at least 57.3%, following a new antibacterial compound treatment reported a significant oxidative stress, thus seriously affecting the vitality of the bacterial culture.

The study results obtained in the treatment of *Salmonella enterica (S.* Abony ГИСК 03/03y*)* reference strain with the definite doses of the two active compounds are presented in Figure 5.

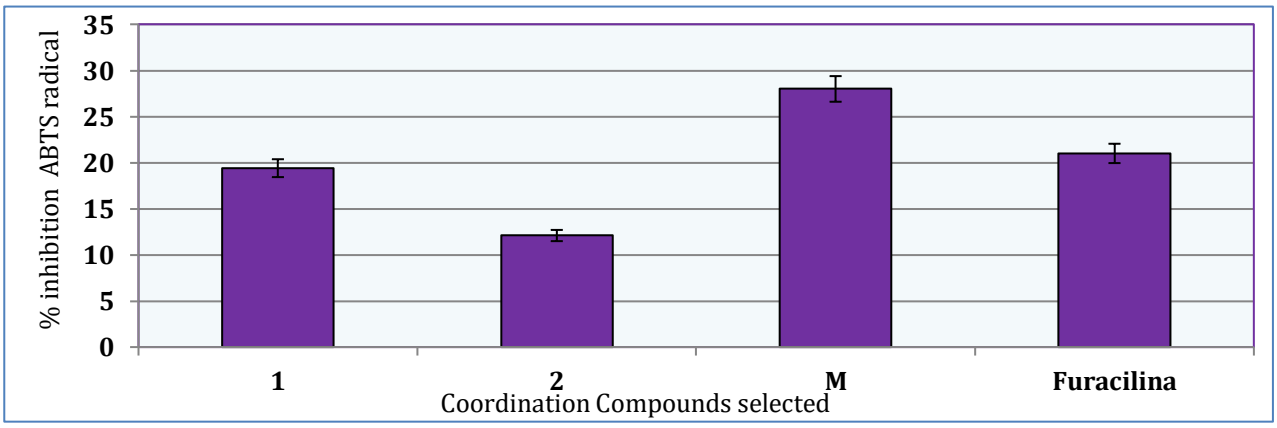

Figure 5. Total antioxidant capacity in standard *Salmonella enterica (S.* Abony ГИСК 03/03 y*)* under the influence of the following new chemical compounds: 1 – $C_{38}H_{38}Cu_2N_{14}O_{10}S_4$; 2 – $C_{44}H_{40}Cl_2Cu_2N_{14}O_4S_6$; M – untreated culture.

Total antioxidant activity of *Salmonella* culture under normal conditions was 28% inhibition of ABTS cation radical, thus showing lower values than the previously studied cultures. The action of Furacillin on the culture leads to a reduced antiradical capacity by 25% compared to the control samples. The selected compounds have a higher impact on the reduction of antioxidant capacity by 30.7-56.8% compared to the control sample.

DISCUSSIONS

The analysis of the obtained results revealed that the newly selected chemical compounds considerably decrease the total antioxidant capacity in the reference cultures studied.

The highest-intensity effect in reducing the antioxidant capacity of *Staphylococcus aureus* ATCC 25923 strains was reported in the group of coordination compounds of Cu (II) with 4-(dimethylphenyl) thiosemicarbazone 2-formylpyridine (compounds 5, 6, 8-10), as well as in di (μ-S) -bis {chloro-[1-(pyridin-2-yl) ethanon-4-methylthiosemicarbazonate (1-)] copper} which produced a significant decrease in antioxidant capacity.

The most pronounced effect in reducing the antioxidant capacity of the *Bacillus cereus* ГИСК 8035 reference strain was found in di (μ -S)-bis {nitrato-[2-picoliden-4-(2,6-dimethylphenyl) thiosemicarbazido-(1-)] copper} tetrahydrate and di (μ-S)-bis {nitrate-[2-picolidene-4-(3,4-dimethylphenyl) thiosemicarbazido - (1-)] copper} tetrahydrate, which are included in the group of coordination compounds of Cu (II) with 4 - (dimethylphenyl) thio semicarbazone 2 -formylpy-ridine.

The most active compounds with antibacterial activity against the *Shigella sonnei* ATCC 25931 reference strain were found in di (μ-S) -bis {nitrate-[2-picolidene-4-(3,4-dimethylphenyl) thiosemicarbazido- (1)] copper} tetrahydrate and di (μ-S) -bis {nitrate-[2-picoliden-4-(2,4-dimethylphenyl) thiosemicarbazido-(1-)] copper} tetrahydrate, which are both included in Cu (II) compounds with 4-(dimethylphenyl) thiosemicarbazone 2-formylpyridine.

It is worth mentioning that most antioxidant compounds against *Escherichia coli* ATCC 25922 strains belong to the group of Cu (II) compounds with 4-phenylthiosemicarbazone 2-formylpyridine and sulfanylamides. The ABTS assay values were comparable for assessing other reference strains.

The study findings have proven that *Salmonella enterica (S.* Abony ГИСК 03/03 y) strain is able to provide antioxidant homeostasis, thus being less vulnerable to the action of compounds, which compared to other reference strains are considered as highly effective antibacterial substances.

CONCLUSIONS

1. The analysis of the study results have proven that the selected compounds might substantially reduce the total antioxidant capacity in the reference cultures studied.
2. Oxidative stress occurs within the reference cultures, namely, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ГИСК 8035, *Escherichia coli* ATCC 25922, *Shigella sonnei* ATCC 25931 and *Salmonella enterica* (*S.* Abony ГИСК 03/03y) under the action of novel antimicrobial chemical compounds.
3. The selected compounds might considerably reduce the total antioxidant capacity in the reference cultures studied. *Staphylococcus aureus* ATCC 2592 and *Bacillus cereus* ГИСК 8035 were found to be the most vulnerable to high-intensity oxidative stress, whereas *Salmonella enterica (S.* Abony ГИСК 03/03y) was the most resistant reference culture.
4. The specific activity of new chemical compounds on primary antioxidant enzymes, which refers as one of the pathogenic factors for disease-causing agents, allows assuming the *in vivo* effectiveness of the new native substances and thus, recommending the selected compounds for further biomedical tests.

CONFLICT OF INTERESTS

Nothing to declare.

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Date of receipt of the manuscript: 17/06/2020
Date of acceptance for publication: 22/07/2020

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

BIOLOGICAL SCIENCES

OPEN ACCESS

CURRENT ISSUES RELATED TO ACCESS AND USE OF INFORMATION ON *EX SITU* CONSERVATION OF PLANT GENETIC RESOURCES IN THE REPUBLIC OF MOLDOVA

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DOI: 10.38045/ohrm.2020.1.17 UDC: 631.52:004(478)

Keywords: *conservation, gene bank, plant genetic resources, information system, documentation.*

Introduction*. The rapid development of information technologies has provided challenges and opportunities for effective documentation of plant genetic resources (PGR). Access to information on PGR is essential for their conservation and sustainable utilization.*

Material and methods. *Information System on PGR in Republic of Moldova – ReGen was created to manage the data on PGR conservation at the national level and represents unified system that includes three basic functional blocks: ex situ, in situ/on-farm.*

Results. *The study resulted in an analysis of existing situation on information management of PGR ex situ conservation at national level, as well it highlighted the major challenges and constraints in this area. The priorities for ex situ conservation activities and related information were proposed.*

Conclusions. *An integrated approach of ReGen information system seems to be the most adequate way to increase the possibility to manage more efficiently the existing information about germplasm collections at the national level, make more easily and available the data for users and use the appropriate conservation strategies.*

Cuvinte cheie: *conservarea biodiversității, banca de gene, resursele genetice vegetale, sistem informațional, documentarea.*

PROBLEME ACTUALE PRIVIND ACCESUL ŞI UTILIZAREA INFORMAȚIEI CU REFERIRE LA CONSERVAREA *EX SITU* A RESURSELOR GENETICE VEGETALE ÎN REPUBLICA MOLDOVA

Introducere. *Dezvoltarea accelerată a tehnologiilor informaționale a oferit noi provocări și oportunități pentru documentarea eficace a resurselor genetice vegetale (RGV). Accesul la informațiile despre RGV este esențial pentru conservarea și utilizarea durabilă a acestora.*

Material și metode. *Sistemul informațional cu privire la RGV din Republica Moldova – ReGen a fost creat pentru a gestiona datele referitoare la conservarea RGV la nivel național, reprezentând un sistem unitar care include trei blocuri funcționale de bază: ex situ/in situ/on farm.*

Rezultate. *Studiul s-a finalizat cu analiza situației existente în domeniul gestionării informațiilor a RGV conservate ex situ la nivel național, a evidențiat provocările majore și constrângerile din acest domeniu. Sunt propuse prioritățile pentru activitățile de conservare ex situ și informațiile aferente acestora.*

Concluzii. *O abordare integrată a sistemului informațional ReGen este cea mai potrivită cale de gestionare eficientă a informațiilor aferente colecțiilor de germoplasmă vegetală existente la nivel național, ceea ce facilitează accesul utilizatorilor la datele de interes, precum și adoptarea de strategii de conservare adecvate.*

INTRODUCTION

Plant genetic resources for food and agriculture (PGRFA) constitute an integral component of biodiversity and are crucial for sustainable agricultural production.

The conservation of PGRFA in gene banks and the promotion of their sustainable use are essential for ensuring global food security and overcoming growing environmental challenges and climate change. PGRFA can be defined as "genetic material of plant origin of actual or potential value for food and agriculture" (1), that comprise modern cultivars, breeding lines and genetic stocks, obsolete cultivars, ecotypes, landraces (LR), crop wild relatives (CWR), as well as weedy races and primitive forms of crops (2). They are the raw material for breeding new plant varieties and constitute a reservoir of genetic diversity.

Today, the problem is that the genetic resources are not managed effectively, and the loss of important germplasm is documented. *Ex-situ* collections are currently kept in about 1750 gene banks worldwide, many of which are in poor physical condition and continue to be degraded as a result of insufficient funding. Another problem is that many of the accessions are duplicates that are a waste of valuable resources. Besides this, poor publicly available information about the stored materials was attested (3). In this context can be mentioned that despite the availability and accessibility of PGR the availability of information and the easy access to it are a necessary utilization prerequisite.

Information access is the ability to identify, retrieve, and use information effectively. Access to information is vital for social, political, and economic advancement. Traditionally, information has been disseminated in a variety of formats that have been widely accessible. However, advances in computer technology revolutionized information access, making vast stores of business, education, health, government, and entertainment information accessible on the World Wide Web (4).

Access to information on PGR is essential for their conservation. The importance of information in conservation and sustainable use of genetic resources is recognized in various global conventions, such as the Convention of Biological Diversity (1992) – Article 17: *Facilitate the exchange of information relevant to the conservation and sustainable use of biological diversity*; Global Plan Action (GPA 1 – 1996 and GPA II – 2011) – Priority Activities 17 & 15: *Constructing and stren-gthening comprehensive information systems for PGRFA* and International Treaty on Plant Genetic Resources for Food and Agriculture – ITPGRFA (2001) – Article 17: *Contracting Parties shall cooperate to develop and strengthen a global information system to facilitate exchange of information…related to PGRFA*.

The basic element of PGR data management in gene banks is the documentation systems. Nearly all gene banks created their own documentation system and computerized the information about their germplasm. The complexity of these systems varies widely and depends on the size of the collection, technical support, funding, and level of integration in PGR community etc. Small collections that allow simple documentation systems used Excel spreadsheets. However, once the collections grow, database management issues such as data integrity, data security or data processing become more important. Thus, more specialized computer database management software programs (*MySQL, Access* or *Oracle*) need to be used (5).

One of database software programs cover only passport data some include genebank management information, and others include characterization and evaluation (C&E) data (data on the distribution and use of the germplasm or other types of related information). Currently, information on PGR is concentrated on passport information that is usually rather similar, and consists of stand ard fields such as scientific name, common name, accession (variety) name, accession number, maintaining institute and country of origin etc. However, germplasm users are usually more interested in the germplasm traits. They would like to be able to select directly the material for the resistant, high yielding, drought tolerant, early maturing, and bright green or sweetest accessions. This information represents the evaluation and characterization data (C&E) and is rarely available in computerized forms of genebanks, so the information interpretation is difficult. Currently molecular marker data, information about QTLs and genes are becoming widely available. This is an important source of information for researchers, genebank staff, and plant breeders for identifying appropriate germplasm for breeding purposes. Farmers and

other users are also interested in genebank activities (6).

Currently, efforts are being made by the scientific community for promotion of open access to scientific information. Open access was initially developed for publications and journals, but now the concept is being extended (7). The accepted definition comes from the Berlin Declaration on Open Access, which states: "We define open access as a comprehensive source of human knowledge and cultural heritage that has been approved by the scientific community. Open access contributions include original scientific research results, raw data and metadata, source materials, digital representations of pictorial and graphical materials and scholarly multimedia material." Open access initiatives offer a great opportunity to make PGR information accessible to anyone, at any time and any place (8, 9). Thus, the information can be accessed through various web page, database etc.

A lot of institutions are encouraged to put their data on-line, promote open access to PGR information, improve the conservation initiatives, and share the data on their germplasm collections.

Recently, many institutions that maintain germplasm collections put the information concerning their holdings online. The information at the accession/sample/species level can be available at the following level (10):

- Genebanks (N.I.Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Russian Federation (www.vir.nw.ru/ data/dbf.htm), Centre for Genetic Resources, Plant Genetic Resources (CGN-PGR), the Netherlands (www.cgn.wur.nl/UK/CGN+ Plant+Genetic +Resources), Kew's Millennium Seed Bank, Wakehurst Place, UK (http://data.kew.org/ sid/about.html); Nordic Gene Bank (https:// sesto.nordgen. org/sesto/) etc.;
- National level (Informationssystem Genetische Ressourcen (GENRES), Germany (www.genres.de/pgrdeu), Japan National Institute of Agrobiological Sciences (NIAS) Genebank (www.gene.affrc. go.jp/ databases_en.php), USDA Agricultural Research Service, National Genetic Resources Program (NGRP), USA (www. arsgrin.gov), Nordic Countries (Denmark, Finland, Iceland, Norway, Sweden) (www. nordgen.org/ngb), National Inventory of Plant Genetic Resources for Food and Agriculture, Austria (www. Genbank.at) etc.;
- Regional/sub regional (EURISCO European search catalogue (https://eurisco. ipk-gatersleben.de/), ECPGR – European Cooperative Programme for Plant genetic resources with various Working Groups (https://www.ecpgr.cgiar.org/), The Southern African Development Community (SADC) countries (https://www.sadc.int/information-services) etc.;
- Global level (GENESYS (www. genesys-pgr.org), Global Biodiversity Information Facility (GBIF) (https://www. gbif.org/) etc.;
- Information at a metadata level (FAO World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS) – http://www.fao.org/ wiews/).

The information systems of these genebanks allow the user to browse, search and view information on their germplasm holdings.

According to Helmut Knüpffer (2007) the information on genebank collections belongs to other different levels (11).

- Collection-level data – metadata about genebanks and the germplasm collections they hold. Such information is available from the FAO WIEWS database etc.
- Accession-level data – information about individual accessions, especially passport data. Most PGR databases on various federation levels are based on accession-level data, such as individual genebanks (IPK'sLeibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany with on line information system GBIS, Nordic Gene Bank's information system SESTO, Germplasm Resources Information Network (USDA-GRIN – https://www.ars-grin.gov/), central crop databases, regional or international "multi-crop" information systems (EURISCO, SINGER etc.). In biodiversity informatics, this is called 'unit-level data'.
- Taxon-level data are related to (plant) species (or other taxa) as a whole, e.g., distribution information, uses, taxonomic classification. Examples of such databases related to crop plants are the taxonomic core of GRIN (U.S. Germplasm Resources Information Network) and Mansfeld's World Database of Ag-

ricultural and Horticultural Crops etc.

The rapid development of information technologies has provided challenges and opportunities for effective documentation of plant genetic resources (PGR). Access to the data from documentation system maintained by different genebanks is not easy, due to several causes. In this context especially, incompatible information systems and languages used by genebanks could be mentioned. One of the simplest starting points in data and information on PGR is to make an existing data available on Internet. An important activity for genebank management has become developing web pages and making the information related to PGR available on-line. Improved access to Internet contributed to sharing the information related to PGR stored germplasm in the genebanks at the national, regional, and global levels (12, 13, 14).

In the context of information management of PGR, a proper activity could result in more efficient application of GIS, statistical analysis, improved access to information and technologies, exchange of information, knowledge, best practices among the experts, enhanced communications through cost-effective electronic networks, construction of a common Internet platform for more efficient access to network national documentation systems, and links to global gateways for PGR information such as WIEWS, GENESYS, GRIN-Global, EURISCO, etc. These information systems were created for managing germplasm-associated information, facilitating genebank workflows, and providing a public interface for users to access and search for relevant information on plant genetic resources.

It is important to mention that, today, there is no single point of access to all genebank collections worldwide at the accession level, but germplasm data portals such as the WIEWS – FAO (World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture), EURISCO (European search portal), numerous Central Crop Databases (CCDBs), the Nordic Genebank (NGB, Northern Europe), and the CGIAR's System-wide Information Network for Genetic Resources (SINGER) among others, show that distributed data on genebank accessions can be reached at global and regional level (15).

Easy accessible, high-quality and comprehensive information about PGRFA is essential for gene banks management.

This paper was focused on current issues of access to PGR data and described ongoing efforts to address these challenges in the Republic of Moldova.

MATERIAL AND METHODS

At national level, there is no program in the field of conservation and use of PGRFA. Thus, it is very difficult to analyze the real situation in area of documentation of PGR at national level, and the mechanism of access and sharing of information related to PGR do not exist. However, in order to monitor and manage the information of PGR at national level, *ReGen* system was created.

The present Information System on plant genetic resources in Republic of Moldova – *ReGen* has been developed since 2002 in the Center for Plant Genetic Resources of Moldova, now Laboratory for PGR of Institute of Genetics, Physiology and Plant Protection. For elaboration of *ReGen* was used programming language *Visual Fox Pro 9.0*. The system was set up for operation system Microsoft Windows 2000 and XP.

This system, created to manage the data on PGR conservation at the national level, represents unified information system that includes three basic functional blocks: *ex situ* (seed collections), *in situ* – maintenance of plant genetic resources in natural habitats, and data on crops and crop varieties grown *on-farm*.

When genetic resource is included into the collection, the national accession number is assigned to each accession as the unique identifier. This number is common for all three parts of *ReGen*.

Data on PGR in a relational database is organized into tables to record the passport, characterization and evaluation datasets. In tables, by indexation, certain relationships that allow fast search of necessary information can be established. Databases containing specific information can be managed separately, or linked when is necessary, to combine information from different database.

Top priority in database system was placed on the passport data as it contains fundamental information related to each germplasm managed by the Laboratory. The passport part includes the basic information on a genetic resource: taxonomy, cultivar name, country of origin, geographical coordinates, status of sam ple, year of

acquisition, breeder and donor institution, etc. Many passport data are coded (institute code, donor code etc.) and all necessary coding tables are incorporated in the passport. Passport data correspond to Multi Crop Passport Descriptors (MCPD) elaborated by the Bioversity International and FAO, and revised in 2017.

Characterization and evaluation descriptors are crop-specific and are described according to Bioversity International, i.e. specific lists of descriptors for the genus with rules for the scoring of each trait.

ReGen is an important source of information for researchers, plant breeders, gene bank staff, farmers etc.

This system is not available on line due to the lack of adequate financial resources, but the datasets on germplasm conservation at the national level are included in big information system at international level such as EURISCO, WIEWS and the date on 6415 accessions conserved *ex situ* was reported in indicator 2.5.1. "*Number of plant and animal genetic resources for food and agriculture secured in either medium or long-term conservation facilities*" for Sustainable Development Goals. In 2017 National Information Sharing Mechanism (NISM) – FAO was established, and the data from our country were published in the NISM reporting system.

The aim of this research is the analysis of the existing situation in field of information management of germplasm collections conserved *ex situ* at national level and put in evidence the possible ways of accession to necessary data from existing *ReGen* documentation system for various purposes. Moreover, information sharing mechanisms between institutions holding *ex situ* germplasm collections will be highlighted in order to develop an integrated system for PGR documentation in our country.

RESULTS

In the Republic of Moldova there are five holder institutions of *ex situ* collections: Institute of Genetics, Physiology and Plant Protection (IGPhPP), through the Laboratory of PGR; Scientific-Practical Institute of Horticulture and Food Technologies (SPIHFT); The Botanical Garden (Institute), Porumbeni Institute of Plant Growing and Selectia Research Institute of Field Crops.

The *ex situ* collection of the Laboratory for PGR is stored at medium-term conditions (+4°C) and cover most crops that were cultivated in Moldova. The largest part of collection is presented by cereal crops such as wheat, maize, barley and triticale, vegetables such as tomatoes, chickpeas, soy beans, beans, and Vigna. These cultures are presented by varieties, hybrids, lines, mutants, local forms, etc.

In the Botanical Garden are preserved active collections and it ensures the medium-term conditions for seed. BG conserves *ex situ* about 10 000 plant species, of which 1200-1300 are food, fodder, aromatic, and spice species.

The collection of fruit crops is kept in the ISPHFT, being represented by apple, plum, apricot, pear, quince, cherry, cherry plum, walnut, almond, etc. The collection is represented by vast advanced/improved cultivars of local and foreign breeding materials, and an important role is assigned the old local forms.

The *ex situ* collection of grapevines is preserved in ISPHFT and is represented both by local varieties and new interspecific and intraspecific breeding.

Porumbeni Institute of Plant Growing stored a huge collection of maize, tobacco, etc. Thus,
four types of collections can be mentioned: varieties collection – 270, mutants – 250, collection of lines – 300 and sources for cytoplasmic androstirility – 200. *Zea mays* and *Nicotiana tabacum* are the two species that focus on *ex situ* conservation at Porumbeni Institute.

The collection of the Selectia Research Institute of Field Crops is presented by the cereal crops – wheat, barley, millet; leguminous and fodder crops-radish, soybeans, beans, sunflower, sugar beet etc.

It is important to mention that each holder institutions of *ex situ* collections have individual method and format to store the information on their collections. So, due to this fact the activities in field of PGR documentation are very difficult and access to this information is limited and the sharing of germplasm data is limited too. Only some of mentioned institutions such as Scientific-Practical Institute of Horticulture and Food Technologies, Porumbeni Institute of Plant Growing have been exported a small part of their datasets in *ReGen.*

A lot of information is documented manually in the registers and only a small part of these data have been computerized. This situation is similar in practically all *ex situ* collection holders in the country.

Thus, at national level, the exchange of information on stored germplasm collections practically does not exist, and there is also no unique form for data storage.

The number of accessions conserved *ex situ* under medium-term condition in the Laboratory PGR for that are included in database is 4405 accessions. In particular, passport data were recorded, according to MCPD and only 4% of characterization and evaluation data were doc umented.

The grapevine collection is fully documented. For this purpose, a grapevine database consisting of 1910 samples was developed. The entire *Vitis* collection is presented in the *ReGen* database by the passport data.

The collection of Porumbeni Institute of Plant Growing consists of 10,000 accessions. The total data on this impressive collection is not available for other institutions. In the *ReGen* database the data of 285 maize accessions from this institution are documented. Also, data on the fruit collection from ISPHFT are stored in *ReGen* and consist of passport data on 280 accessions of apple, apricot, plum, and pear, cherry (tab.1).

Table 1. The number of accessions stored in the institutions holding *ex situ* germplasm collections.

Institution holding *ex situ* collections	Crop	Nr. of accessions
Institute of Genetics, Physiology and Plant Protection	Wheat	487
	Triticosecale	70
	Maize	191
	Tomato	671
	Chickpea	101
	Soybean	482
	Common bean	408
	Pea	169
	Pepper	226
	Eggplant	46
	MAP	994
Porumbeni Institute of Plant Growing	Maize	10,000
	Tabacco	-
Scientific-Practical Institute of Horticture and Food Technologies 1. **Laboratory Gene pool, Breeding and Genetics of fruit trees** 2. **Laboratory Grapevine Genetic resources and breeding**	Apricot	50
	Plum	75
	Common pear	20
	Apple	104
	Cherry	14
	Grapevine	1910
Selectia Research Institute of Field Crops	Wheat	430
	Barley	102
	Leguminous and fodder crops	250
	Beans	220
	Soybean	550
	Sunflower	600
	Sugar beet	120
Botanical Garden (Institute)	Different species	10,000

The information on germplasm collections conserved within holder institutions are shared at national, regional, and international levels. Information on PGR *ex situ* collections at national level is available through EURISCO and WIEWS – FAO.

The European Search Catalogue for Plant Genetic Resources (EURISCO) provides information about 2,023,530 plant accessions comprising 6393 genera and 43,230 species preserved by almost 400 institutes, based on a network of National Inventories of 43 member

countries (16). In 2019, the data of the National Inventory (NI) of Moldova, now comprising 6015 accessions, have been updated in EURISCO.

Many of the present data exchange mechanisms in use in the genebank community rely on laborious and repeated transformations of the original genebank data set into the agreed standard formats. In order to update the datasets on *ex situ* collections in EURISCO is need to comply with the EURISCO data exchange format, based on the Multi-Crop Passport Descriptors.

Thus, the information consisting of passport data from NI of Moldova, in accordance with the revised MCPD (2017) were updated.

The National Information Sharing Mechanism (NISM), on PGRFA in the country, has been established with the FAO support.

FAO WIEWS Reporting System, consisting of the 63 indicators from 18 Priority activities and reporting format, adopted by the FAO Commission on Genetic Resources for Food and Agriculture, the National Information Sharing Mechanism (NISM), which serves as a monitoring tool of the implementation of the Global Plan of Action (GPA), in Republic of Moldova, has been established in 2017. In this context, various activities have been carried out. In order to gather relevant information on state of PGRFA in RM, collaboration between national stakeholders was initiated. As part of the Moldavian National Information Sharing Mechanism on PGRFA, the obtained data were analyzed, compiled, revised, and recorded into the FAO WIEWS Reporting system. Dataset on 1443 accessions stored in the *ReGen* was published in NISM Reporting system that corresponds to the required information.

Furthermore, FAO WIEWS requests, on a regular basis, updated subsets from all genebanks worldwide. The requested format for these subsets is roughly based on the MCPD standard. The record level data unit is however different, as WIEWS request metadata on stratified groups of genebank accessions, rather than the accession level data have been requested by EURISCO, ECCDBs and SINGER.

In 2018, with the reference to indicator 2.5.1 "*Number of plant and animal genetic resources for food and agriculture secured in either medium or long-term conservation facilities*" for Sustainable Development Goals, the data on 6415 accessions conserved *ex situ* at national level was reported, but in 2019, as a result of database updating the dataset on 6015 accessions was exported in WIEWS database.

Distributed germplasm information system will make updated germplasm information more easily accessible to plant breeders, crop scientists and other users, thus providing better access to plant material.

DISCUSSIONS

The Republic of Moldova is part of the UN Convention on Biological Diversity of 1995 and ratified its two Protocols – the Cartagena Protocol on Biosafety (2003), including the Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety and the Nagoya Protocol on Access and Benefit Sharing (2016), and signed International Treaty on Plant Genetic Resources for Food and Agriculture (2001) and the requirement of this official agreements need to be respected. But actually, the lack of easy access to germplasm information at the national level remains an important bottleneck for utilization of plant genetic resources material.

The problem that needs to be solved is insufficient interinstitutional collaboration in the field of exchange of information. Implementation of general information standards on PGR documentation at national level will ensure the interoperability of genebank data sets within institutions holder of *ex situ* collections in the country. In order to achieve this goal, one of precondition is the existence of easily accessible, good quality and comprehensive data on conserved germplasm. Only the collaboration of all *ex situ* holder institutions will contribute to the initiation of a unified database at the national level.

Major challenges and constraints of activities in the field of PGR documentation can be summarizes as:

- There is no collaboration between all maintainers of germplasm;
- All relevant information on *ex situ* collections was not documented in the unique database;

- The lack of common standard format on collection's information of all institutions involved in PGR conservation;
- Historical gaps in the stored infor mation on PGR;
- Insufficient well-trained staff in documentation of PGR;
- Insufficient financial resources for database maintenance.

At the level of scientific institutions, the capacity of supporting *ex situ* collections decreases annually, due to the reduction of financing support from the state budget, the lack of human resources, especially the young specialists, and the lack of performance equipment.

The lack of auxiliary staff, especially field workers, as well as those involved in other activities related to the preservation of *ex situ* collections represents a considerable problem. They are also missing in the field of RGV documentation, and the data on existing collections in each institution were recorded partial, especially in computerized form. There is no collaboration in area of sharing information at national level. Another problem is the professional training of the employees. Thus, it is important to mention the need to train staff in order to make the conservation activities more efficient.

The situation in the Botanical Garden is quite complicated. Supporting of existing *ex situ* collections requires considerable funding, which, being modest, was significantly reduced. Similar situations are attested in other institutions, which lead to the diminution of PGRFA conservation activities and the loss of accessions from existing collections.

One of the problems of Laboratory for PGR of IGPhPP, is the technical modernization of the block for long-term conservation. One part of the equipment was procured from the SeedNet project in 2007, other part with the FAO support in 2017. However, it is still necessary to solve several technical and organizational moments that cannot be done due to some subjective considerations.

The following priorities for *ex situ* conservation activities are proposed:

- Support to existing holder institutions of *ex situ* collections should be strengthened with particular reference to their modernization;
- Regeneration activities should be improved for maintaining the collected germplasm and safeguarding against their loss;
- The support should be ensured in terms of trained staff and finance, particularly for active collections to prevent their loss;
- *Ex situ* conservation system should be developed with the involvement of local farmers/people so: the collection of indigenous germplasm can be strengthened; and information on local knowledge and practices, as well as information on the uses of indigenous PGR can be gathered, documented and preserved.

ReGen is an information system that aims to document PGR at national level. But in the absence of the national program, there is no interinstitutional collaboration in this area. Most of the information on germplasm collections from holder institutions is not included in this database. Thus, it is necessary to develop an official information-sharing mechanism among the institutions, holders of *ex situ* collections with the direct involvement of Ministry of Agriculture, Regional Development and Environment that will coordinate the activities related to access and share the information on PGR.

CONCLUSIONS

1. An integrated approach of the *ReGen* information system on PGR that includes three basic functional blocks that refer to the existing information on *ex situ, in situ/on farm* conservation in the Republic of Moldova seems to be the most adequate way to increase the possibility to: manage more effectively the existing information about germplasm collections at the national level; make more easy and available the data that interest breeders and researchers; and use the most useful conservation strategies.
2. The efficient and effective conservation and sustainable use of the world's plant genetic resources for food and agriculture depends on reliable and accessible information about the resource. An evolving international regulatory framework for assessment and sharing of PGRFA benefits in

cludes the information dimension that needs to be put in evidence worldwide, because "*the information determines the value of germplasm collections".*

3. Access and sharing of information which are essential for plant conservation research are often lacking and it is essential to solve this situation as soon as possible.

CONFLICT OF INTERESTS

The authors do not declare any conflict of interest.

ACKNOWLEDGEMENT

Activities of this research study were performed in the framework of the Project: "Support to the development of National Programme for Plant Genetic Resources for Food and Agriculture in Moldova", Project number: TCP/MOL/3504, jointly funded by FAO. Special thanks to my colleagues from Ministry of Agriculture, Regional Development and Environment, National Agency for Food Safety (ANSA); State Commission for Variety Testing Plant; the National Federation of Farmers from Moldova; from scientific institutions involved in PGR conservation: Scientific-Practical Institute of Horticulture and Food Technologies (SPIHFT); the Botanical Garden (Institute) (BG); *Porumbeni* Institute of Plant Growing (*Porumbeni*) etc., Selectia Research Institute of Field Crops, and the staff of the Laboratory of PGR of Institute of Genetics, Physiology and Plant Protection, who participated in the realization of this study.

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Date of receipt of the manuscript: 26/04/2020
Date of acceptance for publication: 20/07/2020

OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 1, ISSUE 2
2020

CASE PRESENTATION – STUDIU DE CAZ – PRESENTATION DE CAS CLINIQUE – ПРЕЗЕНТАЦИЯ СЛУЧАЕВ ИЗ КЛИНИЧЕСКОЙ ПРАКТИКИ

MEDICAL SCIENCES

OPEN ACCESS

CHALLENGING DIAGNOSIS: COEXISTENCE OF TWO RARE DISEASES – FAMILIAL MEDITERRANEAN FEVER AND LOYEZ-DIETZ SYNDROME TYPE 3

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DOI: 10.38045/ohrm.2020.1.18 UDC: [616.928.8+616.132-007.64]-056.7-07

Keywords: *autoinflammatory diseases, familial Mediterranean fever, children.*

Introduction*. Autoinflammatory diseases are a group of genetically inherited disorders and familial Mediterranean fever is the most common of this group. It is rare in other than Middle East populations. Clinical manifestations of FMF are attacks of fever usually shorter than 24 hours, associated with arthritis, pleuritic chest pain, and abdominal pain.*

Case presentation*. A 15-year-old female patient was included in the study. She complained of recurrent episodes of fever associated with arthritis and abdominal pain. Moreover, the patient presented dysmorphic features like hyperthelorism, prognathia, scoliosis, pectus carinatum, and hypermobility syndrome. The laboratory exam revealed mutations in both MEVF and SMAD 3.*

Conclusions. *An autoinflammatory disorder should be suspected in any patient who has a history of recurrent fever. The attack patterns of FMF varies not just in different patients, but also in the same patient. Mainstay of treatment is colchicine that significantly improves the prognosis of patients with FMF.*

Cuvinte cheie: *boli autoinflamatorii, febră mediteraneană familială, copii.*

DIFICULTĂȚI DIAGNOSTICE: COEXISTENȚA A DOUĂ BOLI RARE – FEBRA MEDITERANEANA FAMILIALĂ ȘI SINDROMUL LOEYS-DIETZ TIP 3

Introducere. *Bolile autoinflamatorii constituie un grup de maladii determinate de activarea aberantă a căilor inflamatorii. FMF este cea mai frecventă afecțiune autoinflamatorie. Cu excepția țărilor din Orientul Mijlociu, FMF se întâlnește rar. Manifestările clinice includ episoade febrile cu o durată ce nu depășește 24 ore, fiind asociate cu artrită, durere abdominală și de tip pleuritic.*

Prezentarea cazului*. Pacientă de 15 ani, inclusă în studiu. S-a adresat cu acuze de episoade febrile recurente, asociate cu artrită și dureri abdominale. La examenul clinic pacienta prezenta dismorfisme: hipertelorism, prognație, pectus carinatum, sindrom de hipermobilitate. La examenul de laborator au fost depistate mutații în genele MEVF și SMAD 3.*

Concluzii. *Un sindrom autoinflamator va fi suspectat la pacienții cu istoric de febră recurentă. Patternul atacurilor în FMF este variabil nu doar la diferiți pacienți, ci și în cazul aceluiași bolnav. Baza terapiei este colchicina, care a ameliorat substanțial prognosticul pacienților cu FMF.*

INTRODUCTION

Autoinflammatory diseases are a group of genetically inherited disorders, caused by inadequate activation of inflammatory pathways it the absence of antigen directed autoimmunity. Periodic fever is the most common manifestation (1, 2).

An autoinflammatory disorder should be suspected in any patient who presents a history of recurrent fever over years and months. Most patients present first symptoms in early childhood. The clinical picture include fever, rashes, serositis, arthritis, meningitis, and uveitis. Clinical patterns should be evaluated to check if it refers to an autoinflammatory condition [2]. Next, genetic tests should be carried out in order to confirm the presumptive diagnosis (3).

Familial Mediterranean fever is the most common autoinflammatory disease. Its prevalence is 1:250-1:1000 in Middle East population, but rare in other populations. The cause is the mutation in MEVF gene located on chromosome 16 (4).

The attack lasts 12-72, showing the following characteristics: fever, aseptic peritonitis, pleuritic thoracic pain, erysipel-like rash, headache (rare), orchitis (rare), and arthralgia/arthritis (children – oligoarthritis). During the febrile episode, a high number of neutrophils and acute phase reactants can be noted. The diagnosis is confirmed by genetic testing, although it remains a clinical one. It is based on a history of recurrent, self-limited attacks of fever and serositis that are prevented by colchicine (2, 4, 5).

CASE PREZENTATION

A 15-year-old female was admitted to the Rheumatology unit, complaining of a recurrent fever (2-6 times per month), up to 40°C, associated with nausea, vomiting, sometimes abdominal pain; fever commonly lasts no more than 24 hours and does not respond to fever medication. In addition, she complained of non-inflammatory joint pain, myalgia after mild physical exertion, fatigability and migraine.

The disease history revealed that the first febrile episode of unknown etiology and increased levels of acute phase reactants occurred during infancy. She had many episodes, interpreted as upper respiratory tract infections and urinary tract infections. Moreover, at the age of 4, the patient had the first episode of arthritis. At the age of 8 she was admitted to hospital for being suspected of peritonitis. At the age of 8, she was diagnosed with juvenile oligoarthritis, but the patient did not return for follow-up monitoring. At the age of 15, the patient presented again at the rheumatologist and a diagnostic plan was set up.

The patient was born from uneventful pregnancy, breastfed until 1 year of age and vaccinated according to the national schedule. Patient did not report any allergies.

Family history was negative for autoimmune, immunodeficiency or genetic diseases. Patient's mother was diagnosed with migraine.

The patient was clinically and paraclinically examined within the Rheumatology unit.

Patient's clinical examination revealed that she was underweight (BMI 16.6, -1.61 SDS), having astenic constitution and deformities of the thorax – pectus carinatum and thoracolumbar scoliosis. On the lower limbs – multiple excoriations and bruises (according to patient's mother the girl presented a delayed wound healing). On inspection, hypertelorism and prognatia were also revealed. Patient wore braces for orthodontic treatment. Musculoskeletal system exam revealed hypermobility syndrome (Beighton score of 8 points) and pes planus. Respiratory and cardiovascular examination were normal (including blood pressure – percentile 50 for age and height). No hepatomegaly or splenomegaly were revealed on abdominal exam.

The laboratory exam recorded normal values of differential CBC, biochemical test (renal function, liver panel, metabolic panel), and urine test. C-reactive protein was positive (6 mg/dL). Serum amyloid was 24 mg/L (normal value <10mg/L). The internal organ ultrasound imaging of kidneys and heart was normal. Antinuclear antibody screening test, as well as immunoblot for autoimmune disease were negative. Also, the immunoglobulin levels were assessed, and flow-cytometry was performed. The study findings are presented in Table 1.

Due to multiple stigma, a genetic test for conective tissue diseases was carried out. Also, considering the recurrent fever, the autoinflammatory panel was added. Finally, the patient was found positive to mutations Chr16:3293407T>C, p. Met694Val and Chr16:3293405T>C, p. Met694Ile on gene MEVF, both having pathologic significance.

Table 1. Immunoglobuline levels and flow-cytometry results.

	Prior to the treatment	After 3 months of treatment	Reference	Units
SAA	24	20	<10	mg/L
Lymphocytes	31.8	29.1	36-43	%
CD3+	78.98	78	66-76	
CD4+	54.61	54	33-41	
CD8+	19.79	20	27-35	
CD16+	3.71	6	9.9-22.9	
Monocytes	9.1	7.9	4-8	
Lymphocytes	1749	1775	2000-2700	cells/uL
CD3+	1381.4	1384	1400-2000	
CD8+	346.1	355	600-1900	
CD19+	243.5	284	300-500	
CD16+	64.8	106	100-500	
CD4/CD8	2.76	2.7		

Furthermore, a pathologically significant mutation in gene SMAD 3 – Chr15: 67477068G>C was revealed.

Therefore, based on EUROFEVER/PRINTO a diagnosis of familial Mediterranean fever was made. Furthermore, based on the presence of confirmatory mutation on the gene SMAD 3, a diagnosis of Loyez-Dietz syndrome was also established.

Treatment with colchicine 1 mg/24h was initiated. Over half a year, the attack intensity of markedly decreased, as well as their frequency.

DISCUSSIONS

Autoinflammatory diseases are a group of genetically inherited disorders, caused by inadequate activation of inflammatory pathways it the absence of antigen directed autoimmunity. Periodic fever is the most common manifestation. The spectrum of the autoinflammatory disorders is extending continuously, and now include not just Il-1 mediated diseases but also those that don't include fever as a major sign (1, 2).

An autoinflammatory disorder should be suspected in any patient who presents with a history of recurrent fever over years and months. Most patients present first symptoms in early childhood. The clinical picture include fever, rashes, serositis, arthritis, meningitis, uveitis. Clinical patterns should be evaluated to check if it is related to an autoinflammatory condition. Next, a genetic test should be ordered in order to confirm the presumptive diagnosis (1, 2, 3, 6).

Differential will include recurrent fever, malignancies, systemic onset juvenile idiopathic arthritis.

Familial Mediterranean fever is the most common autoinflammatory disease. Its prevalence is 1:250-1:1000 in the Middle East population, but rarely occurs in other populations. The cause is the mutation in MEVF gene located on chromosome 16. This mutation is a gain of function mutation, so it stimulates uncontrolled production of Il-1. It has both, autosomal recessive and autosomal dominant transmission (1, 3, 4).

Nowadays, there are around 300 mutations described; all of them are registered in the INFEVERS database. Not all the mutations are associated with FMF phenotype and its significance is not clear. The most common mutations that are associated with FMF are p.M694V, M694I, M680I and V726A. Mutations localized on 10th exon are considered to be the most severe. In our patient's case, there are two different mutations – p.M694V (Ancestry – Jewish Non Ashkenasi), M694I (Ancestry – Maghrebian), both located on 10th exon. Therefore, is expected to have a severe disease, with a chance not to respond to colchicine treatment (4, 6).

Clinical features. Usually, a patient with FMF presents the first symptom by the age of 20. The duration of the attack is 12-72 hours, showing the following characteristics: fever, aseptic peritonitis, pleuritic thoracic pain, erysipel-like rash, headache (rare), orchitis (rare), and arthralgia/arthritis (children – oligoarthritis). During the febrile episode, a high number of neutrophils and acute phase reactants can be noted. The attack patterns vary not just in different patients,

but also in the same patient. The mechanism that triggers the attack is unknown, but many patients complain of physical and psychological exhaustion, associated with the onset of the attack (2,4).

Eurofever/PRINTO Familial Mediterranean Fever criteria
Presence of a confirmatory MEVF genotype and at least one ot the above: • Duration of febrile episodes 1-3 days • Arthritis • Pleuritic pain • Abdominal pain **OR** **Presence of a nonconfirmatory MEVF genotype and at least two of the above:** • Duration of febrile episodes 1-3 days • Arthritis • Pleuritic pain • Abdominal pain
Sensitivity 0.94 *Specificity 0.95* *Accuracy 0.98*

The diagnosis is confirmed by genetic testing but mostly remains a clinical one. It is based on a history of recurrent, self-limited attacks of fever and serositis that are prevented by colchicine.

Treatment. Colchicine licenced by FDA is used for prophylactic use in case of FMF from the age of 4. Continuous use of colchicine prevents or reduces substantially the symptoms of FMF in at least 95% and almost completely excludes the risk of amiloidosis. The mechanism of action of colchicine is unknown, but most of patients have a good clinical response to doses 250 mg-2g/24h (7, 8).

Complications. The treatment is necessary to be continued throughout the whole life, and the prognosis is favorable, having a good life expectancy. Prior to use of colchicine, 60% of patients with FMF develop amiloidosis. Destructive arthritis is rare. Growth and fertility are nearly normal for both sexes (in case of treatment adherence) (5, 7, 8).

The described case is unique – there are no registered patients with FMF in the Republic of Moldova. Furthermore, our patient exhibited clinical features not just of an autoinflammatory syndrome, but also of a hereditary connective tissue disease, that made the clinical diagnosis even more complicated.

CONCLUSIONS

1. Autoinflammatory diseases are a group of genetically inherited disorders, caused by inadequate activation of inflammatory pathways. Familial Mediterranean fever is the most common autoinflammatory disease, with the highest prevalence among Middle East population that rarely occur in other populations.
2. Clinical manifestations of FMF are attacks of fever usually shorter than 24 hours, associated with arthritis, pleuritic chest pain, and abdominal pain. Pattern of attacks varies not just in different patients, but also in the same patient. The mainstay of treatment is colchicine that significantly improves the prognosis of patients with FMF.

CONFLICT OF INTERESTS

All authors declare no competing interests.

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Date of receipt of the manuscript: 23/06/2020
Date of acceptance for publication: 19/09/2020

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OH&RM ONE HEALTH & RISK MANAGEMENT
VOL. 1, ISSUE 2
2020

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

BIOSAFETY ASSOCIATION FOR CENTRAL ASIA AND THE CAUCASUS (BACAC) – REGIONAL COOPERATION

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During the Covid-19 outbreak, there are many questions related to biosafety/biosecurity, raised on how to handle human material, etc. Currently, there is no sufficient knowledge about the pathogenic potential and transmission risks for the novel 2019 Coronavirus, SARS coronavirus-2 (SARS-CoV-2), and its associated diseases. Therefore, biosafety associations worldwide can provide real assistance.

Biosafety Association for Central Asia and the Caucasus (BACAC) was founded in 2009. The initiative came me from Kazakh Scientific Center for Quarantine and Zoonotic Diseases (KSCQZD), and supported by different former Soviet anti-plague institutions.

Soviet Union operated a large and unique network of facilities, called the "anti-plague (AP) system," which main mission was to control life-threatening endemic diseases and to prevent the import of exotic pathogens from other countries. Though the name of the system was "anti-plague", it also studied other dangerous endemic and exotic diseases caused by bacteria or viruses. Throughout the Soviet era, the anti-plague system had worked effectively to prevent major epidemics, leading to death of Soviet citizens in regions, where diseases such as anthrax, brucellosis, plague, Crimean-Congo Hemorrhagic Fever (CCHF), tularemia, etc. are commonly endemic.

The former Soviet Union (FSU) republics shared a common legislation, including biosafety regulations on handling dangerous pathogens. However, after the Soviet Union collapse, the situation worsened and experts in biosafety found it necessary to establish regional biosafety associations.

The common issues related to Biosafety across Central Asia and Caucasus were as following:

- Biosafety was not developed as a separate area.
- The biosafety approaches differed from the international ones.
- Inadequate requirements when handling highly dangerous pathogens.
- Lack of staff responsible for biosafety.
- Inadequate training of personnel in the field of biosafety.
- Low wages and high employee turnover.
- Lack of equipment.
- Poor biosecurity management.
- Risk of unauthorized access to some institutional laboratories.
- Poor legal and regulatory frameworks regarding biosafety and biosecurity.

The BACAC Member countries initially included Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, Armenia, Azerbaijan, Georgia, Turkmenistan, Afghanistan and Mongolia. Today BACAC comprises about 100 members, representing public health, animal health, academia, engineering, and other fields, which is very productive for bringing together different views.

BACAC is member of the International Federation of Biosafety Associations (IFBA), partner – organization for European Biosafety Association (EBSA), American Biosafety Association (ABSA), sister-organization for Georgian Biosafety Association (GeBSA) and Azerbaijan Biosafety Association. Since its establishment, BACAC was supported by international donors and organizations, like Global Partnership Programme, Global Affairs Canada, Defense Threat Reduction Agency (DTRA), US Department of Defense, Ministry of Defense of United Kingdom, International Science and Technology Center (ISTC), Science and Technology Center of Ukraine (STCU), etc.

The major objectives of the Association are as follows:

- To promote biosafety as a regional priority.

- To integrate the best international practices for biosafety when handling infectious microorganisms.
- To assist members in the development of programs, guidelines, standards and regulations.
- To promote research and science development via modern biosafety and biosecurity methods.
- To develop and provide integral training programs.
- To promote biosafety cooperation with other international associations and societies.

Since its establishment, BACAC had held 6 conferences viz. the conference of founders, which included 155 participants from 16 countries and 6 Annual Conferences. During these conferences, there were conducted about 30 trainings, aiming to train more than 1200 specialists. Several biorisk management guidelines were published for various types of institutions. A workshop for Mongolian biosafety/biosecurity experts was conducted in 2014 in Ulaanbaatar, Mongolia, where 64 Mongolian specialists were trained.

In 2013 – 2014, BACAC faced some difficulties, regarding low participation and contribution to various BACAC activities, as well as a lack of adequate financial control mechanisms and accountability, etc. Thus, the International Federation of Biosafety Associations (IFBA), being supported by the UK MoD, implemented the BACAC Sustainability Project, which carried out an assessment of BACAC organizational diagnosis.

In November 18-21, the BACAC Sustainability Workshop was organized in Tbilisi, Georgia, being supported by IFBA and UK MoD, at which the BACAC members discussed challenges and their solutions. The workshop resulted in elaboration of the new Statute and Strategic Plan.

Mission

Spreading knowledge and building capacity for biosafety and biosecurity among professional groups and the population from Central Asia and the Caucasus.

Vision

Professional coordinator and agent for dissemination of knowledge regarding biosafety, employing international best practices, in the countries of Central Asia and the Caucasus.

Strategic Objectives

- To provide the application/implementation of biosafety international best practices among professionals within a region.
- To outline a common scheme/framework for dissemination of knowledge to the population within the region and facilitate awareness-raising on biosafety issues.
- To develop BACAC into a well-functioning organization, as to become a well-recognized leader in biosafety at the local and regional level.
- To establish and strengthen close cooperation with international organizations engaged in the field of biosafety.
- To collaborate with governments, in order to strengthen national and regional biosafety policies and programs.

On September 19-23th 2016, the ISTC in collaboration with BACAC and Regional Biosafety Training Centre (Dushanbe) delivered a regional CCHFV/Ebola Capacity Building Training Seminar for 42 leading and young scientists in the Tajik Research Institute of Preventive Medicine (TRIPM), Dushanbe, Tajikistan. The scientists included participants from Armenia, Azerbaijan, Georgia, Iran, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Tajikistan, Turkmenistan and Uzbekistan.

EU CBRN CoE/BACAC 6th Annual Conference "BACAC: Bridging the Gaps"

The European Union Chemical Biological Radiological and Nuclear Risk Mitigation Centers of Excellence Initiative (or EU CBRN CoE) (www.cbrn-coe.eu) were launched in response to the need to strengthen the institutional capacity of countries outside the European Union to mitigate CBRN risks. These risks may be created intentionally, accidentally or naturally.

The EU CBRN CoE aims to strengthen regional security by increasing local ownership, local expertise and long-term sustainability. The EU CBRN CoE is centred around a worldwide network of local experts and collaborating partners. Currently the Initiative covers 8 regions and 61 partner countries.

The EU CBRN CoE Regional Secretariat for Central Asia is located in Tashkent, Uzbekistan. It works in partnership with Central Asian EU CBRN CoE Partner Countries to encourage local ownership of CBRN action plans, policies and joint preparation of regional project proposals.

The European Union Chemical, Biological, Radiological and Nuclear Risk-mitigating Centers of Excellence Initiative (EU CBRN CoE) and the Biosafety Association of Central Asia and the Caucasus (BACAC) conference "BACAC: Bridging the Gaps" was conducted on 11-15 March 2019, in Tashkent, Uzbekistan. Over 170 participants from Central Asia, the Caucasus, Eastern and Western Europe, United States of America, and various international organization (WHO, OIE, IFBA, EBSA, UNICRI, UNOG, ISTC and STCU) attended the meeting.

The major purpose of the conference was to support cooperation and coordination at the regional and international level, as well as to stimulate discussions on strengthening policies, capabilities and capacities to mitigate Biosafety and Biosecurity risks.

The Conference covered the following topics:

- Harmonization of national regulatory frameworks with respect to the Biological Weapons Convention (BWC), the World Health Organisation's International Health Regulation (IHR), and Codex Alimentarius.
- Regional and international cooperation on:
  - emergency response to biological incidents (either accidental, natural or intentional);
  - disease surveillance, discussing challenges and solutions, including international laboratory standards;
  - improving training standards and supporting creation of regional networks of trainers.
- Outcomes and benefits of EU CBRN CoE Projects regarding Biosafety and Biosecurity, such as from EU CBRN CoE Projects 53, 61, 65 and 67.
- Biosecurity challenges that are being faced by institutions and countries.

Prior to the conference, 7 pre-conference training workshops were organized regarding Laboratory Quality Management Implementation, Grant Writing: Funder Perspective, The Anthropocene and Threats to Human Survival, Biological Spill Response Training, Laboratory Risk Assessment – How to Create a Safe, Sustainable Laboratory, Opportunities to Enhance the Implementation of the BWC, etc. About 300 subject experts from Partner Countries of the Regional Secretariat for Central Asia and other countries were trained. Moreover, the participants had opportunity to take IFBA certification exams.

EU CBRN CoE/BACAC 7th Annual Conference "Challenges of Biosafety/Biosecurity – Lessons Learned from Coronavirus Crisis"

After COVID-19 global crisis ceases or slows down, a thorough review of the lessons learned regarding preparedness, prompt response, and gaps analysis, etc. will be required. EU CBRN CoE/ Biosafety Association of Central Asia and the Caucasus (BACAC) conference might organize a good forum for bringing together representatives' experts from partner countries from different Regional Secretariats of EU CBRN CoE. This forum will be conducted in Tbilisi, Georgia during fall 2020-spring 2021, depending on the COVID-19 pandemic situation.

Date of receipt of the manuscript: 02/04/2020
Date of acceptance for publication: 22/05/2020

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

PROFESORUL OLEG LOZAN – PILONUL SĂNĂTĂȚII PUBLICE

Nu există activitate umană care ia loc în afara domeniului sănătății publice.

William Foege

Determinarea şi efortul, întrunite într-o persoană cu puternice valori morale, asigură calea spre o carieră profesională de succes. Acesta este, indubitabil, cazul Domnului Profesor Oleg Lozan, fondator şi director al Şcolii de Management în Sănătate Publică în cadrul USMF "Nicolae Testemiţanu".

Născut la 10 aprilie, 1970, în oraşul Bălţi, Republica Moldova, Oleg Lozan activează la USMF "Nicolae Testemiţanu" încă din anul 1994, imediat după absolvirea acestei instituţii. Între 1997 şi 2001 a activat în calitate de asistent universitar, iar mai apoi lector superior în cadrul Catedrei Sănătate Publică şi Management, în 2002 i s-a conferit titlul ştiinţifico-didactic de conferenţiar universitar, iar în 2014 de profesor universitar. Acest parcurs ascendent îi denotă perseverenţa înnăscută. Experienţa sa de predare în medicină numără peste 26 de ani şi se extinde la toate nivelele sistemului de educaţie superioară. Între 2013 şi 2019 este vice-rector în cadrul USMF "Nicolae Testemiţanu".

Numele Domnului Oleg Lozan va fi mereu asociat cu mândrie cu Şcoala de Management în Sănătate Publică a USMF „Nicolae Testemiţanu", instituţie pe care a iniţiat-o în 2003 şi în fruntea căreia se află până astăzi. Activitatea sa prolifică în cadrul Şcolii este o dovadă a spiritului inovator şi a consecvenţei, pe care le posedă, fiind consultant al PNUD şi UNICEF, precum şi expert OMS. În calitate de expert naţional a contribuit la implementarea diverselor proiecte internaţionale şi la elaborarea Strategiei Naţionale de Sănătate pentru perioadele 2014-2020 şi 2020-2030.

Activitatea sa publicistică numără astăzi peste 120 de lucrări ştiinţifice şi ştiinţifico-metodice. Apogeul acesteia fiind Premiul Prezidiului Academiei de Ştiinţe din RM pentru ciclul de lucrări „Aspecte medicosociale şi demografice ale sănătăţii populaţiei din Republica Moldova".

Domnul Oleg Lozan, în virtutea calităţilor sale de lider, este, de asemenea, membru al mai multor organizaţii naţionale şi internaţionale, printre care Asociaţia Şcolilor de Sănătate Publică din Regiunea Europeană – ASPHER şi Agenţia pentru Acreditare a Educaţiei în Sănătate Publică – APHEA. Domnul Lozan a fost director şi coordonator de proiecte susţinute de entităţi precum Comisia Europeană, Agenţia Elveţiană de Dezvoltare şi Cooperare şi OMS. Actualmente este Coordonator Naţional de Sănătate Publică în cadrul Comisiei Naţionale Extraordinare în Sănătate Publică.

În perioada 2008-2009 a deţinut funcţia de viceministru al Sănătăţii şi de Medic-Şef Sanitar de Stat în cadrul Guvernului Republicii Moldova. În 2015 i-a fost acordat Premiul Naţional al Republicii Moldova pentru lucrările ştiinţifice asupra implementării noului concept de sănătate publică.

Mulţi ani prosperi, Domnule Profesor!

Cu profund şi deosebit respect, consiliul editorial al Revistei *One Health & Risk Management*

LA MULȚI ANI, DOAMNĂ PROFESOR OLGA TAGADIUC!

Odată trasat drumul, nu se poate să nu mergi înainte.

Antoine de Saint-Exupery

Talentul şi erudiţia, obiectivitatea şi exigenţa, curajul şi tenacitatea, pasiunea şi dăruirea de sine sunt doar câteva dintre calităţile ce concertează pe fundalul imaginii Doamnei Profesor Olga Tagadiuc.

Doamna Profesor Olga Tagadiuc este un exemplu viu de dedicaţie profesională şi perseverenţă, precum şi o imagine clară a faptului că, efortul continuu se încununează cu succes.

Născută în Chişinău pe 25 septembrie 1965, excelenţa academică i-a caracterizat de-a lungul timpului toate etapele formării profesionale. A absolvit Liceul Teoretic „Ion Creangă" cu medalie de aur şi Facultatea de Medicină Generală la Universitatea de Stat de Medicină şi Farmacie „Nicolae Testemiţanu" cu Diplomă de menţiune, cele mai înalte distincţii oferite absolvenţilor celor două instituţii de învăţământ. Iar între anii 1989 şi 2004 a fost doctorandă la Catedra biochimie, experienţă încununată cu susţinerea tezei de doctor în ştiinţe medicale.

Omul cât trăieşte - învaţă, maximă ce descrie perfect ascensiunea academică a Doamnei Tagadiuc, care în 2013 şi-a obţinut diploma de Masterat în Managementul Sănătăţii Publice, iar ulterior a participat la numeroase seminare, stagieri şi întâlniri de experţi, la nivel naţional şi internaţional.

Cunoştinţele acumulate şi-au găsit aplicaţii vaste în cadrul carierei profesionale prolifice a Doamnei Tagadiuc. Începând de la poziţia de asistent universitar, între anii 1992-1998, în cadrul Catedrei biochimie a USMF „Nicolae Testemiţanu", urmată de cea de conferenţioar universitar, mai apoi, din 2000 până în 2005, continuându-şi activitatea în calitate de şef de studii al Catedrei de biochimie şi biochimie clinică ale aceleiaşi instituţii. De atunci şi până în 2012 s-a dedicat cercetării în cadrul Laboratorului Ştiinţific de Biochimie al Universităţii, iar între 2015 şi 2019 a fost Coordonator al Secţiei de ştiinţe medicale a AŞM.

Din 2012, Doamna Tagadiuc se află în fruntea Catedrei de biochimie şi biochimie clinică a Universităţii, în 2018 este numită vice-rector pentru programele de Doctorat şi Postdoctorat în cadrul USMF „Nicolae Testemiţanu", iar din 2020 – Director general al Agenţiei Naţionale pentru Cercetare şi Dezvoltare. Aceste ascensiuni profesionale constituie rezultatul efortului şi al dedicaţiei investite în cariera sa academică şi profesională.

Totodată, a participat la elaborarea manualelor, lucrărilor metodico-didactice şi a publicaţiilor ştiinţifice; precum şi la numeroase consilii şi seminare, atât în calitate de expert, dar şi de consultant, recunoscut la nivel internaţional.

Mulţi ani prosperi, Doamnă Profesor!

Cu profund şi deosebit respect, consiliul editorial al Revistei *One Health & Risk Management*

VASILE DUMITRAŞ – MEDIC MILITAR DE CARIERĂ, PEDAGOG ISCUSIT

În condiţii de campanie, în deosebi, succesul ori eşecul în sprijinul medical al trupelor îi revine administraţiei medico-militare.

Nicolai Pirogov

Meseria de medic militar poate părea neprivilegiată în plan economic. Însă pe lângă umanismul meseriei de medic, ea include şi înaltul ideal de dragoste faţă de Patrie, dorinţa de a o apăra. Ea, de asemenea, presupune ferma hotărâre de a depune toată străduinţa pentru a salva ce este mai scump pentru Ţară - viaţa cetăţenilor ei. Domnul colonel medic Vasile Dumitraş şi-a dedicat întreaga carieră medicinei militare şi medicinei calamităţilor, ramuri înrudite ale medicinei.

Născut la 8 octombrie 1950 în satul Cetireni, raionul Ungheni, Republica Moldova, într-o familie de ţărani cu patru copii, încă din copilărie a fost pasionat de domeniul militar şi a fost educat în spiritul bărbăţiei, disciplinei, stimei şi respectului faţă de oamenii muncitori.

În anul 1967 a fost înmatriculat la Institutul de Stat de Medicină din Chişinău. După anul IV îşi continuă studiile la facultatea medico-militară din Kuibâşev (astăzi Samara, Federaţia Rusă).

Spiritul de a avansa în cariera de medic militar şi de a-şi perfecţiona cunoştinţele, l-a determinat să-şi continue studiile la Academia Medico-Militară din Leningrad (Sankt-Petersburg). După absolvirea Academiei (1982) i se conferă specializarea „Ofiţer cu studii militare superioare – manager în medicina militară". Timp de patru ani a activat în funcţie de comandant (şef) de spital militar cu capacitatea mai mult de 1000 de paturi, care asigura tratamentul spitalicesc a Armatei a 40-a din Afganistan.

Din anul 1986 până în prezent activează în calitate de şef Catedră de medicină militară şi a calamităţilor. În anul 1995 susţine teza de doctor în ştiinţe medicale cu tema „Argumentarea ştiinţifică a conceptului de tratament şi evacuare la trupele Armatei Naţionale în campanie". În anul 1996 i se conferă titlul ştiinţifico-didactic de conferenţiar universitar.

Pe parcursul activităţii în funcţie de şef de catedră (din anul 1992 până în prezent) au fost formaţi peste 1000 de ofiţeri medici în rezervă, inclusiv ofiţeri medici pentru Forţele Armate ale Republicii Moldova. Sub îndrumarea domnului Vasile Dumitraş au fost elaborate 8 proiecte de masterat prin cercetare şi două teze de doctor în ştiinţe medicale. A publicat peste 50 de lucrări ştiinţifice şi ştiinţifico-didactice.

Pentru merite deosebite în activitatea de medic militar a fost decorat cu insigna „Eminent al Ocrotirii Sănătăţii", 5 medalii în Armata Sovietică şi 5 medalii în Armata Naţională, Ordinul „Credinţă Patriei" de clasa I, inclusiv diplome de onoare ale Ministerului Apărării şi Ministerului Sănătăţii.

Mulţi ani prosperi, Domnule Profesor!

Cu profund şi deosebit respect, Consiliul editorial al Revistei *One Health & Risk Management*

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.

For more information see: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

CERINŢE PENTRU AUTORI

Reguli de tehnoredactare
Pregătirea manuscrisului (elaborat în limbile română, engleză, franceză şi rusă) va fi în conformitate cu instrucţiunile 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, spaţiat la interval 1,5, aliniere justificată, câmpurile 2 cm pe toate laturile. Toate paginile trebuie să fie numerotate consecutiv (în colţul de jos, în partea dreaptă) şi să includă numerotarea continuă a paginilor. Abrevierile trebuie să fie explicate la prima apariţie în text şi nu trebuie utilizate excesiv. Manuscrisele nu trebuie să depăşească (fără a număra titlul, afilierea, rezumatul şi referinţele): pentru articole de sinteză/referate – 4500 de cuvinte; pentru articole de cercetare – 3000 de cuvinte; pentru opinii ale experţilor – 2500 de cuvinte; prezentare de caz şi imagini din practica clinică/laborator – 1700 de cuvinte; note experimentale şi clinice – 1300 de cuvinte; recenzii şi prezentări de carte – 2000 de cuvinte; articole didactice – 4000 de cuvinte. Volumul tabelelor şi figurilor nu trebuie să depăşească ⅓ din volumul manuscrisului. Revista îşi rezervă dreptul de a face orice alte modificări de formatare. Manuscrisele respinse nu sunt returnate.

Toate manuscrisele transmise spre publicare trebuie să fie însoţite de două rezumate: în limba de origine al articolului şi în limba engleză.

Titlul şi autorii
Titlul ar trebui să fie cât mai scurt posibil (maximum - 120 de semne cu spaţii), elocvent pentru conţinutul manuscrisului. Numele autorilor vor fi scrise deplin: prenume, nume de familie (*ex:* Ion RUSU). Afilierea va include: Secţia/Departamentul/Catedra, Universitatea/Spitalul, Oraşul, Ţara pentru fiecare autor. Se vor menţiona obligatoriu, mai jos, datele autorului corespondent şi informaţiile 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 cerinţele mai jos)
- **INTRODUCERE**
- **MATERIAL ŞI METODE**
- **REZULTATE**
- **DISCUŢII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULŢUMIRI ŞI FINANŢARE**
- **REFERINŢE**

Rezumatul va conţine până la 1600 de semne cu spaţii şi va cuprinde:

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

În rezumat nu vor fi incluse tabele, grafice şi note bibliografice; informaţii 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 însoţită de titlu şi legendă. Ele vor fi numerotate cu cifre arabe şi vor fi menţionate în text în paranteze (*ex:* fig. 1). Titlul (*ex:* Figura 1) şi 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 însoţită de titlu. Tabelele vor fi inserate în text, fără a depăşi lăţimea unei pagini. Ele vor fi numerotate cu cifre arabe şi vor fi menţionate în text în paranteze (*ex:* tab. 1). Titlul tabelului va fi poziţionat deasupra tabelului centrat (*ex:* Tabelul 1).

Referinţele trebuie să fie numerotate în ordinea apariţiei în text. Citarea sursei de referinţă va fi conform stilului *Vancouver*, plasată la sfârşitul articolului şi va include doar referinţele citate în text (menţionând numărul de referinţă în paranteză rotundă). Dacă aceeaşi referinţă este citată de mai multe ori, ea va fi trecută în text cu acelaşi număr ca la prima citare. Numărul total de referinţe nu va depăşi 50 de surse. Acurateţea datelor ţine de responsabilitatea autorului.

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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 – 4500 mots ; pour les articles de recherche – 3000 mots ; pour les opinions d'experts – 2500 mots ; présentation de cas et photos de la pratique clinique/de laboratoire – 1700 mots ; notes expérimentales et cliniques – 1300 mots ; commentaires et présentations de livres – 2000 mots ; articles pédagogiques – 4000 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 1600 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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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 – 4500 слов; для научных статей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клинической/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 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 1600 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. Источники 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

Conceptul *One Health*

Sănătatea umană

OMS a definit în 1946 sănătatea ca fiind „o stare pe deplin favorabilă atât fizic, mintal cât şi social, şi nu doar absenţa bolilor sau a infirmităţilor", cu o completare ulterioară „capacitatea de a duce o viaţă productivă social şi economic".

Sănătatea animală

OIE defineşte bunăstărea animalelor în 2008: un animal este în bună stare dacă este sănătos, se bucură de confort, este bine hrănit, se află în siguranţă, poate să îşi manifeste comportamentul înnăscut (natural) şi nu suferă din cauza unor stări neplăcute, precum durere, frică şi stres.

Sănătatea plantelor şi mediului

Sănătatea mediului se referă la acele aspecte ale sănătăţii umane ce includ calitatea vieţii determinată de factorii fizici, biologici, socio economici şi psiho sociali din mediul ambiant. Interrelaţiile omului cu mediul preocupă medicina, atunci când un sistem ecologic este în stare de echilibru, prevalează starea de sănătate a populaţiei.

La nivel global conceptul *One Health* este o strategie mondială de extindere a colaborărilor interdisciplinare şi a comunicărilor în toate aspectele legate de îngrijirea sănătăţii oamenilor, animalelor domestice sau a faunei sălbatice, care nu mai poate fi abordată separat ci doar în comun.

One Health se referă nu numai la preocupările legate de bolile ce apar la oameni şi animale, ci şi la aspecte legate de stilul de viaţă, dietă, exerciţiu, impactul diferitelor tipuri de relaţii om-animal şi expuneri de mediu care pot afecta ambele categorii populaţionale. Pentru a se atinge efectele scontate este nevoie şi de o educaţie a populaţiei care să conştientizeze factorii de risc şi beneficiile prevenţiei, dar şi de comunicare şi înţelegere între pacienţi şi furnizorii de servicii de sănătate.


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